DISEASES OF SWINE  10TH EDITION
10TH EDITION
DISEASES OF SWINE

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Editors’ Note

Howard Dunn envisioned *Diseases of Swine* to be a “complete and up-to-date reference on swine” when he and Iowa State University Press released the first edition in 1958. The eight subsequent editions followed the course set by Dunn and provided the definitive resource on swine health for generations of veterinarians and animal health professionals. Our hope is to continue this tradition in the 10th edition of *Diseases of Swine*.

Our mission has been to provide a comprehensive yet concise reference on infectious and noninfectious diseases of swine for use by our colleagues—the veterinarians, veterinary students, swine health researchers, and other health specialists engaged in improving swine health. We have sought to fulfill this mission by bringing together recognized scientific authorities from around the world to provide expert knowledge on the many “-ologies” that converge on the complex topic of swine health and disease. In an era characterized by the exponential growth of data, our challenge has been to distill an overabundance of information into a concise and useful body of knowledge that fits into one volume. Our conscious choice has been to exclude generalist information that is readily accessible on the Internet or other formats, for example, animal husbandry, nutrition, postharvest food safety, and others. To help the reader navigate the plethora of information in the book, the contents have been extensively indexed, the Table of Contents has been expanded, and the topics organized consistently within each section.

We hope the 10th edition of *Diseases of Swine* continues in the spirit of excellence and relevance Howard Dunn originally envisioned.

Jeffrey J. Zimmerman
Locke A. Karriker
Alejandro Ramirez
Kent J. Schwartz
Gregory W. Stevenson

**Acknowledgment**

We wish to thank Ms. Christine Meraz for her contributions and help in seeing this project through to its conclusion.
DISEASES OF SWINE  10TH EDITION
1 Herd Evaluation
2 Differential Diagnosis of Diseases
3 Behavior and Welfare
4 Longevity in Breeding Animals
5 Effect of the Environment on Health
6 Optimizing Diagnostic Value and Sample Collection
7 Diagnostic Tests, Test Performance, and Considerations for Interpretation
8 Analysis and Use of Diagnostic Data
9 Drug Pharmacology, Therapy, and Prophylaxis
10 Anesthesia and Surgical Procedures in Swine
11 Disease Transmission and Biosecurity
12 Preharvest Food Safety, Zoonotic Diseases, and the Human Health Interface
13 Special Considerations for Show and Pet Pigs
INTRODUCTION

With changes in the structure of the swine industry, there have also been changes in the roles of swine veterinarians. Swine veterinarians today focus more on preventive medicine and improving overall herd health rather than responding after disease occurs, the latter common in traditional “fire engine” practices of 20+ years ago. Swine veterinarians now have a proactive role in anticipating problems and preventing disease with a concurrent responsibility to provide care to each pig. This is a challenge as resources (money, labor, and time) are becoming more limited. Consequently, swine veterinarians are highly motivated to be innovative. The use of modern technology, epidemiological principals, biostatistics, and improved diagnostic methods guides them through the diagnosis as well as the prioritization and allocation of resources to improve the health and welfare of pigs. A successful veterinarian is one who not only solves a problem, but also creates opportunities and promotes the financial success of his or her clients.

Before starting any evaluation of a farm, it is important to understand the objectives and goals of each individual involved in the farm operation. This is critical as ultimately the success of any intervention requires actions by the client or those working for the client. Better understanding of the client’s goals and constraints will ensure that recommendations on herd health are made in that context. The context often requires swine veterinarians to innovate because recommendations will often vary between clients and may change for a particular client over time. For example, a client may be focused on improving average daily gain for a period but may transition to reducing cost of gain as his or her facts, business inputs, or understanding changes. The most important question for an owner or manager who is requesting veterinary services to answer is “What is my primary concern?”

Investigation of health or production issues is best approached by site visits—that is, inspection of pigs in their environment. As will be seen in the following discussion, there are many factors that contribute to compromised health and well-being of pigs. Many of the assumptions made by clients or swine veterinarians can only be validated by a well-designed, systematic on-farm site visit.

PREPARING FOR A SITE VISIT

History and Records

History and record evaluation should occur prior to any herd evaluation or investigation. Looking at the operation’s medical records and past diagnostic laboratory reports helps to provide a picture of previous areas of concern and guidance on the expected health status of the herd. It is important to see the actual past reports rather than rely on client’s interpretation of results, particularly when serving a new client or as a second opinion. Experience dictates that even with the best intentions, managers and owners are more likely to recall some results while downplaying or neglecting to mention others based on their particular biases.

Production records, usually computerized, are common in modern swine operations. The value of computerized records lies in the ability to instantly query the data and summarize it in meaningful ways. Morris (1982) is reported to be one of the first to suggest the concept of “performance-related diagnosis.” This

capability to evaluate herd performance and then determine the need for interventions has created a dilemma in regard to the term “subclinical” (Polson et al. 1998). The true definition of subclinical implies not measurable, but today modern records allow for measuring slight differences in productivity (clinical manifestation), which without records would have gone unnoticed (subclinical). All information gathered on a farm, including records, should be evaluated objectively from a perspective of “trust yet verify.” Inaccurate or misinterpreted information and records will often lead to misdiagnosis and inappropriate recommendations.

**Benchmarks**

Benchmarking is a unique tool that allows operations to identify areas of concern or areas where improvements can be made. Many studies have reported different benchmarks to use as targets (see review by Polson et al. 1998). Others have suggested that the best production benchmarks are those set by the herd’s own records (Lloyd et al. 1987). Over time, productivity and processes change such that older benchmarks may no longer be relevant. Depending on the objectives and changing constraints of a specific operation, a particular benchmark may not have the utility or impact that it did under previous conditions. As information suggesting benchmarks becomes more available in the age of the Internet, it is increasingly important to determine the characteristics of the operations from which these benchmarks were derived. Experienced swine veterinarians are able to decipher the intricate methods of data reporting and have insight for which circumstances certain parameters are achievable. For those just starting to learn about swine production medicine, it is best to use benchmarks as means to understand the appropriate magnitudes of different parameters rather than use them as specific goals per se.

From the veterinary and diagnostic perspectives, it is better then to focus on understanding the relationship of different production parameters rather than memorize specific values. A good example of this conceptual thinking can be seen in Figure 1.1. This figure helps show the interrelationship of several different parameters on their impact on a breeding herd’s weaned pig output. Basically, throughput (i.e., pigs weaned) is determined by multiplying capacity (female inventory or facility space) by efficiency (how many pigs are produced per female inventory or facility space). The advantage of understanding this productivity tree is that all factors influencing throughput can be evaluated at the same time and interventions can be implemented in different areas of the tree. Extending this example to the evaluation of the number of pigs weaned, issues like preweaning mortality are obvious, but others such as female removal and replacement rates or lactation length may not initially come to mind. In the case of a producer with a target of >28-day weaning age, the number of litters weaned/female/year will automatically be impacted (fewer) by the system design.

**Reporting Structure**

Reporting structure refers to the organization of workers, management, and owners as it occurs in larger production systems. It also refers to whom a veterinarian is to report findings and recommendations. It is important for swine veterinarians to ask and understand the proper reporting structure for any new client. This is true for operations of all sizes. For the small or family farm, it is important to know what information

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**Figure 1.1.** Weaned pig output productivity tree for investigating variables that impact the number of pigs weaned per year. Adapted from Gary Dial.
the owner wants to share with workers. In a larger corporate setting (corporate ownership or part of a producer cooperative), it is even more important to understand how decisions are made, who makes decisions, and who should get veterinary reports. Understanding reporting structures is critical in ensuring that the veterinarian and managing team are working together and a single consistent message is being delivered to workers. Providing information to the wrong person may actually hinder progress, as many times those closer to the pigs and daily processes may not be fully aware of all considerations influencing a business decision.

Frequently in the United States, the owner of the pigs is different than the caretaker. The caretaker may be focused on minimizing his labor efforts while the owner may be more focused on the cost of a particular treatment or prevention option. The veterinarian is focused on food safety, maximizing pig health and welfare, operational sustainability, and owner profitability. Ultimately, the owner decides what is to be implemented.

Biosecurity
Biosecurity has been a major topic of concern for the swine industry from many years. Protocols to prevent disease transmission into the farm and within the farm are now commonplace. Swine veterinarians need to proactively follow proper biosecurity protocols to ensure the safety and security of our food supply. See Chapter 11 for complete details regarding biosecurity. The key points when performing a herd examination are for the veterinarian to be fully aware, and fully comply, with all biosecurity guidelines for the operation he or she is visiting. To do this, the veterinarian has to be proactive and always ask for biosecurity requirements before visiting the site. Being informed ahead of time will help ensure that the veterinarian is prepared and able to follow proper biosecurity protocols once on site.

SITE VISIT
Introduction to the Four Circles
One of the most important concepts of a proper herd evaluation is to be consistent! It is critical to ensure that herd examinations are performed in a consistent manner so as to be thorough and efficient, and minimize the opportunity for missing something important. Checklists may be helpful for specific, routine evaluations, but many times they are not practical for a complete and thorough investigation. Checklist approaches limit the problem-solving ability of the veterinarian and are especially poor approaches to new problems. There are too many areas of interest as well as too many differences in facility type and design to make a single valid checklist across all farms. Farm-specific checklists or checklists for particular aspects of operation can, however, be useful.

One systematic approach involves the concept of the four circles (Figure 1.2). The overall objective is to be systematic in the evaluation of an operation to make sure that all relevant information is evaluated when looking after pigs’ health and welfare. Each successive circle becomes more focused, culminating in the evaluation of individual pigs. The most important question the veterinarian must be able to answer after going through the four-circle process is, “Is there currently a disease or welfare issue or is one imminent?”

Circle 1: Evaluation of the Outside of the Building
The first circle involves walking around the outside of the building to assess the overall site. This first circle is especially important when visiting a new site. Evaluation of the outside of the building has value both clinically for the pigs, as well as practically with respect to informing the veterinarian about the caretakers’ attention to maintenance and facility management.

As one walks around the site, biosecurity risks for the operation will be better understood. Are there any other hog sites in close proximity? Is the health status of these other operations known? How close are public roads from hog buildings? What appears to be the traffic pattern for this particular site (feed delivery, removal of dead carcasses, employee parking)? How well maintained is the site? If the site is not well maintained, could it be due to lack of attention to details or insufficient staffing? Either of these reasons would suggest that the veterinarian’s recommendations should be tailored to accommodate these realities. For example, a manager who is very attentive to detail is more likely to follow a complex or detailed treatment protocol.

Circle 2: Evaluation of the Inside of the Building
The second circle involves walking through the inside of the building. In this case, the objective is to get a
better feel for the overall environment of the pigs covering all regions of the building. One must walk from one end of the building all the way though the other side. If one takes too long to walk from one end to the other, it becomes more difficult to identify ventilation differences as one starts to become adapted to the new environment.

Stocking density is also evaluated at this time. It is important to note differences in stocking densities between pens as well as between barns. Lower stocking densities may indicate high mortalities in a particular pen or barn. Recommended stocking densities are listed in Table 1.1. Pig sizes are also assessed using the guidelines in Table 1.2 on expected pig weights based on age.

The general health of all pigs in the barn is evaluated at this time. Is there coughing, sneezing, or signs of diarrhea? The magnitude of the problem should be quantified. This is easily done by estimating the number of affected pigs in a pen as well as the total number of pigs in the pen. For example, if there are approximately 5 pigs coughing in every pen and there are around 25 pigs per pen, then it would suggest that approximately 20% of the pigs are affected. On the other hand, if it is found that only one or two pigs are affected in every other pen, then it would suggest the prevalence to be approximately 2–4% of the barn. The quantification of prevalence does not have to be exact, as usually we are more concerned on the size of the magnitude of the problem (60% vs. 10%) rather than knowing the exact prevalence of the clinical sign (8% vs. 12%). Determining prevalence has three main goals. It allows for the correct perspective on the extent of the problems (i.e., is there currently a disease or welfare issue or is one imminent?). It helps to differentiate herd problems from individual pig issues, thus helping to determine the correct level of treatment (i.e., whole herd treatment or individual pig treatments). Finally, it provides a baseline for determining the effect of any intervention. This is especially important as although coughing may still be present after 5 days of treatment, the change in prevalence from 25% to 4% is a good indicator of improvement, suggesting that further intervention may not be warranted.

**Circle 3: Evaluation of Individual Pens**
The third circle is performed by doing an evaluation of individual pens. Based on the second circle, pens identified in the evaluation of the room are selected for further evaluation of the extent of the problem. Veterinarians must get in the pens with pigs. One cannot make a full assessment of the problem by simply walking the alleyway of the barn as many pig issues will be missed. This is the point in time that feeders and waterers are also checked for proper function (Table 1.3). Also, see Chapter 5 for the effect of the environment on swine health.

The overall behavior/attitude within the pen is evaluated, identifying individual pig concerns as well as pen concerns. Differences in sizes of pigs in a pen are again noted at this time (Table 1.2). It is very important to always ask if any type of size sorting (regrouping by size) has occurred as well as knowing the expected age difference for the barn. This is a good time to look closely for evidence of diarrhea. Many times the diarrhea is first noted by the fecal character that may be present on the floor or walls of the facility, and extra observational time is needed to identify the individual pigs that may be affected.

There are no specific recommendations on how many individual pens need to be evaluated. A key point is to make sure several pens from different parts of the building are evaluated to have a true representation of the potential herd issues recognized by the second circle evaluation. Individual pig issues of concern, especially those related to welfare (severe, chronic, or moribund individuals) should also be identified at this time.

**Circle 4: Evaluation of Individual Pigs**
The fourth and final circle involves a complete evaluation of individual pigs. Pigs are evaluated from head to tail. Anomalies are noted as well as suspected chronicity of issue. Rectal temperatures are taken at this time as a measure of presence of infectious disease processes and stage of infection (e.g., acute infections have fever). Table 1.4 provides a summary of the expected normal temperature, respiratory, and heart rates of pigs based on size. A key point to remember is that as the environmental temperature increases, so will the average respiratory rates and body temperatures for healthy pigs.

For breeding herd examinations, the body condition of females should be evaluated periodically (Table 1.5).

---

**Table 1.1.** Recommended space per pig by phase of production

<table>
<thead>
<tr>
<th>Phase</th>
<th>Indoor</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Solid</td>
<td>Slatted</td>
<td>Outdoor</td>
</tr>
<tr>
<td>Gill</td>
<td>1.86 (20)</td>
<td>1.49 (16)</td>
<td>2.32 (25)</td>
</tr>
<tr>
<td>Sows</td>
<td>2.2 (24)</td>
<td>1.86 (20)</td>
<td>2.32 (25)</td>
</tr>
<tr>
<td>Farrow pen</td>
<td>8 (88)</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Farrow crate</td>
<td>4.4 (48)</td>
<td>4.4 (48)</td>
<td>NA</td>
</tr>
<tr>
<td>Boars</td>
<td>NA</td>
<td>1.86 (20)</td>
<td>NA</td>
</tr>
<tr>
<td>Nursing</td>
<td>NA</td>
<td>2.0 (22)</td>
<td>NA</td>
</tr>
<tr>
<td>Nursery 20kg</td>
<td>0.37 (4)</td>
<td>0.28 (3)</td>
<td>0.74 (8)</td>
</tr>
<tr>
<td>Nursery 40kg</td>
<td>0.37 (4)</td>
<td>0.40 (4.4)</td>
<td>0.74 (8)</td>
</tr>
<tr>
<td>Grower 60kg</td>
<td>0.56 (6)</td>
<td>0.53 (5.8)</td>
<td>1.86 (20)</td>
</tr>
<tr>
<td>Finisher 80kg</td>
<td>0.74 (8)</td>
<td>0.67 (7.2)</td>
<td>1.86 (20)</td>
</tr>
<tr>
<td>Finisher 110kg</td>
<td>0.75 (8)</td>
<td>0.75 (8)</td>
<td>1.86 (20)</td>
</tr>
</tbody>
</table>

NA, not applicable.

Sources: Dewey and Straw (2006). Adapted from English et al. (1982), Baxter (1984a,b,c), Patience and Thacker (1989a,b), and Gonyou and Stricklin (1998).
<table>
<thead>
<tr>
<th>Age (Days)</th>
<th>Slow Weight</th>
<th>Daily Gain in the Previous 20 Days</th>
<th>Moderate Weight</th>
<th>Daily Gain in the Previous 20 Days</th>
<th>Ideal Weight</th>
<th>Daily Gain in the Previous 20 Days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>lb</td>
<td>kg</td>
<td>lb</td>
<td>kg</td>
<td>lb</td>
<td>kg</td>
</tr>
<tr>
<td>20</td>
<td>8–10</td>
<td>3.6–4.5</td>
<td>10–12</td>
<td>4.5–5.5</td>
<td>12–14</td>
<td>5.5–6.4</td>
</tr>
<tr>
<td>40</td>
<td>18–22</td>
<td>8.2–10.0</td>
<td>22–26</td>
<td>10.0–11.8</td>
<td>26–30</td>
<td>11.8–13.6</td>
</tr>
<tr>
<td>60</td>
<td>33–40</td>
<td>15.0–18.2</td>
<td>40–47</td>
<td>18.2–21.4</td>
<td>47–54</td>
<td>21.4–24.5</td>
</tr>
<tr>
<td>80</td>
<td>54–64</td>
<td>24.5–29.1</td>
<td>64–74</td>
<td>29.1–33.6</td>
<td>74–84</td>
<td>33.6–38.2</td>
</tr>
<tr>
<td>100</td>
<td>82–95</td>
<td>37.3–43.2</td>
<td>95–108</td>
<td>43.2–49.1</td>
<td>108–122</td>
<td>49.1–55.5</td>
</tr>
<tr>
<td>120</td>
<td>110–126</td>
<td>50.0–57.3</td>
<td>126–142</td>
<td>57.3–64.5</td>
<td>142–160</td>
<td>64.5–72.7</td>
</tr>
<tr>
<td>140</td>
<td>138–157</td>
<td>62.7–71.4</td>
<td>157–176</td>
<td>71.4–80.0</td>
<td>176–198</td>
<td>80.0–90.0</td>
</tr>
<tr>
<td>160</td>
<td>165–187</td>
<td>75.0–85.0</td>
<td>187–209</td>
<td>85.0–95.0</td>
<td>209–235</td>
<td>95.0–106.8</td>
</tr>
<tr>
<td>180</td>
<td>191–216</td>
<td>86.8–98.2</td>
<td>216–241</td>
<td>98.2–109.5</td>
<td>241–271</td>
<td>109.5–123.2</td>
</tr>
<tr>
<td>20–60</td>
<td></td>
<td>0.63–0.75</td>
<td></td>
<td>0.75–0.88</td>
<td>0.88–1.00</td>
<td>398–455</td>
</tr>
<tr>
<td>60–180</td>
<td></td>
<td>1.32–1.47</td>
<td></td>
<td>1.47–1.62</td>
<td>1.62–1.81</td>
<td>735–822</td>
</tr>
<tr>
<td>0–180</td>
<td></td>
<td>1.06–1.20</td>
<td></td>
<td>1.20–1.34</td>
<td>1.34–1.51</td>
<td>609–684</td>
</tr>
</tbody>
</table>

Source: Dewey and Straw (2006).
When making recommendations for feed or feeding changes, the stage in the reproductive cycle must be considered. Females entering the farrowing house should be in their best body condition (target body condition score [BCS] of 3) while gilts exiting the farrowing house (end of lactation) will have lower BCSs. Feed changes are best executed by making small changes (0.5–1.0 kg) in the daily feed allotments.

This is also a good time to identify individual pigs requiring treatment as well as acutely infected animals that would be useful for diagnostic sample collection. Animals appropriate for euthanasia, necropsy, and tissue collection are also identified at this time. When selecting pigs for diagnostic tissue sample collection, there are several important points to consider:

1. An animal’s life will be sacrificed for the good of the herd and due consideration should be placed into selecting the appropriate pig(s).
2. Animals must be selected which truly represent the major clinical signs of concern in the herd.
3. Animals should be in the early stages of the disease process. The selection of acute cases will increase the probability that the primary causative agent and compatible lesion is identified.
4. An animal that has received no antimicrobials or therapy is usually preferred.

The number of animals selected for necropsy and tissue sample collection depends on the objective. As a general rule, animals that are found dead are necropsied first. Mortalities are necropsied until a pattern of disease process is apparent, which suggests the primary herd disease issue rather than unrelated individual animal afflictions. Based on necropsy findings and clinical evaluation, representative live animals are euthanized for fresh tissue sample collection. The number of

### Table 1.3. Recommended water requirements, water flow rate, and feeder space per pig by phase of production

<table>
<thead>
<tr>
<th>Restricted feed</th>
<th>Water Requirements</th>
<th>Feeder Space/Pig</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>l/day l/minute mm (in.)</td>
<td></td>
</tr>
<tr>
<td>Gestating sows</td>
<td>12–25 2 457–610 (18–24)</td>
<td></td>
</tr>
<tr>
<td>Lactating sow</td>
<td>10–30 2</td>
<td></td>
</tr>
<tr>
<td>Boar</td>
<td>20 2</td>
<td></td>
</tr>
<tr>
<td>Nursing</td>
<td>1 0.3</td>
<td></td>
</tr>
<tr>
<td>Nursery</td>
<td>2.8 1 254 (10)</td>
<td></td>
</tr>
<tr>
<td>Grower</td>
<td>7–20 1.4 260 (10)</td>
<td></td>
</tr>
<tr>
<td>Finisher</td>
<td>10–20 1.7 330 (13)</td>
<td></td>
</tr>
<tr>
<td>Ad libitum</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nursery</td>
<td>2.8 1 60 (2.3)</td>
<td></td>
</tr>
<tr>
<td>Grower</td>
<td>7–20 1.4 65 (2.5)</td>
<td></td>
</tr>
<tr>
<td>Finisher</td>
<td>10–20 1.7 76 (3)</td>
<td></td>
</tr>
</tbody>
</table>

Sources: Dewey and Straw (2006). Adapted from Baxter (1984a,b,c), Patience and Thacker (1989a,b), Swine Care Handbook (2003), and Muirhead and Alexander (1997a,b).

### Table 1.4. Temperature, respiration, and heart rate of pigs of different ages

<table>
<thead>
<tr>
<th>Age of Pig</th>
<th>Rectal Temperature (Range ± 0.30°C, 0.5°F)</th>
<th>Respiratory Rate (breaths/min)</th>
<th>Heart Rate (beats/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Newborn</td>
<td>39.0 102.2</td>
<td>50–60</td>
<td>200–250</td>
</tr>
<tr>
<td>1 hour</td>
<td>36.8 98.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12 hours</td>
<td>38.0 100.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>24 hours</td>
<td>38.6 101.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unweaned piglet</td>
<td>39.2 102.6</td>
<td>25–40</td>
<td>90–100</td>
</tr>
<tr>
<td>Weaned piglet (20–40lb) (9–18kg)</td>
<td>39.3 102.7</td>
<td>30–40</td>
<td>80–90</td>
</tr>
<tr>
<td>Growing pig (60–100lb) (27–45kg)</td>
<td>39.0 102.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Finishing pig (100–200lb) (45–90kg)</td>
<td>38.8 101.8</td>
<td>25–35</td>
<td>75–85</td>
</tr>
<tr>
<td>Sow in gestation</td>
<td>38.7 101.7</td>
<td>13–18</td>
<td>70–80</td>
</tr>
<tr>
<td>Sow 24 hours prepantum</td>
<td>38.7 101.7</td>
<td>35–45</td>
<td></td>
</tr>
<tr>
<td>12 hours prepantum</td>
<td>38.9 102.0</td>
<td>75–85</td>
<td></td>
</tr>
<tr>
<td>6 hours prepantum</td>
<td>39.0 102.2</td>
<td>95–105</td>
<td></td>
</tr>
<tr>
<td>Birth of first pig</td>
<td>39.4 102.9</td>
<td>35–45</td>
<td></td>
</tr>
<tr>
<td>12 hours postpartum</td>
<td>39.7 103.5</td>
<td>20–30</td>
<td></td>
</tr>
<tr>
<td>24 hours postpartum</td>
<td>40.0 104.0</td>
<td>15–22</td>
<td></td>
</tr>
<tr>
<td>1 week postpartum until weaning</td>
<td>39.3 102.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 day postweaning</td>
<td>38.6 101.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Boar</td>
<td>38.4 101.1</td>
<td>13–18</td>
<td>70–80</td>
</tr>
</tbody>
</table>

Source: Dewey and Straw (2006).
Table 1.5. Sow body condition scoring

<table>
<thead>
<tr>
<th>Body Condition Score (BCS)</th>
<th>Condition</th>
<th>Back fat mm (in.)</th>
<th>Description</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>BCS 1</td>
<td>Excessively thin</td>
<td>&lt;10 (&lt;0.39)</td>
<td>Ribs, hips, and backbone are easily visible and palpable.</td>
<td>Sow is in poor condition and needs large amounts of muscle and fat gain to maintain productivity. Needs a significant increase in feed.</td>
</tr>
<tr>
<td>BCS 2</td>
<td>Moderately thin</td>
<td>10–15 (0.39–0.58)</td>
<td>Ribs, hips, and backbone can be palpated with slight pressure.</td>
<td>A moderate increase in feed is required.</td>
</tr>
<tr>
<td>BCS 3</td>
<td>Ideal condition</td>
<td>15–22 (0.59–0.89)</td>
<td>Ribs, hips, and backbone can be palpated with firm pressure, but cannot be observed visually.</td>
<td>Monitor feeding to maintain this body condition.</td>
</tr>
<tr>
<td>BCS 4</td>
<td>Moderately fat</td>
<td>23–29 (0.90–1.13)</td>
<td>Ribs, hips, and backbone cannot be palpated.</td>
<td>May be appropriate to cut back slightly on feeding.</td>
</tr>
<tr>
<td>BCS 5</td>
<td>Excessively fat</td>
<td>≥30 (≥1.14)</td>
<td>Ribs, hips, and backbone cannot be palpated.</td>
<td>Sow has excessive amounts of fat tissue. Reduce feeding to bring her back to a BCS 3.</td>
</tr>
</tbody>
</table>

Adapted from Ken Stalder.

animals euthanized depends on the individual case presentation and necropsy findings in the euthanized pig. When considering multifactorial etiologies, it is important to remember that not all animals in the herd will have all pathogens present at any one time point. This suggests that in a large herd, it may be necessary to euthanize sufficient animals to completely represent the full range of clinical and pathological findings and to identify the multiple, interacting disease agents. In other cases where there may be only one primary pathogen of concern, one or two euthanized pigs may be sufficient to answer the diagnostic question. The goal is to sacrifice the least number of animals yet maximize the diagnostic value for the benefit of the rest of the herd, thereby benefitting the current group as well as future groups. Live-animal (antemortem) sampling is commonly done. For some pathogens (e.g., influenza A virus via nasal swabs), simply finding the agent in the herd is all that may be necessary. In other cases, finding a common, endemic potential pathogen of interest (e.g., porcine circovirus type 2) must be in association with compatible lesions to support the role of such agent in the current clinical presentation.

Summary of Four Circles

The concept of the four circles is to obtain a systematic and complete picture of the clinical status of the site. It provides a systematic view that is important in deciding what interventions need to be implemented to mitigate the effects of the current disease. It starts with a big-picture overview and then narrows the focus to individual pigs. It helps separate unrelated individual pig afflictions from whole herd disease problems, both of which need to be addressed, but priorities and recommendations will be different depending on context and the client’s goals and objectives. The role of the veterinarian is to help guide the client to maximize the impact of any intervention. Information obtained from this systematic approach will also help differentiate what issues are primarily due to pathogens and which ones are being confounded or even caused by management practices or management failures. It will help veterinarians formulate a more complete assessment of the prognosis and expected outcomes of the current health situation. Once mastered, the process can be quick and very efficient.

Asking Questions

The process of data collection should not be restricted to the veterinarian’s observations. It is very helpful to ask others working on the farm or within the operation for their perspectives. This should be done not only from upper management individuals (i.e., managers or owners) but also from the workers themselves. Often, the managers make many assumptions as to what they believe is being done on the farm, but the actual workers have a different perspective. This may be due to lack of training, poor communication of protocols, or inadvertent deviations in protocols of which participants are unaware. This is why it is useful to ask the same questions to different people in the same production system for confirmation and to assess consistency. Questions should be formulated as open ended rather than seeking a simple yes or no answer. It is also very helpful to have employees demonstrate how to perform a task (“show me how”) rather than providing an explanation (“tell me how”). This ensures that the actual process and technique are observed and allow evaluation of significantly more details than are
apparent in a verbal description. This has been especially useful in troubleshooting intensive, high-impact procedures such as artificial insemination.

As a site visit is performed, it is also important to examine storage and utility areas and to investigate refrigerators or medicine cabinets. This process should help support and validate the different worker’s answers to questions regarding processes and protocols. For example, an operation that claims routine vaccination of sows prefarrowing and yet has no vaccine on-site may need further evaluation and discussion to ascertain vaccine management and handling procedures.

On-site Records
Production sites should have treatment and mortality records on-site. These records are helpful in determining the total number of pigs in the original lot, number of mortalities, and the chronology of mortalities to date. Caretakers should be instructed to record euthanized animals in a different manner. A good practice is to also record a presumed “death reason” and educate clients on how to properly evaluate mortalities and record such. However, research has shown there are significant differences between recorded and actual death reasons (Lower et al. 2007). To facilitate this process, the focus should be on the actual observations that can be accurately made by caretakers. For example, it is difficult for a caretaker to diagnose *Escherichia coli*-associated diarrhea as cause of death. Instead, the mortality should be recorded as due to diarrhea. There should also be a second code to identify whether the animal died on its own or was euthanized. Practical and more valid mortality records can be collected by simply narrowing down the options provided, focusing on general clinical signs rather than a specific disease etiology, and training all individuals on how to properly categorize mortalities.

Records for farrowing, nursery, and finishing sites may include daily water consumption and daily high and low barn temperatures. This information is easy to collect in today’s modern facilities and can be helpful (especially the water) in predicting a possible respiratory outbreak (Brumm 2006). The high and low barn temperature recording is helpful in identifying possible concerns with the ventilation system. It is best to utilize an independent high–low thermometer to record temperature fluctuations rather than use the barn’s electronic control system in order to validate the proper function of the controller.

For breeding herds, there are many other records that are kept on-site. These records can vary in form and content, from hand notes to an actual computer on-site. Log sheets are very helpful in ensuring that jobs are routinely done. For example, a simple semen log can track the date, time, current temperature of the semen storage unit, and initials of the individual who rotated the semen (e.g., manually resuspended semen in extender by gently rocking the semen bags/bottles back and forth). The advantage of having this type of manual record is that it ensures this important job is done routinely, and having individuals write down their initials facilitates accountability. It is a reality that in operations with multiple workers, duties are sometimes not performed because a worker believes that someone else was doing the job.

Computer records can be accessed either through daily/weekly reports provided to the farm or through direct access to a computer. The number and variety of reports that are available from computerized sow record systems precludes discussion here. It is important for the swine veterinarian to understand and objectively evaluate different herd performance parameters. The greatest advantage of computerized record systems is their ability to summarize relevant data in many different ways, and as previously mentioned, compare with relevant internal or external benchmarks to help identify those performance parameters in need of improvement.

When looking at reports, it is important to remember that data are usually summarized based on time or by cohort. In a time-based report, data are simply attributed to a particular time period. For example, January breeding and farrowing number summarizes data for all the sows that were bred in January as well as the sows that farrowed in January, which are two distinct groups of animals. This information is helpful in monitoring the overall herd’s performance, but it is not helpful in evaluating cause and effect within a particular group. To better evaluate a particular group, a cohort-based report must be used. In this case, all parameters reported are specific to a common group of animals so the breeding and farrowing data pertain to the same group of animals although accumulated at different dates. This cohort-based report is very useful in evaluating the effects of different interventions by keeping all the relevant data associated with the particular group of interest in one report.

The most important part of any data collection is the desire to take action when an abnormality is detected. When a veterinarian requests data to be collected by workers or caretakers, effective communication should outline the importance of the data, how the data will be used, at what threshold they are expected to take action, and the consequences of failing to act. For example, simply recording the daily temperature of the semen storage unit has no value unless action is taken when temperature is outside of the desired range.

**DIAGNOSIS**

Once a site’s evaluation has been performed (four circles) and data have been collected, it is then necessary to interpret all the findings in the context of the
CHAPTER 1  HERD EVALUATION

veterinarian's clinical observations. The Greek word “diagnosis” literally means “through thinking” (Morley 1991). The process of arriving at a diagnosis can vary among individuals and clinical presentations. What is important is to be systematic, once again, to ensure that decisions are focused and objective. Figure 1.3 summarizes the field investigation and case management process. The following brief summaries are a few examples of different approaches/aspects that can be considered.

**Subjective Observations, Objective Data, an Assessment, and the Resulting Plan**

One of the traditional means for summarizing data in the medical profession is to utilize a process in which subjective observations, objective data, an assessment, and the resulting plan (SOAP) are all specified. Four senses (sight, hear, smell, and touch) are generally used when gathering data. Subjective data are focused on identifying issues reported by the owner, manager, or
other workers, as well as any other qualitative observations. The objective section is focused on quantitative data. The assessment is an evaluation or interpretation of both subjective and objective data. Finally, a plan of action is provided in response to the assessment. Using this SOAP approach allows for a complete and thorough thought process to occur before any diagnosis is made. It is a systematic way to ensure completeness. Consistency is king!

**Grouping Observations**

Many times it is helpful to group observations based on commonalities. It is especially helpful to categorize based on organ system relationships. Grouping observations helps apply Occam’s razor (the simplest explanations are more probable). In other words, it is more likely that the pulmonary edema, ascites, and respiratory dyspnea in a pig are caused by circulatory system failure rather than the pig having three completely different pathogens, each independently causing one of the clinical findings noted. After grouping observations, a possible differential list can then be compiled.

**DAMNIT**

This approach focuses on coming up with a complete differential list to ensure all possibilities, so as to avoid too narrow a focus on infectious diseases. The following list helps identify the terms associated with each letter of the acronym:

- **D** = Degenerative
- **A** = Anomaly
- **M** = Metabolic
- **N** = Nutritional or neoplasia
- **I** = Inflammatory, infectious, or immune mediated
- **T** = Trauma or toxicity.

One of the disadvantages of this particular acronym is that it does not help prioritize the list. It also encourages veterinarians, especially those in their early career, to generate a very long list of possible, yet not probable, differentials.

**Five Production Inputs Model**

One other approach in thinking of differential diagnosis and risk factor list is to think more holistically and ensure that all aspects of production are considered. The five production inputs model of integrating cause and risk factors includes consideration of nutrition, environment, disease, genetics, and management. This model is very useful as it helps ensure multifactorial causes contributing to the clinical issue of concern. The nutritional aspect of veterinary medicine has become more important in recent years as feed prices have dramatically increased. High feed prices have promoted the use of alternative feedstuffs including the use of dry distiller grains (DDGs). The effects of these changes in diets and variability in quality of ingredients on the health of pigs have not been fully investigated. Environment also plays a key role in the health and welfare of pigs as is mentioned throughout this book but especially in Chapters 3 (behavior and welfare), 4 (longevity in breeding animals), and 5 (effect of the environment on swine health). The disease component is typically the first focus of veterinarians and is the focus of many chapters in this book. Genetics is an input that many times can be confusing as genotype and phenotype expressions are very complex especially when focused on clinical significance. Finally, management, especially all the people involved, is a very integral part of livestock production and can have a tremendous influence on the health, welfare, and success of raising animals. With the urbanization of the world and increasingly fewer people with an agricultural background, training workers on basic husbandry practices is becoming an integral part of any successful operation. New entry-level workers generally have very limited, if any, experience and knowledge on how to raise pigs.

The five production inputs model works to integrate the interactions of different factors that may be working together and, at the same time, are influencing the health of a pig. The diagram in Figure 1.4 demonstrates the interaction of possible contributing factors associated with a simple example case of piglet diarrhea.

**Determining Interventions and Prioritization**

After observations are made and a list of differentials has been created, the next step is to identify appropriate interventions and prioritize their implementation. This step of the process becomes easier with experience. Personal experiences, client constraints and capabilities, ease, likelihood of success, and impact of intervention all play an important role in helping guide prioritization. It is important to always keep in mind the client’s goals and objectives.

From the pigs’ point of view, the priorities for survival and health are (fresh) air, (clean) water, (wholesome) food, and appropriate vaccination or treatment as needed. A producer’s expectation and a veterinarian’s training sometimes places therapeutic intervention as first priority. Vaccines will not be successful unless the pig is placed in an environment that allows the vaccine to work to its full potential. From the pig’s perspective, the last area of need is vaccination or treatment as compared with having good quality air as the top priority, with access to good-quality feed and water of similar priority.

Many times a diagnostic workup may be necessary to rule different differentials either in or out. Necropsies have been mentioned above and sample collecting (blood and oral fluid) will be discussed at the end of this chapter. Chapter 2 will cover some lists for differ-
ential diagnosis. Further general information on diagnostics is covered in Chapters 6 and 7.

Usually, priority is given to interventions that will have the greatest impact on the greatest number of animals. Because resources (time and money) are always limited, priorities need to be evaluated based on their cost and benefit as well as overall welfare of pigs and sustainability of operations. The benefit does not always have to be financial, although many times this is the primary objective. Priorities that require substantial investment in resources usually will require a justification on the expected return.

Reporting

Once interventions have been identified and prioritized, it is critical to provide this information to the client in a concise and clear manner. A farm report or client letter is a very helpful tool in making sure the correct information is being communicated. Written reports and instructions will minimize miscommunications. Reports should be short (usually) and should include a prioritized list (bullet points) with only two or three top interventions. Personal experience suggests that providing too many recommendations allows for the clients to lose focus. They may select only recommendations that are desired or easiest to implement. The client may feel as though the veterinarian’s recommendations are being followed but in reality have a false sense of security and may be neglecting the most important recommendations. The report should be short (preferably up to one page long and definitely no more than two pages), which helps ensure the client will actually read it. Very long reports are conducive for a quick skimming by the client and thus many important points can be missed. Certainly, there are times when a comprehensive report is needed, but for routine investigations, simpler is better. Client letters need to be provided back to the client in a timely manner (usually within a few days) in order to maximize implementation of recommendations. Integrated or complex production systems also require knowledge and understanding of the farm or company reporting structure.

Veterinarians must understand and follow the proper reporting structure in order to meet clients’ expectations. The structure serves as means for the central entity and decision maker(s) to have an understanding on the issues of the entire system. Following proper reporting structures ensures that everyone is working together as a team.

Client reports are no substitutes for medical records. Veterinarians should keep detailed records on clinical observations and diagnosis. These complete medical records will serve as an excellent reference for future visits and have legal implications, including the justification for the use of any antibiotic per label or in an extra label manner.

MONITORING OUTCOMES

It is important for the client to be able to measure outcomes that can help determine the effectiveness of the intervention plans (Figure 1.3). Veterinarians must demonstrate the value they bring in order to be viewed as an asset rather than just a liability (expense).

SAMPLE COLLECTION

Blood Sampling

Blood sampling is one of the most common sample collecting techniques practiced in the United States today. There are several different techniques used in blood sample collecting in swine. Blood sample collecting requires a good understanding of pig’s anatomy as all major blood vessels are nonvisible, and thus a blind stick is performed. Mastery is achieved though practice. Much of this blood sampling information has been summarized in Dewey and Straw (2006).

Pig Restraint. It is important to properly restrain pigs for safe sample collecting both from the perspective of the pig as well as the person. The size of the pig and the comfort level of the restrainer will dictate the desired method. Figures 1.5 and 1.6 depict two approaches commonly used for restraint. In both cases, the person doing the restraining is just as important as the person collecting the blood sample. Pigs need to be immobilized and held in the correct position to facilitate access to the target veins. In the standing pig, it should have all four feet squarely placed on the ground. Its neck should not be stretched too much otherwise access to the veins will be much more difficult.

Anterior Vena Cava. The pig’s right jugular groove is identified, and the needle is inserted just cranial to the thoracic inlet. The needle is inserted aiming to the top of the opposite shoulder. This is approximately at a 30° angle from the median and 90° angle from the neck line (line from thoracic inlet to the head). Figure 1.7
depicts the approximate location of major veins. The pig’s right side is used for sample collection as the right vagus nerve provides less innervation to the heart and diaphragm than the left vagus nerve. Vagus nerve puncture can cause the pig to start showing signs of dyspnea, cyanosis, and convulsions (Dewey and Straw 2006).

**Jugular Vein.** To reach the jugular vein, the procedure is similar to that of the anterior vena cava with the needle being inserted about 5 cm cranially from the thoracic inlet (Figure 1.5). The right side of the pig is still preferred. The jugular vein is located more superficial than the anterior vena cava but cannot be visualized as in many other species. The process still requires a blind stick.

**Ear Veins.** Ear veins can be raised by using a slight tourniquet (usually a rubber band around the ear or pressure with one’s thumb) as seen in Figure 1.8. Slight slapping of the back of the ear with one’s back of the fingers can help stimulate the raising of the veins. Veins in pigs with colored ears are more difficult to visualize. Venipuncture is done starting at the most distal point (toward the ear tip) of the largest vein, so if a hematoma is formed, a more cranial point can still be used for sample collection. A butterfly catheter and syringe should be used. For a quick polymerase chain reaction (PCR) testing, a simple prick of an ear vein with the tip of a 20-gauge needle can provide enough blood for collection with a Dacron swab.

**Miscellaneous Methods.** Tail bleeding (Muirhead 1981), femoral vein (Brown et al. 1978), cephalic vein (Sankari 1983; Tumbleson et al. 1968), cardiac puncture (Calvert et al. 1977), and orbital venous sinus bleeding (Huhn et al. 1969) have all been described.
Oral Fluids Collection

Oral fluids collection for veterinary testing is becoming a more common practice in swine medicine especially in the United States. Oral fluid is a mixture of saliva and oral mucosal transudates. Oral fluids can contain both organisms and antibodies of interest (Prickett et al. 2008).

The process of oral fluid collection is simple and practical. It involves the following steps:

1. A cotton rope is hung from a pen by using a special bracket, by plastic tie, or simply by tying it with a knot. A cotton rope is used because it is highly absorbent with greater yield. The suggested rope sizes are 1.3 cm (1/2 in.) for nursery or 1.6 cm (5/8 in.) for grow-finish pigs. Ropes should be cut to the correct length so that they reach the top of the shoulders of the pigs. As pigs chew the ropes, the ropes will unravel and stretch, so longer lengths are not recommended.

2. Allow the pigs in the pen to chew on the rope for 20–30 minutes.

3. Extract the oral fluids from the rope by inserting the bottom (wet) end of the rope in a clean plastic bag (or disposable plastic boot). Squeeze the rope so that fluid accumulates in one of the corners of the bag. Cut the corner of the bag and collect the fluid into a sample tube. Ideally, at least a 4-mL sample will be obtained.

The sample then needs to be refrigerated until testing. The sample may be centrifuged for 10 minutes if it contains large number of particulates. The sample needs to be identified as an oral fluid sample when submitting for testing as special testing protocols need to be used by the diagnostic laboratory.

REFERENCES


INTRODUCTION

The objective of this chapter is to provide a list of differentials to consider under various different clinical presentations. They are organized by system affected. Due to the international scope of this book, these lists are designed to be inclusive rather than exclusive as prevalence is relative to geographical location. The World Organization for Animal Health (OIE) continually updates its list of diseases requiring international reporting (www.oie.int) because of their impact in animal and public health worldwide, including trade concerns. World Animal Health Information Database (web.oie.int/wahis/public.php?page=disease) provides a web portal for monitoring outbreaks, disease distribution maps, and detailed country diseases for all OIE-listed diseases.

It is envisioned that readers will refer to this section to remind themselves of possible differentials. It is helpful to be open about all possible causes rather than just focus on the common causes especially when dealing with challenging cases and to ensure that new causes to a particular system or region are not missed. Many times clinical disease outbreaks in large populations are multifactorial, and thus, focusing on single causes can misguide practitioners. A quick review of the respective body system chapter (Section II, Chapters 14–22) can help guide the prioritization of the list. Individual causes can then be better researched (etiology, clinical signs, diagnosis, and prevention) in their respective chapters, which are identified in most of the tables.

It is important to remember that because these lists are inclusive rather than exclusive, there are many causes listed for which commercially available diagnostic test are not available. Chapter 7 reviews many of the different diagnostic tests available including important information on test performance and considerations in interpreting results.

DIGESTIVE SYSTEM

Chapter 15 covers valuable information regarding the digestive system including useful tables summarizing the diagnosis of mechanisms of diarrhea (Table 15.1), some common gastrointestinal conditions (Table 15.2), and pathology and diagnostic confirmation of common conditions (Table 15.3).

The approximate age at which certain causes of diarrhea and vomiting are more common is shown in Tables 2.1 and 2.2, respectively. The approximate age is given solely as guidance to help emphasize certain causes based on the age of pigs and does not imply the cause to be restricted only to that age group. Table 2.3 provides a general list of possible causes of rectal prolapses including a brief explanation.

RESPIRATORY SYSTEM

An overview of the respiratory system is provided in Chapter 21. Tables 2.4 and 2.5 summarize differential diagnosis lists for major respiratory clinical presentations.
<table>
<thead>
<tr>
<th></th>
<th>1-2 days</th>
<th>3-4 days</th>
<th>5-6 days</th>
<th>1 week</th>
<th>2 weeks</th>
<th>3 weeks</th>
<th>1 month</th>
<th>2 months</th>
<th>3 months</th>
<th>4 months</th>
<th>5 months</th>
<th>6 months</th>
<th>Adults</th>
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<td><em>Clostridium difficile</em></td>
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<tr>
<td><em>Clostridium perfringens</em> type A</td>
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<td>Bovine viral diarrhea</td>
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<td><em>Enterococcus</em> spp.</td>
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<td>Teschovirus</td>
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<td>Porcine reproductive and respiratory syndrome virus</td>
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<td>Transmissible gastroenteritis virus</td>
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<td>Porcine epidemic diarrhea virus</td>
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<td><em>Campylobacter</em> spp.</td>
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<td>Hairballs</td>
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<td>15</td>
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<tr>
<td>Foreign body</td>
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</tbody>
</table>

HEV, hemagglutinating encephalomyelitis virus; EEEV, eastern equine encephalitis virus.

### Table 2.3. Causes of rectal prolapses in pigs (also see Chapter 15)

<table>
<thead>
<tr>
<th>Cause</th>
<th>Comments</th>
</tr>
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<tbody>
<tr>
<td>Diarrhea</td>
<td>Abnormally acid stool in the rectum causes irritation, tenesmus, and prolapse. Refer to the section on diarrhea for differentiation between causes of diarrhea.</td>
</tr>
<tr>
<td>Cough</td>
<td>Increased abdominal pressure generated during coughing (especially chronic prolonged bouts) causes displacement of the rectum. Refer to the section on cough for differentiation between causes of cough.</td>
</tr>
<tr>
<td>Piling</td>
<td>Environmental temperatures too low. Abdominal pressure on the pig at the bottom of the pile produces prolapse.</td>
</tr>
<tr>
<td>Zearalenone</td>
<td>Estrogens cause swelling of the perineal area, tenesmus, and prolapse.</td>
</tr>
<tr>
<td>Floor design</td>
<td>Excessively sloped floors for crated sows cause increased pressure on pelvic structures as pregnancy progresses.</td>
</tr>
<tr>
<td>Antibiotics</td>
<td>Rectal prolapse has been reported in pigs in the first few weeks after lincomycin or tylosin has been added to the feed. Prolapses cease later as pigs apparently become accustomed to the antibiotic.</td>
</tr>
<tr>
<td>Inherited predisposition</td>
<td>Sporadic reports in the literature of herd outbreaks that occurred in the offspring of certain boars.</td>
</tr>
<tr>
<td>Postpartum</td>
<td>Complex etiology surrounding farrowing.</td>
</tr>
<tr>
<td>Prepartum</td>
<td>Constipation and pressure of heavily gravid uterus.</td>
</tr>
<tr>
<td>Any condition that is associated with tenesmus</td>
<td>Urethritis, vaginitis, rectal or urethral injury postbreeding, urethral calculi. Excess salt in the diet.</td>
</tr>
</tbody>
</table>

Source: Straw et al. (2006).
<table>
<thead>
<tr>
<th>Cause</th>
<th>&lt;1 week</th>
<th>1-4 weeks</th>
<th>1 month</th>
<th>2 months</th>
<th>3 months</th>
<th>4 months</th>
<th>5 months</th>
<th>6 months</th>
<th>Adults</th>
<th>Chapter</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCMV</td>
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<td>28</td>
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<tr>
<td>Carbon monoxide toxicity</td>
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<td>17</td>
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<tr>
<td>Porcine reproductive and respiratory syndrome virus</td>
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<tr>
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<td><em>Clostridium tetani</em></td>
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<td><em>Arcanobacterium pyogenes</em></td>
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<td>Nitrite toxicity</td>
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<td>Iron deficiency anemia (or blood loss anemia)</td>
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<td><em>Actinobacillus suis</em></td>
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<td>Blue eye paramyxovirus</td>
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<td><em>Salmonella choleraeuis</em></td>
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<td><em>Ascaris suum</em></td>
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<tr>
<td><em>Metastrongylus spp.</em></td>
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<tr>
<td>Vitamin A deficiency</td>
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<td>Vitamin D toxicity</td>
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<td>Organophosphate toxicity</td>
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<tr>
<td>Carbamate toxicity</td>
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<tr>
<td>Chlorinated hydrocarbon toxicity</td>
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<td>Pentachlorophenol toxicity</td>
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<td>Dipyridal herbicide toxicity</td>
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<td>Fumonisin</td>
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<td><em>Erysipelothrix rhusiopathiae</em></td>
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<tr>
<td><em>Mycobacterium spp.</em></td>
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<td>63</td>
</tr>
<tr>
<td><em>Mycoplasma suis</em></td>
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<tr>
<td><em>Mycoplasma hyopneumoniae</em></td>
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<tr>
<td>Hydrogen sulfide toxicity</td>
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<tr>
<td>Gossypol toxicity</td>
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<td>70</td>
</tr>
</tbody>
</table>

PCMV, porcine cytomegalo virus; HEV, hemagglutinating encephalomyelitis virus; CM, coliform mastitis; Puffer, puffer sow syndrome.
Table 2.5. Certain causes of sneezing in pigs (also see Chapter 21 especially Table 21.5)

<table>
<thead>
<tr>
<th>Cause</th>
<th>Chapter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atrophic rhinitis</td>
<td>Chapter 49</td>
</tr>
<tr>
<td>Blue eye paramyxovirus</td>
<td>Chapter 41</td>
</tr>
<tr>
<td>Environmental contaminants</td>
<td></td>
</tr>
<tr>
<td>Ammonia</td>
<td>Chapters 5, 58</td>
</tr>
<tr>
<td>Dust, pollen, irritants</td>
<td>Chapter 5</td>
</tr>
<tr>
<td>Hemagglutinating encephalomyelitis virus</td>
<td>Chapter 35</td>
</tr>
<tr>
<td>Porcine cytomegalovirus</td>
<td>Chapter 28</td>
</tr>
<tr>
<td>Porcine reproductive and respiratory syndrome virus</td>
<td>Chapter 31</td>
</tr>
<tr>
<td>Pseudorabies virus</td>
<td>Chapter 28</td>
</tr>
</tbody>
</table>

**INTEGUMENTARY SYSTEM**

The integumentary system is reviewed in Chapter 17. Table 2.6 helps summarize the approximate age when specific skin diseases are more common. Tables 2.7 and 17.2 help narrow down the differential diagnosis of skin diseases based on location and clinical presentation of the lesions.

**HEMOPOIETIC SYSTEM**

The cardiovascular and hemopoietic systems are reviewed in Chapter 14. Anemia is a common clinical presentation related to the hemopoietic system. Possible causes of anemia are listed in both Tables 2.8 and 14.7.

**NERVOUS AND LOCOMOTOR SYSTEM**

Chapter 19 reviews both the nervous and locomotor system. It is important to note that many times diseases affecting either of these systems have similar general clinical presentations. Table 2.9 lists some causes of neurological signs, while Table 19.8 tries to further differentiate clinical presentations. Lameness conditions are summarized in Table 2.10.

**REPRODUCTIVE SYSTEM**

The reproductive system is summarized in Chapter 20. Possible causes of reproductive losses in pigs are summarized in Tables 2.11 and 20.8. Although not directly related to reproductive performance, but rather related to pregnancy, common congenital anomalies are listed in Table 2.12.

**ZOO NOTIC**

An overview of preharvest food safety and zoonotic diseases is included in Chapter 12. Pig diseases with zoonotic potential are summarized in Table 2.13.

**ACKNOWLEDGMENTS**

The following individuals are acknowledged for their valuable input and guidance provided: Robert Desrosiers, Phil Gauger, Eric Neumann, Kent Schwartz, Ernest Stanford, and Locke Karriker.
<table>
<thead>
<tr>
<th>Weeks of Age</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>8</th>
<th>10</th>
<th>14</th>
<th>18</th>
<th>32</th>
<th>50</th>
<th>100</th>
<th>156</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infection of injury caused by trauma, ischemia, or surgical procedures</td>
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<tr>
<td>Mange and lice</td>
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<tr>
<td>Ringworm</td>
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<tr>
<td>Insect bites from fleas, flies, and mosquitoes</td>
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<tr>
<td>Sunburn or photosensitization</td>
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<td>Necrobacillosis</td>
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<td>Teat and knee erosion</td>
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<td>Thrombocytopenia purpura</td>
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<td>Dermatosis vegetans</td>
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<tr>
<td>Staphylococcal acne</td>
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<tr>
<td>Swine pox</td>
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</tr>
<tr>
<td>Acute generalized exudative epidermitis, local exudative epidermitis</td>
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<td></td>
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<tr>
<td>Pityriasis rosea</td>
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</tr>
<tr>
<td>Ear necrosis</td>
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</tr>
<tr>
<td>Parakeratosis</td>
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<td></td>
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</tr>
<tr>
<td>Callus of the knee, fetlock, elbow, hock, or tuber ischia</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Porcine dermatitis and nephropathy syndrome</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>Bursitis</td>
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<tr>
<td>Dermatosis erythematosa</td>
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</tr>
<tr>
<td>Mastitis</td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>Shoulder ulcer callus</td>
<td></td>
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<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

Source: Straw et al. (2006).
<table>
<thead>
<tr>
<th>Location</th>
<th>Normal Tissue</th>
<th>Proliferative or Nonproliferative</th>
<th>Demarcation of Lesions</th>
<th>Possible Cause</th>
</tr>
</thead>
<tbody>
<tr>
<td>Face</td>
<td>Elevated</td>
<td>Nonproliferative</td>
<td>Discrete</td>
<td>Staphylococcal acne</td>
</tr>
<tr>
<td></td>
<td>Flat</td>
<td>Nonproliferative</td>
<td>Discrete</td>
<td>Necrotic stomatitis</td>
</tr>
<tr>
<td>Face and feet</td>
<td>Elevated</td>
<td>Nonproliferative</td>
<td>Discrete</td>
<td>Vesicular diseases(^a)</td>
</tr>
<tr>
<td>Shoulder</td>
<td>Elevated</td>
<td>Nonproliferative</td>
<td>Discrete</td>
<td>Hematoma, callus</td>
</tr>
<tr>
<td>Knees, elbows, and hocks</td>
<td>Flat</td>
<td>Nonproliferative</td>
<td>Discrete</td>
<td>Ulcer</td>
</tr>
<tr>
<td>Knee erosions</td>
<td>Flat</td>
<td>Nonproliferative</td>
<td>Discrete</td>
<td>Callus</td>
</tr>
<tr>
<td>Callus</td>
<td>Flat</td>
<td>Nonproliferative</td>
<td>Diffuse</td>
<td>Bursitis</td>
</tr>
<tr>
<td>Ear</td>
<td>Elevated</td>
<td>Nonproliferative</td>
<td>Discrete</td>
<td>Hematoma</td>
</tr>
<tr>
<td>Flat</td>
<td>Nonproliferative</td>
<td>Diffuse</td>
<td>Discrete</td>
<td>Greasy spot behind ear</td>
</tr>
<tr>
<td>Flat</td>
<td>Proliferative</td>
<td>Discrete</td>
<td>Discrete</td>
<td>Ear necrosis</td>
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<tr>
<td>Flat</td>
<td>Proliferative</td>
<td>Diffuse</td>
<td>Diffuse</td>
<td>Mange</td>
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<tr>
<td>Ear, eye, and udder</td>
<td>Flat</td>
<td>Nonproliferative</td>
<td>Diffuse</td>
<td>Photosensitization</td>
</tr>
<tr>
<td>Extremities</td>
<td>Flat</td>
<td>Nonproliferative</td>
<td>Diffuse</td>
<td>Cyanosis or reddening secondary to disease(^b)</td>
</tr>
<tr>
<td>Dorsal</td>
<td>Elevated</td>
<td>Nonproliferative</td>
<td>Diffuse</td>
<td>Lumpy skin disease</td>
</tr>
<tr>
<td>Flat</td>
<td>Proliferative</td>
<td>Diffuse</td>
<td>Diffuse</td>
<td>Hyperkeratinization</td>
</tr>
<tr>
<td>Flat</td>
<td>Nonproliferative</td>
<td>Diffuse</td>
<td>Diffuse</td>
<td>Sunburn</td>
</tr>
<tr>
<td>Flat</td>
<td>Nonproliferative</td>
<td>Diffuse</td>
<td>Discrete</td>
<td>Epitheliogenesis imperfecta</td>
</tr>
<tr>
<td>Ventral abdomen</td>
<td>Elevated</td>
<td>Nonproliferative</td>
<td>Discrete</td>
<td>Pityriasis rosea, eosinophilic dermatitis</td>
</tr>
<tr>
<td>Flat</td>
<td>Nonproliferative</td>
<td>Diffuse</td>
<td>Discrete</td>
<td>Urticarial mange</td>
</tr>
<tr>
<td>Flat</td>
<td>Nonproliferative</td>
<td>Diffuse</td>
<td>Discrete</td>
<td>Transit erythema, teat necrosis</td>
</tr>
<tr>
<td>Flat</td>
<td>Nonproliferative</td>
<td>Diffuse</td>
<td>Discrete</td>
<td>Mastitis, benign peripartal cyanosis</td>
</tr>
<tr>
<td>Ventral cervical area</td>
<td>Elevated</td>
<td>Nonproliferative</td>
<td>Discrete</td>
<td>Jowl abscess, tuberculosis</td>
</tr>
<tr>
<td>Ventral cervical area</td>
<td>Elevated</td>
<td>Nonproliferative</td>
<td>Diffuse</td>
<td>Pharyngeal anthrax</td>
</tr>
<tr>
<td>Generalized</td>
<td>Elevated</td>
<td>Nonproliferative</td>
<td>Discrete</td>
<td>Pustular dermatitis, swine pox, infected injuries, neoplasia, abscess</td>
</tr>
<tr>
<td>Flat</td>
<td>Proliferative</td>
<td>Diffuse</td>
<td>Discrete</td>
<td>Dermatosis vegetans</td>
</tr>
<tr>
<td>Flat</td>
<td>Nonproliferative</td>
<td>Diffuse</td>
<td>Discrete</td>
<td>Parakeratosis, demodectic mange, lice, sarcoptic mange, exudative epidermitis</td>
</tr>
<tr>
<td>Flat</td>
<td>Nonproliferative</td>
<td>Diffuse</td>
<td>Discrete</td>
<td>Ringworm, dermatosis erythematousus, thrombocytopenia purpura, erysipelas</td>
</tr>
<tr>
<td>Flat</td>
<td>Nonproliferative</td>
<td>Diffuse</td>
<td>Discrete</td>
<td>Carbon monoxide toxicity, porcine stress syndrome, hypotrichosis, cyanosis or reddening secondary to any bacteremia or viremia</td>
</tr>
</tbody>
</table>

Source: Straw et al. (2006).
\(^a\)Foot-and-mouth disease, vesicular exanthema, vesicular stomatitis, swine vesicular disease, San Miguel sea lion virus, porcine parvovirus, drug eruption.
\(^b\)Salmonellosis, *Haemophilus parasuis*, *Actinobacillus pleuropneumoniae*, porcine reproductive and respiratory syndrome, colibacillosis, organophosphate toxicity, hemagglutinating encephalomyelitis.
Table 2.8. Cause of anemia in pigs (also see Chapter 14 especially Table 14.7)

<table>
<thead>
<tr>
<th>Category</th>
<th>Cause</th>
<th>Chapter</th>
</tr>
</thead>
<tbody>
<tr>
<td>General</td>
<td>Blood loss (acute or chronic)</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>Chronic disease</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>Gastric ulcer</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>Hemorrhagic bowel syndrome</td>
<td>15</td>
</tr>
<tr>
<td>Bacterial</td>
<td><em>Lawsonia intracellularis</em></td>
<td>59</td>
</tr>
<tr>
<td></td>
<td><em>Mycoplasma suis</em></td>
<td>57</td>
</tr>
<tr>
<td></td>
<td><em>Salmonella</em> spp.</td>
<td>60</td>
</tr>
<tr>
<td>Deficiencies or toxicities</td>
<td>Aflatoxin</td>
<td>69</td>
</tr>
<tr>
<td></td>
<td>Anticoagulant toxicity (warfarin, brodifacoum, etc.)</td>
<td>70</td>
</tr>
<tr>
<td></td>
<td>Coal tar toxicity (clay pigeons)</td>
<td>70</td>
</tr>
<tr>
<td></td>
<td>Cobalt toxicity</td>
<td>68</td>
</tr>
<tr>
<td></td>
<td>Copper deficiency and toxicity</td>
<td>68</td>
</tr>
<tr>
<td></td>
<td>Folic acid deficiency</td>
<td>68</td>
</tr>
<tr>
<td></td>
<td>Iron deficiency</td>
<td>68</td>
</tr>
<tr>
<td></td>
<td>Niacin deficiency</td>
<td>68</td>
</tr>
<tr>
<td></td>
<td>Trichothecces</td>
<td>69</td>
</tr>
<tr>
<td></td>
<td>Vitamin B₁₂ deficiency</td>
<td>68</td>
</tr>
<tr>
<td></td>
<td>Vitamin B₆ deficiency</td>
<td>68</td>
</tr>
<tr>
<td></td>
<td>Vitamin E deficiency</td>
<td>68</td>
</tr>
<tr>
<td></td>
<td>Vitamin K deficiency</td>
<td>68</td>
</tr>
<tr>
<td></td>
<td>Zearalenone</td>
<td>69</td>
</tr>
<tr>
<td>Parasites</td>
<td><em>Fasciola hepatica</em></td>
<td>67</td>
</tr>
<tr>
<td></td>
<td>Flea infestation</td>
<td>65</td>
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<tr>
<td></td>
<td><em>Haematopinus suis</em></td>
<td>65</td>
</tr>
<tr>
<td></td>
<td><em>Trichuris suis</em></td>
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<tr>
<td></td>
<td><em>Macracanthorhynchus hirudinaceus</em></td>
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<tr>
<td></td>
<td><em>Strongyloides ransomi</em></td>
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<tr>
<td>Viral</td>
<td>Bovine viral diarrhea virus</td>
<td>38</td>
</tr>
<tr>
<td></td>
<td>Porcine reproductive and respiratory syndrome virus</td>
<td>31</td>
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</tbody>
</table>
Table 2.9. Cause of neurological signs in pigs (also see Chapter 19 especially Table 19.8)

<table>
<thead>
<tr>
<th>Category</th>
<th>Cause</th>
<th>Chapter</th>
</tr>
</thead>
<tbody>
<tr>
<td>General or congenital</td>
<td>Brain or spinal cord injury</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td>Congenital malformations</td>
<td>19.13</td>
</tr>
<tr>
<td></td>
<td>Congenital tremors</td>
<td>19.7</td>
</tr>
<tr>
<td></td>
<td>Hypoglycemia</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td>Hypoxia/anoxia</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td>Middle ear infection</td>
<td>19</td>
</tr>
<tr>
<td>Bacterial or protozoal</td>
<td><em>Clostridium botulinum</em></td>
<td>52</td>
</tr>
<tr>
<td></td>
<td><em>Clostridium tetani</em></td>
<td>52</td>
</tr>
<tr>
<td></td>
<td><em>Escherichia coli</em> (usually 1–2 weeks postweaning)</td>
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<tr>
<td></td>
<td><em>Haemophilus parasuis</em></td>
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<td></td>
<td><em>Listeria monocytogenes</em></td>
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<tr>
<td></td>
<td><em>Streptococcus suis</em></td>
<td>62</td>
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<tr>
<td></td>
<td><em>Toxoplasma gondii</em></td>
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</tr>
<tr>
<td></td>
<td>Other bacterial meningitis</td>
<td>19</td>
</tr>
<tr>
<td>Deficiencies or toxicities</td>
<td>Ammonia salts toxicity</td>
<td>70</td>
</tr>
<tr>
<td></td>
<td>Arsanilic acid toxicity</td>
<td>70</td>
</tr>
<tr>
<td></td>
<td>Arsenic toxicity</td>
<td>70</td>
</tr>
<tr>
<td></td>
<td>Calcium deficiency</td>
<td>68</td>
</tr>
<tr>
<td></td>
<td>Carbamate toxicity</td>
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</tr>
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<td></td>
<td>Carbon dioxide toxicity</td>
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</tr>
<tr>
<td></td>
<td>Carbon monoxide toxicity</td>
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<tr>
<td></td>
<td>Chlorinated hydrocarbon toxicity</td>
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<td></td>
<td>Cocklebur toxicity</td>
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<tr>
<td></td>
<td>Copper deficiency</td>
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<tr>
<td></td>
<td>Dichlorvos toxicity</td>
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<tr>
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<td>Hydrogen sulfide toxicity</td>
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<tr>
<td></td>
<td>Hygromycin toxicity</td>
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</tr>
<tr>
<td></td>
<td>Iron toxicity</td>
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</tr>
<tr>
<td></td>
<td>Lead toxicity</td>
<td>70</td>
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<tr>
<td></td>
<td>Magnesium deficiency or toxicity</td>
<td>68</td>
</tr>
<tr>
<td></td>
<td>Mercury toxicity</td>
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</tr>
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<td></td>
<td>Niacin deficiency</td>
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</tr>
<tr>
<td></td>
<td>Nightshade toxicity</td>
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</tr>
<tr>
<td></td>
<td>Nitrate/nitrite toxicity</td>
<td>70</td>
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<tr>
<td></td>
<td>Nitrofurans toxicity</td>
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</tr>
<tr>
<td></td>
<td>Organophosphate toxicity</td>
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<td></td>
<td>Pantothentic acid deficiency</td>
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<tr>
<td></td>
<td>Pentachlorophenol toxicity</td>
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<td>Phenoxy herbicide toxicity</td>
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<td>Phosphorus deficiency</td>
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<td></td>
<td>Pigweed toxicity</td>
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<td>Riboflavin deficiency</td>
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<td>Sodium chloride deficiency</td>
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<td></td>
<td>Sodium fluoroacetate toxicity</td>
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<td></td>
<td>Strychnine toxicity</td>
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<td></td>
<td>Streptomycin toxicity</td>
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<tr>
<td></td>
<td>Vitamin A deficiency</td>
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<tr>
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<td>Vitamin B6 deficiency</td>
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<td>Vitamin D deficiency</td>
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<td></td>
<td>Water deprivation (salt poisoning)</td>
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</tr>
<tr>
<td>Viral</td>
<td>African swine fever</td>
<td>25</td>
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<tr>
<td></td>
<td>Blue eye paramyxovirus</td>
<td>41</td>
</tr>
<tr>
<td></td>
<td>Classical swine fever</td>
<td>38</td>
</tr>
<tr>
<td></td>
<td>Hemagglutinating encephalomyelitis virus</td>
<td>35</td>
</tr>
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<td>Nipah virus</td>
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<td></td>
<td>Porcine cytomegaloirus</td>
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<tr>
<td></td>
<td>Porcine reproductive and neurological syndrome virus</td>
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<tr>
<td></td>
<td>Porcine technoviruses</td>
<td>42</td>
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<tr>
<td></td>
<td>Pseudorabies virus</td>
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<tr>
<td></td>
<td>Rabies virus</td>
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</tbody>
</table>
Table 2.10. Approximate ages at which diseases causing lameness are more common (also see Chapter 19)

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<tr>
<th>Age in Months</th>
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<th>2</th>
<th>3</th>
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<th>6</th>
<th>18</th>
<th>30</th>
<th>42</th>
<th>54</th>
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</thead>
<tbody>
<tr>
<td>Trauma: muscle bruising, sprains, strains, dislocations, fractures</td>
<td></td>
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</tr>
<tr>
<td><em>Clostridium tetani</em> or septicum infection</td>
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</tr>
<tr>
<td>Vesicular diseases: foot-and-mouth, vesicular exanthema, swine vesicular disease, vesicular stomatitis, San Miguel sea lion virus</td>
<td></td>
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<tr>
<td><em>Streptococcus suis</em> infection</td>
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<td></td>
<td></td>
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</tr>
<tr>
<td><em>S. equisimilis</em> infection</td>
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<td></td>
</tr>
<tr>
<td>Acute <em>Mycoplasma hyorhinis</em> infection</td>
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<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td><em>Haemophilus parasuis</em> infection</td>
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Source: Straw et al. (2006).
Table 2.11. Causes of reproductive losses in pigs (also see Chapter 20 especially Table 20.8)

<table>
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<tr>
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<th>Abortion</th>
<th>Weak Births</th>
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<td>Porcine reproductive and neurological syndrome virus</td>
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*Parovirus can cause abortions under rare and unique situations.*
<table>
<thead>
<tr>
<th>Defect</th>
<th>Prevalence (%)</th>
<th>Etiology</th>
<th>Diagnosis</th>
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<tbody>
<tr>
<td>Microencephaly</td>
<td>0.07%</td>
<td>Heat stress midpregnancy</td>
<td>History of heat stress</td>
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<tr>
<td></td>
<td></td>
<td>Unknown (most cases)</td>
<td>An agent affecting development in early or midpregnancy</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Vitamin A deficiency</td>
<td>Multiple defects in affected litters; heavy neonatal mortality; history; diet analysis; serum and liver vitamin A analysis</td>
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<tr>
<td></td>
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<td>Hog cholera (HC) infection</td>
<td>HC infection in herd; virus isolation; fluorescent antibody test; serology; congenital tremor AI present in herd</td>
</tr>
<tr>
<td>Neural tube defects</td>
<td>0.04%</td>
<td>Heritable</td>
<td>Mode of inheritance uncertain; dominant gene (?)</td>
</tr>
<tr>
<td>(anencephaly, encephalocele,</td>
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<td>Unknown</td>
<td>An agent affecting embryos at 12–16 days of development</td>
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<td>hydrocephalus, spina bifida)</td>
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<td>Vitamin A deficiency (hydrocephalus)</td>
<td>Multiple defects in affected litters; heavy neonatal mortality; history; diet analysis; serum and liver vitamin A analysis</td>
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<tr>
<td>Congenital tremor</td>
<td>0.20%</td>
<td>HC virus (type AI)</td>
<td>History of vaccination during early pregnancy</td>
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<tr>
<td></td>
<td></td>
<td>Type All (unidentified virus)</td>
<td>HC infection in herd; virus isolation; fluorescent antibody test; serology; affects piglets of all breeds and both sexes; hypomyelinogenesis; cerebellar hypoplasia; neurochemical analysis of myelin lipids of spinal cord; small cross-sectional area of the spinal cord</td>
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<tr>
<td></td>
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<td>Type AIII</td>
<td>Hypomyelinogenesis of spinal cord; analysis of myelin lipids of spinal cord; small cross-sectional area of the spinal cord</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Type IV</td>
<td>Monogenic sex-linked gene mutation in Landrace affecting only males and associated with defect in myelin sheath</td>
</tr>
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<td></td>
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<td>Pseudorabies (PR) virus</td>
<td>PR infection in herd; virus isolation; serology</td>
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<tr>
<td></td>
<td></td>
<td>Neguvon (metrifonate, trichlorfon)</td>
<td>History of dosing sows in midpregnancy; hypoplasia of cerebrum and cerebellum; Purkinje-cell loss; changes in neurotransmitters</td>
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<tr>
<td>Arthrogryposis</td>
<td>0.10</td>
<td>Tobacco stalks, jimsonweed, poison hemlock, wild black cherry</td>
<td>History of exposure to plants in early to midpregnancy</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Vitamin A deficiency</td>
<td>Multiple defects in affected litters; heavy neonatal mortality; history; diet analysis; serum and liver vitamin A analysis</td>
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<tr>
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<td></td>
<td>Hog cholera (HC)-attenuated vaccine virus</td>
<td>History of vaccination during early pregnancy</td>
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<tr>
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<td>HC infection</td>
<td>HC infection in herd; virus isolation; fluorescent antibody test; serology; affects piglets of all breeds and both sexes; hypomyelinogenesis; cerebellar hypoplasia; neurochemical analysis of myelin lipids of spinal cord; small cross-sectional area of the spinal cord</td>
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<td>Paramyxovirus infection</td>
<td>Menangle virus infection during pregnancy</td>
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<td></td>
<td>Heritable</td>
<td>Recessive gene (?); autosomal recessive in Yorkshire pigs</td>
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<td></td>
<td>Unknown (most cases)</td>
<td>An agent affecting development in early or midpregnancy</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Unknown</td>
<td>Possibly caused by limb vascular defects in early pregnancy</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Heritable</td>
<td>Possibly a recessive gene; cleft palate in Poland China pigs probably genetic</td>
</tr>
<tr>
<td></td>
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<td>Unknown (most cases)</td>
<td>An agent affecting development in early or midpregnancy</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Possibly heritable</td>
<td>Mode of inheritance uncertain; occasionally urogenital defect associated</td>
</tr>
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<td>Unknown</td>
<td>Often associated with motor defects in hind limbs; vertebral defects</td>
</tr>
<tr>
<td>Micromelia</td>
<td>0.10</td>
<td>Heritable</td>
<td>Most common in Landrace, less in Large White; probably polygenic mode of inheritance; incidence modified by maternal stress, slippery floor, birth weight, maternal nutrition</td>
</tr>
<tr>
<td>Cleft palate/harelip</td>
<td>0.07</td>
<td>Heritable</td>
<td>Higher mortalities than other forms; feed analysis</td>
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<tr>
<td>Deformed tail</td>
<td>0.08</td>
<td>Heritable</td>
<td>Mode of inheritance uncertain; incidence modified by environment</td>
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<tr>
<td>Myofibrillar hypoplasia</td>
<td>1.05</td>
<td>Heritable</td>
<td>Possibly polygenic mode of inheritance</td>
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<tr>
<td>Inguinal hernia</td>
<td>0.40</td>
<td>Fusarium toxin</td>
<td>Possibly polygenic inheritance or an autosomal recessive or autosomal dominant form of transmission</td>
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<td>Umbilical hernia</td>
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<td>Heritable</td>
<td>Possibly polygenic inheritance or an autosomal recessive or autosomal dominant form of transmission</td>
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Table 2.13. Pig diseases with zoonotic potential (also see Chapter 12)

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<tr>
<th>Defect</th>
<th>Prevalence (%)</th>
<th>Etiology</th>
<th>Diagnosis</th>
</tr>
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<tbody>
<tr>
<td>Hypotrichosis</td>
<td></td>
<td>Heritable in some breeds</td>
<td>Mode of inheritance uncertain</td>
</tr>
<tr>
<td>Epitheliogenesis imperfect</td>
<td>0.05</td>
<td>Heritable</td>
<td>Possibly autosomal recessive gene; hydronephrosis</td>
</tr>
<tr>
<td>Dermatosis vegetans</td>
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<td>Heritable</td>
<td>Autosomal recessive; associated</td>
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<tr>
<td>Pityriasis rosea</td>
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<td>Probably heritable</td>
<td>Mode of inheritance uncertain; affects young pigs</td>
</tr>
<tr>
<td>Von Willebrand's disease</td>
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<td>Heritable</td>
<td>Recessive gene in Poland China pigs; excess bleeding</td>
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<td>Navel bleeding</td>
<td>0.14–1.2</td>
<td>Unknown</td>
<td>Cord is edematous, familial linkage</td>
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<td>Cardiac defects</td>
<td>0.03</td>
<td>Unknown</td>
<td>Most cases recognized at 4–8 weeks; mostly males</td>
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<tr>
<td>Cryptorchidism</td>
<td>0.39</td>
<td>Probably heritable</td>
<td>Polygenic transmission; left testicle most commonly</td>
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<tr>
<td>Female genital hypoplasias, duplications</td>
<td>0.68</td>
<td>Probably a heritable component</td>
<td>Mode of inheritance uncertain; genital tract incomplete or duplicated</td>
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<td>Male pseudohermaphroditism</td>
<td>0.2–0.6</td>
<td>Heritable</td>
<td>Mode of transmission uncertain; testicles in abdomen</td>
</tr>
<tr>
<td>True hermaphroditism</td>
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<td>Heritable</td>
<td>Mode of inheritance uncertain; testicular and ovarian</td>
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<tr>
<td></td>
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<td>tissues usually with female tubular tract</td>
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Table 2.12. (Continued)

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<th>Prevalence (%)</th>
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<th>Diagnosis</th>
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<tbody>
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<td>Hypotrichosis</td>
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<td>Heritable in some breeds</td>
<td>Mode of inheritance uncertain</td>
</tr>
<tr>
<td>Iodine deficiency</td>
<td></td>
<td></td>
<td>Stillbirths and high neonatal mortality; enlarged thyroids; skin edematous; feed analysis</td>
</tr>
<tr>
<td>Epitheliogenesis imperfect</td>
<td>0.05</td>
<td>Heritable</td>
<td>Possibly autosomal recessive gene; hydronephrosis</td>
</tr>
<tr>
<td>Dermatosis vegetans</td>
<td></td>
<td>Heritable</td>
<td>Autosomal recessive; associated</td>
</tr>
<tr>
<td>Pityriasis rosea</td>
<td></td>
<td>Probably heritable</td>
<td>Mode of inheritance uncertain; affects young pigs</td>
</tr>
<tr>
<td>Von Willebrand's disease</td>
<td></td>
<td>Heritable</td>
<td>Recessive gene in Poland China pigs; excess bleeding</td>
</tr>
<tr>
<td>Navel bleeding</td>
<td>0.14–1.2</td>
<td>Unknown</td>
<td>Cord is edematous, familial linkage</td>
</tr>
<tr>
<td>Cardiac defects</td>
<td>0.03</td>
<td>Unknown</td>
<td>Most cases recognized at 4–8 weeks; mostly males</td>
</tr>
<tr>
<td>Cryptorchidism</td>
<td>0.39</td>
<td>Probably heritable</td>
<td>Polygenic transmission; left testicle most commonly</td>
</tr>
<tr>
<td>Female genital hypoplasias, duplications</td>
<td>0.68</td>
<td>Probably a heritable component</td>
<td>Mode of inheritance uncertain; genital tract incomplete or duplicated</td>
</tr>
<tr>
<td>Male pseudohermaphroditism</td>
<td>0.2–0.6</td>
<td>Heritable</td>
<td>Mode of transmission uncertain; testicles in abdomen</td>
</tr>
<tr>
<td>True hermaphroditism</td>
<td></td>
<td>Heritable</td>
<td>Mode of inheritance uncertain; testicular and ovarian</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>tissues usually with female tubular tract</td>
</tr>
</tbody>
</table>

Modified from Glenda Dvorak.

REFERENCES

DEFINING ANIMAL WELFARE AND ANIMAL CRUELTY

Animal Welfare

One of the first definitions of animal welfare was written by the Brambell Commission in the United Kingdom (Brambell 1965). From a review of the scientific literature available at that time, the commission proposed several conditions deemed necessary to ensure the welfare of production animals. In 1979, the Farm Animal Welfare Council revised the Brambell Commission recommendations naming these conditions the “five freedoms.” The “five freedoms” serve as the basis for many of the farm animal welfare educational and assessment programs globally, and the plethora of regulations enacted in the European Union. These freedoms are the following:

1. freedom from hunger and thirst by ready access to fresh water and a diet to maintain full health and vigor;
2. freedom from discomfort by providing an appropriate environment including shelter and a comfortable resting area;
3. freedom from pain, injury, and disease by prevention or rapid diagnosis and treatment;
4. freedom to express normal behavior by providing sufficient space, proper facilities, and company of the animal’s own kind; and
5. freedom from fear and distress by ensuring conditions and treatment, which avoid mental suffering.

The five freedoms address three areas: “biological functioning” focused on fitness and health; “nature-based” measures that focus additional value on the behavior of animals under natural conditions; and “feeling-based measures” such as fear and distress, or conversely, pleasure. Humans formulate their own definitions of welfare using different weighted combinations of these areas. The idea of feelings being important for welfare was developed by Duncan (1981), and it has since been suggested that feelings are the only thing that mattered when assessing an animal’s welfare (Duncan and Petherick 1991). Others, such as Warnier and Zayan (1985), Broom (1986), Mormède (1990), and Barnett et al. (1991), focus on the biological functioning of animals as a driving component of welfare.

Over time, definitions presented on animal welfare have become more complex. A widely recognized definition of animal welfare developed by Broom (1986) notes that the welfare of an individual animal is based on “its state as regards its attempts to cope with its environment.” This definition is mainly based on the biological function of the individual animal. Recently, the World Organization for Animal Health (OIE) has defined animal welfare as “how an animal is coping with the conditions in which it lives” and provide examples that contribute to good animal welfare that include a combination of biological function, affective state, and concepts of natural living (OIE 2010). The OIE is recognized by the World Trade Organization as the international standard-setting body for animal health and welfare; therefore, this definition is often referenced in international discussions of animal welfare, including those regarding international trade.

Public, Legal, and Technical Definitions of Animal Welfare

Over time, three types of animal welfare definitions have been identified: public, legal, and technical (Gonyou 1993). Public definitions of animal welfare
reflect society’s view of animals and are constructed from the public’s previous knowledge of and experience with animals, which can be highly variable. The public definition is constantly changing as societal views evolve. Legal definitions, crafted by legislators, must satisfy and be accepted by the general public as well as be clear and concise for interpretation by the judicial system. Technical definitions of animal welfare are based on measures of welfare and influence how scientific data are interpreted. Different sectors of the population have emphasized one type of measure over another when interpreting animal welfare. Producers and large-animal veterinarians tend to focus on the biological function of the animal, whereas consumers tend to focus on what they perceive to be natural living. There is a fundamental need for a multidisciplinary approach to measuring animal welfare that includes evaluation of biological function (immune function, growth, etc.), affective states (fear, pain, hunger, etc.), and living in an animal’s natural environment (Fraser et al. 1997).

Defining Animal Cruelty
Animal cruelty can be classified as animal abuse or animal neglect. Animal abuse is an intentional act by an individual to purposely inflict physical harm or injury to an animal (USLegal 2010) whereas animal neglect is a failure to act by the animal caretaker. Simple neglect, or failure to provide basic sustenance needs of the animal, is often committed due to a lack of knowledge or ability of the owner and can be corrected through education and training (ASPCA 2010). Examples of animal abuse and neglect include but are not limited to malicious hitting or beating an animal; applying electric prods to sensitive areas of the animal; and purposeful failure to provide food, water, or minimal care that result in serious harm or death (NPB 2010).

While there are currently no federal laws in the United States that govern the care of livestock on the farm, there are animal cruelty laws in all 50 states. The language, enforcement, and penalties of these laws vary from state to state, and it is important for all veterinarians and livestock producers to be familiar with these state laws. They should be familiar with how livestock are defined and classified within the state, what acts constitute abuse, what penalties are associated with violations, and any mandatory reporting requirements that may exist specifically for veterinarians.

Due to the implications of animal cruelty on the health and welfare of animals and people, the American Veterinary Medical Association and the American Animal Hospital Association have policy statements that support veterinarians reporting cases of animal cruelty to the appropriate authorities when education of the caretaker is inappropriate or has failed, even if animal cruelty reporting is not legally mandated in a state (AAHA 2009; AVMA 2009). Anyone involved in animal care should be aware that accurate record keeping and documentation of these cases are essential.

Comparisons between the Domesticated and Wild Pig
When a veterinarian is assessing the welfare of an animal, they will seek to determine if it is exhibiting normal behaviors. It is necessary to have a concept of what behaviors a feral or wild counterpart may choose to engage in and how this may be relevant to the domesticated pig. Comparisons between domesticated and wild ancestors of a variety of species indicate that the behavioral repertoire of a species remains relatively static during domestication whereas the quantity of or threshold at which individual behaviors are performed may change (Price 1998); that is, the domesticated pig may perform the same behaviors as the wild ancestor but may not perform those behaviors as frequently, or more frequently. Stolba and Wood-Gush (1989) observed the behavior of adult pigs in a seminatural environment and found that although raised in confinement, adult pigs exhibited many behaviors performed by the European wild boar such as rooting, grazing, and nesting. Because domesticated pigs raised in confinement do have similar behavioral needs to their wild counterparts, the environments in which pigs are raised should be designed with the opportunity to express positive behaviors that they are highly motivated to perform.

Deviations in behavior for domesticated pigs compared with their wild/feral counterparts may indicate impaired animal welfare. For example, the presence of stereotyped behavior, behavior that is performed repeatedly without an obvious function, can be indicative of impaired welfare. Stereotypical behaviors reported in pigs include bar biting, sham chewing, and belly nosing. It has been hypothesized that these behaviors develop when a pig is unable to perform highly motivated behaviors, such as foraging, nest building, or suckling (Fraser 1975). However, not all behavioral deviations result in impaired welfare; anti-predator behavior, for example, is a useful behavioral sequence to have in a wild/feral setting for survival, but is less important in a controlled and protected housing environment.

Scientific Approaches to Animal Welfare

Biological Function: Production, Health, and the Immune System
Various responses to physical and psychological stressors that reflect activation of the sympathetic–adrenal–medullary system (i.e., heart rate, epinephrine, and norepinephrine) and the hypothalamic–pituitary–adrenal axis (i.e., adrenocorticotropic hormone [ACTH]
and cortisol) are used as indicators of animal welfare in laboratory studies. Other physiological measures that have been used to measure the stress response in pigs include endorphin, lactate, and glucose concentrations in the blood; heart rate; respiration rate; and electroencephalography.

Because these responses to stress influence key metabolic, immunological, and reproductive processes governing disease resistance and production performance, health and production performance are also used as indicators of animal welfare. High corticosteroid levels reduce protein synthesis and lean tissue growth (Spencer 1985), resulting in slower growth and poorer conformation. In breeding animals, physiological stress responses also influence the hypothalamic–pituitary–ovarian axis. The effects of psychological stressors on performance have been well established in numerous experiments by Hemsworth et al. (1986, 1987, 1996) on the adverse effects of negative pig handling by caretakers. Unpleasant handling, in comparison with sympathetic handling, resulted in pigs that were more fearful, had chronically elevated corticosteroid levels, slower growth rates, lower pregnancy rates in gilts, and delayed reproductive development in young boars.

Stressful environmental conditions can increase the susceptibility of pigs to infectious diseases through alteration of the immune system (Kelley 1980). Sustained high levels of corticosteroid hormones in the blood can reduce proliferation of lymphocytes and decrease antibody production impairing the ability of the pig to resist infection. Immune challenge techniques provide another potential set of measures that have been used to assess animal welfare. Morrow-Tesch et al. (1994) demonstrated that social status of pigs had an impact on lymphocyte proliferation in response to a pokeweed mitogen. The pigs that were both dominant and subordinate had lower proliferation than the intermediate pigs in the social hierarchy. Welfare can be assessed not only through measures of performance and disease incidences, but also by examining signs of injury (Backstrom 1973; de Koning 1985), indicators of metabolic problems or impaired health such as fatigue or decreased bone strength as well as mortality rates.

Although production parameters have been considered as appropriate measures of animal welfare (Curtis 1987) and poor productivity can be a useful indicator of a welfare problem, high levels of productivity alone are not always indicative of a high standard of welfare. It is a paradigm that a physically healthy animal is “fairing well” but a healthy pig with high productivity could be mentally compromised.

Affective States of Animals
The psychological state of an animal (referred to as the affective or emotional state) is an integral component of its overall welfare status. Although some areas of the scientific community find it hard to accept that animals can experience emotions, neuroscience has indicated that brain structures and neurotransmitters in humans and animals have similar functions and structures (Butler and Hodos 2005; Jerison 1997; Panksepp et al. 2002).

The “triune brain,” a concept described by MacLean (1990), provides a simple illustration of the adaptations between reptilian, mammalian, and human brain regions. The centermost brain region, shared by all groups, is the limbic brain region. The limbic system is at the top of the spinal cord deep within the cortex and includes structures such as the amygdala, hippocampus, and parts of the diencephalon. The limbic system is the emotional center of the brain for both humans and animals (Panksepp 1998). Emotional circuits controlling anger and fear have been mapped in the limbic system (Panksepp 1990; Siegel 2005). Emotions motivate an animal’s behavior. When studying how certain management and production systems impact animal affective states, researchers, veterinarians, and producers usually focus on the negative emotions. For example, scientists have tried to mitigate panic (i.e., separation anxiety) by studying different weaning methods. Additionally, caretakers try to ameliorate any sort of practices that cause fear such as mixing, transport, and handling. Frustration is another emotion that is studied and often manifests itself in the expression of abnormal behaviors. For example, pigs are highly motivated to perform certain behaviors such as rooting and when they are prevented from doing so they may begin to develop oral stereotypies. Lay et al. (1999) assessed both behaviors expressing positive (play) and negative (aggression and stereotypies) affective states in pigs housed in hoop structures as compared with an environmentally controlled slatted floor building. They observed a lower incidence of abnormal behaviors and a higher incidence of play behaviors in the pigs housed in the outdoor hoop structures.

The emotional state of an animal is characterized by changes not only in behavior but also in certain physiological parameters via activation of the hypothalamic–pituitary–adrenal axis and the sympathetic–adrenal–medullary system, that is, a “stress response.” These changes occur to prepare the animal for the stressor with which they are confronted, that is, “fight or flight.” It is important to note that many of the physiological changes associated with a stress response are found in response to both negative and positive stressors, and therefore, caution needs to be taken when interpreting physiological parameters (Dawkins 1998).

Ethologists have designed a variety of experiments that can be used to determine how animals feel about various housing conditions and management systems. Preference tests can also be used to measure an animal’s motivation for resources or environments with the
underlying assumption that animals approach what they find positive and avoid what they find aversive. When given a choice between different circumstances, pigs can express their relative preference on matters such as diet, floor type, thermal environment, and degree of social contact. Refer to Elmore (2010) for studies detailing how provision of various resources can impact sow motivation and behavior.

**Welfare Monitoring and Assessment**

Monitoring and assessing animal welfare provide the producer with methods for benchmarking welfare. These benchmarks can then be used for decision making regarding best management practices and provides a way for producers to demonstrate that their pigs are receiving a certain level of care. On-farm measures of animal welfare typically fall into two categories: resource-based or animal-based measures.

Resource-based measures are also called input-, management-, or design-based measures. Examples include space allowance; stocking density; feed and water quantity and quality; frequency of inspections and stockperson training; and other caretaker characteristics such as attitudes, knowledge, and competency. The disadvantage of resource-based measures is that they are indirect indicators of animal welfare and therefore do not provide a true evaluation of how the animal is coping with its environment (Barnett and Hemsworth 2009). However, the advantage of resource-based measures is that they can identify potential causes of poor animal welfare prior to the welfare of an animal being negatively impacted. Therefore, resource-based measures can be considered “lead” indicators because corrective and preventative actions can be taken for the pigs being evaluated (Manning et al. 2007).

Animal-based measures are also called output- or outcome-based measures. Examples include mortality, morbidity, culling rates, lameness, injuries, body condition, stereotypic behaviors, aggressive behaviors, and fear behaviors. The advantages to using animal-based measures are that they serve as a direct indicator of animal welfare and they allow for variation in system design and management (Blockhuis et al. 2003). The disadvantage of these measures is that they tend to “lag” meaning that any existing welfare issues have already occurred for the pigs being evaluated and changes can only be made for future production cycles (Manning et al. 2007). Emphasis should be given to animal-based measures to identify the actual welfare of the animal and to resource-based measures to identify potential causes of poor welfare. Using thermal comfort as an example, we consider a group of nursery pigs that are huddling, shivering, and piling as an animal-based measure and a thermostat reading of 27°C (80°F) as a resource-based measure. We could conclude that the animals are experiencing cold stress and that the cause was a ventilation draft or faulty sensory equipment. Both types of measures are needed to understand the animal’s welfare and to identify potential causes.

**MATERNAL BEHAVIORS**

**Prefarrowing Behaviors of the Sow**

Gilts and sows exhibit a specific pattern of behaviors prior to farrowing (Widowski and Curtis 1989, 1990). In nonconfined sows (i.e., outdoor arks, indoor huts or pens), nest building occurs during the last 24 hours preparturition and is most intense 6–12 hours before farrowing (Jensen 1986). During the same time period, sows housed in farrowing stalls have an increased number of posture changes, indicating restlessness, and nest-building behavior is redirected at pen fixtures with the absence of suitable material (Haskell and Hutson 1996).

**Preweaning Mortality, Overlay, and Trauma**

Preweaning mortality is a welfare and economic problem in all swine housing systems. Piglet survival is due to a variety of complex interactions involving the sow, the piglet, and the environment (University of Minnesota 2002). The causes of piglet mortality, including crushing, starvation, disease, and savaging, can be affected by nutrition, experience, age, health, and injury status (Barnett et al. 2001). Crushing of the piglet by the sow is the predominant cause of preweaning mortality, accounting for 70–80% of total deaths (English and Morrison 1984). Historically, crushing has been viewed as involuntary, mainly caused by the physical environment (Andersen et al. 2005). Recently, it has been hypothesized that differences in maternal behavior play a role in the variation of piglet mortality (Johnson et al. 2007). Crushing can be viewed as a sow’s failure to protect her offspring. Among sows, there is a large variation in piglet mortality, even within one farrowing environment. Andersen et al. (2005) found sows that did not crush any of their piglets (“noncrushers”) showed a more protective mothering style than those that crushed several piglets (“crushers”). Noncrushers performed more nest-building activity, responded sooner to piglet distress calls, initiated nose contacts sooner after distress calls, and nosed more piglets during a posture change. These studies suggest that it may be possible to decrease preweaning mortality by focusing on maternal behavior.

Housing design heavily influences preweaning mortality. For sows housed in farrowing stalls, most crushing is reported when the sow lies down, and almost none when she rolls over (Weary et al. 1996). The design of the stall can reduce these types of crushing events. In loose farrowing systems, piglets are crushed when the sow lies down and when she rolls over (Damm et al. 2005).

Considering the reciprocal relationship between sow and litter, newborn piglets are dependent on the sow
for nutrition, but at the same time, the sow is the greatest threat to piglet welfare due to the possibility of crushing (Grandinson et al. 2003; Lay et al. 1999). Malnourished or starved piglets are more vulnerable to crushing (Grandinson et al. 2003; Lay et al. 1999). The inability to communicate pain rather than verbal language. Pain is a complex phenomenon, and it involves multiple nerve cells, types of nerve chemicals, and different nerve cell receptors to which the nerve chemicals bind in order to propagate a pain signal to the spinal cord and brain (Coetzee et al. 2008a,b). Savaging is a persistent kind of pain that may or may not be associated with injury, but is generally associated with inflammation, changes to nerve cells, and hyperexcitability of the nerve cells in the spinal cord and brain (Gudin 2004). This hyperexcitability phenomenon, or “wind up,” is a physiological increase in sensitization of excitable nerve cells. Because the brain and spinal cord are wound up to detect pain, they are hypersensitive to future painful stimuli; thus, normally mild pain becomes intense pain after repeated physical insults. Prolonged inflammation caused by damaged tissue helps perpetuate the wind-up phenomenon and plays a large role in chronic pain. In addition, the changes in the spinal cord and brain associated with wind-up make pain resistant to treatment with analgesics (Coetzee et al. 2008a). Preventing the wind-up phenomenon is an important human presurgery consideration; studies have shown that if analgesic or anti-inflammatory drugs are given to a patient prior to surgery, less analgesic or anti-inflammatory drugs are needed to control pain after surgery. Pigs are commonly teeth clipped, tail docked, and castrated without analgesia or anaesthesia on commercial pig farms in the United States (AVMA 2010; FDA 2010). Scientific information describing effective pain management for these procedures is limited.

**Savaging**

Aggression directed to newborn piglets by a sow (referred to as savaging) can be defined as an attack using the jaws that results in serious or fatal bite wounds (Chen et al. 2008). Although the cause is poorly understood, the incidence of savaging has been reported to range from 5% to 12% (Harris and Gonyou 2003; Knap and Merks 1987; van der Steen et al. 1988). Sows do not exhibit clear behavioral cues indicating that they will savage in advance, although it has been found that sows that savage had a greater frequency of posture changes beginning before parturition and through the expulsion phase (Chen et al. 2008). Pain and fear are hypothesized to predispose gilts to savaging (Pomeroy 1960). Other possible suggestions for causation include inability of sows to isolate themselves and perform nesting behavior, climatic stress, and human interference during parturition (Luescher et al. 1989). Savaging almost always occurs during farrowing or directly afterward (Chen et al. 2008) and has been found to be more common in primiparous sows (Harris and Gonyou 2003). Spicer et al. (1985) found that sows who savage often direct their aggression to only the first-born piglet and are more likely to have been mated at a low body weight.

Harris and Gonyou (2003) suggested that the savaging of piglets born outside of working hours can be reduced by keeping farrowing rooms continuously lit. If savaging occurs and a caretaker is on hand, there are a few steps that can be taken in order to calm the sow: massage the udder, inject a tranquilizer (English et al. 1984), and remove the piglets from the sow until farrowing is complete. However, Chen et al. (2008) pointed out that sedation cannot prevent the behavior before it is administered or guarantee no return of the behavior after recovery from sedation.

**INVASIVE PROCEDURES**

**How Can We Recognize Pain in Swine?**

Pain is defined by the International Association for the Study of Pain (IASP) as “an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage.” The IASP adds, “The inability to communicate verbally does not negate the possibility that an individual is experiencing pain and is in need of appropriate pain-relieving treatment.”

Acute pain is a protective mechanism that makes one notice an injury, move away from the danger that caused the injury, and then take care of the injury; thus, it is generally short lived. Pain associated with more severe trauma, like surgery, begins as acute pain but can become chronic with prolonged inflammation. Chronic pain is a persistent kind of pain that may or may not be associated with injury, but is generally associated with inflammation, changes to nerve cells, and hyperexcitability of the nerve cells in the spinal cord and brain (Gudin 2004). This hyperexcitability phenomenon, or “wind up,” is a physiological increase in sensitization of excitable nerve cells. Because the brain and spinal cord are wound up to detect pain, they are hypersensitive to future painful stimuli; thus, normally mild pain becomes intense pain after repeated physical insults. Prolonged inflammation caused by damaged tissue helps perpetuate the wind-up phenomenon and plays a large role in chronic pain. In addition, the changes in the spinal cord and brain associated with wind-up make pain resistant to treatment with analgesics (Coetzee et al. 2008a). Preventing the wind-up phenomenon is an important human presurgery consideration; studies have shown that if analgesic or anti-inflammatory drugs are given to a patient prior to surgery, less analgesic or anti-inflammatory drugs are needed to control pain after surgery. Pigs are commonly teeth clipped, tail docked, and castrated without analgesia or anaesthesia on commercial pig farms in the United States (AVMA 2010; FDA 2010). Scientific information describing effective pain management for these procedures is limited.

**Tail Docking**

In North America, the majority of pigs are tail docked (Hunter et al. 1999) to prevent tail biting (refer to tail biting in the “Oral and Locomotory Behaviors” section).
Tail docking in pigs is usually performed within the first week of life and can be performed with teeth clippers, cutting pliers, scissors, scalpel blade, and gas or electrical cautery iron. The length of the tail stump varies depending on the producers’ standard operating procedures, although generally, the remaining stump needs to be at least 2 cm (1 in.) long so that the tail stump covers the vulva in females.

Tail docking using a heated cautery iron did not affect ACTH, cortisol, or lactate concentrations in young pigs (Prunier et al. 2005; Sutherland et al. 2008), but cortisol concentrations were greater in pigs tail docked using side clippers 60 minutes after cutting compared with nondocked controls (Sutherland et al. 2008). The behavioral response to tail docking can include tail jamming (clamping of tail stump between the hind limbs without side-to-side movement; Torrey et al. 2009), tail wagging (Noonan et al. 1994), and posterior scooting (Sutherland et al. 2008). Furthermore, tail-docked piglets produced more grunts (Noonan et al. 1994) and peak vocal frequencies during the procedure (Marchant-Forde et al. 2009; Torrey et al. 2009) compared with control piglets.

There is relatively little research comparing various methods of tail docking or methods of pain relief for tail docking in pigs. Tail docking using cutting pliers increased the cortisol response compared with docking with a cautery iron (Sutherland et al. 2008). In contrast, Marchant-Forde and others (2009) found that docking using cautery iron had a tendency to increase the number of squeals during docking compared with docking using cutting pliers. Administering local anaesthetic immediately prior to tail docking or inducing general anaesthesia using carbon dioxide gas reduced the percentage of stress vocalizations performed by pigs during tail docking but did not reduce the cortisol response (Sutherland et al. 2011). Administering anaesthetic locally or topically to the wound or inducing general anaesthesia using carbon dioxide gas did not reduce the cortisol response to tail docking in pigs (Sutherland et al. 2011).

Tail docking is routinely conducted to help prevent tail biting in pigs, and currently, there is no alternative to tail docking except to not tail dock. However, strategies can be put in place to help prevent tail-biting behavior such as providing enrichment in pens (refer to tail biting in the “Oral and Locomotory Behaviors” section).

**Teeth Clipping**

Born precocious, pigs have their deciduous canines and third incisors fully erupted at birth. These eight sharp “milk” or “needle” teeth function as weapons during sibling rivalries for preferred teats during the first 2–3 days after birth (Fraser and Thompson 1991). As the incidence of facial injuries and udder wounds is higher when needle teeth are left intact, some or all of the teeth may be clipped or ground within a day of birth (Fraser 1975).

When clipping is carried out, different techniques may be used with regard to the portion of the tooth being removed and the instruments used to do so (electric grinder vs. side-cutting pliers). To prevent exposure of the vascularized and innervated pulp chamber to infection, it is preferable to remove only the tip of the tooth as opposed to the entire tooth (Heinritz et al. 1994). Maintaining the appropriate equipment and utilizing good technique also helps prevent sharp fragmentation or shattering of the tooth, two conditions that can lead to tongue and gingival lacerations and possible mouth infections (Brown et al. 1996; Meunier-Salaün et al. 2002). As a litter will establish a consistent teat order within 72 hours of birth, removal of the needle teeth beyond this time period is unnecessary and may in fact increase the chances of infection.

Teeth clipping of young pigs did not affect ACTH, cortisol, or lactate concentrations (Prunier et al. 2005); however, β-endorphin concentrations were greater in pigs after teeth grinding compared with clipping (Marchant-Forde et al. 2009). The behavioral response to teeth clipping or grinding includes increased grunting, escape attempts, and squeals (Marchant-Forde et al. 2009; Noonan et al. 1994).

No research has identified a chemical intervention to reduce or eliminate the behavioral or physiological reactions associated with teeth clipping in piglets. As the procedure is commonly performed within a day of birth when piglets are still immature, giving any pain medication that impedes piglet motor skills could increase the risk of crushing when in the presence of the sow. The extra costs and time associated with administering drugs to individual piglets is also considered to be unreasonable for most producers.

This once routine procedure has become less practiced by producers in North America as the labor costs and possible risk of oral injury and infection associated with clipping are weighed against the often superficial and limited injuries resulting from piglet fighting.

**Castration**

Surgical castration of male piglets is a common management practice carried out on commercial swine farms to reduce the performance of aggressive and sexual behaviors and to prevent the development of boar taint. Boar taint is used to describe the unpleasant smell and flavor that can occur in pork from intact mature male pigs.

Castration is usually performed surgically by making one or two incisions on either side of the scrotum using a scalpel and then removing the testes. The spermatic cords are severed by cutting or pulling. Pigs are usually castrated within the first week of life. Reduced suckling behavior was observed in pigs in the 6-hour period following castration (McGlone et al. 1993); therefore, it
may be preferable not to castrate pigs within the first 24 hours of life so as not to affect colostrum intake or establishment of teat order.

Pigs surgically castrated without pain relief have increased cortisol (Carroll et al. 2006; Prunier et al. 2005), ACTH and lactate concentrations (Prunier et al. 2005), mean arterial blood pressure (Haga and Ranheim 2005), heart rate (Haga and Ranheim 2005; White et al. 1995), and respiration rates (Axiak et al. 2007) compared with noncastrated control animals. Behavioral changes include reduced nursing, walking, and lying, and increased pain-related behaviors (Carroll et al. 2006; Hay et al. 2003; McGlone and Hellman 1988; Moya et al. 2007; Taylor et al. 2001). Castration has also been shown to increase the duration and percentage of stress vocalizations (Puppe et al. 2005) and performance of defense behaviors (Leidig et al. 2009) in pigs.

Orally administered aspirin and butorphanol have been reported as ineffective at reducing the behavioral response to surgical castration in pigs (McGlone et al. 1993). General anesthetics including an injectable anesthetic consisting of xylazine, ketamine hydrochloride, and glyceryl guaiacolate administered intravenously (McGlone et al. 1993); ketamine, cloramphol, and azaperone administered intramuscularly or intranasally (Axiak et al. 2007); and gaseous anesthetics including isoflurane (Hodgson 2006, 2007; Walker et al. 2004), sevoflurane (Hodgson 2007), and carbon dioxide gas (Gerritzen et al. 2008) have been used to reduce the pain-induced distress caused by castration in pigs with varying levels of success. McGlone et al. (1993) observed an increase in mortality in piglets anesthetized using a general anesthetic and piglets that survived showed suppressed nursing behavior. The sedentary effects of an injectable or induced anesthetic (i.e., ketamine, cloramphol, and azaperone, isoflurane) can last from 2 to 50 minutes (Axiak et al. 2007; Hodgson 2006, 2007; Walker et al. 2004). A prolonged recovery period from anesthesia could increase the risk of crushing of the piglet by the sow and reduce feeding opportunities. Pigs given local anesthetic prior to surgical castration had reduced mean arterial blood pressure (Haga and Ranheim 2005), slower heart rate (White et al. 1995), and behavioral changes (McGlone and Hellman 1988; White et al. 1995) compared with pigs surgically castrated without pain relief. Administration of local anesthetic subcutaneously into the scrotal sac (Haga and Ranheim 2005; White et al. 1995) or intratesticularly (Haga and Ranheim 2005; Leidig et al. 2009) has been shown to reduce the behavioral and physiological response to castration in piglets. Furthermore, Haga and Ranheim (2005) demonstrated that injecting local anesthetic intratesticularly or intrafunicularly equally reduced indications of nociception. Ranheim et al. (2005) recommended injecting local anesthetic into the testes as the local anesthetic is then rapidly transported up the spermatic cords and the animal receives the benefit of analgesia at two anatomical sites, but only one injection is required. Ranheim et al. (2005) demonstrated that the highest concentration of local anesthetic is available in the testicular tissues 3 minutes after injection into the testes. Pigs given local anesthetic 2, 3, or 5 minutes prior to castration showed a reduction in frequency, duration, or number of vocalizations as compared with piglets castrated without any pain relief (Leidig et al. 2009; White et al. 1995). Local anesthetic administered to the testes at least 3 minutes prior to surgical castration appears to provide the most effective pain relief.

Alternatives to castration include slaughtering pigs before they reach sexual maturity (Dunshea et al. 2001), using immunocastration techniques, sperm sexing for selection of female offspring, and genetic selection for pigs with low levels of boar taint. Slaughtering pigs before they reach sexual maturity means harvesting pigs at a lower body weight. However, at a body weight of 80–90 kg (176–198 lb), 5% of carcasses still exhibit boar taint (Bonneau et al. 1994). Furthermore, the average weight of pigs at slaughter is increasing and light carcasses are less profitable for commercial swine processors (EFSAs 2004). Immunocastration involves immunizing boars against gonadotropin-releasing hormone (GnRH), which uses the boars’ own immune system to suppress GnRH, consequently shutting down the stimulus to the testes resulting in a temporary inhibition of testicular function (Thun et al. 2006). Currently, alternatives such as sperm sexing are still in the experimental stages and are not ready for implementation on farms (EFSAs 2004; von Borell et al. 2009).

**Tusk Trimming**

Boars with tusks pose a potential risk to both human handlers and to other pigs. Current Codes of Practice for Western Australia recommend that trimming be carried out in situations where injury is likely to occur, and legislation within Canada prohibits the transportation of tusked boars in the presence of other animals (Health of Animals Act 1990). Current research by Paetkau and Whiting (2008), however, suggests that injuries are not reduced with tusk trimming, for boars either in transit or held in lairage. Though aggression is common among newly mixed boars, neither the length of time assembled in pens, stocking density, size of boar, nor the presence of tusks was found to be influential in the skin injuries sustained in fighting.

Removal of the distal end of boar tusks is often carried out twice per year or prior to transport using one of two methods: clipping (using hoof trimmers or bolt cutters) or sawing (with hack saw or orthopedic/embryotomy wire). Although more restraint is required, sawing is the preferred method as it provides more precision and less chance of pulp exposure or fracturing. Based on research by Bovey et al. (2008), the length of pulp chamber extending into the tusk beyond the
gingiva varies greatly and is not related to boar age. Recommendations for the length at which tusks should be trimmed are approximately 1.5 cm (0.59 in.), as this was found to be slightly beyond the longest extending pulp chamber within their sample group.

Immunohistochemical and histological evaluation of commercial boar tusks by Bovey et al. (2008) indicates that the pulp chamber of boar tusks frequently extends into the tusk beyond the gumline and is innervated in both vascularized and nonvascularized areas. Such innervation suggests the possibility of pain; however, more research is required to determine the type of innervation present (autonomic or sensory). Exposure of the pulp cavity to bacterial infection was also a common condition associated with trimming too close to the gingiva and can progress into gingivitis and pulpitis, two conditions presumed to be painful. Little work has been scientifically conducted to understand the pain associated with tusk trimming. Housing or transporting boars singly reduces the need to trim tusks.

FEEDING AND DRINKING BEHAVIORS

Dental Development in the Pig

The beginning of initial food mastication is highly dependent on the level of cheek teeth development (Langenbach and Van Eijden 2001), and this is one physical feature often overlooked in the ontogeny of the pig. Having a diphodont dentition, pigs have all deciduous teeth replaced by their permanent counterparts by approximately 2 years of age (Tonge and McCance 1973). Their deciduous dentition numbers 28 (2 × incisors, 3 upper incisors canines 1/1, premolars 3/3, molars 2/0) with their permanent set increasing to 44 (2 × incisors, c/1, p/1, m/3), the most comprehensive for any eutherian mammal (Tonge and McCance 1973). The majority of teeth used for masticating food (i.e., the deciduous premolars) erupt between the first and fifth week of life (Tucker and Widowski 2009) and influence preweaning feeding behavior in an age-dependent fashion. Initial premolar eruption often induces bleeding and localized inflammation of the surrounding gingiva and is associated with lower feed-oriented behavior prior to 18 days of age (Tucker et al. 2010a). By 21 days of age, piglets engage in more feeding behavior as premolars continue to erupt and occlude (make contact in opposing jaws). Increased levels of occlusion result in more efficient feeding (Huang et al. 1994). At weaning, piglets having both their p3 and p4 erupted (i.e., the two premolars required for initial occlusion) and have higher weight gains in the following 3 weeks (Tucker et al. 2010b). In addition to eruption, growth of the masticatory muscles and learning of the motor patterns involved with chewing are also essential for feeding development. Factors influential to the timing of premolar eruption include piglet birth weight and average daily gain in the first 2 weeks of life (Tucker and Widowski 2009).

Development of Feeding and Drinking Behaviors

The development of independent ingestive behaviors (i.e., feeding and drinking) follows different trajectories and is controlled by different motivational systems in the young pig (for review, see Widowski et al. 2008a). Drinking has been defined as voluntary oral ingestion of liquids (Hurnik et al. 1995) and refers to the total consumption of water, which includes water that is often contained in feed (Fraser and Broom 1997). The discovery and consumption of water after birth can be facilitated by supplying bowl versus nipple or press lever drinkers, and is significantly more effective if auditory bubbling cues are present. Drinking behavior can develop within several hours of birth when piglets require supplemental nutrition or hydration, particularly in response to high environmental temperatures (Fraser et al. 1990; Phillips et al. 2001). Between birth and 4 weeks, water intake increases as a function of age but consumption per kilogram body weight remains constant at about 50–65 mL/kg (Phillips and Fraser 1990).

Immediately after weaning, piglets often increase their time at the drinker, possibly to alleviate feelings of hunger (by increasing gastric fill) that develop in response to low feed intakes, or to relieve gastrointestinal discomfort associated with a sudden shift in feed composition and form (i.e., from a high-fat and lactose-rich liquid diet to a high-protein and starch solid diet). As feeding becomes established after weaning and consistent meal patterns develop, drinking becomes prandial and most water is consumed around meal times.

The transition from suckling to independent feeding requires development in both the piglet’s peripheral features (i.e., eruption of teeth, gastrointestinal maturation) and central features (i.e., shifting of motivational systems; Huang et al. 1994). The time course of this development often varies greatly between individuals and litters but is always gradual in nature. Abrupt artificial weaning in modern intensive systems therefore presents one of the most difficult periods to manage as piglets experience nutritional, emotional, and environmental challenges simultaneously. Exploration and social facilitation help piglets during the earlier stages of feeding development, with nutritional benefits becoming increasingly important as maternal inputs decline (Appleby et al. 1992; Delumeau and Meunier-Salaün 1995; Morgan et al. 2001). Overall, physical maturity is the best indicator for when piglets develop independent feeding, with larger, more robust individuals ingesting more feed at earlier ages relative to their smaller, less mature littermates (Appleby et al. 1991).
Providing creep diets prior to weaning can familiarize piglets with solid feed and entice earlier consumption, particularly if those diets are complex or offered in gruel form (Fraser et al. 1994; Toplis et al. 1999). They can also help prepare the gastrointestinal system for the postweaning diet by stimulating the production of certain digestive enzymes (de Passillé et al. 1989), a necessary step in the complex digestive transition accompanying weaning. Because there is significant variation in feeding, both within and across litters, with the majority of piglets not consuming significant quantities until after 19 days of age (Fraser et al. 1994), the effectiveness of using creep feeding as a management practice in preparation for weaning needs to be carefully considered with weaning age in mind.

Troubleshooting to Enhance Feeding and Drinking Behaviors
Farm animals form a social hierarchy or rank order that can affect accessibility to key resources within their pen (Bouissou 1965). In competitive situations, higher-ranked animals might have more access to feed and water. If the producer considers the placement of drinkers within a pen and/or the ratio of drinkers to pigs, then lower-ranking animals might have more success obtaining water. Likewise, by increasing feeder space and feeding times (e.g., by using multiple trickle feeders for group-housed sows), aggression surrounding this limiting resource will be reduced.

ORAL AND LOCOMOTORY BEHAVIORS
Tail Biting
Tail biting is a behavior in which one pig takes the tail of another pig into its mouth, sucking or chewing on the appendage. The behavior can lead to infection, spinal abscess, disease transmission, carcass damage, and in some cases, cannibalism and death (Kritas and Morrison 2001; Schroder-Petersen and Simonsen 2001).

Recommended as an animal-based measure for on-farm welfare audits (Goossens et al. 2008; Whay et al. 2003), the frequency of tail biting is a serious welfare and production issue. Although tail docking may reduce tail biting, it does not eliminate it, with upward of 2% of tail-docked pigs exhibiting signs of having been tail bitten by the time they arrive at the packing plant (Moinard et al. 2003; Smulders et al. 2008).

While there appears to be no single factor that results in tail biting (Schroder-Petersen and Simonsen 2001), numerous management, environmental, and individual factors have been implicated. There is increasing evidence that crowded environments lead to tail biting (Moinard et al. 2003; Randolph et al. 1981), although it may be space allowance in the postweaning period that is most critical (Bovey et al. 2010; Smulders et al. 2008). Barren growing environments are also implicated with increased tail biting (Bolhuis et al. 2005; Moinard et al. 2003). However, not all pigs tail bite, and it appears that some pigs may be predisposed to performing injurious oral behavior. Pigs with a predisposition to tail bite may be lighter at weaning (Beattie et al. 2005), be more active, and perform more nosing behavior (Keeling et al. 2004) compared with other pigs.

Adequate space allowance in the nursery and grower barns can help reduce the behavior, as can the installation of chains other chew toys, or the provision of straw (Day et al. 2008; Zonderland et al. 2008). These enrichments may work to draw the pigs’ oral attention away from one another. Tail dock length may also play a role in tail biting; however, the ideal length is still undetermined. In an epidemiological study, Moinard et al. (2003) found that tail docking to one quarter of the original tail length was associated with a 10-fold increase in tail biting. Recently, Bovey et al. (2010) found that a longer (4.5 cm [1.8 in.]) docked tail led to more tail biting than a shorter (1.2 cm [0.5 in.]) docked tail. There also appears to be a genetic component to the tail biting, with the behavior correlated with lean tissue growth and back-fat thickness (Breuer et al. 2003). Removal of the tail biter and tail-bitten pigs is also important to reduce the probability of social facilitation of the behavior, and stem increased harm to the injured pig.

Belly Nosing
Belly nosing was first described over 30 years ago as the distinctive, rhythmic up-and-down movement of one piglet rubbing the belly of another with its snout (Fraser 1978). This behavior, when performed persistently, can result in skin lesions on the belly and flank of the receiver and may ultimately lead to ulceration (Straw and Bartlett 2001).

Although belly nosing is most often associated with weaning at an early age (Fraser 1978; Worobec et al. 1999), the motivation behind the behavior has yet to be determined. It has been suggested that this behavior is the result of discomfort and stress in newly weaned piglets (Dybkjaer 1992). However, since the motor patterns performed during belly nosing appear similar to those used in suckling, many researchers have hypothesized that belly nosing is redirected suckling behavior (Fraser 1978; Metz and Gonyou 1990; Widowski et al. 2008).

Environmental enrichment (EE) (Rodarte et al. 2004; Waran and Broom 1993), suckling devices (Rau 2002; Widowski et al. 2005), and alternative drinkers (Torrey and Widowski 2004) have been successful in reducing, but not eliminating, the behavior. Although most piglets perform some belly nosing, not all do, and there is a wide variation in the amount of belly nosing individual piglets perform. There appears to be a link between age and weight for age (Gardner et al. 2001; Torrey and Widowski 2006), but it is unclear whether
there is an optimum age or weight at which to wean piglets. There also appears to be some genetic component to belly nosing, with Landrace pigs performing the behavior more than Duroc pigs (Bench and Gonyou 2007; Breuer et al. 2003).

Lameness
Lameness and hoof problems can affect pig behavior. Stienzen (1996) observed sows prior to farrowing and through lactation for overgrown hooves. The authors reported no behavioral (percentage standing, dog sitting, or lying) differences between normal sows and sows with overgrown hooves in the 6 hours leading up to the first piglet being born but found some differences when observing sows immediately before, during, and after their morning feed. Phenotypically normal (control) sows spent more time feeding and standing than sows with overgrown hooves. There were also some differences in the number of rear leg slips and rising attempts between control and overgrown hoof sows. In addition, sows with overgrown hooves tended to produce smaller-sized litters compared with control sows. In another study, Leonard et al. (1997) found that time spent feeding and standing decreased and weight shifts and slipping increased in sows with overgrown rear hooves. These results indicate that sows with overgrown rear hooves exhibited discomfort and thus decreased the amount of weight-bearing time spent on the overgrown hooves.

Veterinarians and caretakers can use practical on-farm scoring methods to determine the level of lameness within their herd. The implemented scoring system needs to be quick and affordable yet accurate. Two subjective scoring systems, the numerical rating scale and visual analog scale, have been applied to characterize lameness in animals (Quinn et al. 2007). The numerical rating scale uses 4–6 ordinal categories to score lameness (1 being a sound animal and 6 being an animal that is unable to rise). Alternatively, the visual analog system utilizes an observer’s perception of lameness. An observer is asked to place a mark on a 100-mm (4-in.) line between two end points of normal and “could not be more lame” for an individual’s level of lameness (Quinn et al. 2007).

HUMAN AND ANIMAL INTERACTIONS
The Role of the Caretaker and the Interaction of People and Pigs
There is a prevalent and long-held belief that the caretaker has a more important influence on pig welfare than the choice of production system (Brambell 1965). Humans play a number of important roles for the pig. They act indirectly through their responsibility for the design of the environment and development of husbandry and management regimens. They also act directly by providing the day-to-day care of the animals.

The human as caretaker is the critical factor in the success or failure of a housing system, and his or her role in pig welfare has received increasing attention over recent years (Hemsworth et al. 1989, 1993, 1994). There are three important factors that will determine whether or not an individual will be a successful caretaker: (1) the caretaker’s knowledge and expertise; (2) the caretaker’s personality, attitude, and beliefs (Broom and Johnson 1993); and (3) the caretaker’s situational variables (personnel details; Spoolder and Waiblinger 2009), all of which may be interrelated.

Fear of Humans
Animals are “neophobic”; that is, they are fearful of novel or unfamiliar things (Rushen 1996), and excessive fear is of concern to animal producers from a production and management perspective. Fear is defined as the general susceptibility of an individual to react to potentially threatening situations (Boissy et al. 2007), and has been posited as a personality trait in a variety of animal species (Gosling 2001). Fearful animals are likely to grow more slowly and less efficiently than nonfearful animals and have reduced reproductive output (Hemsworth et al. 1987, 1989, 1993).

Fatigued Pigs
Ritter et al. (2009) published a comprehensive review on the marketing process and transport losses in the swine industry. Transport losses refer to the number of pigs that die or become fatigued during the marketing process. A fatigued pig is a pig that refuses to walk or keep up with its contemporaries and is without obvious injury, trauma, or disease (Ritter et al. 2005). Fatigued pigs are often characterized by open-mouth breathing, muscle tremors, blotchy skin, and abnormal vocalizations, and often, if given enough time, a fatigued pig can recover (Ellis et al. 2003; Ritter et al. 2005). Fatigued pig etiology is thought to be stress induced. The fatigued pig syndrome is impacted by many factors such as individual characteristics of the pig, caretaker, facility design, transportation, and the environment (Anderson et al. 2002; Ellis et al. 2003; Johnson et al. 2010). In North America, research has addressed the effects of facility design (Berry 2007; Ritter et al. 2007a), truck design (Faucitano et al. 2010), season (Hayne et al. 2009), number of animals moved in a group at one time (Correa et al. 2010), and management strategies such as presorting pigs ahead of transport (Johnson et al. 2010; Ritter et al. 2007b) on pig transport losses at marketing.

AGGRESSION
Aggression is often reported at times when pigs are mixed or group housed. During production, this can occur when young pigs are mixed after birth to equalize litter size, after weaning, during transport, and at the
packing plant. Sows are mixed when returning from farrowing to the breeding herd. Postmixing aggression, in order to settle intragroup disputes, deter intrusions into the group, and establish a social hierarchy, although stressful, is relatively short lived and typically decreases quickly over the first few hours postmixing, reaching basal levels within 1–2 days (Pritchard 1996). Aggression to establish hierarchies is very difficult to control or prevent. Chronic aggression over a longer period of time is usually over competition for resources, such as feed, water, breeding mates, and/or nesting sites, and is easier to control (Barnett et al. 1994; Csermely and Wood-Gush 1987; Edwards et al. 1994; Ségui et al. 2006). Interestingly, stability of the social hierarchy is established without the need for all pigs to fight with each other. Mendl and Erhard (1997) mixed four pigs from one established group with four pigs from another group 11 times and in no single case did all 16 possible unacquainted pairs fight before stability was reached. There is a mechanism by which domestic pigs are able to assess their relative fighting ability or relative place in the hierarchy based on information gained from their own interactions and probably from interactions of other pairs. Pre-exposure that permits a mixture of visual (e.g., physical size), auditory (e.g., frequency or duration of vocalizations), and olfactory (e.g., information conveyed by pheromones) (McGlone 1985) cues could reduce fighting postmixing (Durrell et al. 2003).

Persistent aggression can decrease welfare as indicated by increased stress hormone concentrations (Otten et al. 1999), increased heart rates (Marchant et al. 1995), increased injuries, and restricted access to resources (O’Connell et al. 2003) in animals that are aggressive or ones that are being attacked. Aggression can also increase costs by slowing growth (Stookey and Curtis 1985). Therefore, may be a way of reducing fighting among newly regrouped prepubertal pigs and, therefore, may be a way of reducing fighting among newly regrouped prepubertal pigs. Gonyou et al. (1988) compared levels of aggression, when injected with amperozide (1.0 mg/kg IM), azaperone (2.2 mg/kg IM), or saline (0.1 mL/kg IM) immediately prior to mixing. Both drugs reduced total fighting. Amperozide resulted in fewer fights involving two pigs than did azaperone or saline. Injuries to the ears and total injuries were less severe in amperozide-treated pigs than in those on the other treatments. Amperozide-treated pigs spent less time eating on day 1 than did saline- or azaperone-treated pigs but compensated on day 2 such that total eating time in 2 days did not differ. Both drugs reduced agonistic behavior but had no effect on performance. Similar effects have been found using anti-aggression (amperozide; Barnett et al. 1993, 1996) and sedative (azaperone; Luetscher et al. 1990) drugs. With both of these, aggression appears to be reduced while the effects of the drug last, but once the effects have worn off, aggression rebounds to that seen with untreated animals.

Boar presence can also impact aggression. Grandin and Bruning (1992) compared barrows and gilts at the packing plant with or without a mature boar in their
lairage pens. The authors reported that boar presence reduced both the incidence and the intensity of fighting. Docking et al. (2001) found that aggressive interactions, skin damage, and flight distance for sows were all reduced by at least 28% over a 28-hour postmixing period by boar presence. However, Séguin et al. (2006) found that mixing sows in the presence of a boar following the breeding period was minimally effective at reducing fighting and scratches compared with controls, and that sows showed a greater stress response in the presence of a boar.

Early social experience may also play a role as a longer-term solution to reduce aggression at mixing (Pitts et al. 2000). Mixing as piglets prior to weaning has been shown to benefit social skills in the longer term. Socialized piglets are able to form stable dominance hierarchies during future encounters with unfamiliar pigs quicker than piglets mixed after weaning (D’Eath 2005). Early socialization also increases consistency of behavior during social encounters (D’Eath 2004). However, the amount of aggression at mixing can still be reduced later in life by practicing repeated mixing, premixing, or pre-exposure with and to other pigs. With repeated mixing, pigs that are remixed three or four times postweaning subsequently show reduced aggression when mixed at 5 months of age, compared with pigs mixed just once or twice (Durrell et al. 2003; van Putten and Buré 1997). Lastly, and with largely untested potential, is the practice of pre-exposing pigs prior to mixing. Kennedy and Broom (1996) placed groups of five gilts in a small pen within a large pen and let the resident sows have olfactory, auditory, visual, and limited physical contact with them for 5 days before mixing. Once mixed, aggression was reduced by 60% over the course of the mixing day and the following 2-week period compared with gilts that were mixed into the resident group without pre-exposure. Jensen and Yngvesson (1998) have also reported this pre-exposure effect on aggression in nursery pigs and a reduction in interaction nosing phase.

EE for the pig may also be considered to redirect aggression onto “another” item rather than a pig within that pen (Jensen and Pedersen 2010). Elmore (2010) proposed that EE be defined as biologically relevant (i.e., have meaning for the animal in terms of its natural biology). Additions or modifications to the environment that allow coping with stressors (Moberg 2000) by promoting species-specific (i.e., “natural”) coping behavior may be linked to the experience of positive affective states in animals (Boissy et al. 2007; Young 2003). Schaefer et al. (1990) compared EE on aggressive behavior in newly weaned pigs. Six-week-old gilts were divided into two treatment groups and each pen either had a car tire suspended on a chain or no device. Pigs offered the tire and chain device displayed a lower frequency of total aggressive acts. Most notable was the reduced frequency of head-to-head knocks. In a further experiment, the authors compared –28-day-old barrows and gilts that were assigned nothing, a pacifier (sugar–mineral block suspended in a metal basket), or a teeter-totter (metal bar with rubber belts on the ends). Pigs offered a play device committed fewer total aggressive acts (compared with the control pigs). The authors concluded that aggression frequency in intensively raised pigs can be modified by enriching their environment with play objects.

Aggression may also be managed through selection of pigs that display low levels of aggression; although still in its infancy for application selection of pigs based on levels of aggression is being considered (Erhard et al. 1997). Turner et al. (2000a) determined the genetic contribution to aggressiveness in pigs by correlating the relationship of aggression with skin lesions. Postmixing aggressiveness of pigs was assessed to have a heritability of 0.22. The response to selection, when all selection pressure was placed on the lesion score (LS) trait, was a 25% reduction in LS per generation. Further work by Turner et al. (2000a) used a Bayesian approach to estimate the heritability of three traits associated with aggressiveness in pigs during the 24-hour postmixing: duration of reciprocal aggression, and whether in receipt of, or delivery of nonreciprocal aggression (NRA). The authors concluded that, based on the estimated genetic parameters, the selection of breeding values for reduced LS (especially LS for the central region of the body) is expected to reduce reciprocal aggression and the delivery of NRA, but will not change the receipt of NRA directly.

**INFLUENCE OF DISEASE ON BEHAVIOR**

Little scientific attention has been directed at understanding what pigs need during states of acute disease and convalescence (Millman 2007). At the group level, diseased and injured pigs represent a vulnerable population with unique needs and preferences (Millman 2007). Housing and management of pigs are generally designed to meet the needs of healthy pig populations efficiently, whereas suffering of compromised pigs may be exacerbated by any inadequacies of the standard commercial environments where behavioral responses may be thwarted and where bullying may occur (Millman 2007). During acute stages of disease, pigs alter their behavior such that activity, social interaction, feeding, and drinking are reduced whereas huddling, shivering, and resting increase. For a comprehensive review of the concept of “sickness behavior,” please refer to Hart (1998). The expression of behavior during disease is context dependent, affecting the likelihood of clinical signs being expressed in certain social environments. Pigs present subtle behavioral indicators of disease and pain and are viewed as “stoic” due to evolutionary niche (Flecknell 2000;
Millman 2007). However, behavioral responses may be readily expressed in familiar environments and among familiar conspecifics.

Hospital pens provide opportunities to segregate diseased individuals and to tailor husbandry to the needs of this potentially vulnerable population. However, optimal management of these hospital, treatment, and/or recovery pens remains elusive. Questions that remain unanswered are how often these pigs should be checked daily, how long should they be in these pens, what is the optimal flooring type, and what is the ratio of hospital pens needed for the population (Millman 2007). A decision about euthanasia needs to also be included in the treatment plan by the herd health veterinarian.

EUTHANASIA

Timely euthanasia is required for severely injured, non-ambulatory and emaciated pigs and for compromised pigs that are in pain or that have little possibility of recovery. A standardized euthanasia protocol can improve overall welfare of the herd and reduce the economic costs of providing continued care for compromised pigs (Morrow et al. 2006). Euthanasia of low birth weight piglets (<0.9 kg [2 lb]), for example, may be recommended because they have higher mortality preweaning and in the nursery (Smith et al. 2007), and have higher likelihood of being of poor quality at weaning, in the nursery, and into the finishing period (Fix et al. 2010). Additionally, a euthanasia protocol for pigs entering the nursery that are identified as weak, lame, and suffering from prolapse or from two or more concurrent conditions (e.g., injury, damaged digits, hernia) has been shown to significantly improve herd welfare scores (Morrow et al. 2006). For pigs of all sizes, euthanasia should be carried out in a manner that minimizes pain and distress while rendering the animal immediately insensible. The process of death should begin with loss of consciousness in conjunction with or followed shortly by full loss of brain function and full respiratory and cardiac arrest (AVMA 2007). Some euthanasia techniques are effective at rendering the animal insensible and causing death in one step, whereas two-step methods effectively stun the animal but require a secondary step such as exsanguination to achieve death.

There are three primary means of achieving death: chemical depression of the central nervous system (CNS), hypoxia, and physical destruction of neuronal tissue essential for life (AVMA 2007). Direct depression of the CNS by anesthetic overdose initiates unconsciousness by induction into a deep state of anesthesia followed by cardiac and respiratory failure. Hypoxia limits oxygen delivery to the brain, ultimately shutting down vital centers for heart and lung function. Physical destruction of the brain, whether by concussion, depolarization by electrocution, or direct injury, targets the cerebral cortex and brain stem to damage critical pathways and regions of the brain essential for life. The prefrontal cortex and brain stem along with their connections in the thalamus are the brain regions associated with consciousness and arousal (Seth et al. 2005). In order to ensure that death occurs without the perception of pain, signs of insensibility in the animal should be monitored throughout the process until death is confirmed.

Observations of the brain stem and spinal or nociceptive reflexes, similar to those used to determine effective stunning prior to slaughter or depth of anesthesia during surgery, are the most practical measures for determining insensibility during euthanasia of animals on farms (Erasmus et al. 2010). Key brain stem reflexes include the corneal, palpebral, and pupillary light reflexes; when insensible, the animal does not exhibit a blink response when either the eyelid or cornea is touched, and the pupil remains fixed and dilated in the presence of light (Gregory 2008). Any natural blinking without stimulus indicates that the animal is sensible (Grandin 2010). However, ocular reflexes are not reliable indicators of anesthetic depth in pigs (Smith and Swindle 2008), and weak corneal reflexes can be observed during unconsciousness following damage to the cerebral cortex when the brain stem remains intact (e.g., head-only electrical stunning; Grandin 2010). Therefore, spinal or nociceptive reflexes, such as the pedal reflex, response to nose prick, or anal reflex, are important for assessing insensibility (Kaiser et al. 2006). Absence of withdrawal responses to painful stimuli indicates that the animal no longer perceives pain.

In addition to the sensory reflexes, several behavioral observations are important for assessing effectiveness of the euthanasia technique. These include absence of rhythmic breathing, absence of vocalizations, and loss of muscle tone (Gregory 2008). The return of rhythmic breathing is one of the earliest signs of return to consciousness (Anil 1991). Vocalizations are a sign of pain or distress and should not be present at any time during the euthanasia process (Warriss et al. 1994). Loss of posture and muscle tone occurs with the onset of unconsciousness, and a limp jaw or tongue is a reliable indicator of insensibility in pigs. Clonic muscle spasms, characterized by kicking or paddling movements, and tonic spasms, characterized by rigid extension of the limbs, are associated with some euthanasia techniques. These are involuntary muscle spasms and should not be confused with voluntary movements or deliberate escape attempts (Grandin 2010).

To date, there has been little research as to the most appropriate methods for euthanasia of pigs, and veterinarians should work with the caretaker to design a euthanasia protocol using the most up-to-date guidelines. Human safety, costs, the technical skill required,
and personnel preferences should also be taken into account. The recommended methods of euthanasia currently include anesthetic overdose, carbon dioxide, gunshot, penetrating and nonpenetrating captive bolt, blunt trauma, and electrocution (head-to-heart and head-only), depending on the size of the pig (NPB 2009).

Euthanasia by carbon dioxide (CO$_2$) requires exposure to 90% CO$_2$ for at least 5 minutes in either a pre-charged or gradual fill system (NPB 2009). Carbon dioxide induces unconsciousness by altering the pH of cerebrospinal fluid and ultimately causes death by hypoxia (Raj 1999). Although CO$_2$ is effective for causing death, this method is controversial; loss of consciousness is not immediate (Chevillon et al. 2004), and vocalizations, signs of breathlessness, and active avoidance are observed during the inhalation phase (Raj and Gregory 1996).

Gunshot, penetrating captive bolt, and blunt force trauma should inflict sufficient physical damage to both the cerebral cortex and brain stem to cause immediate and irreversible brain damage leading to death. A gunshot to the head can be effective for pigs greater than 5 kg (12 lb; AVMA 2007; NPB 2009), but the trajectory of the bullet should follow the angle of the spine ideally passing through the brain and lodging in the brain stem (Woods et al. 2010). Captive bolt guns rely on concussion and destruction of brain tissue to render the animal immediately insensible. Sufficient transfer of energy from the gun to the head is required, and effectiveness depends on both the diameter and velocity of the bolt (see Gregory 2007). The effects of various combinations of bolt diameter and velocity on evoked potentials indicative of neuronal activity in the cortex have been investigated for ruminants, but not for swine, and recommendations for pigs are based on practical experience. Penetrating captive bolt is currently considered to be acceptable without a secondary step for pigs weighing more than 5 kg (12 lb); nonpenetrating captive bolt guns do not cause sufficient brain damage to effectively stun or kill larger pigs (NPB 2009).

Electrocution is another physical method of euthanasia, although concerns for human safety make it a less favorable option in some circumstances (AVMA 2007). Only commercially available electric stunners should be used. It is important to note that both head-to-heart and head-only methods are acceptable. However, head-only electrocution causes loss of consciousness but not cardiac arrest; therefore, it is reversible and must be followed by a secondary step within 15 seconds (Anil 1991; Blackmore and Newhook 1981). Tonic and clonic neuromuscular spasms should be present in the head-only method (McKinstry and Anil 2004), but these are not seen in association with head-to-heart electrocution, which also causes cardiac fibrillation (Gregory 2008; Wotton and Gregory 1986).

For suckling piglets, physical methods of euthanasia may be the most practical for the caretaker. Although aesthetically objectionable, blunt trauma either by a blow to the head with a heavy instrument (Chevillon et al. 2004) or by striking the cranium against a flat hard surface (Widowski et al. 2008a) has been shown to be effective at causing immediate insensitivity followed by cardiac arrest within 10 minutes. A nonpenetrating captive bolt can be effective without a secondary step for suckling piglets, but the shape of the percussive bolt head and depth of depression of the bolt head into the cranium may be important. Widowski et al. (2008a) found that some piglets showed signs of return of sensibility and had a variable time to cardiac arrest when a round-headed percussive bolt was used for neonate piglets. When the bolt head was modified to a conical shape, which resulted in a greater depth of depression, the method proved highly effective (Casey-Trott et al. 2010). Clonic convulsions (kicking and paddling) should be expected following application of blunt trauma and percussive bolt; brain stem reflexes as well as behavioral indicators should be checked to ensure insensibility.

REFERENCES


Longevity in Breeding Animals
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Poor sow longevity or sow productive lifetime in commercial breeding herds can lead to economic inefficiency and animal welfare concerns. Thus, effective culling strategies should be an essential part of herd health management. Culling policies influence herd economic performance in many different ways. A high sow turnover is generally associated with a shift toward younger females, which are less productive, and with an increase in the number of nonproductive sow days. Additionally, the offspring from gilt litters have greater mortality, reduced growth rate, and poorer feed efficiency in the nursery and grow-finish phases of production. A high removal rate requires larger replacement gilt pools, which will increase disease risks and the cost of production. Difficulty in supplying replacement animals may also lead to a suboptimum population that will decrease the herd output and disrupt pig flow. On the other hand, an excessively low removal rate will be associated with a higher proportion of older sows, which are more prone to certain diseases and may have lower production levels.

Evaluation of a culling program should include the determination of the average longevity in the herd, reasons for culling and death, nonproductive days associated with removal, lifetime productivity, and risk factors for poor longevity.

MEASURES OF LONGEVITY

Determining the sow productive lifetime is the first step in assessing a herd culling program, and several measures have been used to define sow productive lifetime: removal rate, culling rate, replacement rate, percent gilts in the herd, mean parity of females in inventory, and mean parity at removal. Others have proposed the use of economic indicators, such as pigs weaned per day of life, number of herd days per pig weaned, or parity at which a positive net present value is attained (Culbertson and Mabry 1995; Lucia 1997; Stalder et al. 2000, 2003).

The term “removal rate” covers all types of removal, including culling, natural death, and euthanasia. This rate is defined as the number of animals removed from the herd during a year, divided by the average inventory, and multiplied by 100. Culling and death rates should be analyzed separately, and the number of animals euthanized should be distinguished from natural mortalities. Annual removal rates of 35–55% for sows have been reported in different surveys (Boyle et al. 1998; Dagorn and Aumaitre 1979; D’Allaire et al. 1987; Dijkhuizen et al. 1989; Engblom et al. 2007; Friendship et al. 1986; Knauer et al. 2007; Marsh et al. 1992; Mote et al. 2009; Paterson et al. 1997a; Pattison et al. 1980). High removal rates seem to be more frequent than excessively low rates. A target of 39–40% is recommended: 35–36% for culling and 3–5% for death (Dial et al. 1992; Muirhead 1976). The removal rate is influenced by many factors, such as herd size, genetic turnover desired, breeds, definition of the average inventory, and population dynamics. Higher rates are to be expected for seed-stock herds to facilitate rapid genetic improvement. Market trends and economic conditions also influence a producer's culling decision and timing (Brandt et al. 1999; Stalder et al. 2004).

Comparisons of sow removal rates between herds or studies are often difficult because the definition of “average female inventory” may be different. The inventory may refer to sows only or to sows and gilts, with gilts being introduced at different times in their production cycle. For a better standardization of
terminology, only mated females should be considered in the calculation of the annual removal rate.

Longevity of sows in the herd can also be evaluated by the replacement rate, which is defined as the total number of animals entering the herd divided by the average inventory and multiplied by 100. The removal rate and replacement rate should be similar in a herd if the inventory remains constant.

The mean parity of sows at removal indicates the average length of time that sows stay in the herd. Because the mean can be influenced by extreme values, a parity distribution of removed sows is usually more informative. Studies reveal that the average parity at removal is between 2 and 5.6, but it can vary from 2 to 8 for a particular herd (Arganosa et al. 1981b; D’Allaire et al. 1987; Koketsu et al. 1999; Lucia et al. 2000; Pedersen 1996; Stein et al. 1990). Sows are removed from breeding herds at a very young age considering that the “natural” longevity would most likely be 12–15 years (Pond and Mersmann 2001).

Understanding culling reasons and when they occur are critical components that allow producers to identify management factors that will have a positive impact on herd productive lifetime. Interventions that improve sow productive lifetime will likely improve economic efficiency and animal well-being of commercial pork production operations. A sow remaining in the breeding herd for fewer parities is likely to produce fewer pigs in her lifetime, which reduces the opportunity of a sow to be sufficiently productive to recoup the investment costs associated with the replacement gilt (Stalder et al. 2000, 2003).

Gilts and first-parity females often represent a large proportion of removals. Many authors have reported that 50–69% of the removals occur before the fourth litter (Arganosa et al. 1981b; Dagorn and Aumaitre 1979; D’Allaire et al. 1987; Kangasniemi 1996; Lucia 1997; Paterson et al. 1997a). The probability of being removed is highest for parity 7 and over, followed by parities 0 and 1 (Tiranti et al. 2004). The risk of being removed for some specific reason is also different among parity groups. There is a higher risk of culling for reproductive and locomotor problems in first-parity females and for inadequate performance and old age in advanced-parity sows (Boyle et al. 1998; Lucia et al. 1996; Paterson et al. 1997a,b).

Ideal Parity Distribution Recommendations

The ideal parity structure for a commercial sow breeding herd is necessary to maintain consistent production and to avoid huge swings in replacement gilt needs. As stated by Dial et al. (1992), it is difficult to recommend a standard parity distribution. The optimum parity distribution for litter size on one farm may be different from that on another farm, considering the breed, the cost of replacement, the type of facilities, and the husbandry skills. Ideal parity profile for a breeding herd is a function of the maximum number of parities that sows are allowed before automatic culling occurs and annual culling rate (Abell et al. 2010). Once these two values are known, the distribution or percentage of sows in each parity is a linear function. A good rule of thumb to obtain an optimum herd parity profile is that 90% of the gilts that are started in the herd should reach parity 1, and 90% of the parity 1 animals should reach parity 2 (Leman 1992).

REASONS FOR SOWS LEAVING THE HERD

Causes for Sow Culling

Sows are culled when they are considered unsuitable for further production. The specific reasons for removal can identify diseases or management deficiencies. Data from different studies reveal a general pattern of removal in which reproductive failure is the main reason for culling, followed by several other factors including old age, inadequate performance, locomotor problems, death, and milking problems. The culling reasons vary over time, among countries, among herds, and among parities.

Some authors have used the terms “voluntary” (planned) and “involuntary” (unplanned) removal. A strict definition is difficult but voluntary removal refers to culling for old age, inadequate performance, poor progeny, and poor milking, for which the producer makes the decision concerning the cause and the time of removal. Involuntary removal refers to other causes, such as locomotor and reproductive problems, for which the producer does not have as much control. Voluntary culling allows minimization of nonproductive days and better planning of the introduction of replacement animals.

Reproductive Failure Effects on Sow Culling

Reproductive failure is used to define a variety of conditions: no observed puberty in gilts, no observed post-weaning estrus, regular and irregular returns to estrus, negative pregnancy diagnosis, failure to farrow, and abortion. Reproductive failure is the predominant reason for culling, representing between 13% and 49% of all removals. The greatest number of nonproductive days since the last weaning is observed for this category (Paterson et al. 1997a). Thus, good reproductive management with an increased awareness of the reproductive state of each sow at all times should be emphasized.

Young females are more likely than older sows to be culled for reproductive failure (Dagorn and Aumaitre 1979; D’Allaire et al. 1987; Dijkhuizen et al. 1989; Lucia 1997; Paterson et al. 1997a; Stein et al. 1990). The average number of litters produced by these culled sows is between two and four. Inefficient estrous detection, mating at an early age, improper male stimulation, use of young boars that are less mature and can more easily
be overused, improper nutrition, infectious or toxic agents, management practices, and environment may be responsible for these high levels of culling for reproductive failure in young females. Older sows that have stayed in the herd have also undergone a selection process and may be less prone to reproductive failure. Females that did not conceive are often found only late into their presumed gestation. Sows culled for return to estrus stayed in the herd for an average of 75–79 days, whereas sows removed because they failed to farrow remained for 121–132 days after weaning (Paterson et al. 1997a; Pattison et al. 1980). It is important to decrease this period of nonproductive days because of the extra feed and labor required as well as the underutilization of production facilities. Management must differentiate between late loss of pregnancy and late detection of nonpregnant females. These two variables indicate different problems that necessitate different solutions on a farm.

Gilts and first-parity sows are more likely to be removed for anestrus than older sows (Lucia 1997; Paterson et al. 1997a). The acceptable period between introduction or weaning and mating differs among farms and may be partly responsible for the variation in proportion of animals culled. The length of the period allowed is worth investigating; on some farms, it might be too short, especially for younger females, which usually have a longer interval from weaning to breeding, thus unnecessarily increasing the number culled. Abortions do not seem to be a major reason for removal, generally representing less than 3% of all cullings, except in herds experiencing an outbreak of disease such as porcine reproductive and respiratory syndrome (PRRS).

When the proportion of culling for reproductive failure is high, a slaughter check may be useful to compare the reasons given by the producer and the physiological status of the reproductive tracts. Josse et al. (1980) examined 338 reproductive tracts and compared the findings with the reasons for culling reported by producers. The reason for removal could not be substantiated in 36% of the cases. Einarsson et al. (1974) found similar results in a study of genital organs in gilts: Of 54 gilts culled for anestrus, 23 had apparently active corpora lutea and 2 were pregnant. Possible explanations for these discrepancies are inefficient estrous detection or pregnancy testing, silent heat, or physiological changes occurring between the decision of culling and slaughtering.

Age Effects on Sow Culling. Old age is another reason for removal, accounting for 3–33% of all removals; the average parity at culling varies between 7 and 9. As the proportion of sows removed for other reasons decreases, the percentage of sows culled because of old age increases. Overlapping between “old age” and “inadequate performance” are likely to occur, for old sows may experience a decrease in productivity. Some producers routinely cull sows as soon as the fifth or sixth parity. According to a model developed by Dijkhuizen et al. (1986), the economic optimal herd life for average-producing sows is generally 10 parities. Rarely is it economically beneficial to cull sows before parity 8, considering the economic losses associated with the cost of replacement, the lower litter size and farrowing rate of gilts, and the longer interval from weaning to mating in younger sows. Their model took into account the annual replacement rate, the average parity of farrowing and removed sows, the average slaughter price of culled sows, and the cost of replacement gilts.

Inadequate Performance Effects on Sow Culling. This category includes a variety of reasons: small litter size at farrowing or weaning, high preweaning mortality, and low piglet birth or weaning weights. Inadequate performance is usually ranked as the second or third most common reason for culling sows, with a range of 4–21% of removals attributable to this category. Pomeroy (1960) reported that inadequate performance was the main reason for removal, accounting for 33% of all cullings. However, management was different in those years relative to modern herds; herds were smaller, usually fewer than 10 sows, and were farrowing outside. In a herd with a high level of culling for inadequate performance, a parity analysis is useful. If too many young animals are culled for this reason, intervention is warranted because it is well known that litter size increases with parity until the third litter. In such a herd, the benefit of culling sows for the purpose of improving productivity might be lost because of a decrease in herd productivity associated with a high proportion of young sows. Culled females will have to be replaced by gilts, which are not very predictable. Dijkhuizen et al. (1986) asserted that parity 1 sows with a litter size of even 50% below average should not be culled on economic grounds. The repeatability of litter size is low, which means that predicting the next production from the previous one is very inaccurate. These factors indicate that sows should not be culled for poor performance earlier than after weaning the third parity.

Locomotor Problems. Locomotor problems refer to a variety of conditions, including osteochondrosis, osteoarthritis, osteomalacia, arthritis, leg weakness, posterior paralysis, foot rot, foot and leg injuries, and fracture. Dewey et al. (1993) examined 51 sows culled for lameness and found that osteochondrosis, followed by infectious arthritis and foot lesions, was the major underlying cause. The proportion of sows removed for locomotor disorders varies between 9% and 20%; some reports, however, have indicated a percentage as high as 45%. Knauer et al. (2007) surveyed a large number of sows at harvest and reported that over 80% of the sows had one or more feet that had a lesion. Sows
removed for locomotor problems represent the highest economic losses related to culling (Dijkstra et al. 1989). In a study by Paterson et al. (1997b), 25% (range 0–62%) of the sows removed for locomotor problems had to be humanely euthanized for welfare reasons. This has an important economic impact due to the loss of the sale value of the animal.

Milking Problems. Milking problems include mastitis, agalactia, low milk production, and poor mothering abilities. This category may overlap with inadequate performance, for milk failure can influence weaning weights and preweaning mortality. The proportion of sows culled for these reasons ranges from less than 1% up to 15%. In some reports, milking problems are included with peripartum problems. In a study from Minnesota, sows culled because of milking problems produced an average of 4.6 litters (D’Allaire et al. 1987). Svendsen et al. (1975) reported that parity 2 sows were more prone to culling for mastitis. Halgaard (1983) observed that the risk of mastitis increased with increasing age up to the third or fourth litter.

Reasons for Sow Mortalities
Annual death rates of 3–10% are frequently observed; however, they can reach 20% on some farms (Abiven et al. 1998). Mortality risks in breeding herds appear to be increasing, especially in the United States (Deen and Xue 1999; Koketsu 2000). In addition to welfare and economic concerns, breeding herd mortalities can affect worker morale (Deen and Xue 1999). High levels of death or euthanasia in a herd result in some of the greatest losses per sow (Dijkstra et al. 1989, 1990; Paterson et al. 1997a) because of the increased number of nonproductive days, the extra costs (such as veterinary costs before removal and rendering costs), the lack of income associated with unslaughtered sows, or the loss of whole litters, since sow death often occurs around farrowing.

In most recording systems, natural death and euthanasia are both included in the mortalities. It is recommended in herds with high mortality that euthanasia rates and reasons be analyzed separately since euthanasia may contribute significantly to the death losses. In a study of 59 herds, the combined death/euthanasia rate was 9%, with 5% for natural deaths and 4% for euthanasia (Paterson et al. 1997a). Employees lacking pig experience and having limited husbandry skills are less likely to recognize sows at risk quickly and institute a prompt and effective treatment before a problem becomes life threatening (Loula 2000). Inappropriate treatments of sows with a product having a long withdrawal period may also preclude the sending of animals to slaughter, hence increasing the euthanasia rate if the health condition of the animals deteriorates. Some level of breeding herd euthanasia is appropriate. It is reflective of effective animal well-being plan implementation when sows that are not likely to respond to treatment are humanely euthanized in a timely manner.

The mean parity at death varies between 3.4 and 4.2; the variation among studies and possibly among herds may be a reflection of the relative incidence of certain causes of sow losses, as some of them appear to be age related. Cystitis–pyelonephritis occurs more frequently in older sows (Madec 1984; Paterson et al. 1997a), whereas some locomotor problems seem to be more prevalent in young breeding stock (D’Allaire et al. 1991; Dewey et al. 1993; Doige 1982; Spencer 1979). Many conditions responsible for death in sows are often reported by the producers as sudden deaths or rapid deaths associated with some rather nonspecific premonitory clinical signs. When trying to ascertain the causes of death in a particular herd, it is imperative to have a significant number of sows necropsied in order to identify the general pattern of causes of death. Standardized protocols and diagnostic approaches have been proposed to help determine the causes of mortality in herds (Chagnon et al. 1991; Pretzer et al. 2000).

Although sows die from a variety of causes, some of the causes seem to have a greater incidence. Torsion and other accidents involving abdominal organs, heart failure, and cystitis–pyelonephritis are overall the major causes of natural death in sows, whereas locomotion- and leg-related problems are the most frequent causes for euthanasia.

Torsion and Abdominal Organ Accidents. Torsions and accidents involving abdominal organs are probably among the most significant causes of death in breeding stock. Gastric, splenic, and hepatic lobe torsions are the most common conditions reported within this category. Lethal gastric dilation can also occur without concurrent torsion (Sanford et al. 1994; Ward and Walton 1980). In studies on sow mortality published before 1980, torsions of abdominal organs are not reported as significant causes of death. In one study, the proportion of deaths attributable to torsions of abdominal organs was 20.5% for herds kept in indoor units compared with 4.1% for outdoor units (Karg and Bilkei 2002). Torsions of abdominal organs are often found in older sows (Christensen et al. 1995; Morin et al. 1984). Although affected sows are usually pregnant, sows may die at any stage of the reproductive cycle. Rough movements and manipulations, noise, and excitement among sows have been incriminated in the pathogenesis of torsion of abdominal organs (Morin et al. 1984). Feeding management and possibly housing type can influence the incidence of these conditions (Abiven et al. 1998). It has been suggested that any factors that provoke a rapid intake of food and water in excited animals predispose to gastric dilation or torsion; such factors include the number of meals...
Heart Failure. Heart failure has been reported as being among the main causes of death in sows (Abiven 1995; Chagnon et al. 1991; D’Allaire et al. 1991; Karg and Bilkei 2002; Maderbacher et al. 1993; Senk and Sabec 1970; Smith 1984; Svendsen et al. 1975), accounting for up to 31% of the mortalities. However, in several other studies, heart failure per se is not even reported among the causes of death or is considered of negligible incidence in sows (Hsu et al. 1985; Jones 1967, 1968; Madec 1984; Ward and Walton 1980). The diagnosis of heart failure can be difficult to make, particularly in acute cases and has to rely on all current diagnostic procedures available. Diagnosis should be based on the presence of lesions indicative of heart failure such as cutaneous cyanosis; transudate in the pericardial, thoracic, and abdominal cavities; cardiac chamber changes; pulmonary edema; and passive congestion of lungs and liver, along with the absence of other gross, microscopic, and microbiological findings to carefully exclude other diseases.

Some of the predisposing factors for this condition have to be regarded in light of the way pigs often overreact to exogenous factors and, probably more important, of their particularly delicate cardiovascular system. The porcine heart has many anatomical and physiological peculiarities, namely, low volume and small weight, abnormal systolic-to-diastolic ratio, and exceptional myocardial sensitivity to oxygen deficiency. In swine, the heart weight-to-body weight ratio decreases as the size of the animal increases (Stünzi et al. 1959), and in adults, this ratio is considered to be among the smallest of domestic animals (Lee et al. 1975). The swine heart weight-to-body weight ratio, which is about 0.3% in sows, is much smaller than that of the less sedentary or more athletic species such as the dog, which is about 0.8% (Bienvenu and Drolet 1991). This precarious situation may easily lead to irreversible overload of the circulation and to acute heart failure (Thielscher 1987).

The lack of exercise in sows raised in total confinement may also affect cardiovascular fitness. Thus, any factor that requires increased effort from the cardiovascular system in sows may be considered to predispose to heart failure: obesity, parturition, high ambient temperature, and stressful events such as mating, fighting, and transport (Drolet et al. 1992).

Drolet et al. (1992) and Christensen et al. (1995) found that sows that died of heart failure were heavier and fatter than sows dead of other causes. Therefore, in gestation units, sows should be fed properly and not be allowed to put on excessive weight and fat. In a study involving 137 dead sows of which 43 had heart failure, more than 60% of the heart failure deaths occurred during the peripartum period, suggesting that parturition is a demanding event for the cardiovascular system of the sow (Chagnon et al. 1991). Cardiovascular failure associated with high ambient temperatures may be responsible for significant losses in some herds. In a recent study involving 130 breeding herds in which sows were housed indoors, 11% of the yearly deaths occurred during 3 consecutive days of warm weather, which represent only 0.8% of a year (3/365 days) (D’Allaire et al. 1996). Interestingly, only three dead sows were submitted for necropsy to the nearby diagnostic laboratory during the week. Causes of death that can be readily identified by producers, such as those occurring on hot days, are underrepresented in submissions to diagnostic laboratories (Sanford et al. 1994). Sows in total confinement are particularly susceptible to heat stress; they are not allowed to wallow in mud to decrease their body temperature nor are they exposed to winds that would decrease the ambient humidity and increase heat dissipation. Proper ventilation obviously plays a major role within these confinement systems, especially for periparturient females in the farrowing house. When the ambient temperature and humidity are high, the use of portable fans or other cooling system in the farrowing houses may be beneficial for sows. Other precautions may include removing heat sources (e.g., infrared lamp), less handling of animals to limit stress, and modifying the feeding schedule to avoid the heat of the day. It is important to distinguish heart failure from malignant hyperthermia (porcine stress syndrome).

Cystitis–Pyelonephritis. The proportion of all deaths attributable to cystitis–pyelonephritis generally varies between 3% and 15% (Christensen et al. 1995; Jones 1968). However, in some studies, urinary tract infection represented the major cause of mortality, accounting for up to 40% of all deaths (Jones 1967; Karg and Bilkei 2002; Madec 1984; Smith 1984). Bacteria most commonly isolated from cases of cystitis–pyelonephritis are Escherichia coli and Actinobaculum suis (Carr and Walton 1993; D’Allaire et al. 1991; Madec and David 1983; Smith 1984). Other bacteria commonly associated with urinary tract infection include Proteus spp., streptococci, enterococci, micrococci, klebsiellae, and Arcanobacterium pyogenes.

The determination of urea concentration in ocular fluids can be a useful aid in diagnosing cystitis–pyelonephritis in dead sows, particularly when a complete necropsy is not possible or when it is difficult to ascertain that the lesions found in the urinary tract are responsible for death (Drolet et al. 1990). A
significantly higher aqueous humor urea concentration was found in sows dead of cystitis–pyelonephritis (45–52 mmol/L) than in those dead of other causes (9–10 mmol/L) (Arauz and Perfumo 2000; Chagnon et al. 1991).

The risk of cystitis–pyelonephritis increases with age (Jones 1967; Paterson et al. 1997a). The underlying reasons for this age-related susceptibility have not yet been fully investigated. Lack of exercise, limb injuries (Madec and David 1983), and obesity (Smith 1983) appear to be more frequent in old sows and predispose to urinary tract infection. These factors are associated with a decreased frequency of micturition, hence leading to a decreased flushing of bacteria and potentiating microbial growth (Smith 1983). It has also been reported that restricted water intake is one of the major risk factors for cystitis–pyelonephritis (Madec and David 1983). A flooring type that does not allow easy cleaning and good elimination of urine and feces can also lead to urogenital diseases and serious reproductive problems (Madec and David 1983; Muirhead 1983).

Infectious Diseases. Proliferative enteropathy and PRRS, among other infectious diseases, may also increase breeding herd death rate. But usually, they will appear as outbreaks or will be of short duration. The mortality can reach up to 10% during these episodes (Halbur and Bush 1997; Yates et al. 1979). In most studies, endometritis represents less than 9% of all deaths. This condition can be associated with concurrent urinary tract infection or, less frequently, with mastitis (the latter is an uncommon cause of death in sows).

Pneumonia is not a major cause of mortality; it rarely represents more than 5% of all deaths. Pneumonia is often more severe in young growing pigs than in full-grown pigs (Pijoan 1986). This could partly explain why pneumonia is not a frequent cause of death in adult sows and is more likely to affect younger females (Chagnon et al. 1991).

Sow deaths have also been attributed to Clostridium novyi in some countries (Abiven 1995; Walton and Duran 1992). The diagnosis of C. novyi sudden death (clostridial hepatopathy) is difficult mainly because this organism is a common and early postmortem invader, especially of adult swine in warm weather (Taylor and Bergeland 1992). Different aspects of this disease have been examined by Duran and Walton (1997). They reported that affected sows had generalized edema, subcutaneous infiltration with bubbles, and foul-smelling bloody fluid in body cavities. The liver was enlarged and the parenchyma was infiltrated with gas bubbles, giving a spongy appearance. Several deaths in periparturient sows have been attributed to Clostridium difficile; sows had been previously treated with enrofloxacin (Mauch and Bilkei 2003). Septicemia and endocarditis have been observed as sporadic causes of death in some earlier studies.

Uterine Prolapse and Complications at Parturition. Uterine prolapses are generally responsible for fewer than 7% of all deaths. This condition is mostly observed in old sows, and the reasons for this increased frequency are unclear. Among possibilities are large pelvic inlet, long and flaccid uterus, and excessive relaxation of the pelvic and perineal region, which are probably mostly encountered in full-grown females (Roberts 1986). Although dystocia or complications at parturition are occasionally reported as causes of death, caution should be exercised so that every death occurring in the peripartum period is not ascribed to these causes. As mentioned previously, sows are most at risk of dying during the peripartum period, due to a variety of causes. They may be apathic or suffer from exhaustion or downer sow syndrome, which may be interpreted as a difficult farrowing by the producer.

Herd Size Effects on Sow Mortality. Christensen et al. (1995) observed that the risk of death for sows was three times higher in herds with more than 100 sows than in herds with fewer than 50 sows. In a study of 130 herds, the death rate was significantly higher in herds with 200 or more sows than in those with fewer than 200 sows: 8% versus 6% (S. D’Allaire and R. Drolet, unpublished data). Koketsu (2000) observed that as herd size increases by 500 females, the mortality risk increases by 0.44%.

Seasonal and Gestational Phase Effects on Sow Mortality. Seasonal patterns in sow losses have been observed. In the United Kingdom, Jones (1967, 1968) observed that more than 55% of the dead sows had died during the winter months. These sows were kept inside during winter and outside during summer. A higher incidence of mortality during summer months is reported in studies where herds were kept mainly in total confinement (Chagnon et al. 1991; Deen and Xue 1999; Drolet et al. 1992; Koketsu 2000). A significant percentage of yearly deaths may be associated with thermal stress during periods of warm weather in sows housed indoors (D’Allaire et al. 1996). The annual mortality in Hungarian breeding herds was 5.1% and 12.2% for indoor and outdoor production units, respectively (Karg and Bilkei 2002).

Sows appear to be at risk most during the lactation period (Abiven 1995), and particularly during the peripartum period (Chagnon et al. 1991; Deen and Xue 1999; Duran 2001; Madec 1984). Therefore, particular attention and care should be given to sows during this period of the reproductive cycle to limit sow losses.
Older sows are in general at higher risk of dying (Deen and Xue 1999; Koketsu 2000).

**GENETIC FACTORS INFLUENCING LONGEVITY**

Rydhmer et al. (1994) and Bidanel et al. (1996) have shown that genetics can impact age, weight, and back fat at puberty, which are associated with sow productive lifetime. Additionally, crossbreeding or heterosis impacts sow productive lifetime (Serenius et al. 2008). Živković et al. (1986) reported that crossbred sows averaged 5.3 litters, while purebred sows averaged 4.4 litters at culling, a difference of 12%. They also noted that 55.2% of culling in purebred sows occurred in the first three parities compared with 40.4% in the crossbred sows. Jørgensen (2000) reported that mean age and number of litters at removal were lower in purebred Yorkshire sows when compared with crossbred sows. Specifically, the purebred sows had higher culling for locomotion problems and reproductive failure.

Longevity may also be influenced by the breed makeup of crossbred breeding females. Hall et al. (2002) noted that sows that were one-quarter Meishan had significantly higher mean days of productive lifetime (778 days) when compared with sows that were one-eighth Duroc or one-quarter Duroc (674 and 639 days, respectively). This resulted in a greater mean parity at culling for the one-quarter Meishan sows (4.54) compared with the one-eighth Duroc (3.79) or the one-quarter Duroc sows (3.67) and a greater lifetime number of pigs born alive of 55.0 compared with 42.7 and 42.3, respectively.

Improving sow productive lifetime involves choosing the correct genetic line. Johnson (2000) reported that results from the National Pork Board’s Maternal Line Project demonstrated that traits contributing to longevity and attrition are heritable. The same report noted line differences for percentage of sows producing longevities and attrition are heritable. The same report

**Foot and Leg Soundness**

Weak pasterns have a positive influence on longevity (Grindflek and Sehested 1996; Serenius et al. 2001), while buck-kneed front legs, swaying hindquarters, and upright pasterns on rear feet were all associated unfavorably with longevity (Jørgensen 1996). Numerous studies have shown that foot and leg conformation scores are moderately heritable traits (Bereskin 1979; de Koning 1996; Huang et al. 1995; Lundeheim 1987; Rothschild and Christian 1988; Serenius et al. 2001), and some of these conformation traits have even been associated with sow longevity (de Sevilla et al. 2008, 2009; Serenius et al. 2004; Tarrés et al. 2006; Tiranti and Morrison 2006). Rothschild and Christian (1988) demonstrated that selection for improved front leg structure was quite successful in only five generations. This seems to suggest that lines with poor leg soundness scores, poor structure, or having a substantial number of foot and leg problems could be improved through proper selection. In a related paper, Rothschild et al. (1988) indicated that leg weakness problems are antagonistically correlated with back fat. Selection against back fat has been employed by most seed-stock suppliers for a number of years and may help explain some of the foot and leg problems that many commercial producers see in the females in the breeding herd. It is clear that a few soundness traits are extremely important on early culling and mortality. These include buck-kneed front legs, straight rear pasterns, swaying hindquarters, rear legs up under the body; all have a negative impact on sow productive lifetime.

**Gilt Development**

Effective gilt development management strategies allow producers to raise replacement females that achieve desired body (weight, back fat) and physiological (age, estrous cycle) parameters for gilts at first service, while maintaining the economic efficiencies of a small, well-managed, gilt pool. Achieving desired body and physiological goals is an essential component to maximize sow productive lifetime (Williams et al. 2005). Based on their experimental data and cost/benefit analysis, gilts should be bred at a target weight of 135–150 kg (300–330 lb). Gilts weighing less than 135 kg at breeding had fewer pigs born over three parities than gilts weighing over 135 kg. Additionally, their work suggests that producers can ignore body tissue reserve levels (specific back fat and loin muscle area or depth reserves). However, it is likely that achieving minimum levels of back fat in the area of 16–19 mm (0.62–0.75 in.) at the time of selection is adequate, and beyond this level, weight is the determining factor.

Gilt pool management should start by providing adequate space so puberty attainment is not delayed. A general formula can be used to calculate space for developing gilts where floor space is calculated by using the equation $A = kW^{0.667}$. $A$ represents square meters, $k$ is a constant of 0.036, and $W$ is the live weight (in kilograms) of the gilts (Levis 1997).

Boar exposure should begin between 185 and 200 days of age. The boar exposure should involve placing a mature boar in the pens for 5–15 minutes per day (Levis 1997). This time has been shown to be adequate in inducing puberty in prepubertal gilts. The activities that a mature boar should possess include production of adequate froth, vocalization, and aggressive nosing of the flanks of the gilts and attempts to mount gilts that are coming into estrus. Gilt management activities should include recording the dates that each gilt attains puberty so that heat–no service can be recorded and gilts can subsequently be mated on either the second or the third estrous cycle, which has been shown to
maximize number of pigs born alive in the first litter. Ensuring that a sufficient number of replacement gilts are produced when needed is a key component to effective gilt pool management. Once a producer is forced to mate gilts that are either too young or have insufficient isolation and acclimation time in order to maintain production at capacity, it becomes difficult to correct so that gilts are the correct age and weight and have proper development time before entering the herd. At some point, there is likely to be a “hole” in production.

Average Lactation Length Effects on Sow Productive Lifetime
The average length of lactation for the herd may also influence the annual removal rate. Herds with shorter lactation periods tend to have higher removal or death rates even though the mean parity at culling is similar (D’Allaire et al. 1989; Koketsu 2000; Paterson et al. 1997a). A reasonable explanation is that the number of litters per sow per year is higher when the lactation period is shorter. Because a sow has a certain probability of being removed during each farrow-to-farrow interval, more farrowings per year result in a higher annual probability of being removed.

BOAR LONGEVITY
In herds where artificial insemination (AI) is not used extensively, good boar-culling policies are important, for they facilitate the replacement program. Planning problems associated with boar introduction may be considerable, because boars should be kept in quarantine for a certain period and their full workload is attained only at 1 year of age. High boar removal rates seem to be more frequent than excessively low rates. In a study involving 84 commercial herds, the annual removal rate for boars averaged 59% (D’Allaire and Leman 1990). The lifetime breeding expectancy for boars is estimated at 15–20 months but varies considerably between 0.3 and 38.5 months (Arganosa et al. 1981a; D’Allaire and Leman 1990). In a study by Senk and Sabec (1970), the causes of death in 30 boars were found to be heart failure (50%), locomotor problems (23%), splenic torsions (10%), gastric ulcer (7%), endocarditis (3%), and unknown (7%).

REFERENCES
Effect of the Environment on Health

Joseph M. Zulovich

Buildings are commonly used for housing pig production. The building system should create a desirable environment for the pig. Several building/facility issues must be addressed that provide for the environmental needs of the pig. The welfare aspects of pig production are discussed in Chapter 3. Facility issues affecting the needs of the pig include (1) air temperature, (2) humidity level, (3) air velocity or air movement, (4) indoor air quality, (5) space, (6) manure removal or handling, (7) feed and water access, and (8) structural design and construction. These issues affect the pig’s environment individually as well as interactively. How well a swine facility performs can be expressed as its effectiveness. The effectiveness of a swine building/facility is defined as how well a building can provide conditions needed for optimum pig performance and how well these conditions are maintained throughout the year. The conditions that need to be provided include the first seven facility issues listed above. To date, a numerical value for building effectiveness has not been developed, but examining and comparing pig performance between building systems can provide a relative comparison of building effectiveness. For a swine building to be effective, the building needs to have had all five of the following criteria addressed:

1. properly sited to minimize any off-site impacts;
2. designed to incorporate any site limitations and management preferences;
3. constructed to meet design specifications;
4. operated to incorporate selected management preferences; and
5. maintained to meet design requirements and equipment recommendations.

All five of these criteria must be integrated and implemented for the building to provide for an optimal environment for profitable swine production.

The overall objective of this chapter is to provide a review of the pig’s environment; a basic understanding of building operational characteristics; and a foundation for one to integrate the pig’s environment with building operation such as ventilation so that one can begin to assess what facility operational parameters need to be addressed to improve pig performance and health.

FUNDAMENTALS

Several fundamental processes and concepts serve as the foundation to quantify and qualify aspects of the pig’s environment as well as building operational characteristics such as the operation of ventilation systems. A basic understanding of these fundamentals is required to better understand how and why ventilation systems interact with a swine facility. The heat transfer basics described below provide a foundation to begin understanding the processes of how a pig interacts with its environment and the building interacts with the rest of the world.

Psychrometrics

Knowledge of air–vapor mixtures provides a key fundamental understanding for quantifying the air around a pig and for designing and operating ventilation systems. Psychrometrics provides for both an understanding and a quantification of the physical and thermodynamic properties of air–vapor mixtures. Seven major properties of air–vapor mixtures include (1) dry bulb...
temperature, (2) wet bulb temperature, (3) dew point temperature, (4) relative humidity, (5) humidity ratio, (6) enthalpy (total heat content), and (7) specific volume. A psychrometric chart can be used to determine all seven properties of a particular air–vapor mixture when two independent properties are measured. The state point of an air–vapor mixture will have a unique set of values for each of the properties. The basic configuration of a psychrometric chart is given in Figure 5.1. The dry bulb temperature of the air–vapor mixture is plotted along the bottom of the chart, increasing in value from left to right. The chart gets taller from left to right, because warmer air can hold more moisture than cooler air. So the vertical dimension indicates the quantity of moisture within an air–vapor mixture. Of the seven properties of air–vapor mixtures, only three properties, dry bulb temperature, relative humidity, and dew point temperature, are needed to understand the basics of how air–vapor mixtures interact within a facility and with a ventilation system.

**Dry Bulb Temperature.** The dry bulb temperature, or air temperature, is the basic temperature of the air–vapor mixture and is easily measured with a thermometer. Dry bulb temperature is typically the most common property measured to obtain the state point of an air–vapor mixture. The state point of a given air–vapor mixture will be located on the vertical line of a psychrometric chart that intersects the bottom axis at the given dry bulb temperature, which increases in value from left to right.

**Relative Humidity.** Relative humidity can be measured directly using some of the electronic sensors that are now available. The relative humidity, typically expressed as a percentage, is technically defined as the ratio of the actual water vapor pressure to the vapor pressure of saturated air at the same dry bulb temperature. Or, in simpler terms, the relative humidity measures or indicates how saturated or “full” the air is of moisture at a particular temperature. Relative humidity lines on a psychrometric chart are the curved lines running from the lower left to the upper right of the chart. A 100% value of relative humidity is shown as the top curved line on a psychrometric chart. Since warmer air can hold more moisture than cooler air, the actual amount of moisture in an air–vapor mixture cannot be determined from only a relative humidity value. The state point of a given air–vapor mixture can be found on a psychrometric chart where the measured dry bulb temperature intersects the measured relative humidity. Once the state point is found, the quantitative amount of moisture in the air can be determined.

**Dew Point Temperature.** Dew point temperature had been a common property measured to find the state point of an air–vapor mixture before the availability of relative humidity measurements. Dew point temperature is defined as the temperature at which moisture starts to condense from air cooled at constant pressure and humidity ratio. On a psychrometric chart, dew point temperatures for various state points are found on the horizontal lines. A particular dew point temperature is found where the dry bulb temperature intersects with the 100% relative humidity line. The dew point temperature equals the air temperature when the relative humidity is 100% for a given air temperature. The dew point temperature increases and decreases in value along the vertical direction of the psychrometric chart; dew point temperature does give a true indication of the amount of moisture in the air.

**Wet Bulb Temperature.** Wet bulb temperature is a state point property and is a commonly measured property when using a basic psychrometer. A psychrometer is a measurement device that has two identical thermometers. One thermometer is used to measure dry bulb temperature. The other thermometer has a sock placed over the bulb of the thermometer. The sock is wetted and when air is moved over the thermometer bulb, a cooling effect is created from water evaporating from the sock, which lowers the temperature of this thermometer. The amount the temperature is lowered is proportional to the moisture level in the air. When a stable reading can be seen on the thermometer with the sock, the wet bulb temperature is then read from the thermometer. To find the state point of the air–vapor mixture, the wet bulb temperature can be located on a psychrometric chart showing all properties. Many psychrometers provide a slide rule-type device that can be used to determine relative humidity given the dry bulb and wet bulb temperatures. Then the relative
humidity can be used to find the state point of the measured air–vapor mixture.

**Heat Transfer Basics**

Heat transfer defines the processes of transferring heat energy from one body to another. Two major types of heat energy transfer exist within swine housing systems. Sensible heat transfer is the first type of energy transfer and is caused or driven by a temperature difference between two bodies. Latent heat transfer is the second type of energy transfer and is caused by a vapor pressure or moisture concentration difference between two bodies. Swine produce heat energy from metabolic activity that must be dissipated to their environment. Either enhancing or reducing both the sensible and latent heat transfer to match the rate of metabolic heat produced obtains thermal comfort of swine. If too much heat transfer is created, swine will be chilled and health may be compromised. If not enough heat transfer is created, swine will be heat stressed and production will be reduced. A large enough unbalance between heat transfer and metabolic heat production can cause swine to die from being either too cold or too hot. A more comprehensive discussion of livestock energetics can be found in DeShazer et al. (2009).

**Conduction.** Conduction provides the first mode of sensible heat transfer and is generally considered the easiest sensible mode to understand. Conductive heat transfer is the exchange of heat energy between two bodies touching each other and at two different temperatures. Heat flow is from higher temperature to lower temperature. For example, a pig lying on a cold floor will lose heat energy to the floor due to the temperature difference between the pig and the floor. Heat energy also flows through a solid by conduction if two surfaces of the body are at different temperatures. For example, heat energy will flow through a wall if the inside temperature is warm and the outside temperature is cold. The rate of conductive heat flow is dependent on both the temperature differential and the resistances to heat flow either between the two solid objects or through the mass of a solid object.

**Convection.** Convection provides the second mode of sensible heat transfer. Convective heat transfer is the exchange of heat energy between a solid body and either a gas or a liquid flowing over the solid body when a temperature difference between the solid body and the fluid exists. Heat flow is from higher temperature to lower temperature. For example, a pig standing in a flowing, relatively cool airstream will lose heat energy to the air due to the temperature difference between the pig’s skin temperature and the air temperature. The rate of convective heat transfer is dependent on both the temperature differential and velocity or speed of the fluid across the surface of the solid.

**Radiation.** Radiation provides the third mode of sensible heat transfer and is usually considered the most complex sensible heat transfer mode to understand. Radiative heat transfer is the exchange of thermal energy between objects by electromagnetic waves. Radiative energy transfer follows the same laws as light: It is transmitted through space; it follows a straight-line path; and it can be reflected, absorbed, or transmitted. An object must absorb radiant energy in order to be converted to a heat gain for the object. Radiative heat transfer occurs between all objects with at least a three quarters of an inch separation distance between them, but cooler objects will have a net radiative heat energy gain compared with warmer bodies with which the cooler object is exposed. Some supplemental heating tasks incorporate the characteristics of radiative heat transfer to benefit swine facility operation. One example uses heat lamps for creep heating in farrowing facilities. Heat lamps will radiate energy to heat piglets and flooring exposed to the lamps but will not heat other surfaces or the air within the room. Radiant heat exchange can have negative impacts on swine thermal comfort. For example, a pig exposed to a cold surface can have a significant amount of heat loss to the cold surface even when the air temperature around the pig is acceptable. This kind of negative impact can occur during cold weather in facilities having cold inside surface temperatures due to poor or low insulation levels.

**Evaporation.** Evaporation provides the first type of latent heat transfer. Latent heat transfer occurs by moisture evaporating from the warm, moist surface of a pig and blending into the surrounding air–vapor mixture. The rate of evaporative heat transfer is determined by the vapor pressure potential between the moist surface and the surrounding air. Heat energy transfers by evaporation from high vapor pressure conditions to low vapor pressure conditions. Dew point temperature is directly correlated with the vapor pressure of air or moist surface. So, heat energy will transfer by evaporation from a surface with a relatively high dew point temperature to the surrounding air with a relatively low dew point temperature. If the temperature of a surface is equal to or lower than the dew point temperature of the surrounding air, no evaporation will occur from the moist surface.

**Condensation.** Condensation provides the second type of latent heat transfer. Latent heat transfer occurs by moisture condensing from relatively warm, moist air on to a relatively cool surface. The rate at which condensation will occur is determined by the vapor pressure differential between the air and the surface. As with evaporation, the dew point temperature is directly correlated with the vapor pressures of both the air and surface. So, heat energy will transfer by condensation from the surrounding air with a relatively high dew point temperature.
point temperature to an object or surface with a relatively low dew point or surface temperature. If the temperature of an object or surface is higher than the dew point temperature of the surrounding air, no condensation will form on the object or surface.

**PIG’S ENVIRONMENT**

The pig’s environment can be separated into three major categories—(1) thermal environment, (2) gaseous environment, and (3) physical environment. Each of these categories must be provided in an adequate manner to ensure good pig performance and to maintain good swine health. These categories will be reviewed separately in the following sections. For the most part, these categories generally do not interact with each other but closely interact with various building operational characteristics. The exception is humidity because it has an impact with the thermal environment and its level is controlled within the gaseous environment.

**Thermal Environment**

The air temperature surrounding the pig is typically considered the primary component of the thermal environment. The desired temperature range for pigs at various sizes is presented in Table 5.1 (MWPS 1990). The temperature range in Table 5.1 can be referred to as the thermoneutral zone (Hillman 2009). The low end of the thermoneutral zone is normally considered the lower critical temperature (LCT), which is defined as the lowest air temperature that a given animal can maintain homeothermic conditions without increasing metabolism to maintain body temperature (Hillman 2009). The exact LCT for any given pig or group of pigs will depend on its feed intake and body size (DeShazer and Yen 2009). Pigs of any given size that consume more feed will have a lower LCT as compared with pigs consuming less feed. The upper end of the thermoneutral zone is normally considered the upper critical temperature (UCT) or the evaporative critical temperature (Hillman 2009). The UCT can be defined as the temperature at which pigs begin to pant or perform other behaviors in order to dissipate heat to the environment in order to maintain body temperature.

Air temperature is not the only component of the pig’s thermal environment. Other components can influence the sensible heat exchange between the pig and its environment. These other components include but are not limited to the air velocity surrounding the pig or the type of floor pigs may lie on. In production, the challenge is how to account for these other environmental components affecting the pig’s thermal comfort. The effective environmental temperature (EET) adjusts the air temperature to account for these other components of the thermal environment. For example, if a draft is occurring on a pig, the pig will feel cooler than the air temperature than if no draft was occurring and would have a lower EET than a pig not in a draft. For the pig to feel as warm in a draft as without a draft, the air temperature would need to be increased to compensate for the chilling effect caused by the draft. When the air temperature is adjusted for these other factors affecting the pig’s thermal environment, this temperature is often referred to as the setpoint temperature. The setpoint temperature is the air temperature a control sensor must read in order to provide the necessary thermal comfort to the pig. The setpoint temperature is often designated for the air temperature. A comprehensive review of the literature was completed by Gonyou et al. (2006) providing a suggested setpoint temperature for swine at various stages of production.

**Gaseous Environment**

The following section addresses in some detail a few of the indoor air quality issues related to swine confinement housing. The targets for indoor air quality have been based in recent years on what is required for worker health and safety. Based on very limited recent research data available, targets being proposed for worker health are well within air quality requirements for swine health.

Air quality in swine confinement housing is addressed with swine housing facilities for the 21st century. Modern swine housing facilities are designed to address many of the air quality concerns seen in the literature. Design considerations along with accompanying management and operational procedures are combined to greatly improve air quality in swine confinement facilities. As a result, swine facilities for the 21st century have some significant design and management characteristics that improve indoor air quality.

### Table 5.1. Recommended air temperature ranges at animal level for pigs at various sizes and ages

<table>
<thead>
<tr>
<th>Animal Weight</th>
<th>Optimum Temperature</th>
<th>Temperature Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactating sow</td>
<td>35°C (95°F)</td>
<td>32–35°C (90–95°F)</td>
</tr>
<tr>
<td>Litter—newborn</td>
<td>27°C (80°F)</td>
<td>24–29°C (75–85°F)</td>
</tr>
<tr>
<td>Nursery 5–14 kg (12–30 lb)</td>
<td>24°C (75°F)</td>
<td>21–27°C (70–80°F)</td>
</tr>
<tr>
<td>Nursery 14–23 kg (30–50 lb)</td>
<td>18°C (65°F)</td>
<td>16–21°C (60–70°F)</td>
</tr>
<tr>
<td>Nursery 23–34 kg (50–75 lb)</td>
<td>16°C (60°F)</td>
<td>13–21°C (55–70°F)</td>
</tr>
<tr>
<td>Grower 34–82 kg (75–180 lb)</td>
<td>13°C (55°F)</td>
<td>10–21°C (50–70°F)</td>
</tr>
<tr>
<td>Finisher 82 market kg (180 market lb)</td>
<td>10–21°C (50–70°F)</td>
<td></td>
</tr>
<tr>
<td>Gestating sows</td>
<td>10–21°C (50–70°F)</td>
<td></td>
</tr>
<tr>
<td>Boars</td>
<td>10–21°C (50–70°F)</td>
<td></td>
</tr>
</tbody>
</table>
Design characteristics of 21st-century swine confinement facilities address manure management systems, ventilation systems, and overall facility management to improve indoor air quality. Ammonia levels are often used as an indicator of air quality. Midwest Plan Service (MWPS 1990) recommends 10 ppm of ammonia as an excellent long-term target level for ammonia, and a 10-ppm ammonia level could be maintained for many facilities with well-designed and -managed ventilation systems. For industrial workers, the daily 8-hour time-weighted average (TWA) exposure limit for ammonia is 25 ppm as recommended by the National Institute for Occupational Safety and Health (NIOSH). The threshold limit value for TWA exposure concentration for ammonia during a normal 8- to 10-hour workday is 25 ppm as recommended by the American Conference of Governmental Industrial Hygienists. The only current industrial worker air quality standard for ammonia is the 15-minute average short-term exposure limit of 35 ppm as regulated by the Occupational Safety and Health Administration (OSHA). Swine health has not been shown to be affected by ammonia levels less than 10 ppm.

Deep pit systems, where the manure is stored in a basement-type tank located under the pen flooring, can maintain long-term ammonia level averages below 10 ppm but have had ammonia levels as high as 50 ppm or higher for several hours or potentially days. As stated before, the OSHA short-term industrial worker exposure limit for ammonia is 35 ppm average measured for a time duration of 15 minutes. A worker entering a building with an elevated ammonia level should only spend 15 minutes in the building for the entire day to stay within the industrial worker standard for ammonia.

Another air quality concern with deep pits located under buildings is the potential hazard due to hydrogen sulfide. Hydrogen sulfide is the gas that smells like rotten eggs. Most people can smell hydrogen sulfide at a 1-ppm concentration. As the concentration increases, people tend to lose the ability to smell hydrogen sulfide when it reaches 50–100 ppm. Hydrogen sulfide can become lethal at concentrations above 500 ppm. The major hazard with hydrogen sulfide is one cannot detect by smell that hydrogen sulfide has reached lethal levels. Hydrogen sulfide levels in swine buildings with deep pit storage are normally very low if detectable. However, when manure is pumped from the storage under the building, hydrogen sulfide levels can be elevated and can reach lethal levels in some cases. To minimize the risk due to hydrogen sulfide during manure agitating and pumping activities, the ventilation provided to the pig space should be operating as high as feasible for weather conditions and pig age. A person should never enter a building when manure is actively being pumped from the under building storage.

The ventilation system for swine facilities is another key system that directly affects indoor air quality. Ventilation has been required in swine facilities for a number of years. The key to success lies in the actual design and operation of the ventilation system. A minimum amount of air exchange (given in Table 5.2) is required throughout the year and regardless of season.

The target minimum ventilation rates, shown in Table 5.2 for pigs of various sizes, should be provided on a continuous basis and in a uniform manner. The ventilation rate needs to be constant and at the required rate for the season. The required ventilation rate will vary depending on outside weather conditions. The ventilation system needs to have controls and be managed such that the system can vary the ventilation rate uniformly throughout the building.

The ventilation system can have a significant effect on dust levels within the building. If the air exchange is greater than is required for moisture control, the building will tend to dry out and result in higher dust concentrations. Moisture levels should range between 40% and 70% relative humidity.

During winter conditions, the relative humidity target should be 60–70% during most weather conditions. This range still remains in the “healthy” range while minimizing supplemental heat requirements due to minimum ventilation rates.

High dust levels have been shown to adversely affect swine health as well as human health. Dust levels are decreased with proper ventilation rates and control.

Dust levels in swine confinement are directly affected by feed and feed handling. Feed handling systems have options available to minimize feed dust generation during handling. Feed processing techniques also affect dust levels in swine confinement. Adding oil to feed during processing can reduce dust generation potential. Adding oil to feeds can reduce total dust on a mass basis due to handling by 75% or more.

Finally, the facility management practice of all-in/all-out (AIAO) production has been shown to improve swine health and performance. AIAO affects air quality because the animal space is emptied and completely

### Table 5.2. Target minimum ventilation rates for pigs at various sizes

<table>
<thead>
<tr>
<th>Weight</th>
<th>Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sow and litter 4-14 kg (10–30 lb)</td>
<td>0.0094 m³/s-crate (20 cfm/crate)</td>
</tr>
<tr>
<td>14–23 kg (30–50 lb)</td>
<td>0.0014–0.0019 m³/s-pig (3–4 cfm/pig)</td>
</tr>
<tr>
<td>23–34 kg (50–75 lb)</td>
<td>0.0026–0.0033 m³/s-pig (5–7 cfm/pig)</td>
</tr>
<tr>
<td>34–82 kg (75–180 lb)</td>
<td>0.0033–0.0042 m³/s-pig (7–9 cfm/pig)</td>
</tr>
<tr>
<td>82 adult/market kg (180 adult/market lb)</td>
<td>0.0047–0.0057 m³/s-pig (10–12 cfm/pig)</td>
</tr>
</tbody>
</table>

Unit: cfm, cubic feet per minute.
cleaned before a new group of pigs are moved into the space. By starting with a clean area, dust levels during a given production cycle are reduced because all residual dust from the previous production group was removed from the animal space.

**Physical Environment**

The physical environment is not only somewhat separate but also integrated with the thermal and gaseous environmental components. The number of pigs within a given airspace such as a single room or building impacts how the ventilation system must be designed and operated to help ensure a quality thermal and gaseous environment for the pig.

**Space.** Space is considered a physical component of a pig’s environment. However, insufficient space during hot weather can result in more heat stress for the housed pigs than if sufficient space is provided. General space recommendations for growing pigs of various sizes are shown in Table 5.3. The recommendations in Table 5.3 are for the largest size the pig will achieve before it is moved from the pen. Pens should be sized for the number of pigs per pen (generally 10–25 pigs) at the largest they will be in the pen. If a nursery is being considered to optimize growth of pigs from weaning to 27 kg (60 lb), 0.33–0.37 m²/pig (3.5–4 ft²/pig) should be used to size the pens. Nursery pens sized at 0.23 m²/pig (2.5 ft²/pig) or less are generally intended for pigs that consistently do not get larger than about 18 kg (40 lb).

**Water and Feed Access.** Water and feed access is part of the physical environment. Water is an important component for swine growth and should be offered continuously without limited access. Waterers should be provided in at least two locations within a given pen. A watering system that has two nipple waterers on a single drop pipe really does not provide two waterer locations because one “boss” pig can control access to the two nipples attached to the single drop. During hot weather, water consumption is an important means for heat stress relief.

Feeder space is another important component of the physical space. Feeder space recommendations are presented in Table 5.4. The pigs per feeder space recommendations are the long-standing recommendations. New research data on pigs per feeder space indicates that up to 10 pigs per feeder space is acceptable at the feeder space sizes indicated in Table 5.4. This increased feeder space size better reflects the amount of space required for one pig to fit in front of a feeder. The new feeder size recommendation is a result of experiences with the new wean-finish facilities.

**Equipment and Penning.** The long-standing recommendation for pigs per pen has ranged from 10 to about 25 or 30 pigs per pen. New experiences with wean-finish facilities have shown that much larger numbers of pigs per pen will still allow good pig performance. Pens with 50 up to 200 pigs per pen have allowed for good pig performance. The feeder space recommendations for large group pens are the feeder space per pig values in Table 5.4. Feeders for large group pens are typically located in the center of the pens on a feeder platform (about 75 mm [3 in.] thick) with the waterers located on each end of the feeders. The amount of space per pig for large group pens can be reduced to 0.67–0.70 m² (7.25–7.5 ft²) per finishing pig. The space per pig can be reduced because pigs will lie around the edge of the pen. With the feeder and waterer in the center of the pen, fewer disturbances occur when an individual pig wants to eat or drink. Locating and treating individual pigs is a significant challenge with large group pens.

**TRANSPORT ENVIRONMENTS**

This chapter thus far has been focused on the environment within a given room or building of pigs. Transport environments are defined as the environment within any given truck or vehicle used to move pigs from one location to another. The physical environment components of feed and water are rarely provided as part of a transport environment. The amount of space per pig provided during transport on a vehicle is normally less than what is provided within a pen. The thermal and gaseous environment aspects within transport environments are typically not closely controlled and are typically created through operation of the various openings.

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**Table 5.3.** Space recommendations for growing pigs

<table>
<thead>
<tr>
<th>Animal Weight (lb)</th>
<th>Space per Pig</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wean-14 kg (wean-30 lb)</td>
<td>0.16–0.23 m² (1.7–2.5 ft²)</td>
</tr>
<tr>
<td>14–27 kg (30–60 lb)</td>
<td>0.28–0.37 m² (3–4 ft²)</td>
</tr>
<tr>
<td>27–45 kg (60–100 lb)</td>
<td>0.46 m² (5 ft²)</td>
</tr>
<tr>
<td>45–68 kg (100–150 lb)</td>
<td>0.56 m² (6 ft²)</td>
</tr>
<tr>
<td>68 market kg (150 market lb)</td>
<td>0.74 m² (8 ft²)</td>
</tr>
</tbody>
</table>

**Table 5.4.** Feeder space recommendations for growing pigs

<table>
<thead>
<tr>
<th>Animal Weight</th>
<th>Pigs per Feeder Space</th>
</tr>
</thead>
<tbody>
<tr>
<td>4–14 kg (10–30 lb)</td>
<td>2</td>
</tr>
<tr>
<td>14–23 kg (30–50 lb)</td>
<td>3</td>
</tr>
<tr>
<td>23–34 kg (50–75 lb)</td>
<td>4</td>
</tr>
<tr>
<td>34 market kg (75 market lb)</td>
<td>4–5</td>
</tr>
<tr>
<td>Animal Weight</td>
<td>Feeder Space per Pig</td>
</tr>
<tr>
<td>---------------</td>
<td>-----------------------</td>
</tr>
<tr>
<td>4–23 kg (10–50 lb)</td>
<td>15–18 cm (6–7 in.)</td>
</tr>
<tr>
<td>23 market kg (50 market lb)</td>
<td>30–35 cm (12–14 in.)</td>
</tr>
</tbody>
</table>

Note: Up to 10 pigs per above feeder space size.
of a truck compartment. For a complete discussion on the specifics of how to provide reasonable conditions for pigs in transport, one should obtain a copy of the latest version of the Transport Quality Assurance materials from the National Pork Board.

REFERENCES


Optimizing Diagnostic Value and Sample Collection

Jerry L. Torrison

DEVELOPING THE DIAGNOSTIC PLAN

The Diagnostic Question and Response

Veterinarians who submit samples to a diagnostic laboratory are looking for accurate and timely information to help them make medical and management decisions for swine herds in their care. This process of producing helpful diagnostic information is very much a collaborative effort involving the producer, practitioner, and diagnostician. The process can be straightforward and routine or may require considerable discussion and planning, depending on the complexity of the problem and the availability of suitable tests to address it.

Optimizing the value of diagnostic effort begins with a clear statement of the diagnostic question. The question might be “Why did this pig die?” or “What is causing the clinical signs in this group of pigs?” The diagnostic approaches to address these questions are different from addressing questions such as “When were these pigs exposed to [a disease agent]?” or “Did we successfully eliminate (or are these pigs still free of) a disease agent?” Developing a clear diagnostic question is the first step in developing an efficient and effective diagnostic approach plan (Cannon 2002).

A second question must also be addressed clearly before details of a diagnostic plan can be determined: What will be done with the results of the diagnostic testing? A cardinal rule of diagnostic testing is that no test should be run unless there is a plan for interpreting and responding appropriately to the results. A series of additional questions are bundled into this basic question: Will further testing be required, depending on the results? Is a plan in place for this, including sufficient and appropriate samples for additional testing? Is the diagnostic laboratory aware of this plan? Who should receive the results?

An example of this preparation for a diagnostic result is a formal diagnostic algorithm for testing boars in a boar stud for porcine reproductive and respiratory syndrome virus (PRRSV) using serum samples collected at the time of semen collection. There is great concern that PRRSV might infect a boar stud and result in transmission of the virus to sow herds using semen from infected boars (Yaeger et al. 1993). Routine testing in boar studs has generated the need for diagnostic algorithms for what is in essence an emergency response plan for a potential introduction of PRRSV into a boar stud population (Reicks et al. 2006a). Algorithms illustrate the important concepts of first establishing a clear purpose for submitting samples for testing and then having an understanding of how the results will be used for making decisions.

A third question regarding the design of a sampling protocol is the financial structure of the diagnostic question being addressed. In boar stud testing, there is an attempt to optimize costs through an understanding of the risks and benefits of pooling samples. The effects of pooling samples on the relative sensitivity of diagnostic tests on an individual basis and population basis need to be considered carefully when testing protocols are being designed (Reicks et al. 2006b; Rovira et al. 2008). The budget in question is not simply the expenses for sampling and testing. The budget equation must also consider the probability of correctly determining the health status of a pig or pigs and the consequences of incorrectly determining the status.

Diagnostic Sample Selection

Once the diagnostic question has been established, a plan for diagnostic testing can be designed. Selection of samples is dictated by the tests available (Chapter 7)
to address the question, the dynamics of the disease, and the biology of the etiologic agent or toxin. Several factors have beginning and end points relative to each other. These factors are clinical signs (including fever), agent replication, antigen expression, agent shedding, and antibody development.

The stage of a pig or a population of pigs on the disease timeline must be considered to optimize sample selection. For example, if the objective of the diagnostic workup is isolation of a virus or bacteria, the sampling must be done during the patent period of the infection when the infectious agent is present. In contrast, if the objective is to detect antibodies against a specific disease agent, sampling must be done after sufficient time has passed to allow antibodies to develop to detectable levels, often after the virus or bacteria has been cleared. This dynamic is variable by disease agent and even by specific serology test for the same agent (Chapter 7).

The onset and duration of the factors on the timeline varies by disease, host factors such as age and immune status, and disease agent factors such as dose and virulence. For example, the clinical course of foot-and-mouth disease in pigs varied by challenge virus strain using a contact method of challenge (Alexandersen et al. 2003). This report illustrated a common phenomenon in which the presence of infectious agent is highly correlated with fever, and the clearance of the agent coincides with the development of circulating antibodies.

Within a population of pigs, there is typically a distribution along the continuum that provides an opportunity to identify individual pigs that may be at different stages of the disease process, allowing a variety of sampling from within the population. In general, fever can be used as a reasonable predictor of success for detecting disease agents in live pigs; if the pig has a fever, the odds of finding the agent increase. Thus, if the objective is to detect antibodies against a specific disease agent, sampling must be done during the patent period of the infection when the infectious agent is present. In contrast, if the objective is to detect antibodies against a specific disease agent, sampling must be done after sufficient time has passed to allow antibodies to develop to detectable levels, often after the virus or bacteria has been cleared. This dynamic is variable by disease agent and even by specific serology test for the same agent (Chapter 7).

The biology of the etiologic agent must also be considered in diagnostic planning. For example, *Haemophilus parasuis* survives poorly at warm temperatures, so isolating the bacteria is unlikely unless samples are collected soon after a pig dies and chilled immediately until culture is attempted. Specifically, Morozumi and Hiramune (1982) demonstrated that from a starting concentration of $10^8$ viable *H. parasuis* bacteria, no viable bacteria remained in saline after 1 hour at 42°C, 2 hours at 37°C, and 8 hours at 25°C. In contrast, the concentration of *Escherichia coli* was essentially unchanged over an 8-hour period at the same range of temperatures. Thus, if the objective is to culture live *H. parasuis* from infected pigs, sample collection and handling methods must consider the temperature sensitivity of the bacteria. This phenomenon plays out as reduced incidence of *H. parasuis* during the warm weather months in terms of frequency of isolation of the bacteria from diagnostic case submissions (Figure 6.1).

Another aspect of the agent biology is the location in which it is most likely to be found. For example, the enteric bacteria *Brachyspira hyodysenteriae* is found exclusively in the large bowel, so sampling for this agent requires submission of the large bowel or feces. If only the small intestine is submitted, the agent cannot be identified.

A final aspect of the agent biology to consider is the distinction between strains of bacteria and location from which they may be isolated. For example, common flora of the gut or respiratory tract may be nominally the same as pathogenic bacteria, but may differ in virulence or consequence from their counterparts that cause disease (Hanson 1988). To extend the examples of *H. parasuis* and *E. coli*, both are found in healthy pigs, from the respiratory tract in the case of *H. parasuis* and the intestinal tract in the case of *E. coli*. Culturing *H. parasuis* from normal lungs or nasopharynx, or *E. coli* from the intestine of a healthy pig, may not be clinically significant. If the purpose of an investigation is to find an *H. parasuis* isolate responsible for causing polyserositis or meningitis, cultures should be attempted from fibrinous exudates and brains rather than lung parenchyma (Oliveira 2004). Similarly, if the intent is to find virulent strains of *E. coli*, samples should be taken from clinically affected pigs rather than random fecal samples. Isolates can be further characterized by
virulence genotype testing for possible understanding of their clinical significance (Moon et al. 1999).

**THE PIG NECROPSY**

Swine practitioners are among the few medical professionals who routinely perform postmortem examinations (necropsies) of their patients (King and Meehan 1973; Pinto Carvalho et al. 2008). The term “autopsy” used in the human medical field is translated as “seeing for one's self.” The postmortem examination is a valuable opportunity for clinicians not only to understand the disease processes involving the individual pig under examination, but also to start the process of identifying newly emerging health threats. The postmortem examination is the first step in an inquiry that goes beyond “dissection and microscopic examination of tissues” to include a vast array of assays and techniques to find disease agents or evidence of their presence (Dada and Ansari 1996).

Producers are generally very interested in participating in the process, and the necropsy provides an excellent client education opportunity. It is also quite helpful to have an assistant to lift heavy pigs, open sample bags, and record findings.

It is important to develop a routine that is consistent, thorough, logical, and systematic. The routine will vary among individuals, but should be consistent for an individual.

**THOROUGH**—There may be situations in which all that is needed is a single organ sample such as the lungs to address a specific diagnostic question, but normally there is merit in taking full advantage of a complete necropsy to extract the full value out of the dead pig, the prosector's time, and the expenditure of time and money in submitting samples to a diagnostic laboratory.

**LOGICAL**—The necropsy routine will also have variations dictated by the clinical problem under investigation and, to a certain degree, the size of the pig being necropsied, and the physical location where the necropsy will be performed. It is important to be systematic while still logical and practical in each particular case.

**Table 6.1.** Suggested necropsy kit components

<table>
<thead>
<tr>
<th>Essentials</th>
</tr>
</thead>
<tbody>
<tr>
<td>Knife, sharpening steel, examination gloves, sterile sample bags, permanent marker, blood collection tubes, culture swabs, syringes and needles, scalpel blades and handle, 10% buffered formalin in nonspilling containers</td>
</tr>
</tbody>
</table>

**Recommended**

Preprinted sample bag labels or bags, euthanasia solution (appropriately secured) or captive bolt gun, hacksaw or hatchet, rib cutter (tree pruner), obstetrical wire and handles, palpation sleeves, pH paper (range from 3 to 8), microscope slides, flashlight or headlamp, surgical scissors and forceps

---

**Table 6.2.** Porcine septicemia—specimen collection

<table>
<thead>
<tr>
<th>Tissue/sample</th>
<th>Fresh (chilled—not frozen)</th>
<th>Fixed (10% buffered formalin)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum</td>
<td>5 mL</td>
<td></td>
</tr>
<tr>
<td>Whole blood</td>
<td>3 mL in EDTA</td>
<td></td>
</tr>
<tr>
<td>Swabs</td>
<td>Brain, epicardium, joint (periarticular tissue including synovium)</td>
<td>Three pieces per pig from affected areas with different gross appearance (2 x 2 x 1 cm)</td>
</tr>
<tr>
<td>Lung</td>
<td>6 x 6 x 6 cm—two sections per pig with visible airways</td>
<td></td>
</tr>
<tr>
<td>Heart</td>
<td>4 x 4 x 4 cm piece</td>
<td>2 x 2 x 0.5 cm</td>
</tr>
<tr>
<td>Liver</td>
<td>4 x 4 x 4 cm piece</td>
<td>0.5-cm slice through center</td>
</tr>
<tr>
<td>Kidney</td>
<td>Half of a kidney</td>
<td></td>
</tr>
<tr>
<td>Spleen</td>
<td>5-cm piece</td>
<td></td>
</tr>
<tr>
<td>Lymph nodes</td>
<td>Mandibular, sternal, tracheobronchial, mesenteric, and superficial inguinal</td>
<td>Mandibular, sternal, tracheobronchial, mesenteric, and superficial inguinal</td>
</tr>
<tr>
<td>Ileum</td>
<td>10-cm segment</td>
<td>2-cm segment</td>
</tr>
</tbody>
</table>


Animal selection—three euthanized pigs with typical signs, acutely affected, and untreated (if available), or three freshly dead pigs. Sample submission—package and identify specimens from pigs individually.

Note: Pigs dying from *S. suis* infection often have acute meningitis but no gross thoracic or abdominal visceral lesions. EDTA, ethylenediaminetetraacetic acid.

**SYSTEMATIC**—The practice of using checklists routinely to reduce mistakes was borrowed from the aviation industry and applied in human medicine (Pronovost et al. 2002). Using a checklist or a necropsy form to record observations ensures that the distractions in the field do not prevent a systematic approach to a routine procedure. Most diagnostic laboratories provide a submission form that will provide space for pertinent information, including clinical history, signalment, and vaccine and treatment history.

One means of developing a systematic approach is to have necropsy kits prepared in advance (Table 6.1), including labeled specimen containers for complete sample selection and correct grouping of samples. Specimen containers can be preprinted using permanent markers. Another approach is to carry packets of preprinted labels that can be placed on the containers at the time of collection. Printing labels according to the clinical condition being investigated and the type of samples that are then required allows more flexibility in sampling protocols (Tables 6.2–6.7 at the end of the chapter). Caution is needed as preprinted labels can
vatively inexpensive such that multiple tissues and lesions can be examined to confirm a role for suspected agents or disease as well as suggest the presence of alternative insults that have not been considered. For some disease processes, immunohistochemistry is a very useful tool for demonstrating causative agent within compatible lesions.

Samples for histopathology should rarely exceed 1 cm in thickness. One hundred percent formalin is the aqueous solution of dissolved formaldehyde gas avail-

### Table 6.3. Porcine respiratory disorders—specimen collection

<table>
<thead>
<tr>
<th>Tissue/sample</th>
<th>Fresh (chilled—not frozen)</th>
<th>Fixed (10% buffered formalin)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum</td>
<td>5 mL</td>
<td></td>
</tr>
<tr>
<td>Swabs</td>
<td>Caudal nasal passage</td>
<td></td>
</tr>
<tr>
<td>Nasal turinate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lung</td>
<td>6 × 6 × 6 cm—two sections per pig with visible airways</td>
<td>1 cm thickness Three pieces per pig from affected areas with different gross appearance (2 × 2 × 1 cm)</td>
</tr>
<tr>
<td>Lymph nodes</td>
<td>Mandibular, sternal, tracheobronchial, mesenteric, and superficial inguinal</td>
<td>Mandibular, sternal, tracheobronchial, mesenteric, and superficial inguinal</td>
</tr>
<tr>
<td>Tonsil</td>
<td>½</td>
<td>½</td>
</tr>
<tr>
<td>Heart</td>
<td>4 × 4 × 4 cm piece</td>
<td>2 × 2 × 1 cm including L and R ventricles and septum</td>
</tr>
<tr>
<td>Liver</td>
<td>4 × 4 × 4 cm piece</td>
<td>2 × 2 × 0.5 cm</td>
</tr>
<tr>
<td>Kidney</td>
<td>Half of a kidney</td>
<td>0.5-cm slice through center</td>
</tr>
<tr>
<td>Spleen</td>
<td>5-cm piece</td>
<td></td>
</tr>
</tbody>
</table>


Animal selection—three euthanized pigs with typical signs, acutely affected, and untreated (if available), or three freshly dead pigs. Sample submission—package and identify specimens from pigs individually.

### Table 6.4. Porcine neurological disorders—specimen collection

<table>
<thead>
<tr>
<th>Tissue/sample</th>
<th>Fresh (chilled—not frozen)</th>
<th>Fixed (10% buffered formalin)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Swabs of:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. meninges</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2. brain</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brain</td>
<td>Cut brain in half longitudinally, slightly off the midline. Submit larger half fresh/chilled.</td>
<td>Fix the smaller half in formalin.</td>
</tr>
<tr>
<td>Spinal cord</td>
<td>If clinical signs indicate, submit 5-cm segment of the lumbosacral and cervicothoracic cord.</td>
<td>If clinical signs indicate, submit 5-cm segment of the lumbosacral and cervicothoracic cord.</td>
</tr>
<tr>
<td>Lymph nodes</td>
<td>½ of the sternal, tracheobronchial, mandibular, superficial inguinal, and mesenteric</td>
<td>½ of the sternal, tracheobronchial, mandibular, superficial inguinal, and mesenteric</td>
</tr>
<tr>
<td>Tonsil</td>
<td>½</td>
<td>½</td>
</tr>
<tr>
<td>Lung</td>
<td>5 × 5 × 5 cm pieces. If there are different gross lesions, submit a chilled specimen from each area that includes a 0.3-cm-diameter bronchial. If similar gross lesions throughout, submit two specimens.</td>
<td>2 × 2 × 1 cm sections (two or three)—include gross lesion if present</td>
</tr>
<tr>
<td>Pleura or pericardium</td>
<td>Swabs or fluid if indicated</td>
<td></td>
</tr>
<tr>
<td>Heart</td>
<td>4 × 4 × 4 cm piece</td>
<td>2 × 2 × 1 cm section</td>
</tr>
<tr>
<td>Liver</td>
<td>4 × 4 × 4 cm piece</td>
<td>2 × 2 × 0.5 cm</td>
</tr>
<tr>
<td>Spleen</td>
<td>Half of a kidney</td>
<td>0.5-cm slice, including cortex and medulla</td>
</tr>
<tr>
<td>Jejunum</td>
<td>5-cm piece</td>
<td>1-cm piece</td>
</tr>
<tr>
<td>Ileum</td>
<td>10-cm segment</td>
<td>Two sections, 2 cm in length</td>
</tr>
</tbody>
</table>


Animal selection—three euthanized pigs with typical signs, acutely affected, and untreated (if available), or three freshly dead pigs. Sample submission—package and identify specimens from pigs individually.
Table 6.5. Porcine abortion—specimen collection

A. Optimum specimens, chilled
1. Three intact fetuses and placenta each from affected litters or different parities—include the freshest fetus
   Note: If there are mummified fetuses, submit nine mummies, three smallest, three medium, and three largest (freezing the fetuses is acceptable if they cannot be sent immediately to the laboratory).
2. Sow serum (5 mL)—If attempting to diagnose PRRSV, sera are best collected when sows are acutely affected (off-feed and febrile).

B. Alternate specimens—Note: Pooling tissues from multiple fetuses is acceptable.

<table>
<thead>
<tr>
<th>Tissue/sample</th>
<th>Fresh (chilled—not frozen)</th>
<th>Fixed (10% buffered formalin)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heads</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Thoracic fluid</td>
<td>2 mL</td>
<td></td>
</tr>
<tr>
<td>Stomach content</td>
<td>3 mL</td>
<td></td>
</tr>
<tr>
<td>Lung</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Heart</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Kidney</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Spleen</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Placenta</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Superficial inguinal lymph nodes</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Mummies (intact; if available)</td>
<td>9 (see above)</td>
<td></td>
</tr>
</tbody>
</table>


Table 6.6. Porcine diarrhea (birth to 4 weeks)—specimen collection

<table>
<thead>
<tr>
<th>Tissue/sample</th>
<th>Fresh (chilled—not frozen)</th>
<th>Fixed (10% buffered formalin)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum</td>
<td>5 mL</td>
<td></td>
</tr>
<tr>
<td>Brain</td>
<td>Cut brain in half longitudinally, slightly off the midline. Submit larger half fresh/chilled.</td>
<td>Fix the smaller half in formalin.</td>
</tr>
<tr>
<td>Tonsil</td>
<td>½</td>
<td></td>
</tr>
<tr>
<td>Lung</td>
<td>5 × 5 × 5 cm piece</td>
<td>2 × 2 × 1 cm</td>
</tr>
<tr>
<td>Liver</td>
<td>4 × 4 × 4 cm piece</td>
<td>2 × 2 × 0.5 cm</td>
</tr>
<tr>
<td>Kidney</td>
<td>Half of a kidney</td>
<td>0.5-cm slice, including cortex and medulla</td>
</tr>
<tr>
<td>Spleen</td>
<td>5 cm piece</td>
<td>1-cm piece</td>
</tr>
<tr>
<td>Jejunum</td>
<td>10-cm segment</td>
<td>Four sections, 2 cm long, unopened</td>
</tr>
<tr>
<td>Ileum</td>
<td>10-cm segment</td>
<td>One section, 2 cm long, unopened</td>
</tr>
<tr>
<td>Mesenteric lymph node</td>
<td>Entire lymph node</td>
<td>1-cm piece</td>
</tr>
<tr>
<td>Spiral colon</td>
<td>Approximately 1⁄4 to 1⁄2 of colon bagged separately from the small intestine</td>
<td>Two sections, 2 cm long, unopened</td>
</tr>
<tr>
<td>Fluid contents from cecum or colon</td>
<td>In leakproof container</td>
<td></td>
</tr>
</tbody>
</table>


Animal selection—three euthanized pigs with typical signs, acutely affected, and untreated (if available), or three freshly dead pigs. Sample submission—package and identify specimens from pigs individually.

Note: Ideally, fix intestines within 15 minutes of death for best preservation.

### Necropsy Safety

Safety is always a concern when performing postmortem examinations on pigs. Pigs harbor microorganisms that can potentially infect humans (Tucker 2006), floor surfaces can become slippery from body fluids, and the sharp tools used in the course of the examination can cut the prossector as well as the pig. The environment in which field necropsies are performed is often not ideal from a safety standpoint, so care should be taken to reduce the risk of injuries when planning postmortem examinations. Personal protective equipment should be worn that is appropriate for the risk posed.

Refer to the table and text for detailed specimen collection methods and protocols.
Some excellent resources are available for individuals who want to learn more about knife sharpening. Griffen (2007) has written a short but thorough pamphlet on postmortem knife care directed toward veterinarians that covers the fundamentals of knife and sharpener equipment selection and sharpening techniques. A complete and readable treatise on all matters related to knife selection and maintenance, including seven chapters on sharpening, is also available (Ward and Regan 2008).

The principles of knife sharpening can be reduced to three basic points: (1) be gentle, (2) be consistent, and (3) be relatively obtuse.

Gentle pressure is preferred when sharpening knives, whether against a stone, steel, or electric grinder. This reduces the risk of damaging the edge by uneven sharpening along sections of the blade or by overheating the knife blade and altering the temper of the metal. It also makes it easier to maintain the consistent angle needed to develop a consistent edge.

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Gentle pressure is preferred when sharpening knives, whether against a stone, steel, or electric grinder. This reduces the risk of damaging the edge by uneven sharpening along sections of the blade or by overheating the knife blade and altering the temper of the metal. It also makes it easier to maintain the consistent angle needed to develop a consistent edge.

Maintaining a consistent angle while sharpening relates to the angle the knife is held relative to the sharpening device. Electric grinders generally have set angles that may or may not be adjustable. For sharpening knives by hand, there are devices that can be attached to the back edge of the blade to maintain the appropriate angle or the knife is mounted in a sharpening device that maintains the angle for the sharpening surfaces to pass along the edge of the knife. Alternatively, a consistent angle can be maintained with practice by carefully controlling the angle of the knife by hand with practice.

The correct angle for sharpening a postmortem knife is subject to personal preference, but generally appears...
CHAPTER 6
OPTIMIZING DIAGNOSTIC VALUE AND SAMPLE COLLECTION

limb. Making the cuts through the skin from the subcutaneous tissues outward reduces the dulling of the knife. A second incision similarly reflects the right hind limb. Raise the foot to abduct the hind limb and cut starting at the right flank at the juncture of the leg with the abdomen, through the interior thigh musculature and hip joint capsule (severing the ligament) posteriorly through the proximal ham to just lateral to the tail.

Examine and collect samples of the inguinal lymph nodes at this time. Reflect the skin past the midline ventrally and dorsally by cutting the subcutaneous connective tissue and muscles.

Open the abdominal cavity, being careful not to puncture any of the alimentary tract, by making an incision along the posterior margin of the ribs. Start at the sternum and continue dorsally to the vertebral column. Continue the cut to the posterior limit of the abdominal cavity and then continue ventrally to the midline. The pig should now look like Figure 6.2. Make sure not to touch any abdominal organs at this time as to minimize contamination.

Thoracic Cavity. The rib cage is opened next by one of several methods, depending on the size of the pigs and the tools available. In immature pigs, the costochondral junctions between the ribs and sternum can usually be cut fairly easily using a knife by cutting through the cartilaginous bone at the costochondral junction. Alternatively, if a pair of pruning shears is available, the pruners can be used to snip the ribs individually along the sternum up to the ventral limit of the thoracic inlet. To reflect the ribs and if the pruner is available, the ribs can be snipped along the vertebral column beginning at the last rib and extending to the dorsal...
limit of the thoracic inlet, and the ribs can be cut free by incising the diaphragm posteriorly and the muscles and vessels at the thoracic inlet anteriorly. Alternatively, the ribs can be reflected individually or in groups of two or three by incising the intercostal muscles lengthwise between every one, two, or three ribs, and then each section of ribs can be manually reflected back over the vertebrae to expose the thoracic cavity.

The ribs can be examined at this time for evidence of rickets or prior fractures. Assess bone density by trimming a single rib out entirely and bending it back against the greater curve of the rib manually until it breaks. In growing pigs, the break should produce a pronounced snapping noise and sensation when the rib cracks. Lack of this snap is suggestive of a mineral and/or vitamin imbalance that warrants further investigation. In adults, the rib should be very difficult to break and should produce a sharp snap if it can be broken manually. This is an important step to include routinely as part of a complete necropsy. While metabolic or nutritional bone disorders are rare in pigs, this assessment is simple, certainly inexpensive, and has proven to be quite sensitive with practice. The practice gained by routinely snapping ribs from normal pigs provides the basis for comparison when bone density is low.

Check with your diagnostic laboratory to determine which bone is preferred to evaluate bone density. Submitting an entire unfixed long bone such as the humerus or femur is preferred to evaluate bone density. It is low.

The next step depends on the primary clinical problem to be addressed. In most cases, the necropsy will proceed with examination and collection of tissues from the thoracic cavity. However, if neurological disease or lameness is the primary concern, there is merit in collecting samples to investigate these problems prior to contaminating equipment and surfaces with thoracic or other tissues.

Examination of thoracic organs involves removal of the tongue, trachea, and lungs—“the pluck”—by first making extending incisions bilaterally along the medial aspects of the jawbones, extending from the angle of the jaw rostral to the apex. Using blunt dissection, the tongue is lifted from the jaw and carefully dissected away, especially noting the large tonsils of the soft palate. Excise the tonsils and place half in formalin and retain half unfixed. The trachea and esophagus are further exposed by pulling the tongue ventrally along with a combination of blunt dissection and careful trimming through to the thoracic inlet. The trachea can be incised and examined grossly at this time. The lungs and heart are then lifted out of the thoracic cavity with continued careful trimming, with final cuts through the esophagus and blood vessels at the diaphragm. The lungs and heart can then be placed back into the thoracic cavity for further examination.

The mediastinal lymph nodes are located near the dorsal aspect of the trachea proximal to the bifurcation into the lungs. These and the sternal and submandibular lymph nodes should also be examined and collected if enlarged or hemorrhagic.

Fixed and unfixed sections of the lungs should be collected from as many unique lesions as are present. When lesions appear the same in several different lung lobes, one to two samples are sufficient. However, if there are areas that differ in texture, color, or appearance, then each unique lesion should be sampled as both fixed and unfixed sections. Fixed and unfixed section samples should always include at least one section of major airway especially when Mycoplasma hyorhinis is suspected. Lung lesions in pigs are often most severe in the anteroventral lobes. Color can be a deceiving indicator of pneumonia, especially with hypostatic congestion or even modest autolysis. Lung texture is a more reliable indicator of lung disease, with firm, rubbery, or noncollapsing areas of particular interest.

Collecting samples for fixation was described previously, but in the specific case of the lungs, there is an added caution to handle the lung tissues very gently during examination and sample collection. If too much pressure is applied to the lungs prior to fixation, historical examination is compromised due to the compression of airways and alveolar spaces.

Unfixed lung samples should be of sufficient size to allow for bacterial culture in addition to several other tests (polymerase chain reaction [PCR], virus
isolation, etc.). Generally, unfixed pieces that are at least 5-cm (2-in.) cubes are adequate. Fixed and unfixed lung samples should include pleura. If pleuritis is present, these areas of the lungs should be sampled specifically.

The heart is examined by first cutting through the pericardium to expose the heart. Collect excess or abnormal pericardial fluid in a syringe, if present. Fibrinous exudates can be collected with swabs. Examine the surface of the heart for evidence of inflammation or hemorrhage. Incise both ventricles and the septum and examine the valves for inflammation and the muscle for hemorrhage or fibrosis. If indicated by clinical or pathological examination, collect sections of any lesions as well as septum, papillary muscle, and right and left ventricles for fixation and an unfixed piece of ventricle.

Abdominal Cavity. The location of intestinal organs should be noted so as to make sure no volvulus or torsions are present. The spiral colon should be located in the pig’s left side with the apex of the cecum on the pig’s right side. If indicated, fibrinous exudates can be collected with swabs and urine with a syringe. Solid organs including liver, spleen, lymph nodes, and kidneys are sampled next. Once again, fixed and unfixed samples should be collected. In adult animals, the female reproductive tract and bladder can be examined for gross pathology as routine sample collection of these organs is not done. The ovaries are also evaluated grossly for abnormalities as well as matching the findings to the animal’s recorded stage in the reproductive cycle.

Intestinal lymph nodes (especially the mesenteric and gastrohepatic) are examined next for size, texture, and hemorrhage. The ileum is the next section sampled. The ileum is easily located by finding the cecum and following the ligament to the ileum. Fixed samples are collected from both the proximal and distal portions of the ileum, along with a 15-cm section fresh. The jejunum and duodenum are sampled similarly, along with all areas with suspected lesions. When enteric disease is suspected, longitudinal incisions of multiple sections of small and large intestines to examine mucosa should be routine.

Fixed and unfixed samples are also collected from the cecum and spiral colon. When sampling the spiral colon, samples should be taken from the proximal, middle, and distal portions. A quick cut into the lumen of the descending colon or rectum will help determine fecal consistency at the time of death.

Once all abdominal organs have been sampled, the stomach can be opened. This is done by cutting along the greater curvature. Presence or absence of feed is noted as well as presence or absence of ulcers or hemorrhage. In pigs, gastric ulcers are located in the nonglandular portion of the stomach also known as the pars esophagea. The pars esophagea should be specifically and carefully examined to rule out gastric ulcer since hemorrhage in stomach is not a reliable indicator of gastric ulcer. The stomach is rarely sampled for routine diagnostic purposes.

Neurological Case Examination and Sampling. In cases where neurological signs are to be investigated, collecting samples of the brain and/or spinal cord is an absolute necessity. To implicate bacterial meningitis, a swab of the brain is simple to collect from pigs of any size. The atlas-axis junction can be exposed by tilting the head dorsally while incising just below the pharyngeal area. The spinal cord is exposed as the head is tilted more acutely, and a swab can be inserted directly into the foramen magnum and pushed back into the brain cavity until it stops. The swab will stop at the junction of the cerebrum and cerebellum, a common site for recovering bacteria that cause meningitis in pigs. The first cervical vertebrae can then be cut off with the spinal cord in place for histological examination. This can be cut using a knife through the intervertebral disk or by using a pair of pruning shears or a hacksaw. Submit the swab for aerobic culture and the vertebrae with spinal cord fixed in formalin.

For a complete neurological investigation, collect and submit the brain and spinal cord. The brain is removed by first skinning the skull in the area bounded by the eyes rostrally, the ears laterally, and the foramen magnum caudally. The calvarium is removed (Figure 6.3) by making a circle of cuts through the skull from

6.3. Pig brain necropsy—pig head with the calvarium removed for complete access for brain gross pathology and sampling.
the occipital condyle to the base of the ear, from the base of the ear to the eye socket, across the skull to the other eye socket, back to the base of the other ear, and finally back to the occipital condyle. These cuts can be made using a saw or hatchet, depending on availability and preference. The calvarium is pried free, exposing the meninges and brain, which can be dissected free by cutting cranial nerve attachments. The brain can be split longitudinally, with half preserved in formalin and half submitted unfixed.

In younger pigs, the skull can be split using a necropsy knife that is thrust through the nasal cavity, with the tip of the knife extending to the apex of the mandible to secure the tip and the knife blade facing the back of the head. Leverage is applied carefully to the knife handle to cut through the skull longitudinally and the brain can be cut loose from the cranial nerves as before.

For cases in which some or all of the vertebral column is needed, remove the entire pluck and offal from the carcass and trim away the muscle from the region to be sampled. If thoracic vertebrae are needed, the ribs can be disarticulated from the spine by cutting with a knife or pruner. If only cervical or lumbar vertebrae are needed, these sections can be cut away from the thoracic vertebrae with a knife, saw, or pruner, and submitted intact. Alternatively, the spinal cord can be excised in situ using a Barnes dehorner as a bone rongeur to scoop out the ventral centrum portion of excised in situ using a Barnes dehorner as a bone rongeur to scoop out the ventral centrum portion of the vertebrae to expose the vertebral canal.

Arthritis and Lameness Sampling. If arthritis or lameness is the primary concern, sampling joints prior to taking thoracic tissue samples is preferred to avoid contamination of joint samples with bacteria such as *Streptococcus suis* that may be present as normal flora in the lungs. The important steps for obtaining optimal joint sample from pigs are sterile technique and thorough examination of joints.

Obtaining clean samples from joints can be a challenge in field settings. As a result, some practitioners prefer to submit intact joints for careful dissection at the diagnostic laboratory. This approach can be successful, but some bacteria that cause infectious arthritis are fastidious and less likely to be cultured a day or two after joints are collected. Therefore, careful dissection of joints and collection of joint fluid and tissue improves the probability of yielding the pathogens involved.

Clean samples can be obtained from joints by first reflecting the skin away from the joints to be sampled and then using a new #22 scalpel blade to trace the outline of the bones through the joint capsule. Making this cut is simpler on the medial aspect of the joint where less muscle is present. The clean scalpel blade is less likely to contaminate the joint than the necropsy knife. Leverage applied to the distal portion of the limb exposes the joint so that fluid and joint sample materials can be collected. With practice, each joint can be opened with minimal cutting and potential for contamination.

It is important to examine multiple joints on pigs suspected of having lameness or arthritis problems. In particular, specific joints are of interest depending on the age of the pig and clinical signs present.

**FINAL COMMENTS**

It is important to remember that a correct diagnostic submission requires three main points: (1) the correct pig(s) sampled, (2) the correct samples submitted (Tables 6.2–6.7), and (3) a complete submission form is submitted along with appropriate history and differential list. All fixed samples should be placed in 10% buffered formalin solution quickly to minimize autolysis and maximize diagnostic results. Unfixed tissues should be chilled immediately to minimize secondary bacterial overgrowth.

**REFERENCES**

INTRODUCTION

Diagnostic testing is used to determine the cause of disease and for surveillance of pathogens that may cause disease. There are many agents that cause disease including viruses, bacteria, protozoa, other parasites, and toxins. However, just detecting the presence of these agents or exposure to them does not necessarily indicate they are the etiologic agent of the particular clinical disease at hand. Therefore, an accurate diagnosis of each specific case is based on the total picture including the herd history, clinical signs, gross and microscopic pathology (histopathology), and results of diagnostic tests. In addition, some organisms may only cause disease at specific thresholds. Since no single test is 100% sensitive (the test correctly identifies 100% of all infected pigs indicating there are no “false-negative” results) or 100% specific (the test correctly identifies 100% of all noninfected pigs, indicating there are no “false-positive” results), an incorrect diagnosis could result if only one test is used and the stage and current context of the disease is not taken into consideration. To determine whether a specific test is identifying the cause of disease, multiple tests or repeated testing over time may be required, and when results of diagnostic testing are received, evaluations of the outcomes in the context of history, clinical signs, and pathology (if available) are critical (Figure 7.1). Please refer to Chapter 8 for information on how to interpret multiple tests and employ sensitivity and specificity at the herd level.

This chapter describes common tests used for the diagnosis of swine diseases or surveillance of swine pathogens and is intended to help determine the appropriate test and interpretation of results for swine diseases. The tests are described in alphabetical order along with their diagnostic strengths and weaknesses (Table 7.1).

Agar-Gel Immunodiffusion

Agar-gel immunodiffusion (AGID) is a serological test for measuring the presence of antibodies to a specific antigen. It can be used to detect host exposure to a pathogen or to serotype field isolates. It has been routinely used for swine influenza virus (SIV) serological testing and to serotype *Haemophilus parasuis* field isolates (Del Rio et al. 2003). Although AGID continues to be used in some laboratories for *Haemophilus parasuis* typing due to the ease of use and low cost, the method has largely been replaced by indirect hemagglutination inhibition (IHA) and enzyme-linked immunosorbent assay (ELISA) due to their greater specificity and sensitivity.

The test is performed in petri plates coated with agar and a seven-well pattern (center well surrounded by six equally spaced test wells). Test antigen is used to fill the center well, and positive control serum is placed in alternating test wells around the center well. Sera to be tested are placed in the remaining wells, and the test is incubated for 1–2 days. Test plates are examined with a bright indirect light source to visualize the specific lines of identity (white precipitate) between the antigen well and positive control serum wells after diffusion of the antibody and antigen from their respective wells. A positive result is recorded when a test serum produces a line of identity in the agar between the serum and reference positive control serum. Test specificity is determined by the quality of the antigen used.
7.1. Possible interpretations of diagnostic test results using serum and tissues for PRRSV diagnosis. *Blood swabs and oral fluids can be tested by PCR. **VN = serum virus neutralization assay.
**Table 7.1.** Diagnostic tests for analyte types: infectious agent, antigen, antibody, or nucleic acid detection

<table>
<thead>
<tr>
<th>Antigen Specific Tests</th>
<th>Antibody Specific Tests</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antigen ELISA</td>
<td>Agar-gel immunodiffusion (AGID)</td>
</tr>
<tr>
<td>Bacterial isolation</td>
<td>Antibody ELISA</td>
</tr>
<tr>
<td>Bioassay</td>
<td></td>
</tr>
<tr>
<td>Clinical pathology*</td>
<td>Buffered <em>Brucella</em> antigen test (BBAT)*</td>
</tr>
<tr>
<td>Complement fixation (CF)</td>
<td>Complement fixation (CF)</td>
</tr>
<tr>
<td>Electron microscopy (EM)</td>
<td></td>
</tr>
<tr>
<td>Fluorescent antibody (direct FA or indirect FA)</td>
<td>Fluorescent microsphere immunoassay (FMIA)</td>
</tr>
<tr>
<td>Fluorescent microsphere immunoassay (FMIA)</td>
<td>Fluorescent polarization assay (FPA)</td>
</tr>
<tr>
<td>Immunohistochemistry (IHC)</td>
<td>Hemagglutination inhibition (HI)</td>
</tr>
<tr>
<td>In situ hybridization (ISH)</td>
<td>Immunoperoxidase monolayer assay (IPMA)</td>
</tr>
<tr>
<td>Parasite identification</td>
<td>Indirect fluorescent antibody assay (IFA)</td>
</tr>
<tr>
<td>Polymerase chain reaction (PCR)</td>
<td>Microscopic agglutination test (MAT)*</td>
</tr>
<tr>
<td>Virus isolation (VI)</td>
<td>Virus neutralization (VN) or serum VN (SVN)</td>
</tr>
</tbody>
</table>

*For detection of *Brucella* sp. antibodies only.

*Indirect method of determining whether an antigen is present.

*For detection of *Leptospira* sp. antibodies only.

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**Table 7.2.** Guidelines for interpretation and troubleshooting of positive and negative bacterial isolation results

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Analyte</th>
<th>Test</th>
<th>Outcome</th>
<th>Interpretation</th>
<th>Additional testing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacteria</td>
<td>Tissues</td>
<td>Positive</td>
<td>Bacterial agent is present in the sample.</td>
<td>Identify agent to the species level</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Body fluids*</td>
<td>Culture in liquid or solid media</td>
<td>Bacterial agent is absent in the sample.</td>
<td>Pig was treated with antibiotics prior to sample submission.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Blood</td>
<td>Negative</td>
<td>Bacterial agent is present but was overgrown by the commensal flora or fast-growing coinfecting pathogen.</td>
<td>Submit samples for PCR resting IHC on histological sections</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Urine</td>
<td></td>
<td>Bacterial agent is present but no longer viable.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Feces</td>
<td></td>
<td>Bacterial agent is present but not isolated on the appropriate media.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Cerebrospinal, thoracic, peritoneal, synovial.

**Bacterial Isolation/Culturing**

Bacterial isolation refers to the recovery and identification of bacterial microorganisms present in clinical samples. This technique is performed routinely by veterinary diagnostic laboratories and is typically considered the gold standard for unambiguous and specific detection of bacteria that may be involved in morbidity and mortality cases.

Most swine bacterial pathogens can be easily isolated from clinical specimens using aerobic or anaerobic culturing. The majority of clinically relevant agents grow aerobically in conventional solid or liquid media, but some clinically relevant species will require both special media and specific atmosphere for growth. For example, *H. parasuis* requires media supplemented with nicotinamide adenine dinucleotide and 5% CO₂ for growth, which are not used for routine bacterial isolation. Therefore, a complete clinical history and detailed description of the lesions associated with the sample submitted for bacterial isolation are critical to guide the pathologist and bacteriologist to use the necessary conditions for isolating fastidious pathogens and for interpreting results (Table 7.2). Not all relevant swine pathogens are routinely cultured by diagnostic
laboratories. For example, *Mycoplasma hyopneumoniae* requires a specific media, is extremely fastidious to grow, and can take up to 4 weeks to reach measurable levels (Thacker 2004).

Once a bacterial isolate is obtained, there are several additional tests that will be performed to confirm its identity. Morphology of the colony, Gram staining, and a variety of biochemical tests are performed to properly identify the isolate. Phenotypic and biochemical characteristics are usually sufficient to identify most swine bacterial pathogens to the species level. However, when biochemical tests are not sufficient to define a bacterial species, 16S rRNA gene sequencing could be used such as for *Mycoplasma* sp. (Cai et al. 2003).

The isolation of a bacterial species from a tissue should always be critically evaluated and analyzed in association with clinical signs and lesions to assess the relevance of such an isolate in morbidity and mortality. Often, bacterial isolation is unable to differentiate commensal, nonvirulent isolates from virulent pathotypes. For example, commensal organisms that could be isolated as either virulent or nonvirulent variants include *Pasteurella multocida*, *Streptococcus suis*, *Escherichia coli*, *H. parasuis*, and *Clostridium perfringens*. This differentiation requires additional characterization of isolates by serological or molecular-based techniques (Osek 2001). It is not uncommon for swine veterinarians to submit samples from clinically affected pigs with lesions characteristic of a specific bacterial pathogen and obtain a negative isolation result. Antibiotic treatments and lack of refrigeration following sample collection are two of the most frequent causes of negative isolation when the organism of interest was originally present in the sample (Oliveira 2007). Negative isolation may also result from overgrowth by the commensal flora or contaminants particularly in respiratory and intestinal samples (Fittipaldi et al. 2003). Additionally, if clinical signs, lesions, and a tentative diagnosis are not properly disclosed by field veterinarians during submission, clinical samples may be cultured in traditional media and conditions only, which impairs the isolation of bacterial species with specific growth requirements.

**Bioassay (Swine Bioassay)**

A bioassay is a test performed using a live animal to determine the infectivity or potency of a particular pathogen or substance. Pigs have been used to measure the infectivity of various viruses (e.g., porcine reproductive and respiratory syndrome virus [PRRSV], porcine circovirus type 2 [PCV2], classical swine fever virus [CSFV], hepatitis E), and the conclusions are then compared to polymerase chain reaction (PCR) results (Christopher-Hennings et al. 1995). The swine bioassay determines whether the detection of nucleic acid corresponds with the presence of live virus. Naïve pigs are inoculated with the infectious agent and monitored at regular intervals for the presence of viremia and/or seroconversion, which would indicate the presence of live virus in the inoculated material. Since live animals are used and need to be housed for several weeks before results can be obtained, the disadvantages of this test are cost, additional labor, and prolonged evaluation time. However, bioassays are one of the most sensitive and conclusive methods to determine whether a particular sample is infectious and could be transmitted to other swine. Swine bioassays have been utilized to determine the relative bioavailability of lead in soil (although this practice is being replaced by nonanimal assays) (Casteel et al. 1997), and mouse bioassays have been used to detect the presence of infectious *Toxoplasma gondii* in swine sausages (De Oliveira Mendonça et al. 2004).

**Buffered Brucella Antigen Test**

Buffered *Brucella* antigen test (BBAT) is a primary binding assay that directly detects the presence of antibodies in serum to *Brucella* sp. antigens. The current BBAT used to screen pigs for exposure to *Brucella suis* contains antigens obtained from the bovine pathogen *Brucella abortus*, since there is extensive cross-reaction between the two species (Nielsen et al. 1999). The antigen utilized in the BBAT is a *B. abortus* suspension, which is mixed with serum on a glass plate, incubated, and rotated. Any agglutination is considered positive. Variations of the test antigen or format can be used to determine if agglutination occurs to specify the herd serostatus. These include the card, tube, or plate (e.g., Rose Bengal test) agglutination or rivanal test.

The BBAT is easy and quick to perform with results obtained in approximately 8 minutes. However, it lacks sensitivity and specificity. This test is not recommended for individual pig testing since it is a screening test to identify herd infections. Breeding swine herds can be determined if agglutination occurs to specify the herd serostatus. These include the card, tube, or plate (e.g., Rose Bengal test) agglutination or rivanal test.

The BBAT is currently being used for presumptive serodiagnosis of swine brucellosis in the United States but several other tests have been proposed as alternatives. One of the tests is the fluorescence polarization assay (FPA), which is reportedly more sensitive and specific than BBAT (Nielsen et al. 1999).

**Clinical Pathology**

Performing complete blood counts (CBCs) may indicate anemia, which could indirectly implicate an infectious agent such as *Eperythrozoon suis* (*Mycoplasma suis*). However, for direct identification, PCR tests might be used, since there are many other noninfectious causes of anemia. CBCs and clinical chemistries could also be useful in determining the presence, severity, and/or location of inflammation, organ dysfunction, an infectious agent, or toxicant. Values of CBC or clinical chemistry parameters should be compared against
normal ranges identified specifically for swine and may be dependent on age, sex, and breed (Evans 2006). Normal pig values within a specific farm are useful for comparisons.

Complement Fixation

This is an immunological method used to detect antigens in infected tissues and fluids, measure antibody responses in naturally or experimentally infected pigs, and assay antigenic relationships among different strains or types of the same pathogen species (Rice 1960). For example, this test was commonly used to detect antibodies against *Actinobacillus pleuropneumoniae* (Enøe et al. 2001) until a commercial ELISA test became available. Complement fixation (CF) testing is based on the ability of antigen–antibody complexes to bind to complement (plasma proteins that combine with antibody to destroy pathogens) and cause hemolysis of sheep red blood cells (sRBCs). A known concentration of sRBC and anti-sRBC antibodies is added to the assay and allowed to react with complement. In samples containing specific antibodies against the antigen of interest, antigen–antibody complexes will form and will consume the available complement prior to the addition of sRBC. Hence, a sample positive for the antibodies of interest will show minimum hemolysis. Serum samples lacking specific antibodies to the target antigen will show maximum hemolysis of sRBC.

The CF assay detects antibodies against any antigen and has been used as a regulatory test for interstate or international movement of animals. However, it is rarely used in diagnostic laboratories since it takes 2 days to complete, has extensive requirements regarding standardization of the necessary reagents, and in most laboratories, has been replaced by ELISA testing.

Electron Microscopy

Electron microscopy (EM) is used to visualize pathogens, particularly viruses, where diagnostic reagents are not available. For example, EM can be used for identifying nongroup A (groups B and C) rotaviruses in swine since most current ELISA reagents will only detect group A (Janke et al. 1990). With transmission EM (TEM), electrons are generated from a heated tungsten filament, accelerated through an evacuated column, and focused through the specimen using an electromagnetic “lense” for projection of the specimen onto a fluorescent screen or photographic substrate. To better visualize the specimen, a variety of staining techniques have been employed. Negative staining with phosphotungstic acid (PTA) is widely used for studying viral morphology. EM is moderately sensitive with a lower limit of detection with negative stain methods of approximately $10^6$ virus particles per milliliter of sample. EM is especially useful for detecting viruses in enteric cases because crude fecal suspensions have routinely greater than $10^6$ particles of pathogenic viruses per gram of feces. The size, distinct morphology, and high numbers of viral particles are important in identifying viruses by EM. It is also useful to visualize viruses causing lesions of the skin and mucous membranes such as poxviruses, caliciviruses, and picornaviruses (e.g., foot-and-mouth disease [FMD], vesicular stomatitis virus). The negative stain EM procedure can be used with tissue homogenates and virus isolation (VI) samples that do not react with reference reagents and for identifying new or emerging pathogens. EM provides a rapid diagnosis but works best with viruses that are present in high numbers and have a relatively fixed morphology such as rotaviruses or large poxviruses, which have a very distinct morphology and are readily identified. Some enveloped viruses may not be as distinctive and may look like debris. EM was used extensively before high-throughput ELISA and PCR assays were available. Due to the high expense in purchasing and maintaining the EM scope and employing specialized EM technologists, some diagnostic laboratories do not offer this service, but may refer EM services to a specialized laboratory.

Enzyme-linked Immunosorbent Assay (ELISA) (Antibody ELISA and Antigen ELISA)

A variety of ELISA-based tests are routinely used in herd health monitoring and disease diagnosis. ELISA technologies are particularly useful for rapid, high-volume sample analysis and many ELISA kits are commercially available for agents associated with major disease syndromes in swine. Variations of ELISA technology can be used for the detection of antibodies against a given pathogen (antibody ELISA) or for the detection of the actual pathogen (antigen ELISA). The diagnostic sensitivity and specificity of ELISA tests are highly dependent on the selection and quality of reagents used in the assay and the intended purpose of the assay. A highly sensitive assay may be more desirable when monitoring for a reportable disease of low prevalence, and a highly specific assay may be more desirable as a confirmatory test. When used for the detection of antibodies against a particular pig pathogen, the most common ELISAs are the indirect and competitive or blocking assay. Indirect assays typically utilize a purified antigen that is coated on test wells, and unreacted well areas are subsequently coated by a protein solution to minimize nonspecific antibody attachment. Typically, a single dilution of test sera is then placed in the test wells and incubated. If antibody is present, it will bind to the test antigen. Next, an enzyme-labeled indirect or secondary antibody directed against swine antibodies is added, and when the substrate of the enzyme label is added, a color change results. The intensity of the color is measured as an optical density (OD), which is evaluated in the context of the OD of a positive and negative control. A formula is then used to obtain a sample-to-positive (S/P) ratio. For example, on some
PRRSV ELISAs, a positive and negative control and sample are each placed on two separate wells: one well coated with normal host cell (NHC) antigens and one well coated with PRRSV antigens. After the ELISA procedure is performed, the S/P is calculated as (the sample OD on the PRRSV coated well minus the sample OD on the NHC coated well) divided by (the positive control OD on the PRRSV antigen coated well minus positive control [OD] on the NHC). A "cutoff" level is designated for positive and negative results (e.g., binary result). The S/P is not generally considered a "titer" since it does not use a serial dilution of the serum to obtain a result that is immunologically meaningful, whereas a serum titer is primarily defined as the reciprocal of the greatest dilution in a dilution sequence that produces an immunological response. For example, the titer of a serum neutralization assay (serum virus neutralization [SVN]) or hemagglutination inhibition (HI) antibody assay measures an amount of antibody in serum that neutralizes the virus or that prevents hemagglutination, respectively. In some cases, the S/P may be loosely correlated to a titer if a linear relationship can be established.

Sera may be screened for antibodies with an indirect ELISA. When unexpected positive findings are obtained, a blocking ELISA might be used to determine specificity of the findings for confirmatory purposes (Erlandson et al. 2005). A competitive or blocking ELISA is performed by coating test plates with an antigen lysate followed by blocking as for the indirect ELISA. Then, the diluted test serum is added to allow it to react with the test antigen. After washing, a specific enzyme-labeled antibody directed against the test antigen is added, resulting in competition with the test serum antibodies. Negative serum samples result in maximum color development, whereas samples with specific antibodies show less color development with increasing antibody levels. This type of assay has been used for differentiation between pseudorabies virus G1- or gE-neutralizing antisera or monoclonal antibody (mAb)-based reagents which uses a nucleoprotein antigen.

The greatest strengths of ELISA for antibody testing are speed of testing and sensitivity and specificity of the test. Antibody ELISAs are useful as herd screening assays; however, if ELISAs are used to determine an individual pig status, false-positive reactions have been observed in some cases and can be difficult to interpret. Repeating the test, obtaining a second serum sample for testing, or using another serological test for confirmation may be useful (O’Connor et al. 2002). However, other assays for antibody detection such as the indirect fluorescent antibody (IFA), immunoperoxidase monolayer assay (IPMA), HI, virus neutralization (VN or SVN), and CF tests are more complex and typically require more time for antibody confirmation.

In addition to numerous applications in antibody detection, ELISA technologies can also be used for the detection of antigens. Antigen detection ELISAs may utilize various assay formats including traditional ELISA plate formats or lateral flow devices, often called immunochromatographic strips. Antigen detection ELISAs use test wells or plates coated with specific antibodies rather than antigens as would be used in an antibody detection ELISA. Lateral flow tests typically use a solid-phase membrane with test and control lines coupled with absorbent pads. The strips may be placed into a test sample or the sample may be added to a designated area of the strip. These test formats may be used with serum or whole blood samples, tissue homogenates, or fecal samples, depending on localization of the targeted pathogen, available processing methods, and quantity of target antigen present in a given specimen. A variety of immunoenzymatic assays for swine diagnostics are available for pathogens such as SIV, classical swine fever (CSF), group A rotaviruses, and PCV2. The primary strengths of antigen detection immunoassays are that they are generally rapid, simple to perform, and require minimal laboratory infrastructure relative to VI, PCR, or EM. Some lateral flow devices have been adapted for on-site application in field or farm settings. However, assay sensitivity may present challenges for the detection of some pathogens, and timing of sample collection may be critical. The antigen of interest must be present in adequate quantity to allow direct detection by these methods and appropriate high-quality antisera or monoclonal antibody (mAb)-based reagents must be available for assay development.

**Fluorescent Antibody or Indirect Fluorescent Antibody for Antigen Detection**

Detection of virus-infected cells from frozen tissues of diseased animals is a classical diagnostic technique that is very rapid and specific. It is used as a presumptive test to quickly identify if a given pathogen is present, since a diagnosis is often completed in less than 6 hours of sample receipt. Another important use of fluorescent antibody (FA) is identifying viruses that may not cause a cytopathic effect (CPE) in cell culture. When monospecific antisera are used, immunological confirmation of the infectious agent and precise identification is rapidly confirmed. There are two basic FA procedures: direct and indirect. The direct FA utilizes a fluorescent-labeled primary antibody, while the IFA uses an unlabeled primary antibody followed by a labeled antispecies antibody that binds to the primary antibody. Both assay formats should use antibodies that are monospecific and do not react with other pathogens. Due to the stoichiometry/geometry of the assay systems, the indirect assay tends to be more sensitive than the direct assay. However, a properly prepared direct FA conjugate will provide brilliant fluorescence that is easily read with a fluorescent microscope. The direct staining procedure is usually shorter (about 45 minutes), whereas the indirect staining procedure takes longer (1–2 hours).
Frozen section testing for specific pathogens is accomplished by mounting target tissue from a diseased animal onto a cryostat specimen holder (chuck), freezing the tissue in the cryostat, and skillfully cutting serial sections for FA staining (usually for multiple pathogens). Once the sections have been collected on glass slides, they are fixed in acetone to keep the tissue on the slide during the staining procedure and permeabilize cells so primary antibodies can react with viral antigens in infected cells. Staining of the sections is accomplished by rehydrating the tissue, reacting with the primary antibody (with or without a fluorescent label) against the pathogen of interest, washing excess reagent from the slide, and in the case of direct FA, mounting and adding a coverslip immediately prior to viewing with the FA microscope. If the indirect staining procedure is used, the washed section is reacted with the secondary antibody with a fluorescent label, washed, mounted, and coverslipped for microscopic exam. For ease of viewing frozen section samples, counterstains like Evans blue are sometimes added to the conjugate. FA staining for pathogen detection in cell cultures is similar to the processes described above except aqueous acetone is used to fix the assay plates when the cells are grown on plastic. Staining of VI cultures is usually done at the first appearance of CPE or at a fixed time, usually 3–5 days postinoculation to detect noncytopathic viruses or cultures with minimal infection. FA staining for viruses is used almost daily in some diagnostic virology labs since it provides a quick, inexpensive, presumptive diagnosis. The quality of the primary antibody is critical to obtain accurate results, so these reagents should be fully characterized for their specificity and sensitivity. False-negative results may not use PCR as an initial step prior to detection (Mahony et al. 2007). Currently, the assay has been developed to detect swine pathogens and immune proteins (Deregt et al. 2006; Lawson et al. 2010). This assay may be highly relevant in the future for herd profiling and management decisions, since it simultaneously detects antibodies against multiple pathogens (Khan et al. 2006).

Fluorescence Polarization Assay
The FPA technology has been developed and validated for the serological diagnosis of brucellosis in cattle, swine, sheep, goat, bison, and cervids. Sufficient cross-reactivity of the common epitopes of B. abortus, Brucella melitensis, and B. suis allows for the use of a single antigen for all species of Brucella. FPA is based on the rotational differences between an antigen labeled with fluorescein isothiocyanate and the antigen complexed with its antibody. A small molecule (e.g., the labeled antigen) will rotate randomly at a rapid rate, resulting in rapid depolarization of light, while a larger molecule (e.g., the antigen–antibody complex) rotates slower allowing for depolarization of light at a reduced rate. This difference in light depolarization is expressed in millipolarization units (mP). A positive reaction is indicated by a significant elevation of the fluorescence polarization (FP) reading over the negative control. FPA has a sensitivity of 99.02% and specificity of 99.96% when a cutoff of 90 mP is used (Nielsen et al. 1999).

The FPA is a straightforward test that provides results in approximately 5 minutes. The accuracy of the FPA equaled or exceeded those obtained using other serological tests such as the buffered antigen plate agglutination test (BPAT). The FPA can also be performed in the field with portable equipment, eliminating the need for sample submission and reducing costs (Nielsen et al. 1999).

FPA testing for serodiagnosis of swine brucellosis is still recommended as a herd screening test. Although this test is reportedly more sensitive and more specific than the current World Organization for Animal Health (OIE)-recommended BBAT, the fact that some pigs do not generate antibodies following B. suis infection restricts the use of these techniques for individual testing (Nielsen et al. 1999).

Hemagglutination Inhibition
Some viruses have the ability to hemagglutinate red blood cells (RBCs). This activity can be used to detect the presence of antibodies that bind to hemagglutination-associated epitopes on the virus and thereby inhibit the virus’ ability to hemagglutinate RBCs (e.g., adhere to RBCs). HI testing is currently used most extensively for SIV serological testing, evaluation of SIV strains for autogenous vaccine formulations, and detection of antibodies to porcine parvovirus (PPV) and hemagglutinating encephalomyelitis virus. In the HI test, if the
hemagglutinating portion of the virion is bound by specific antibodies in the test serum, hemagglutination of RBCs (which are added to the test) is blocked, resulting in a “button” of RBCs at the bottom of a microtiter plate well (e.g., positive result, indicating antibodies to this antigen are present). If the RBCs do agglutinate with the virus after serum is added, this would indicate that the sera do not have antibodies, and a uniform mat of RBCs is observed at the bottom of the test well (e.g., negative result, indicating antibodies to this antigen are not present). Swine serum samples must be tested in a “button” of RBCs at the bottom of the test well that the sera do not have antibodies, and a uniform mat of RBCs is observed at the bottom of the test well (e.g., negative result, indicating antibodies to this antigen are not present). Swine serum samples must be pretreated to remove nonspecific hemagglutinins and/or hemagglutination inhibitors. Generally, an initial antigen is present. If the RBCs do agglutinate with the virus after serum is added, this would indicate that the sera do not have antibodies, and a uniform mat of RBCs is observed at the bottom of the test well (e.g., negative result, indicating antibodies to this antigen are not present). Swine serum samples must be pretreated to remove nonspecific hemagglutinins and/or hemagglutination inhibitors. Generally, an initial serum dilution of 1:10 is used for the assays, and serial dilutions are then made to determine a titer, which is the highest dilution at which there is no longer sufficient antibody present to inhibit agglutination.

For SIV, the test is subtype specific, which means the H1 or H3 type of the viral test antigen must be cross-reactive with the type of SIV present in swine herds. For North America, in 2010, a total of no less than six HI test antigens should be available for test purposes: alpha H1N1, beta H1N1, gamma H1N1, delta H1N1, novel (pandemic) H1N1, and H3N2. Fortunately, the 2001 beta H1N1 test antigen provided by the United States Department of Agriculture to veterinary diagnostic laboratories cross-reacts well with sera from swine that have been infected with either beta or gamma H1N1 viruses. Sera from pigs naturally infected by novel H1N1 can be reliably tested with gamma H1N1 or homologous test antigens. For nonvaccinated swine, HI antibody titers of 1:40 or higher are considered to be indicative of previous infection with H1N1 viral strains. A higher titer cutoff is used for H3N2, 1:80, and suspect titers of 1:40 in combination with other pigs having titers of 1:80 or higher are considered to be indicative of natural infection. However, results on acute and convalescent sera are more meaningful than a single HI result, and the timing of serum collection will affect the magnitude of the HI titer. For PPV, HI titers of 1:256 or greater are usually considered to be indicative of natural exposure. Gilts vaccinated with a killed virus vaccine will commonly develop HI titers up to 1:128. It is very common to obtain titers of 1:2048 or 1:4096 for naturally infected swine.

**Immunohistochemistry**

Immunohistochemistry (IHC) involves the detection of pathogen-associated antigens in formalin-fixed, paraffin-embedded tissues using specific antibody and an enzyme or fluorochrome label. It can be a highly sensitive and specific technique and is widely used in research and diagnostic laboratories. IHC is also of great value in retrospective studies using formalin-fixed, paraffin-embedded tissues. Excellent, detailed reviews of IHC methodologies and applications in the diagnosis of swine infectious diseases are available (Ramos-Vara et al. 1999). The basic steps of most IHC procedures include tissue preparation with formalin fixation, paraffin embedding, and sectioning. Since formalin cross-links proteins, which can limit binding of antibodies to specific antigenic sites, various antigen retrieval methods are then used to unmask or uncover antigens for better recognition by antibody reagents. Common methods include enzymatic digestion or heat-induced antigen retrieval. Blocking agents may be required to reduce background staining due to endogenous enzyme activity. Next, the staining steps may involve direct or indirect procedures. Indirect staining protocols are the most common due to their greater sensitivity. A specific primary antibody is typically followed with a labeled secondary antibody. An avidin–biotin complex (ABC) method is commonly used whereby an unlabeled primary antibody is followed by a biotinylated secondary antibody, and then an avidin–biotin peroxidase reagent reacts with a substrate to produce a colored product.

A major strength of IHC is that it allows clear association of antigen detection with specific histological lesions. This is particularly useful in identifying whether a specific pathogen (e.g., PCV2) is the etiology for a given disease (e.g., postweaning multisystemic wasting syndrome [PWMS]), since some pathogens are detected more frequently than the syndrome. It may also allow for some level of antigen quantitation; however, the antigen may not be evenly distributed throughout a given tissue, and selection of appropriate specimens can be critical. IHC requires the availability of high-quality antibody reagents and highly optimized fixation and staining methods with the use of appropriate controls.

**Indirect Immunofluorescence (Indirect Immunofluorescence or Indirect Fluorescent Antibody) and Immunoperoxidase Monolayer Assay for Antibody Detection**

IFA and IPMA are used to detect the presence of antibodies against some infectious agents. IFA assays utilize a fluorescent-labeled secondary antibody and require a fluorescent microscope. IPMA utilizes a peroxidase-labeled secondary antibody and appropriate chromogen and can be read using a standard light microscope. The colored reaction of the IPMA is more stable than fluorescence. Two of the most common IFA assays routinely used in swine diagnostics are for PRRSV and PCV2 (Magar et al. 2000; Yoon et al. 1992). The basic procedure involves preparation of infected monolayers of permissive host cells on glass slides or 96-well cell culture plates, typically with paired wells of noninfected host cells as controls. Cell monolayers are fixed with acetone or acetone/methanol to permeabilize cell membranes and allow antibody access to internal viral proteins. Dilutions of test swine sera and control sera are incubated with the cells; then, a secondary labeled...
antiswine immunoglobulin G (IgG) or immunoglobulin M (IgM) antibody is added. After washing, cell monolayers are viewed under a fluorescent microscope, and antibody titers are reported as the highest serum dilution showing virus-specific fluorescence. Similar assays have been developed for porcine enteroviruses (Auerbach et al. 1994), *Lawsonia intracellularis* (Knittel et al. 1998), and other pathogens. Most IPMA procedures utilize a similar protocol except the secondary antibody is labeled with peroxidase and followed by a chromogen to provide color development (Guedes et al. 2002). Both the IFA and IPMA measure the binding of specific antibody to antigens in infected host cells, but the IPMA results can be detected without a fluorescent microscope. The IPMA has been used for detection of PRRSV, particularly European strains, since they grow well in porcine alveolar macrophages (PAMs) rather than a continuous cell line, and the IPMA staining is easily observed in PAMs. One advantage of IFA and IPMA methods is that they can provide relative antibody titers by using serial dilution of samples. However, interpretation can be somewhat subjective and dependent on the experience of the technician. These assays also require replication of the indicator virus or intracellular bacterium in cell culture. When dealing with antigenically variable viruses, such as PRRSV, assay sensitivity can be affected by antigenic differences between the virus strain used in the assay and the strain infecting a given group of animals. Table 7.3 demonstrates the effect of strain variation on PRRSV IFA results.

**Microscopic Agglutination Test**

The microscopic agglutination test (MAT) is the reference test for serological diagnosis of leptospirosis in swine. This test is based on the reaction of specific antibodies and live *Leptospira* sp. bacterial cells. A mixture of test serum with live *Leptospira* sp. cells results in agglutination, which can be visualized using dark field microscopy. Results are reported in titers, indicating the highest dilution that resulted in agglutination of 50% of the live *Leptospira* cells compared with the control (Chappel et al. 1992).

The MAT is easily performed by laboratories that are able to maintain live *Leptospira* cultures for clinically relevant serovars affecting swine. It is a fairly sensitive test, inexpensive and quick to perform, and the agreement between high MAT titers (>1:1024) and isolation of *Leptospira* from infected pigs is significant (Chappel et al. 1992). A limitation of the MAT is the subjective definition of a positive result, which varies among technicians and laboratories. Additionally, one single reading is of low diagnostic value. Different laboratories utilize different cutoff titers to define a positive sample, so two consecutive tests within a 2-week interval are recommended to detect convalescent titers indicative of infection. Considering that MAT detects both IgM and IgG, cross-reactions among serovars are commonly observed in acutely infected pigs, whereas the second test provides more specific results regarding the serovar affecting the herd (Ahmad et al. 2005).

**In Situ Hybridization**

In situ hybridization (ISH) uses either a radioisotope, fluorescent, or enzyme-linked nucleic acid (DNA or RNA) to hybridize to a specific complementary DNA or RNA sequence of a specific pathogen in a tissue section. This technique is distinct from IHC, which identifies protein antigens (rather than nucleic acid) in tissue sections. ISH can be used in infectious disease diagnosis

### Table 7.3 Effect of strain variation on PRRSV IFA results

<table>
<thead>
<tr>
<th>Pig #</th>
<th>Days Post Challenge</th>
<th>SD-23983</th>
<th>Ingelvac PRRS MLV</th>
<th>Ingelvac PRRS ATP</th>
<th>Lelystad Isolate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 0</td>
<td>&lt;20</td>
<td>&lt;20</td>
<td>&lt;20</td>
<td>&lt;20</td>
</tr>
<tr>
<td></td>
<td>Day 4</td>
<td>&lt;20</td>
<td>&lt;20</td>
<td>&lt;20</td>
<td>&lt;20</td>
</tr>
<tr>
<td></td>
<td>Day 7</td>
<td>40</td>
<td>40</td>
<td>&lt;20</td>
<td>&lt;20</td>
</tr>
<tr>
<td></td>
<td>Day 11</td>
<td>640</td>
<td>&lt;20</td>
<td>&lt;20</td>
<td>&lt;20</td>
</tr>
<tr>
<td></td>
<td>Day 14</td>
<td>&lt;20</td>
<td>&lt;20</td>
<td>&lt;20</td>
<td>&lt;20</td>
</tr>
<tr>
<td></td>
<td>Day 28</td>
<td>1280</td>
<td>1280</td>
<td>2560</td>
<td>2560</td>
</tr>
</tbody>
</table>

*Endpoint titers expressed as the reciprocal of the greatest serum dilution showing detectable PRRSV-specific fluorescence.*
since DNA or RNA probes specific for pathogen sequences allow direct visualization of the site of pathogen replication in a tissue. It is possible to use radioactive and nonradioactive probes to simultaneously detect multiple transcripts. The most common technique used in the veterinary diagnostic laboratory utilizes digoxigenin-labeled probes. A positive hybridization signal is visualized in tissue sections after using IHC staining methods. The general method for ISH involves permeabilization of the cells with proteinase K, binding of the labeled DNA or RNA probes, antibody–phosphatase binding to the probe, and staining of the antibody with alkaline phosphatase. ISH is particularly useful for pathogenesis investigations and the precise identification of target tissues where the pathogen is replicating. The ISH procedure is very useful when the nucleic acid sequence of the pathogen is known, but no antibody-based reagents are available. The technique is extremely sensitive and has been used to study a number of pathogens including PRRSV, PCV2, and torque teno virus. Typically, ISH is more sensitive than IHC, which requires higher numbers of target molecules to produce a positive reaction. In addition, for pathogenesis studies, ISH signals may be present at longer times postinfection (since the RNA or DNA of the organism is still present) when antigen production is below the levels of detection. ISH is not performed in every diagnostic laboratory and has been used primarily as a research tool rather than a standard diagnostic test.

Parasite (Internal) Identification

Fecal flotation is used to identify specific parasitic egg morphology since adult worms are often not readily speciated. Feces are mixed with a solution (e.g., sugar solution) that has a specific gravity higher than the parasite egg. With centrifugation or passage of time, the eggs float to the surface of the solution, a microscopic coverslip can be applied, and the egg morphology is evaluated via a light microscope (Corwin 1997). This is a quick, low-cost test. To distinguish eggs from other debris, it is also important to evaluate egg size. Very small parasitic eggs such as cryptosporidia may not be identifiable via morphology, and FA or fecal ELISAs might be used. For zoonotic parasitic agents such as *Trichinella* and *Toxoplasma* sp., serological antibody ELISAs have been utilized (Gebreyes et al. 2008).

Polymerase Chain Reaction

DNA and RNA Extractions for Detection of Pathogens by Polymerase Chain Reaction. Prior to PCR detection, the nucleic acid (RNA or DNA) of the pathogen is extracted from the specimen. Extraction is the chemical, physical, or mechanical process needed to recover, concentrate, and purify the RNA or DNA from a mixture of other proteins, lipids, carbohydrates, or other materials that might be found in the clinical specimen, and it allows the PCR reaction to proceed without interference and inhibition from these substances. There are rare circumstances where extraction may not be needed prior to PCR, but a comparison with and without extraction would need to be performed verifying that the PCR gives the same specificity and sensitivity. There are several commercially available extraction protocols designed for specific specimens (e.g., serum, tissues, cells, whole blood), for the specific nucleic acid that is being extracted (e.g., total RNA, viral RNA, messenger RNA, DNA, total nucleic acid), and that can be performed either manually or with automation. These extractions may differ in the mechanical processes used for extraction (e.g., boiling, vortexing, sonicating, physical disruption using glass beads or enzymes) and separation processes whereby the nucleic acids are separated from other substances with organic solvents (e.g., phenol–chloroform) or by binding to silica or magnetic beads. Since various sample types are being used for swine diagnostics such as oral fluids, semen, or blood swabs (whole blood in saline) where commercial kits may not be specifically designed for these specimens, a comparative study between these protocols needs to be performed to ensure the best sensitivity and specificity. In addition, extraction protocols are frequently improved and further refined for various specimens, so the most current, well-validated extractions need to be used. In evaluating various extraction protocols, the quality, quantity, and how well the extracted nucleic acid works in PCR need to be tested using a wide range of amounts of extracted nucleic acid in the PCR assay.

Polymerase Chain Reaction Process. PCR is a technique that utilizes the necessary reagents and conditions to exponentially amplify DNA or RNA in vitro. In a diagnostic laboratory setting, PCR is mainly associated with the amplification of species-specific nucleic acid sequences from clinically relevant viruses and bacteria present in clinical samples. Amplification of nucleic acid from selected pathogens can be followed by sequencing of target segments to improve pathogen identification or define strain groups.

The basic concept of nucleic acid amplification starts with RNA or DNA extraction, followed by exponential amplification of the DNA through thermal cycling at various temperatures. The temperature variations provide for enzymatic reactions that cause conversion of the RNA to DNA (a reverse transcriptase reaction, if RNA is the starting material) followed by denaturation of the DNA, primer binding, and elongation of the copy of DNA with the Taq polymerase enzyme. The temperature cycles are then repeated approximately 30–40 times so that theoretically, doubling of the DNA occurs (100% efficiency) during each temperature cycle, and billions of DNA copies can be obtained from one copy. Traditional detection of amplicons using gel-
based methods is still used by many laboratories worldwide; however, newer highly sensitive and specific automated detection systems such as real-time PCR are rapidly substituting gel-based methods.

Independent of the detection system, PCR is today the gold standard for sensitive and specific detection of viral and bacterial pathogens in clinical samples. It has major advantages compared with isolation, since it detects fastidious and nonviable pathogens (some may have been previously treated with antibiotics), so they are detectable by PCR, but not by culture. In addition, it may take many days to grow certain viruses or fastidious bacterial pathogens, whereas PCR can be performed within a day.

**Gel-based Polymerase Chain Reaction Techniques.** Gel-based PCR uses agarose gels for detection of amplicons produced during PCR. The PCR reaction that precedes gel-based detection utilizes a pair of species-specific primers that will anneal to the target nucleic acid and initiate the replication of target sequences by the polymerase enzyme. Once amplicons are produced, PCR products are loaded onto precast wells in an agarose gel, and an electric current is applied to the system (electrophoresis). PCR products will migrate through the gel and will be separated by size with the smaller fragments migrating faster through the gel and identified with a lower base pair (bp) size. A known positive control is used in every PCR reaction to assure that the amplicon obtained from clinical samples has the expected bp size for the pathogen of interest. A specific band should have the same size as the band observed for the positive control. The absence of a band is interpreted as a negative result. Amplicons are visualized on agarose gels by utilizing an intercalating fluorescent dye that binds to double-stranded nucleic acid and fluoresces under ultraviolet light. Gel-based PCR methods can be adapted to detect multiple targets (multiplex PCR). The sensitivity of this method can be considerably improved by performing a two-step amplification method known as nested PCR. In nested PCR tests, an external set of specific primers is used for the initial detection and amplification of the target sequence in the clinical sample followed by a second amplification utilizing a nested (internal) set of primers. Gel-based PCR can also be used to genotype bacterial (Oliveira and Pijoan 2004) and viral isolates (Wesley et al. 1998).

Gel-based methods are easily developed and standardized, do not require expensive equipment, and have a lower cost compared with real-time PCR. The main limitations of gel-based PCR tests are the lower sensitivity (if nested PCR is not used), the subjective interpretation due to visual inspection of bp sizes on the gel, and the time required to obtain final results, since it requires four steps: extraction of the RNA or DNA from the clinical sample, a PCR reaction, gel electrophoresis, and visualization of the gel under UV light for detection. Another main limitation of gel-based methods is the need to open the PCR tubes after amplification for electrophoresis. The millions of amplicons that are produced during PCR can aerosolize and contaminate the laboratory, especially when nested PCR tests are used where tubes are opened more often. Nested PCR tests may cause contamination resulting in false-positive reactions unless the laboratory has stringent requirements for prevention. These would include the use of aerosol-resistant pipette tips, dedicated rooms, instrumentation and equipment for setup rooms, and rooms where the PCR is performed, adding positive controls after samples are setup and adjusting positive control samples to be at approximately the same quantities as to what might be in clinical samples. The limited number of samples that can be performed on an agarose gel is another drawback of gel-based methods (e.g., approximately 14–28 samples can be evaluated on a single gel) compared with real-time protocols (e.g., approximately 96–384 samples can be evaluated on a single instrument).

Gel-based PCR protocols are reported in the literature for virtually all clinically relevant swine viral and bacterial pathogens. Examples of gel-based PCR tests commonly used by most diagnostic laboratories to detect and characterize bacterial pathogens include detection of toxin genes in *P. multocida* (Lichtensteiger et al. 1996) and *C. perfringens* (Meer and Songer 1997); adhesins and toxins in *E. coli* (Osek 2001); detection of *H. parasuis* (Oliveira and Pijoan 2004), *A. pleuropneumoniae* (Schaller et al. 2001), and *S. suis* (Okwumabua et al. 2003); speciation of *Brachyspira* sp.; and genotyping of a variety of bacterial species (Veralovic et al. 1991).

**Real-time Polymerase Chain Reaction.** Real-time PCR uses an automated system that allows for detection and quantification of PCR products as they are amplified ("real-time" detection), without the need for gel-based detection ("endpoint" detection). Production of double-stranded nucleic acid amplicons is reported as it occurs by fluorescence, which is captured, analyzed, and reported by a computer attached to the real-time thermal cycler. Two main signaling systems are utilized by most diagnostic laboratories to identify swine pathogens in clinical samples: double-stranded DNA intercalating dyes and labeled hydrolysis probes (Hoffmann et al. 2009).

Intercalating dyes such as SYBR Green® (Life Technologies, Carlsbad, CA) bind specifically to double-stranded nucleic acids (amplicons in positive samples) resulting in fluorescence, which is captured and reported in real time by the computer-based detection system. A melting curve analysis, which compares the temperature needed to separate the double-stranded amplicons produced in positive samples and that of the
positive control, is performed at the end of the reaction to confirm the specific detection of the target sequence.

TaqMan® probes (Life Technologies, Carlsbad, CA) (a specific type of hydrolysis probe) can also be used to report the presence of pathogen nucleic acid in clinical samples using real-time PCR. Probes are short oligonucleotides labeled with a fluorescent dye at one end and a quencher at the other end. The quencher is responsible for inhibiting light emission by the fluorescent dye in intact probes. The probe, forward, and reverse primers are specific and complementary to the nucleotide sequence of the pathogen of interest. Once the probe binds to the target DNA (if the target is present), it will be cleaved by the DNA polymerase during the amplification process, the quencher will separate from the fluorescent dye and the fluorescence will be captured and reported by the real-time equipment confirming the presence of the target pathogen in the sample.

Real-time PCR has several advantages compared with gel-based methods. It is usually more sensitive, since detection of positive samples is based on computerized recognition of light emission instead of visual inspection, and highly specific, considering that positives are confirmed based on melting curve analysis or by species-specific probes. Real-time assays can be quantitative, allowing the characterization of pathogen load in the sample. Other advantages of the real-time PCR include high throughput, fast turnaround time (which can be as low as 1 hour compared with several hours required by gel-based methods), and the absence of contamination by aerosolization of amplicons, since tubes remain closed during the process. As with any PCR method, real-time detection can be influenced by the presence of inhibitors in the clinical samples (Hoffmann et al. 2009). Although real-time is fast, sensitive, specific, and quantitative, it utilizes more expensive reagents and equipment than gel-based PCR, which may restrict its use in some laboratories.

Real-time PCR is used by most laboratories to detect and quantify swine pathogens such as PRRSV, SIV, PCV2, rotavirus, and M. hyopneumoniae.

Quantitative Polymerase Chain Reaction. Quantitative PCR in swine diagnostics is typically performed through a real-time PCR assay, whereby a standard curve is derived using a known amount of serially diluted RNA or DNA. The amount of nucleic acid in the clinical samples is then extrapolated from this standard curve. Since the nucleic acid is being amplified in a PCR assay, the number of DNA copies would be a standard method of reporting. When the DNA from a sample is amplified during real-time PCR, the fluorescent intensity that occurs will cross a specific threshold at a given cycle number during the PCR thermal cycling process. This cycle threshold (Ct) will be obtained, and the Ct is inversely proportional to the amount of DNA present in the sample (e.g., a sample that has a Ct of 25 typically has a higher amount of DNA present than a sample that has a Ct of 35). Quantitative PCR results are often used as a measure of the amount of infectious pathogen present within the individual or swine population. It has also been useful in research studies to determine the efficacy of vaccines (Zuckermann et al. 2007) and virulence of various strains of PRRSV (Johnson et al. 2004). However, PCR is only measuring the amount of nucleic acid present (RNA or DNA), and there may not be any infectious (replicating) pathogen within the sample, even though the nucleic acid is detected. As one scientist stated, “we can detect and measure the amount of DNA present in King Tut, but that doesn’t mean he is alive and well and running around.” For example, PRRSV may be detected in serum by PCR but may not grow in cell culture in all samples or be infective in pigs (Figure 7.2).

This needs to be considered when PCR results are obtained and used to evaluate the “infectivity” of clinical samples such as environmental samples or serum used for inoculations. However, in cases where there is a fresh, well-maintained sample submitted, there will most likely be some relationship between the amount of nucleic acid detected and the amount of infectious pathogen detected. When serial dilutions of PRRSV were detected by VI and PCR, there was approximately a 3 log higher concentration by PCR (copy/mL) than results by VI (tissue culture infective dose 50 [TCID50]/mL) (Figure 7.3).

However, the difference between infectious dose and DNA copies obtained through PCR can be variable depending on cell culture and PCR conditions used by different laboratories. The higher levels in copies/mL versus TCID50/mL have also been observed with PCV2 (Gilpin et al. 2003). A higher copy number can be observed since the sample may have some noninfectious or replication defective virus present; there may be a greater amount of subgenomic viral nucleic acid measured since purified virus is not typically obtained from clinical samples; cell culture does measure the presence of infectivity, but it is still an “artificial” system since the virus is grown on a cell monolayer that may not be porcine derived and on a plate or flask. Therefore, VI “may not count all particles present in a preparation, even many that are in fact infectious” (Condit 2007). Factors that could affect the infectious titer in cell culture include pH, the cell culture media used in the isolation, incubation time, cell type used, viral strain, sample submission and handling, and in vivo antibodies, which may neutralize virus. Therefore, some caution is indicated in extrapolating results from PCR and equating them with the amount of infectious virus (TCID50).

Multiplex Polymerase Chain Reaction. Multiplex PCR refers to the simultaneous detection of multiple targets
somewhat limiting the number of targets that can be detected simultaneously. Multiplex PCR assays have been used in swine diagnostics to determine *C. perfringens* toxin genotypes (Meer and Songer 1997), *E. coli* toxin and fimbriae types (Zhang et al. 2007), and multiple viruses or viral genotypes (e.g., PCV1 and PCV2; type 1 and type 2 PRRSV; multiple SIV subtypes) within a single sample.

7.2. Viral load in serum, in TCID$_{50}$/mL, as determined by virus titration and in number of copies/mL as determined by quantitative real-time PCR. Values are for the first 10 days postinoculation for each PRRSV-inoculated boar. Reprinted with permission from Wasilik et al. (2004).

7.3. Serial 10-fold dilution of PRRSV showing the relationship between VI in TCID$_{50}$/mL and real-time PCR results in copies/mL.

Sequencing (DNA). Nucleic acid sequencing is a powerful tool that adds specificity and discriminatory power to veterinary molecular diagnostics. Sequencing complements detection by PCR by further confirming the detection of the pathogen of interest and by characterizing it to the strain level such as for fastidious bacterial pathogens (e.g., *Mycoplasma hyorhinis*, *Mycoplasma hyosynoviae*, *Brachyspira* sp.). It is also used to type bacterial and viral isolates such as *M. hyopneumoniae*, PRRSV, PCV2, and SIV DNA or c-DNA (produced by PCR from RNA). Sequencing was first reported
in the early 1970s and was initially performed by gel electrophoresis. This system was time-consuming and expensive, hence its exclusive use for research purposes. The recent automation of DNA sequencing and consequent reduction of associated costs has allowed many diagnostic laboratories to apply this method routinely for bacterial and viral strain typing (Voelkerding et al. 2009).

The current nucleic acid sequencing method adopted by most diagnostic laboratories utilizes PCR amplicons obtained from clinical samples, which are purified and sequenced. Usually, the same gene utilized for detection of a specific pathogen is also sequenced for strain typing. Amplicons produced by PCR are purified and sequenced using an automated dye-terminator method. Each of the four dideoxynucleotide chain terminators (adenine, guanine, cytosine, and thymine) is labeled with a different fluorescent dye with distinct wavelengths. The sequencing reaction functions as a regular PCR, with the incorporation of labeled dideoxynucleotides being reported to a fluorescence reader. Each fluorescence peak is recorded in a chromatogram, which reports the sequence for the ampiclon of interest. The chromatogram is useful for visual inspection of the quality and purity of the sequence. It can be edited and trimmed prior to reporting of the final sequence as a text file. However, sequencing procedures are rapidly evolving, so next generation sequencers may use different technologies for obtaining longer reads and faster turnaround times.

DNA sequencing is mostly used as a tool to investigate and characterize the molecular epidemiology of the pathogen of interest. It provides information on strain variability and allows swine veterinarians to troubleshoot biosecurity breaches, to determine whether vaccination or treatment was successful, and to identify the emergence of new strains. Although sequencing a single gene is feasible for diagnostic laboratories and affordable for swine veterinarians, it does provide limited information, and the significance of the differences between strains should be carefully evaluated. For example, at this time, there is little information in the literature regarding the biological importance of sequence data regarding virulence and cross protection. Interpretation of sequencing information is unique for each pathogen, and inference of virulence and protection will depend on the gene that is being sequenced. Another limitation of sequencing is that it usually requires a higher pathogen load in the sample to generate accurate data compared with detection PCR. It is not uncommon to have samples positive for a pathogen by PCR, which can generate unreliable or no sequence data.

Typing of bacterial strains has been traditionally performed using gel-based methods (Versalovic et al. 1991). Genomic fingerprinting, which displays a series of bands on a gel characteristic of a specific strain, can be easily performed in most laboratories; however, the poor specificity and reproducibility of this method has generated interest for sequence-based typing systems by diagnostic laboratories. Currently, multilocus sequence typing (MLST) is considered the gold standard of bacterial sequence-based genotyping. It consists of amplifying and sequencing seven housekeeping genes that generate a species-specific sequence type (ST). MLST schemes have recently been published for several clinically relevant swine pathogens, including *H. parasuis* (Olvera et al. 2006) and *M. hyopneumoniae* (Mayor et al. 2008). Although MLST is highly discriminatory, sequencing of seven genes for each strain is laborious and expensive for routine testing. Similar to viruses, routine bacterial strain typing by sequencing should ideally be based on the direct amplification and sequencing of a single discriminatory gene from submitted clinical samples. This is the case, for example, for *M. hyopneumoniae* strain typing based on P146 gene sequencing. P146 is an adhesion-like protein that is expressed on the surface of *M. hyopneumoniae* and is highly variable among different strains. *M. hyopneumoniae* P146 sequencing is almost as discriminatory as MLST (Mayor et al. 2008). This method was used to prove aerosol transmission of *M. hyopneumoniae* and can also be used to monitor introduction of new strains into the herd (Otake et al. 2010).

Specific genomic regions of several swine viruses have been sequenced including those for swine influenza, porcine reproductive and respiratory syndrome (PRRS), PCV2, CSF, African swine fever (ASF), PPV, transmissible gastroenteritis (TGE), Teschen virus, swinepox, vesicular stomatitis, vesicular exanthema, and FMD. The purposes of sequencing whole genomes or portions of the viral genome are to determine genetic relatedness between various viral strains, to provide information on changes in a virus over time, to identify mutations and recombination events, to develop molecular diagnostic assays to identify conserved areas of the genome, to confirm diagnostic testing results, to determine whether a vaccine or wild-type virus is present, and to identify novel viruses. It is important to know the limitations of DNA sequencing information for specific pathogens such as PRRSV (Table 7.4).

In addition, DNA sequencing of the pig genome is advancing through a multicollaborative Swine Genome Sequencing Consortium (SGSC). This may allow for possibilities in identification of genes associated with disease resistance or other biologically relevant genetic markers (Lunney 2010).

**Chemistry Testing**

Toxicities and vitamin or mineral deficiencies can be major contributors to disease syndromes, and diagnostic chemistry testing along with history, clinical signs,
CHAPTER 7  DIAGNOSTIC TESTS, TEST PERFORMANCE, AND CONSIDERATIONS FOR INTERPRETATION

and postmortem findings is important in considering a diagnosis. Toxicities are more thoroughly covered in Chapter 70 “Toxic Minerals, Chemicals, Plants, and Gases,” and excellent references are available (Osweiler et al. 1976; Sachana and Hargreaves 2007).

Virus Isolation

Since viruses are intracellular parasites, VI is used to grow and identify viruses from clinical samples within cells maintained in the laboratory. The variety of cell lines maintained by most laboratories depends on the permissiveness of various cell lines to viruses expected to be encountered. Primary and secondary cell cultures are often prepared from trypsinization of fresh tissues, including primary PAM cultures such as used for PRRSV diagnostics. Alternatively, established continuous cell lines can be used, since they are stable, easier to manage in laboratory settings, and very sensitive for many viruses. Embryonated eggs were widely used for viral diagnosis in the past but are now generally replaced by continuous cell lines. However, they are still used in some laboratories to grow certain strains of influenza A.

Received specimens are processed and inoculated onto susceptible cell cultures, and monitored for CPE, which are characteristic morphological changes in a given cell type, such as lysis, syncytium formation, and inclusion body formation. CPE may be characteristic of certain virus infections but often are not definitive, so immunofluorescent staining or other methods are often used for verification. VI is a very sensitive procedure to detect certain viruses that readily replicate in cell culture. It can be highly definitive and may provide a viral isolate for further analysis such as for use in sequencing or production of autogenous vaccines. It can also be used as a test to detect new viruses where PCR is not available due to limited or no sequence information being available for PCR primer design.

Many viruses are very fastidious or may not replicate in the available cell cultures. VI requires freshly submitted samples maintained under refrigeration, and it may require an extended period of time (2 or more weeks) to obtain results. VI requires specialized equipment and skills and high-quality reagents to confirm isolation of a given virus. Additional challenges occur when dealing with specimens that have high levels of bacterial contamination.

Virus Neutralization (or Serum Virus Neutralization)

SVN assays can be used to measure the presence of neutralizing antibody to a given virus, indicating previous exposure to the virus. Classical SVN involves the binding of antibodies to virions, preventing infection of susceptible host cells. A variety of SVN assays can be applied to swine diagnostic serology. The assay can provide quantitative results, and testing of paired serum samples (acute and convalescent) can provide an indication of recent exposure. SVN can also be used as a tool in identification of an isolated virus or to determine the serotype or strain of an isolated virus, provided that appropriate monospecific antisera or mAb is available. The identification of group A rotavirus G and P types (which have importance in vaccine selection or development) is one example of this application.

SVN assays are generally highly sensitive and specific due to the very specific nature of antigen/antibody interactions. However, the assays may be too specific for useful general diagnostic applications when dealing with extremely diverse viruses, such as PRRSV, where limited cross-reactivity may be seen among different strains of the virus. Some viruses, such as PRRSV, may not induce robust levels of detectable neutralizing antibodies, or the neutralizing antibody response may be delayed until several weeks or more after initial infection, thus limiting the practical utility of VN assays in routine diagnostics.

When used for the evaluation of virus-specific neutralizing antibodies in serum samples, most SVN assays involve adding a constant amount of virus to dilutions of the serum samples to be tested, along with appropriate control sera. These mixtures are then incubated to allow any neutralizing antibodies present to bind to the virus. Susceptible host cells are then added to the
serum/virus mixture, or the mixture is added to existing monolayers of host cells and incubated for 3–5 days until replicating virus induces a visible CPE in the host cells. If no neutralizing antibody is present, CPE will be apparent. If virus-specific neutralizing antibody is present in a given serum sample, no CPE will be apparent at lower serum dilutions and an antibody titer or end point can be determined at a point in the dilution series where CPE becomes apparent.

Variations of the traditional CPE-based VN assays include the plaque reduction neutralization (PRN) assay. This assay is similar to the traditional approach except cell monolayers are overlayed with a soft agar prior to the 3- to 5-day incubation. Any non-neutralized infectious virions present at a given test serum dilution will infect host cells and a “plaque” of lysed cells will develop. These plaques can then be visualized by staining cell monolayers with crystal violet, neutral red, or similar stains and by counting the clear plaques at selected dilutions of test and control sera. Endpoint titers are typically determined as the highest serum dilution resulting in a 50–90% reduction in plaque forming units.

Another variation is the fluorescent focus neutralization (FFN) assay that is of particular value when dealing with viruses that can infect a host cell but do not induce obvious CPE. The test is performed in a manner similar to the traditional CPE-based VN except cell monolayers are fixed with acetone or similar fixative at a selected time point after inoculation, generally 24–48 hours depending on the replication rate of the selected virus. Cells are then stained using standard FA staining techniques, and foci of infected cells in test serum and control wells are counted. Endpoint titers are determined as the highest serum dilution, resulting in a 50–90% reduction in fluorescent focus forming units (FFUs).

REFERENCES


Veterinarians use diagnostic tests to assess the health, productivity, and reproductive status of individual pigs and herds. Although tests can take many forms, including history taking, physical examination, and pregnancy testing, the most frequently used tests are those that involve the submission of samples to a laboratory. Laboratory tests are used to

- detect pathogens or toxins that are responsible for disease outbreaks or suboptimal production;
- evaluate the infection/exposure status of individual pigs;
- determine whether a herd was infected with or exposed to a pathogen and, if so, which age or production groups (subpopulations) were affected;
- estimate the percentage of herds or pigs with antibodies to an infectious agent;
- monitor a herd’s serological response to vaccination;
- monitor the progress and success of disease control and eradication programs.

The optimal approach for each of these objectives may differ; a different test, number of samples, and diagnostic strategy may be indicated depending on the information needed. Choice of tests to answer specified objectives is in part determined by the quality and types of sample that are submitted and in part by the availability of tests at the receiving laboratory or at other collaborating laboratories. Additional factors such as cost, laboratory capacity, turnaround time for test results, and test accuracy (sometimes termed validity) are important considerations. For many tests that are used for swine diseases, estimates of accuracy (commonly measured as sensitivity and specificity) are not published, although it is common for developers to claim that their tests are both highly sensitive and specific. Also, data on the reproducibility (repeatability) of different tests among (within) laboratories are often not available.

Although technical modifications to tests (e.g., antigen purification, use of monoclonal vs. polyclonal antibodies, and use of selective culture media) often improve the ability of the test to discriminate infected from noninfected pigs, it is clear that all tests are imperfect.

In this chapter, the principles that are necessary for rational use of diagnostic data are described, and examples of testing strategies and sample sizes that might be appropriate for different purposes are provided. The increasing availability of rapid serological, microbiological, and parasitological test kits; polymerase chain reaction (PCR); nucleic acid probes; immunohistochemistry and in situ hybridization; and the continuing expansion of tests offered by laboratories necessitate an understanding of the diagnostic principles of each test and a careful evaluation of its strengths and weaknesses. Most of our examples involve serological tests for infectious diseases because these tests are commonly used in swine practice. Issues relevant to test interpretation in both a general (Tyler and Cullor 1989) and a food animal setting (Martin 1988) are described elsewhere, but there are few papers that focus specifically on swine disease. Advantages, disadvantages,
and characteristics of specific tests are discussed in Chapter 7.

VARIATION IN TEST RESULTS

Some tests yield only a positive or a negative result (e.g., bacterial and viral isolation), and others (including many hematological, clinical chemistry, and serological tests) yield a quantitative result that varies among pigs. Results from quantitative serological tests are of two types:

- those, such as serum neutralization (SN), for which results are typically reported as a finite number of doubling dilutions or titers; and
- those, such as enzyme-linked immunosorbent assays (ELISAs), in which an infinite number of optical density (absorbance) values or sample-to-positive (S/P) ratios theoretically can occur.

Variability in results of a quantitative serological test arises from two sources:

- biological variation in the response of infected and noninfected pigs, and
- variation inherent in the test system or assay.

Animal Sources of Variation

For infected pigs, the serological response depends on the duration of infection, challenge dose of organisms, whether the infection is subclinical or clinical, whether disease is systemic or mild and localized, other concurrent infections, and host factors including age. For acute infectious diseases where the agent is removed by the immune system, pigs that were previously infected might no longer be infected when tested, and therefore, it is often more appropriate to describe infected pigs as “exposed.” For noninfected pigs, exposure to cross-reacting organisms, vaccination against the agent, or vaccination against other agents through non-specific immune stimulation might cause elevated responses in some pigs and lead to false-positive serological results.

Laboratory Sources of Variation

Sources of variation attributable to the test include variation in the way that different laboratories or technicians perform (e.g., use reagents) or interpret the test (interlaboratory or interobserver variability) and variation in interpretation by the same person at different times (intraobserver variability). As evidenced by a comparison of test variables for the immunoperoxidase monolayer assay (IPMA) for porcine reproductive and respiratory syndrome (PRRS) virus among eight European laboratories, test conditions are often not standardized from laboratory to laboratory (Drew 1995).

SENSITIVITY AND SPECIFICITY

Reference Standard

We assume that the infection or disease status of each pig can be defined by a reference standard (sometimes termed a gold standard when the true status can be determined with perfect sensitivity and specificity). For most swine diseases, there is no antemortem gold standard. Typically, the reference standard is considered to be the most accurate test or combination of tests for a specific disease and may change over time with technological advances in diagnosis.

When culture or antigen detection is used as the reference standard for evaluation of a new test, a negative culture result might be viewed with some suspicion depending on the methods used and whether there is other evidence of absence of infection. Confidence in the use of a negative culture result as a standard can usually be increased by inclusion of larger amounts of tissue or material and by culture of more sites from within the same pig. Confidence in a negative culture result as a standard also might substantially increase if other criteria are incorporated in the definition of negativity. For definition of absence of Mycoplasma hyopneumoniae infection, a negative culture result on a pig from a herd without clinical or pathological evidence of infection would be a more appropriate standard than a negative culture result from a pig from an infected herd or from a herd of unknown status.

For some viral diseases, SN or other serological tests are used as the standard against which new serological tests are compared. For example, Weigel et al. (1992) compared the performance of two ELISAs with SN for the detection of antibodies to pseudorabies virus (PRV) glycoprotein X, and Lanza et al. (1993) compared a monoclonal antibody-capture ELISA with SN for the serodiagnosis of transmissible gastroenteritis (TGE). The problem with use of a serological test as the standard against which the sensitivity and specificity of a new serological test are estimated is that if the original serological test is of poor accuracy and results of the two tests disagree, it may be difficult to determine whether the new test is more accurate. Statistical approaches that do not require a reference standard (Enøe et al. 2000; Hui and Walter 1980) offer a promising alternative for obtaining sensitivity and specificity estimates for chronic diseases. These methods have been applied to evaluation of serological tests for Actinobacillus pleuropneumoniae serotype 2 (AP2) (Enøe et al. 2001), evaluation of the accuracy of detection of slaughter lesions in Danish pigs (Enøe et al. 2003), and the detection of classical swine fever virus in tonsillar tissue (Bouma et al. 2001).

Definition of Terms

Results of a quantitative serological test such as an ELISA used on samples from known infected and
noninfected pigs can be displayed graphically as two overlapping frequency distributions (Figure 8.1).

Typically, pigs with test results exceeding a predetermined threshold or cutoff value are classed as positive, and pigs with values less than the cutoff are considered negative. In contrast, for some tests such as particle concentration fluorescence immunoassay (PCFIA) and blocking ELISA, a low test value is more indicative of infection.

Because the distributions of test results of infected and noninfected pigs overlap, the designation of a cutoff value results in misclassification of the infection status of some pigs. Four mutually exclusive categories of results are possible: true positives (test positive and infected), false negatives (test negative but infected), false positives (test positive but noninfected), and true negatives (test negative and noninfected).

Sensitivity, when used with its diagnostic or epidemiological meaning, is the probability that the test correctly identifies infected pigs: true positives/(true positives + false negatives). For example, a test with 80% sensitivity would correctly identify an average of 80% of infected pigs as test positive and would incorrectly identify 20% as noninfected because they tested negative (false negatives). The diagnostic definition of sensitivity differs from the use of the term in an analytic context (Saah and Hoover 1997). In the latter context, the term sensitivity is often used interchangeably with the minimal or lower detection limit of the test: the smallest number of bacteria or amount of DNA, toxin, antibody, or residue that can be detected with high probability, for example, >95%. An immunologically more sensitive test (ELISA compared with SN) would be expected to detect antibodies earlier in the course of infection in an individual pig, but for herd diagnosis where prevalence is moderate to high and pigs are at different stages of infection, the need for high sensitivity might not be as great.

Specificity is the probability that the test correctly identifies noninfected pigs: true negatives/(false positives + true negatives). A test with 90% specificity would correctly classify 90% of noninfected pigs on average as negative and would falsely classify 10% of pigs as infected (false positives). The analogous term in an analytical context is the cross-reaction profile or analytical specificity (Saah and Hoover 1997), which indicates the likelihood of cross-reactivity with related pathogens or diseases that present with similar signs. The cross-reaction profile relies substantially on

8.1. Frequency distributions of ELISA results for noninfected (left curve) and infected pigs (right curve). A positive result is an absorbance (optical density) exceeding the cutoff value. Although frequencies are depicted as following a normal (Gaussian) distribution, often the distributions are skewed (non-normal). FN, false negatives; FP, false positives.
laboratory and clinical experience of the test developers or researchers. For example, a validation study of eight PCR tests for detection of *A. pleuropneumoniae* in tonsillar tissue of chronically infected pigs considered cross-reactions with *Actinobacillus suis*, *Actinobacillus minor*, *Actinobacillus equuli*, *Actinobacillus lignieresii*, “*Actinobacillus porcitonsillarum*,” and two other pathogens often isolated from tonsils, *Streptococcus suis* and *Haemophilus parasuis* (Fittipaldi et al. 2003).

In most field situations, high diagnostic sensitivity and specificity are both desirable although it might be difficult to have both attributes with a single test. Lowering the minimal detection limit of the test will often improve diagnostic sensitivity, depending on the numbers of bacteria, concentration of antibody, and so on, typically found in infected pigs, but such a change might lower the specificity of the test. Buyers of pigs and regulatory officials in importing states and countries usually want tests of almost 100% sensitivity to minimize the risk of introducing new pathogens. A similar line of reasoning can also be applied to tests for agents of public health concern, for example, *Salmonella* spp., *Trichinella* spp., and antibiotic residues. Typically, owners of breeding herds want tests of high specificity to maximize their chance of selling replacement boars and gilts. High specificity is also desirable for commercial producers participating in eradication programs based on test and slaughter, where economic losses from false-positive results can be substantial.

One consequence of using multiple imperfectly specific tests is an increased chance of abnormal results in otherwise healthy and nonexposed pigs. The probability of at least one abnormal test result increases as the number of independent tests is increased. For example, suppose that a sow was screened for 10 unrelated bacterial and viral infections. If the sow was never really exposed to any of the agents (unknown to the veterinarian) and each test had a specificity of 95%, the probability that all 10 tests are negative would be 0.95^{10}, or 60%. Hence, the probability that at least one test would yield a positive result is 40%.

**Estimation of Sensitivity and Specificity**

Diagnostic sensitivity and specificity are determined from experimental and field studies, although it is quite common that an experimental study for an infectious disease will overestimate the sensitivity and specificity of the test when used in the field. One advantage of an experimental study is that it is easier to establish unequivocally a pig’s infection status, and the associated serological response can be followed temporally. Even when an experimental infection is used to evaluate a test initially, samples from representative infected and noninfected pigs (age, clinical status, stages of infection, etc.) from commercial herds should be assessed to ensure that test performance is adequate for naturally acquired infections. Test results should be compared with the reference standard (reference test) in a blinded fashion to avoid introducing bias. Sensitivity and specificity and their respective confidence intervals are calculated. As sample sizes to derive these values increase, sensitivity and specificity estimates become more precise, as reflected by narrower confidence intervals. Epidemiological considerations for test evaluation studies are described in detail elsewhere (Greiner and Gardner 2000a).

**Evaluation of a Test at Various Cutoff Values**

Sensitivity and specificity values are useful for determining a test’s diagnostic limitations and comparing the accuracy of two or more tests. Because many cutoff points are possible for a quantitative test, comparison of tests over a range of cutoff values is often more appropriate than comparison at a single value. The trade-off between sensitivity and specificity as the cutoff value is changed may be represented graphically as a receiver-operating characteristic (ROC) curve (Greiner et al. 2000; Zweig and Campbell 1993). ROC curves, which are graphs of sensitivity (y-axis) versus specificity or 1-specificity (x-axis) for all possible cutoff values of a test, are well accepted in human medicine as a method to quantify test performance. Nodelijk et al. (1996) used an ROC curve to represent the accuracy of a commercial ELISA for PRRS virus, and Elbers et al. (2002, 2003) used ROC analysis to evaluate the use of clinical signs and gross lesions at postmortem to detect outbreaks of classical swine fever in the 1997–1998 epidemic in The Netherlands.

**Selection of Test Cutoff Values**

Several factors are considered in the selection of test cutoff values, including the purpose of the testing (e.g., screening vs. confirmation), the relative costs (economic, social, or political) of false-positive and false-negative diagnoses, the availability of confirmatory tests of high specificity, and the prevalence of disease (Greiner et al. 2000). Indeed, various cutoffs might be appropriate given different circumstances for testing and consequences of misclassification (costs of a false-positive result compared with costs of a false-negative result). For simplicity, many diagnostic laboratories report results of ELISAs and other tests as positive or negative at a single cutoff. There are two drawbacks to this approach. First, when the cutoff is chosen by the laboratory or test kit manufacturer, and the result is reported only as positive or negative, information is lost to the practitioner. When an S/P ratio for an ELISA or a titer value is well beyond the cutoff value used for test interpretation, a practitioner usually has a stronger belief that a pig is truly infected than if the test result is close to the cutoff value. Likelihood ratios, which range from 0 to infinity, quantify how many times more frequently a specific titer value is obtained from infected compared with noninfected pigs (Gardner and
Values. Suspicious or indeterminate values might be as suspicious or indeterminate if they are between these greater than the cutoff value, that is, 100% specific, and value, that is, 100% sensitive, as positive if they are reported as negative if they are less than the cutoff negative results occur. Using this approach, results are late range of values where false-positive and false-negative occur. These cutoffs define an intermediate set the cutoff value for individual pig diagnosis is to specify two cutoffs: one value that is 100% sensitive (no false negatives) and another that is 100% specific (no false positives). These cutoffs define an intermediate range of values where false-positive and false-negative results occur. Using this approach, results are reported as negative if they are less than the cutoff value, that is, 100% sensitive, as positive if they are greater than the cutoff value, that is, 100% specific, and as suspicious or indeterminate if they are between these values. Suspicious or indeterminate values might be clarified by additional tests, if warranted.

**USE AND INTERPRETATION OF MULTIPLE TESTS**

To improve diagnostic accuracy, tests might be repeated or additional tests might be included in the diagnostic workup. Indeed, most diagnoses are based on the use of multiple tests (e.g., history, physical examination, laboratory tests, etc.). Multiple tests can be used either simultaneously or sequentially, and results can be interpreted in series or parallel. The sensitivity and specificity of a combination of tests differ from the individual sensitivity and specificity values. Interpreting combinations of tests in parallel results in higher sensitivity than any of the individual tests, and interpretation in series results in higher specificity than any of the individual tests. Sometimes, the change in sensitivity and specificity of the combination of tests is less than theoretically predicted because the test results are correlated (also termed dependent) for infected or noninfected pigs (Gardner et al. 2000). Correlated results would be expected for serological tests that measure the same class of antibody but would be far less likely with two tests that measure different biological responses (e.g., histopathology and serological testing).

**Parallel and Series Interpretation**

When two tests are used, one of four results is possible: both tests positive, test 1 positive and test 2 negative, test 1 negative and test 2 positive, and both tests negative. In parallel interpretation, a pig is considered positive if it reacts positively to either test—This increases sensitivity but tends to decrease the specificity of the combined tests. This parallel testing strategy works well when neither test has a particularly high sensitivity but each detects a different type of disease (e.g., early vs. late, rapidly progressing vs. slowly progressing). Culture for a pathogen might be more sensitive than serological testing early in the course of an infection, but the latter might be more sensitive later in the infection as the pathogen load decreases. Parallel testing with culture and serology should therefore lead to higher sensitivity, although specificity of the combination would be lower than if culture was used alone.

In series interpretation, a pig must be positive on both tests to be considered positive; this increases specificity at the expense of sensitivity. The use of two tests in series might lead to a diagnosis in the following way. The first test might be very sensitive and inexpensive, and pigs that test positive are retested with a second test of high specificity to identify false positives. As a cost-saving measure, pigs testing negative on the first test are considered negative and are not tested with the second test. This testing strategy allows veterinarians to rule out disease using fewer tests, although serial testing often takes more time. The probability of disease after both tests are positive is calculated by regarding the predictive value of a positive after the first test as the same as the prevalence of disease before performing the second test. In the example of the modified agglutination test (MAT) for Toxoplasma gondii, the positive predictive value was 67.9% when the test was used in a population with 20% prevalence. If pigs with a positive MAT result were tested with an additional test, for example, a latex agglutination test (LAT) with sensitivity of 45.9% and specificity of 96.9% (Dubey et al. 1995), the value of 67.9% would become the new prevalence before running the LAT test. Substitution of these values in the Bayes’ theorem formula would yield a positive predictive value of 96.9% after the second test, assuming that MAT and LAT results were uncorrelated. If the assumption of no correlation was correct, positive results on both the MAT and the LAT would be more indicative of infection than a positive result on the MAT alone.

**Choosing among Testing Strategies**

To establish a diagnosis when two tests are available, a veterinarian might decide to use a single test or use both tests. The latter choice incurs additional costs, which need to be passed on to the client. If both tests are used, parallel or series interpretation can be selected depending on the need to emphasize sensitivity or
specificity, respectively. As demonstrated in the brucellosis example that follows, the benefits of multiple serological tests to detect the same pathogen are often less than expected because of correlation between test results. Factors that need to be considered in the final selection of a testing strategy include the sensitivities and specificities of the individual tests, the sensitivities and specificities of the combination of tests when interpreted in series or parallel, costs of false-positive and false-negative diagnoses, prevalence of infection, and the additional costs incurred by adding more tests.

Example. Ferris et al. (1995) estimated the sensitivity and specificity of six serological tests for brucellosis in 231 swine using bacteriological culture results from multiple lymph nodes as the reference standard. Sensitivities ranged from 57% (automated complement fixation test [CFT]) to 85% (PCFIA with a cutoff value of 0.81), and specificities ranged from 62% (standard tube test [STT]) to 95% (rivanol test). Sensitivities of the PCFIA and STT were estimated as 85% and 83%, respectively, and specificities were 74% and 62%, respectively. When results of the PCFIA and STT were interpreted in parallel (a positive on any test being considered positive), the sensitivity of the combined tests was 87% and the specificity was 54%. The use of both tests interpreted in parallel yielded a 2% improvement in sensitivity over the higher of the two tests and an 8% loss in specificity compared with the less specific test. Assuming that the two test results were uncorrelated, the sensitivity of the combined tests theoretically should have been 98% and the specificity should have been 46%. The most likely explanation for the difference between the observed and expected values is that the test results were correlated. In this case, use of PCFIA and STT together would increase diagnostic cost for little gain in information. Indeed, even when results of the four additional tests were considered and interpreted in parallel, the sensitivity of the combination did not further increase (40/46 were positive on one or more tests).

HERD-LEVEL INTERPRETATION OF TEST RESULTS
Assessment of the health status of a population unit (herd, barn, litter, or other grouping of pigs) is often more important than that of an individual pig in the group. A key point that is not widely understood is that herd-level tests must be interpreted differently from individual tests. Interpretation of herd test results is often more complicated, especially when tests are imperfectly specific.

Herd Infection Status
Correct classification of herd status, with respect to one or more pathogens, is important in specific pathogen-free (SPF) and other health certification schemes, in assessments of disease introduction risk associated with purchase of pigs, and in studies of risk factors for disease. Similar to the situation for test interpretation for individuals, data are needed on the herd-level sensitivity and specificity of the test being used to determine herd status. The likely performance of a herd test is usually extrapolated from published individual sensitivity and specificity values as described in Martin et al. (1992). With the exception of M. hyopneumoniae in the Danish SPF scheme (Sørensen et al. 1992, 1993), there are few published field studies that have estimated the performance of herd tests used for swine diseases.

Herd Sensitivity and Specificity
Herd-level sensitivity is the probability that an infected herd yields a positive herd-test result, and herd-level specificity is the probability that a noninfected herd yields a negative herd-test result. The respective false-negative and false-positive herd proportions can be calculated by subtracting herd sensitivity and herd specificity values from 1. Herd-level sensitivity and specificity depend not only on the respective individual-level sensitivity and specificity values but also on other factors: the number tested, the prevalence of infection within infected herds, and the number of positives (1, 2, 3, etc.) used to classify the herd as positive (Christensen and Gardner 2000; Martin et al. 1992). Usually, the individual and herd-level estimates differ. Based on findings from an experimental infection study of 200 SPF pigs (Sørensen et al. 1997), the blocking ELISA for M. hyopneumoniae has an individual sensitivity and specificity of 100% and 100%, respectively, at the cutoff of 50% blocking. When samples from 20 pigs are used for herd diagnosis of M. hyopneumoniae infection in the SPF scheme, the herd-level sensitivity and specificity were 93% and 96%, respectively, when at least one ELISA-positive pig designated a positive herd test (Sørensen et al. 1992). The estimates of herd sensitivity were imprecise because only 15 new infections were detected during the study period. A follow-up study yielded similar estimates (Sørensen et al. 1993).

Some important relationships among factors affecting herd-level sensitivity and specificity warrant comment. First, as the number tested increases, herd-level sensitivity increases. Consequently, the probability of a false-negative herd diagnosis decreases with increasing sample numbers at all values of within-herd prevalence. An increase in sample numbers from 10 to 20 for a perfectly specific test with sensitivity of 50% produces a greater reduction in the probability of a false-negative herd diagnosis if the underlying prevalence is moderate rather than low (30% vs. 1% in Figure 8.2).
Second, as the number of pigs used to classify the herd as positive is increased, there is a corresponding increase in herd-level specificity with a decrease in herd-level sensitivity. A change in the number of test-positive pigs in a sample of 20 from at least 1 to at least 2 decreased herd sensitivity for *M. hyopneumoniae* in the Danish SPF system from 100% to 69% but increased herd specificity from 85% to 98% (Sørensen et al. 1993). Third, when testing a fixed number of samples, it is easier to discriminate infected from non-infected herds as within-herd prevalence increases (refer to Figure 8.2). Fourth, as the number of pigs tested with an imperfectly specific test increases, the probability of detecting at least one false-positive pig increases, leading to a lower herd-level specificity (Figure 8.3).
This is the same effect that was noted when multiple tests of imperfect specificity were used to evaluate the infection or pathogen exposure status of an individual pig. Testing of pooled rather than individual samples can be used for herd diagnosis (e.g., culture of fecal pools for Salmonella spp.; Christensen et al. 2002). Factors affecting the herd sensitivity and specificity of pooled tests are described elsewhere (Christensen and Gardner 2000).

Issues to be considered in the trade-off between herd sensitivity and herd specificity are as described for individual test interpretation. For SPF schemes, herd-level sensitivity is considered more important than herd-level specificity because the costs of failing to detect infection usually outweigh the costs of a false-positive diagnosis in some herds.

**Herd-Level Predictive Values**

Herd-level predictive values, which are analogous to individual test predictive values, are dependent on herd sensitivity and specificity and the prevalence of infected herds. In this context, prevalence of infected herds means the best guess of the probability that a herd is infected before doing the herd test. Although such estimates might be obtained from state or national surveys, local data based on knowledge of the infection status of herds visited by a veterinarian would be more appropriate. In Denmark, it has been estimated that about 10–15% of SPF herds become reinfected with M. hyopneumoniae each year. If 1% is used as the likely prevalence of infected herds as detected by a single monthly sampling of 20 pigs, and then using Bayes’ theorem and the herd-level sensitivity and specificity estimates of 93% and 96% reported by Sørensen et al. (1992), the positive and negative herd-level predictive values are 19% and 99.9%, respectively. These calculations indicate that only about one in five test-positive herds is confirmed as infected when followed up by reference standard methods, yet a negative herd test would be highly suggestive of infection in the herd.

Because of the lack of sensitivity and specificity data for many individual tests, herd-level sensitivity and specificity and predictive values are usually unknown. Frequently, veterinarians are required to interpret herd results with incomplete knowledge of individual test performance. The number of positive pigs and the apparent prevalence of infection (0–100% test-positive pigs) are both considered in determining the status of the herd. If the seroprevalence is high, the answer to the question about the exposure status of the herd is clear. But what if a low seroprevalence (e.g., <20%) is detected when the herd is tested? In these situations, knowledge of specificity alone would facilitate judgments about the herd’s infection status. This question has important practical implications because it is more difficult to use laboratory tests to discriminate herds with a low, rather than a high, prevalence of infection from noninfected herds.

**Example.** If all sows in a 100-sow nonvaccinated breeding herd were tested by CFT for AP2 and five test-positive sows were detected (i.e., seroprevalence is 5%), what can we conclude about the AP2 status of the breeding herd? Without knowing test characteristics, one approach in the absence of a detailed herd history might be to slaughter test-positive sows and culture the upper respiratory tract, including tonsils and nasal cavities, to determine whether AP2 was present. A positive culture would confirm infection, but a negative culture would not rule out infection since culture is imperfectly sensitive. Recently, various PCR assays have been shown to be more sensitive than standard isolation methods on tonsillar tissue (biopsies and whole tonsil collected at slaughter) and would offer an alternative diagnostic strategy for evaluation of the microbiological status of seropositive sows (Fittipaldi et al. 2003).

If a published estimate of CFT specificity were available, however, a more rational judgment could be made that might avoid unnecessary slaughter and culture of test-positive sows. First, if the CFT had specificity of 95%, the expected number of positives in a noninfected herd of 100 sows would be 5, exactly what was found on the test. Second, if specificity was <95%, the belief that these were false positives would increase since the expected number of false positives even in a noninfected herd would be >5. Third, if the specificity of the test was approximately 99% (as estimated in Enoe et al. 2001), detection of five test-positive pigs would be highly suggestive of infection in the herd.

The conclusions that were made in the example would be less well met in the situation where interpretation was based on a small sample of the herd rather than the whole herd. Even when 30 samples are randomly collected in a large herd, the proportion of positive samples may not always reflect the underlying proportion of positive pigs in the herd (Carpenter and Gardner 1996). Guidelines for selection of an appropriate sample size for evaluation of herd status are described in a following section.

**PREVALENCE ESTIMATION**

An estimate of the proportion of infected pigs is often necessary as part of national and regional health-monitoring schemes and for making decisions about vaccination and other disease control and eradication programs. If a random sample of pigs is tested for exposure to an infectious agent, the proportion of positive test results (number positive/number tested) is an estimate of the apparent (test-based) prevalence of infection. If the test is serological, the term seroprevalence is used interchangeably with apparent prevalence. The
apparent prevalence may over- or underestimate the true prevalence, depending on the sensitivity and specificity of the test that is used. True prevalence can be estimated from apparent prevalence by correcting for the imperfect sensitivity and specificity of the test (Rogan and Gladen 1978):

\[
\text{true prevalence} = \frac{\text{(apparent prevalence + specificity} - 1)}{(\text{sensitivity + specificity} - 1)}
\]

Confidence intervals for the estimate of true prevalence should be calculated, and for this, the reader is referred elsewhere (Greiner and Gardner 2000b). The precision of the estimate or a practitioner’s confidence in the accuracy of the estimate is primarily dependent on sample size, with larger samples yielding more precise estimates. Occasionally, the calculation yields a negative or zero estimate for true prevalence: Such a finding might suggest that the herd is not infected. If sensitivity and specificity are unknown, however, this formula cannot be applied and the true prevalence of infection in the population cannot be calculated directly.

**Examples.** Assume that a seroprevalence estimate of 15% was obtained when sows in a herd were screened for *T. gondii* using the MAT of sensitivity = 82.9% and specificity = 90.2%. What is the true prevalence of infection in the herd? By substitution of the values in the equation, true prevalence = \((0.15 + 0.902 - 1)/(0.829 + 0.902 - 1) = 0.071\) (7.1%). This estimated true prevalence is only about one-half of what the test suggests is present, indicating that about 50% of the positive tests are false positives.

Baggesen et al. (1996) found an apparent prevalence of *Salmonella* spp. infection of 6.2% in Danish slaughter pigs based on culture of 5 g of cecal contents. Although the sensitivity of culture can depend on the volume of contents examined and choice of selective medium, the procedure was estimated to be 50% sensitive and 100% specific. Because only 50% of the infected pigs would have been detected by culture, prevalence would have been underestimated twofold. Hence, true prevalence would be 6.2% \(\times 2 = 12.4\%\).

Bayesian methods can also be used for true prevalence calculations and offer the advantage of incorporation of uncertainty into the true prevalence estimates because of the finite sample sizes used in the sampling procedure and in published test accuracy studies (Branscum et al. 2004).

### SELECTING APPROPRIATE SAMPLE SIZES

As part of disease investigations and health monitoring of herds, several sample size questions need to be addressed before sample collection. Although collection of too few samples is the most common mistake, the additional cost of submission of more samples must be weighed against the economic cost of the disease and the importance of establishing a correct diagnosis.

**Detection of Infection if Present in the Herd**

Veterinarians often need to determine whether an infection is or has ever been present in the herd or a subpopulation of the herd. For tests of 100% specificity, a single positive is usually considered sufficient to class the herd as positive, although for serological tests of imperfect specificity, more than one positive might be necessary. To estimate required numbers to detect infection, two values are necessary: the required level of confidence, usually 95%, and the likely prevalence of infection in the herd or in the specific group of pigs being evaluated. The selected prevalence value should be realistic, but if there is doubt, erring toward a lower prevalence is preferable to ensure that adequate numbers of pigs are sampled. If the calculated sample size is large relative to the total population size, these numbers can be adjusted downward.

If a veterinarian’s only goal is to detect infection, sampling does not need to be random but can be directed to higher-prevalence groups, for example, different age groups when there is an age-related risk of infection or clinically affected versus otherwise healthy pigs. If there is an age-related risk of exposure, for example, *Salmonella* spp. in pigs in Denmark (Christensen and Gardner 2000), this information can be used to target risk groups for sampling. To detect *T. gondii* in a herd, sows are a better population to sample because prevalence is likely to be higher than in grower-finisher pigs. To detect PRRS virus, samples from older nursery pigs (6–8 weeks old) would be better than samples from sows or finisher pigs. To detect enteric pathogens by fecal culture or antigen detection methods, preference should be given to sampling pigs with diarrhea rather than pigs with normal feces.

A benefit of nonrandom, or targeted, sampling is that a diagnosis can often be established with fewer samples. In an outbreak investigation when samples are selected for culture from typical lesions identified at necropsy (prevalence close to 100%), few samples will be necessary. In other situations, where infection is subclinical and prevalence is lower, more samples should be submitted. For example, a sample size of 30 will give 95% confidence of detecting at least one positive in the sample if the prevalence of infection is at least 10% and the test is perfectly sensitive. When sensitivity is less than 100%, numbers should be increased. For example, if culture of feces for *Salmonella* spp. was only 50% sensitive, a sample size of about 60 (double the number needed for a 100% sensitive test) would be needed to satisfy the specified criterion.

Despite adequate planning of sample sizes, laboratory results are sometimes negative. If zero positive test
results occur for a sample of pigs from the herd, this should be interpreted differently from negative results based on testing the entire herd. Appropriate interpretation is shown in the example on prevalence estimation that follows.

**Prevalence Estimation**

Detection of infection and estimation of prevalence can often be done with the same sample of pigs, provided that the sampling is truly random. Usually, the random sample for prevalence estimation is collected at a single point in time (cross-sectional sample). When prevalence is unknown and a study is planned, we recommend calculating a sample size at 50% prevalence, which represents the maximum number that could be needed. Selection of error limits will be more subjective, although ±10% and ±20% are used more frequently than ±5% because of the substantially increased cost when a more precise estimate is needed.

We emphasize two points with respect to prevalence estimation. First, at moderate prevalences (30–70%), much larger sample sizes are necessary to estimate prevalence accurately than at either low or high prevalences. For a fixed sample size, the precision of the estimate is greater the more the prevalence differs from 50%. Second, small sample sizes can result in sample test results that are very different from the true population values. For example, Gardner et al. (1996) compared samples of 5 and 30 pigs to estimate the prevalence of porcine parvovirus in a large herd and found that sample sizes of 5 frequently failed to reflect herd prevalence, and even a sample size of 30 yielded poor estimates of herd prevalence on some occasions.

Sometimes, even though the sample size is calculated a priori, no positives are found on a random sample of pigs when tested with a perfect test. What conclusions, if any, can be drawn? If there are no positives, the upper 95% confidence limit for prevalence is approximately \( \frac{3}{n} \) where \( n \) = number sampled (DiGiacomo and Koepsell 1986). Therefore, if 30 pigs were tested and all were negative, the upper 95% confidence limit would be \( \frac{3}{30} \), or 10%. Although a veterinarian initially might conclude that a herd was not infected (prevalence = 0%), a more appropriate interpretation would be that the herd has a prevalence of ≤10% with 95% confidence.

This issue of how to interpret negative results correctly extends to health certification schemes. Only if all pigs are tested in a herd with a test of 100% sensitivity and negative results are obtained is there sufficient evidence, based on the test results alone, to certify freedom from a pathogen. In practice, certifications are usually based on a combination of herd history and repeated testing of samples from the herd and are made in the context of knowledge that many herds become infected over time by the spread of pathogens from neighboring herds. Experience from the Danish SPF scheme indicates that the annual reinfection rate of herds with *M. hyopneumoniae* is about 10–15% (Sørensen et al. 1992, 1993).

**Detection of a Difference in Prevalence or Incidence between Two Groups**

For some investigations, a veterinarian might want to determine whether one group of pigs has a higher prevalence or incidence of infection than another group. The grouping factor could be age, reproductive status (pregnant vs. nonpregnant or aborting vs. nonaborting), production system or husbandry type, or any other comparison factor. If this diagnostic approach was used and a significant association was detected between an infectious agent and an outcome, such as clinical disease, reproductive failure, or the prevalence of emaciated pigs, this would provide additional evidence for a causal role of the agent in the syndrome. This comparative approach is often used in serum-profiling schemes where different age groups in the herd are evaluated for exposure to one or more infectious agents.

If a comparison of prevalences is the primary goal of the submission, the required sample size is determined by the level of confidence and the best a priori estimates of prevalence in the groups. As the percentage point difference between the groups decreases, much larger sample sizes become necessary (Table 8.1).

For example, to detect a significant difference at the 5% level between 40% and 10% prevalence with 80% power would require 38/group, whereas 91/group would be needed to detect a difference between 40% and 20% prevalence. These calculations indicate that the small sample sizes (5–10 per age group) that are often recommended for serum profiling of herds are typically too small for unequivocal comparisons. Sample sizes necessary to compare prevalences are listed in book tables (Fleiss 1981) or can be calculated with public domain computer software such as Epi Info (available at www.cdc.gov/epiinfo/).

**CONCLUSIONS**

Laboratories can assist in establishing a diagnosis, but ultimately, practitioners need to assess the laboratory results in the context of other herd information and existing knowledge about the relative importance of infectious agents and other factors in disease occurrence. To maximize the benefits of laboratory testing, veterinarians should do the following:

1. Clearly define the goal of the submission, for example, confirm a diagnosis, screen for a pathogen, and estimate prevalence of a pathogen.
2. Select a laboratory with good internal and external quality control procedures and with experience working with the agent or test of interest.
3. Give the laboratory the maximum chance to achieve the goals desired by
   A. selecting appropriate sample types, for example, tissue versus serum;
   B. using the correct method of submission, for example, chilled versus frozen versus room temperature;
   C. ensuring samples are from pigs that are truly representative of the problem under investigation and are collected from pigs at the appropriate stage of disease;
   D. submitting adequate numbers of samples to meet the specified submission goal, balancing the additional cost of more samples versus the need to establish a diagnosis correctly;
   E. considering inclusion of a comparison group of samples (controls) if interpretation without them might be equivocal or previous attempts to establish diagnosis have been unproductive.

4. Be knowledgeable about the strengths and limitations of available tests.

5. Interpret results taking into account the predictive values of positive and negative test results, and for quantitative tests, the magnitude of the test result in relationship to the cutoff value.

REFERENCES


Table 8.1. Sample sizes necessary to detect a significant difference in prevalence or incidence of infection or disease between two groups (one with and one without the risk factor) with 95% confidence and 80% power

<table>
<thead>
<tr>
<th>Prevalence in Risk-Factor Negative Group (%)</th>
<th>0</th>
<th>10</th>
<th>20</th>
<th>30</th>
<th>40</th>
<th>50</th>
<th>60</th>
<th>70</th>
<th>80</th>
<th>90</th>
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</thead>
<tbody>
<tr>
<td>Prevalence in Risk-Factor Positive Group (%)</td>
<td>10</td>
<td>93</td>
<td>20</td>
<td>44</td>
<td>30</td>
<td>19</td>
<td>27</td>
<td>14</td>
<td>11</td>
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<tr>
<td>20</td>
<td>27</td>
<td>71</td>
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<td>107</td>
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</table>

Note: By convention, the risk-factor positive group is assumed to have a higher prevalence than the risk-factor negative group. Sample sizes assume independence of groups and random sampling. Tabulated numbers are those required in each of the two groups.

This chapter is an overview of the major considerations specific to drug therapy in swine. Major drug classes and biological agents used in swine, with a particular focus on antimicrobial drugs and the basic principles on which effective drug use is based, are included. Readers seeking a general review of antimicrobial therapy principles are referred to excellent reviews elsewhere (Boothe 2006). Anesthetic drug properties, tranquilizers, and anesthetic protocols are thoroughly discussed in Chapter 10 “Anesthesia and Surgical Procedures in Swine” of this book. A useful additional reference for anesthetic information in swine is the comprehensive bibliography by Smith (2011).

MAJOR CONSIDERATIONS FOR DRUG THERAPY

Managing the effective use of drugs or biological agents for prevention and treatment of disease is an important responsibility of swine veterinarians, which involves detailed knowledge of these agents, including the risks involved in their use and the applicable national and international regulations. In swine, priority is placed on the production of safe meat and protection of animal welfare. Additional considerations include cost, efficacy, and ease of application. Within each of these broad categories exists several swine-specific issues that must be managed by the animal caretaker and veterinarian (Table 9.1).

All decisions to initiate drug therapy must recognize that any chemical addition to the animal has the potential to negatively impact homeostasis. Energy, enzyme pathways, and metabolic substrates must be diverted from growth and homeostasis to eliminate exogenous chemical additions including therapeutic drugs. Therefore, clear evidence that the potential benefit to the animal is greater than the negative impact on homeostasis is a prerequisite to treatment. Demonstrating a clear benefit is predicated on correct identification of existing or potential clinical compromise, most commonly infectious disease. All drug use involves a calculation that benefits of use exceed the risks involved. The goal is to minimize use of drugs in pork production while ensuring the production of healthy animals in a humane, cost-effective, safe, and environmentally sustainable manner. Changes in swine production practices have reduced reliance on antimicrobial drugs and improved pork safety in modern production.

Selecting appropriate drug therapy consists of several specific steps: (1) determine treatment objective using the sum of clinical and diagnostic evidence available, (2) identify the best option while considering swine-specific physiology, (3) establish treatment regimen in light of production logistics and regulation constraints, (4) facilitate implementation and compliance, and (5) assess the outcomes and modify protocols as indicated. The most complex treatment decisions in swine are generally those where an antimicrobial is used for treatment of clinical disease. A useful organization scheme for the swine veterinarian when making a comprehensive antimicrobial selection is the “S.P.A.C.E.D.” mnemonic described in Table 9.2.

Determining Treatment Objective

The current standard for determining a treatment objective in the context of antimicrobial therapy is a diagnosis that includes bacterial culture and identification accompanied with antimicrobial sensitivity testing of that isolate. In swine cases, this isolate should be the
### Table 9.1. Considerations in drug use in swine

<table>
<thead>
<tr>
<th>Major Consideration</th>
<th>Further Considerations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human safety</td>
<td>Direct drug toxicity to user, toxicity to consumer through tissue residues</td>
</tr>
<tr>
<td>Animal welfare</td>
<td>Prevention or reduction of disease, ease of administration for animal</td>
</tr>
<tr>
<td>Host damage and adverse effects</td>
<td>Direct toxicity to pig, tissue damage, adverse drug interactions, indirect adverse effects: resistance in microorganisms, disruption of microflora</td>
</tr>
<tr>
<td>Regulations</td>
<td>Availability of products, national regulations on use; international regulations for export, extra-label drug use (AMDUCA in United States), veterinary–client relationship, withdrawal period</td>
</tr>
<tr>
<td>Efficacy and cost</td>
<td>Assessment of efficacy, cost: benefit of treatment</td>
</tr>
<tr>
<td>Drug dosage and application</td>
<td>Route, ease of administration, physicochemical properties, pharmacokinetic properties, pharmacodynamic properties</td>
</tr>
<tr>
<td>Principles of treatment</td>
<td>Dosage, dosage modification, duration, clinical evidence, drug trial data</td>
</tr>
<tr>
<td>Principles of prophylaxis</td>
<td>Dosage, duration, clinical evidence, drug trial data</td>
</tr>
<tr>
<td>Record keeping</td>
<td>Drug use records</td>
</tr>
<tr>
<td>Stability of drug</td>
<td>Storage conditions</td>
</tr>
</tbody>
</table>


### Table 9.2. Antimicrobial selection considerations (S.P.A.C.E.D.)

<table>
<thead>
<tr>
<th>Spectrum</th>
<th>Drug efficacy against pathogen, pathogen Gram staining, pathogen metabolism (aerobic or anaerobic)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pharmacokinetics and pharmacodynamics</td>
<td>Drugs ability to reach the pathogen at the site of infection in high enough concentrations to be effective, plasma antimicrobial concentration versus time profile, concentration dependent or time above MIC dependent or AUC above MIC dependent, volume of distribution, protein binding</td>
</tr>
<tr>
<td>Adverse reactions</td>
<td>Safe to use in this disease state, breed, age group, class of animal; potential contraindications/toxicities; potential risks to the person administering the drug</td>
</tr>
<tr>
<td>Compliance</td>
<td>Caretaker ability to provide the full course of treatment, legality of use in this class of animal</td>
</tr>
<tr>
<td>Environment</td>
<td>Site of infection characteristics, potential for resistance development</td>
</tr>
<tr>
<td>Diagnostics</td>
<td>Pathogen identification, actual or estimated susceptibility profile</td>
</tr>
</tbody>
</table>

AUC, area under the plasma drug concentration versus time curve.

Consequence of a complete diagnostic investigation that confirms lesions consistent with the pathogen are present in appropriate tissues. Furthermore, the case needs to be representative of the primary problem in the target swine population. It is often necessary to confirm culture and sensitivity data for several representative cases in a specific population as pathogens are not necessarily a homogenous clone in a swine population. Several pathogenic bacteria can coexist with nonpathogenic strains of the same genus and species in the pigs such as the case with intestinal *Escherichia coli*.

When culture and sensitivity confirmation is not available or the urgency of intervention on behalf of swine welfare requires initiating therapy before results are available, several sources of information are available to supplement empirical treatment. Practitioners have the capability with some diagnostic laboratory cooperation to maintain antimicrobial sensitivity summaries for their clients or practice region compiled from a series of diagnostic cases. Several diagnostic laboratories summarize the percentage of isolates that are susceptible to selected antimicrobials on a routine basis. These summaries are available online (www.vads.org) and may represent a more current and broad view of microbial susceptibilities than even randomized, controlled trials using single isolates.

### Treatment Options and Specific Impacts of Swine Physiology

#### Major Classes of Antimicrobial Drugs

A brief overview of some key aspects of the major classes of antimicrobial drugs, their antimicrobial activities, pharmacokinetic properties, toxic or other adverse effects, and major clinical applications is given in Table 9.3. Further details are available through manufacturer's package inserts and through pharmacology and related textbooks (Prescott et al. 2000).

Antimicrobials may be classified as bactericidal or bacteriostatic based on the outcome of specific in vitro drug–bacteria interactions. Typically, a drug is considered bactericidal when there is a 3-log reduction in bacterial cultures over a 24-hour period. It has been suggested that bactericidal drugs are preferred for treatment of serious life-threatening infections, when host defenses are impaired, and infections of vital tissues such as meninges, endocardium, and bones where host defenses are also not fully functional. In other cases, bacteriostatic agents may be equally useful. However, it should be recognized that this classification is only one of many aspects that should be considered when making appropriate antimicrobial selections.

Where feasible, a narrow-spectrum drug may be more appropriate than a broad-spectrum antibacterial because the narrow-spectrum drug interferes less with the normal microbial flora and is potentially less likely to select for widespread resistance.
<table>
<thead>
<tr>
<th>Drug Class</th>
<th>Specific Agent or Example of Agents</th>
<th>Antibacterial Activity, Resistance</th>
<th>Pharmacokinetic Properties</th>
<th>Toxic or Adverse Effects</th>
<th>Major Applications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sulfonamides</td>
<td>Sulfamethazine intermediate acting; others also used</td>
<td>Bacteriostatic; broad-spectrum, gram+, gram− aerobes; anaerobes; acquired resistance very widespread; active intracellular bacteria, protozoa</td>
<td>Rapidly absorbed from intestine, well distributed in tissues</td>
<td>Violative kidney residues from feed use through recycling, feed contamination if feed not withdrawn 15 days before slaughter</td>
<td>Minor value; largely growth promotional, possible disease prevention</td>
</tr>
<tr>
<td>Sulfonamide–diaminopyrimidine combinations</td>
<td>Sulfamethazine–trimethoprim</td>
<td>Bactericidal; gram+, gram− aerobes; anaerobes; <em>Mycoplasma, Leptospira</em> resistant</td>
<td>Rapidly absorbed from intestine, well distributed in tissues; crosses uninflamed blood–brain barrier</td>
<td>Wide safety margin</td>
<td>Largely IM use for acute infections (pneumonia, streptococcal meningitis); in feed for atrophic rhinitis</td>
</tr>
<tr>
<td>Beta-lactam</td>
<td>Penam penicillins, group 1: penicillin G</td>
<td>Bactericidal; highly active many gram+, some fastidious gram− aerobe, for example, <em>H. parasuis, P. multocida</em>; anaerobes; <em>Leptospira</em>, enteric bacteria, and <em>Mycoplasma</em> resistant</td>
<td>Poorly absorbed from intestine, relatively poorly distributed in tissues; crosses only inflamed blood–brain barrier</td>
<td>Safe drug; possible anaphylaxis or procaine-induced excitement</td>
<td>Excellent for IM use in erysipelas, streptococcal infections including meningitis, clostridial infections; some bacterial pneumonias</td>
</tr>
<tr>
<td>Beta-lactam</td>
<td>Penam penicillins, group 4: ampicillin, amoxyillin</td>
<td>As penicillin G, broader activity against gram− aerobes, but resistance widespread</td>
<td>As penicillin G, but better absorbed orally and distributed through tissues</td>
<td></td>
<td>Safe drug</td>
</tr>
<tr>
<td>Beta-lactam</td>
<td>Group 4, “third-generation” cephalosporins; ceftiour</td>
<td>Bactericidal; gram− aerobes especially, including <em>E. coli</em>, <em>Salmonella</em>, gram+ aerobes, anaerobes; <em>Mycoplasma</em> resistant</td>
<td>Poorly absorbed from intestine, relatively poorly distributed in tissues; crosses only inflamed blood–brain barrier</td>
<td>May predispose to <em>Clostridium difficile</em> colitis if used in neonatal pigs; resistance emerging in <em>Salmonella</em> may represent human health hazard</td>
<td></td>
</tr>
<tr>
<td>Aminoglycoside</td>
<td>Gentamicin, neomycin</td>
<td>Bactericidal; gram− aerobes, including enterics</td>
<td>Poorly absorbed from intestine, relatively poorly distributed in tissues</td>
<td>Nephrotoxic with prolonged parenteral use; persistent kidney residues</td>
<td>Gentamicin IM for neonatal <em>E. coli</em> infections; neomycin orally for <em>E. coli</em> infection</td>
</tr>
<tr>
<td>Aminocyclitol</td>
<td>Apramycin, spectinomycin</td>
<td>Bactericidal; gram− aerobes, including enterics</td>
<td>Poorly absorbed from intestine, relatively poorly distributed in tissues</td>
<td>Nephrotoxic with prolonged parenteral use; persistent kidney residues</td>
<td>Orally for <em>E. coli</em> infection</td>
</tr>
<tr>
<td>Lincosamide</td>
<td>Lincomycin</td>
<td>Bacteriostatic; gram+ aerobes, anaerobes including *B. hyodysenteriae; Mycoplasma</td>
<td>Well absorbed from intestine and well distributed in tissues</td>
<td>Safe drug in swine</td>
<td>Oral use for control of <em>Brachyspira</em>; oral or IM use for control of <em>Mycoplasma</em></td>
</tr>
<tr>
<td>Drug Class</td>
<td>Agent</td>
<td>Activity</td>
<td>Pharmacokinetic Properties</td>
<td>Toxic or Adverse Effects</td>
<td>Major Applications</td>
</tr>
<tr>
<td>-----------------</td>
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<td>-----------------------------------------------------</td>
<td>------------------------------------------------------------------------------------------</td>
<td>----------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Macrolide</td>
<td>Tylosin</td>
<td>Bacteriostatic; gram+ aerobes, anaerobes, some gram– aerobes; <em>Mycoplasma</em></td>
<td>Well absorbed from intestine and well distributed in tissues</td>
<td>Safe drug in swine; IM irritant, may cause edema, pruritis, anal protrusion. Perianal edema and rectal eversion may occur in treated animals but resolves after discontinuation of treatment</td>
<td>Oral use for control of proliferative enteropathy, atrophic rhinitis, possibly leptospirosis</td>
</tr>
<tr>
<td>Phenicols</td>
<td>Florfenicol</td>
<td>Primarily bacteriostatic</td>
<td>Well absorbed from the intestine and well distributed in tissues</td>
<td>Injectable solution is indicated for the treatment of swine respiratory diseases associated with <em>Actinobacillus pleuropneumoniae</em> and <em>Pasteurella multocida</em>.</td>
<td>Oral use for control of swine respiratory disease (SRD) associated with <em>Actinobacillus pleuropneumoniae</em>, <em>Pasteurella multocida</em>, <em>Streptococcus suis</em>, and <em>Bordetella bronchiseptica</em> in groups of swine.</td>
</tr>
<tr>
<td>Pleuromutilin</td>
<td>Tiamulin</td>
<td>Bacteriostatic; gram+ aerobes, anaerobes, some gram– aerobes; <em>Mycoplasma</em>; more active than tylosin</td>
<td>Well absorbed from intestine and well distributed in tissues</td>
<td>Generally considered a safe drug in swine, although cases of lameness after injection have been reported</td>
<td>Oral use for control of <em>Brachyspira</em>, <em>Mycoplasma</em>, chronic pneumonias, proliferative enteropathy, leptospirosis</td>
</tr>
<tr>
<td>Quinolones</td>
<td>Enrofloxacin</td>
<td>Bacteriocidal</td>
<td>Well absorbed and distributed in tissues</td>
<td>Injectable enrofloxacin is indicated for the treatment and control of SRD associated with <em>Actinobacillus pleuropneumoniae</em>, <em>Pasteurella multocida</em>, <em>Haemophilus parasuis</em> and <em>Streptococcus suis</em>. Extralabel use in food animals in the United States is prohibited.</td>
<td>Injectable enrofloxacin is indicated for the treatment and control of SRD associated with <em>Actinobacillus pleuropneumoniae</em>, <em>Pasteurella multocida</em>, <em>Haemophilus parasuis</em> and <em>Streptococcus suis</em>. Extralabel use in food animals in the United States is prohibited.</td>
</tr>
<tr>
<td>Tetraacylins</td>
<td>Oxy-, chlor-, tetracycline</td>
<td>Bacteriostatic; classically broad-spectrum, gram+, gram– but acquired resistance extremely widespread; <em>Erysipelothrix</em>, <em>Haemophilus</em>, <em>Leptospira</em>, and <em>Pasteurella</em> are exceptions</td>
<td>Well absorbed from intestine and well distributed in tissues</td>
<td>Oral use as “feed” drugs for growth promotion and nonspecific disease prophylaxis in countries where allowed; used in feed, occasionally IM, for treatment of infections caused by bacteria listed as being susceptible</td>
<td>Oral use as “feed” drugs for growth promotion and nonspecific disease prophylaxis in countries where allowed; used in feed, occasionally IM, for treatment of infections caused by bacteria listed as being susceptible</td>
</tr>
</tbody>
</table>
To some extent, drug dosage can be tailored to the susceptibility of the organism, the site of infection, and the pharmacokinetic and pharmacodynamic properties of the selected antimicrobial agent. However, in vitro susceptibility data are laboratory derived, and the standardized conditions under which the susceptibility data are generated do not exist at the site of infection. Factors involved in tailoring a dosing regimen include, among other things, the susceptibility of the pathogen in terms of minimum inhibitory concentration (MIC), the concentration of the antimicrobial agent at the site of infection in active form (pharmacokinetic properties of the drug), and the pharmacodynamic properties of the antimicrobial agent. Some antimicrobials (aminoglycosides, fluoroquinolones) are concentration dependent (optimum action of the drug depends on concentration of the drug above MIC), whereas others (beta-lactams, lincosamides, macrolides, trimethoprim–sulfamethazine) are time dependent (optimum activity depends on time above MIC). The complex issues involved in optimal antimicrobial therapy are beyond the scope of this chapter, although it can be concluded that some dosage recommendations for drugs licensed in the past have not taken modern understanding into account and are suboptimal or inappropriate. In the United States, the U.S. Food and Drug Administration’s (FDA) professional flexible labeling approach allows veterinarians to adjust the dose based on the MIC of the pathogen. Although a number of factors determine optimal dosage, the factor that most frequently limits dosage is toxicity. The upper level of the recommended dosage should not be exceeded, because this is often determined by toxicity. In some instances, a drug’s antibacterial effects may be limiting and may determine the upper level of dosage. For example, the killing rate of beta-lactam drugs has an optimal concentration, whereas that of the aminoglycosides or fluoroquinolones is proportional to drug concentration. Penicillin G is virtually nontoxic in nonallergenic patients, but its dosage is limited by its antibacterial action. By contrast, the dosage of aminoglycoside is limited not by antibacterial effects but by its toxicity.

In terms of duration of treatment, the variables affecting length of treatment have not been adequately defined in swine. Responses of different types of infections to antimicrobials vary, and clinical experience with many infections is important in assessing response to treatment. For acute infections, it will usually be clear within 2 days whether or not therapy is clinically effective. If no response is seen by that time, both the diagnosis and treatment should be reconsidered. Treatment of acute infections should be continued for at least 2 days after clinical and microbiological resolution of infection. For serious acute infections, treatment should probably last 7–10 days. For chronic infections, treatment will be considerably longer.

**Drug Selection for Specific Diseases.** It is beyond the scope of this text to discuss all product indications, cautions, or regulations nor is it within the scope of this text to include all possible treatment options. Readers are referred to the relevant specific disease chapter for further suggested treatments. Additionally, the FDA provides a searchable, online database of approved product indications that can be searched by species. This database allows access to the information compiled by the FDA (known as the “Green Book”) and can be found by searching the web for “animal drugs at FDA.” The address at the time of this chapter’s publication was www.accessdata.fda.gov/scripts/animal-drugsatfda/ or inquiries can be directed to the FDA at US FDA, 5600 Fishers Lane, Rockville, MD 20857-0001, telephone 1-888-INFO-FDA (1-888-463-6332).

A few swine-specific treatment applications and drug characteristics are noted here to guide veterinary practice. For example, parenteral tilmicosin is fatal to swine even at very low concentrations. However, oral administration is approved for *Actinobacillus pleuropneumoniae* and *Pasteurella multocida* (Backstrom et al. 1994).

Bimazubute et al. (2010) demonstrated that concentrations of oxytetracycline could be achieved in nasal secretions of swine that were greater than the MICs for *P. multocida* and *Bordetella bronchiseptica*, key pathogens in atrophic rhinitis. However, this could only be achieved with intramuscular (IM) injection at 40 µg/kg body weight. This dose level was four times the swine label recommendation. Although peak concentrations in plasma and nasal secretions both occurred 4 hours after administration, the maximum concentration of the drug in nasal secretions was 6.29 µg/mL compared with 19.4 µg/mL in plasma. Efficacious levels were not achieved with lower IM doses nor feed formulations containing 400 µg/kg oxytetracycline.

In many species including pigs, enrofloxacin is metabolized to ciprofloxacin, which also has potent antimicrobial activity. Variations in the amount of enrofloxacin converted to ciprofloxacin occur with age in swine. None of the original dose of the parent enrofloxacin was detected as ciprofloxacin in 10 kg pigs compared with 52% of the original enrofloxacin detected as ciprofloxacin in 76 kg pigs (Bimazubute et al. 2009).

Cornick (2010) demonstrated that fewer pigs fed subtherapeutic levels of tylosin or chlorotetracycline shed *Escherichia coli* O157:H7 for longer than 2 weeks compared with pigs fed no antimicrobials. Experimentally, antimicrobial-free pigs can be infected and shed for longer than 2 months. However, field studies rarely recover the organism from domestic pigs. Potentially, feed medication protocols used in commercial swine production have reduced the presence of this food safety risk.

Most pharmacokinetic parameters are studied in healthy, disease-free pigs. There are few studies evaluat-
ing how disease impacts pharmacokinetics of drugs and the implications for modifying treatment regimens. In one study, the mean of maximum plasma tetracycline concentrations was lower and achieved significantly later postinjection in pneumonic pigs relative to healthy pigs (Pijpers et al. 1991). Pigs infected with porcine reproductive and respiratory syndrome virus (PRRSV) had decreased plasma ceftiofur concentrations relative to healthy pigs (Tantituvanont et al. 2009).

Establishing Treatment Regimens Considering Production Logistics and Regulatory Constraints

Routes of Drug Treatment. In general, individual animal treatment through injection is preferred for serious, rapidly developing infections such as acute systemic infections (septicemia, acute pneumonia, or streptococcal meningitis), but mass medication is preferred when ease of administration can be considered, identifying sick individuals in population is challenging, or there is a lack of necessity to handle and disturb animals. IM injection is preferred for serious infections since it usually results in more complete absorption of drug and higher tissue concentrations than oral administration. Parenteral therapy of individual animals by IM injection is administered just behind the ear on the lateral side of the neck. This site is chosen in case the drug preparation causes local tissue damage as well as to prevent the possible additional effect of sciatic nerve damage if the ham muscles were used. Needleless injection devices are available for vaccines but are infrequently used for treatment due to dose limitations imposed by the design of the devices.

Oral medication is easier to apply to groups of pigs and reduces injection related problems of broken needles, abscesses, and tissue damage. Oral medication for infections outside the gastrointestinal tract generally represents a less efficient use of the total antimicrobial applied to a population. For example, the range of reported bioavailabilities of amoxicillin is 11–47% (Hernandez et al. 2005). Water medication is a more rapid method of treating a group of sick pigs than feed medication, with the advantages of immediate implementation and that sick pigs will drink when they will not eat. However, the disadvantages are that not all drugs are water soluble, that water may be spilled or wasted, and that some drug carriers may block nipple watering systems. Administration of drugs by water is through an in-line proportioner containing a concentrated drug solution or by a water tank containing the appropriately dosed drug. Pigs drink about 8–10% of their body weight daily, depending on environmental temperature and palatability of the drug.

As an example, amoxicillin has been demonstrated to achieve therapeutic levels in swine (Agero et al. 1998) via water delivery. Mean plasma levels fluctuated between 0.5 and 1.3 µg/mL during the treatment period and were suspected to reflect pig drinking behavior. Consumption of amoxicillin-containing water also increased as pen temperature increased during the study. Several studies have demonstrated relatively large individual animal variation (Agero et al. 1998) relative to group plasma means. This variation in effective dose, and corresponding variation in response to therapy, must be considered when setting treatment expectations for water medication protocols. In fact, when tetracycline plasma levels were evaluated in pigs administered water medicated at labeled concentrations, plasma levels were highly varied and generally lower than 0.3 µg/mL, which suggests that therapy would have questionable value for most target pathogens (Dorr et al. 2009). Another study of tetracycline water medication in swine revealed that the oral bioavailability is very low (Mason et al. 2009).

In-feed medication is the most common route of administration of anthelmintic and antimicrobial drugs. The disadvantage for treatment of acute infections is not only that sick pigs may not eat but also that existing nonmedicated feed needs to be either removed or eaten. For this reason, in-feed medication is often reserved for long-term use in the prevention or treatment of chronic infections.

Facilitating Implementation and Compliance Principles of Prophylaxis. Antimicrobial drugs are administered to swine for the prevention of particular diseases. The generally accepted principles of antimicrobial prophylaxis are the following:

- Medication should be directed against specific pathogens or diseases.
- Prophylaxis should be used only where efficacy is established. Prophylaxis should be of a duration that is as short as possible consistent with efficacy.
- Dosage should be the same as that used therapeutically.
- Adverse effects itemized earlier should be minimized.
- Prophylactic use of antimicrobials should be restricted for the treatment of animals that are most likely to respond to treatment.
- Alternatives to these antimicrobial use practices need to be found and employed when possible.

One prophylactic practice in swine is that of “pulse medication,” whereby a therapeutic level of a specific drug is included in the feed at therapeutic concentrations periodically for a short duration to prevent clinical disease while allowing the animal to develop natural immunity as a consequence of infection by the pathogen. This approach requires sufficient diagnostic history from the population to anticipate a predictable onset of the disease on a particular farm. When applied
correctly, this approach can limit total antimicrobial use while preserving animal welfare. A comparison of pulse dosing to continuous dosing in feed demonstrated improved performance and survivability in the presence of naturally occurring disease for both treatments. However, pulsing permitted sufficient natural exposure to stimulate active humoral immunity to *Mycoplasma hyopneumoniae* while continuous treatment did not (Walter et al. 2000). The implications of this approach for the development of antimicrobial resistance are not completely understood.

The timing of prophylaxis is especially critical and may not vary significantly from the optimum for treatment except that prophylaxis implies clinical signs are not yet occurring. In a study of ceftiofur administered as a single injection of the crystalline-free acid, pigs injected 13 and 10 days prior to challenge did not have removal rates significantly different than untreated controls after challenge with *A. pleuropneumoniae* (Crane et al. 2006). However, treatment 7, 4, and 1 days prior to challenge resulted in fewer removals than the control group.

**Regulation.** The use of antimicrobial drugs in food animals is regulated by law in many countries, and veterinarians need to know and abide by the regulations. The regulations involve an approval process of drugs produced by a particular manufacturer only if they meet human and animal safety standards as well as being shown to be efficacious at specified dosages for particular purposes. In the United States, general limits to how antimicrobials are used exist. Extra-label use is allowed when drugs are approved for use in humans or food animals, and the specific requirements of the Animal Medicinal Drug Use Clarification Act are met. However, extra-label use via feed delivery is not allowed, and there are specific drugs that are banned in food animals in the United States. None of these circumstances are swine specific and will not be discussed further here, but the swine veterinarian is cautioned to be aware of the most current regulations.

The logistics of swine production, specifically the need to inject large numbers of animals in a population in a short time period and the resistance of pigs to restraint, creates a desire to combine or compound drugs into a single injection. Compounding is closely regulated in the United States by the FDA to ensure that permitted compounding for legitimate treatment needs does not provide an opportunity to circumvent the drug approval process and consumer protections. The FDA provides specific Compliance Policy Guides that are available electronically as a single document, which includes guidance on what activities are considered inappropriate compounding that veterinarians practicing in the United States should review. In the United States, failure to comply with the regulations may result in fines or imprisonment.

**Drug Withdrawal.** Most drugs must not be used near slaughter, to avoid any significant residues in meat products. While generally understood for antimicrobials, it is often overlooked that most vaccines have required withdrawal times as well. The use of vaccine near market in growing animals is unlikely, but timing the vaccination of sows that might be candidates for culling requires observation of the withdrawal time. The precise period varies with the drug and the dosage. For drugs used at the labeled dosage, this will be specified on the package insert. For extra-label drug use, withdrawal information may be obtained from the manufacturer or, in some cases, from national or international databases such as, in the United States, the Food Animal Residue Avoidance Databank (toll-free number in the United States, 1-888-USFARAD; www.farad.org). Veterinarians should be aware that there is robust international trade of pork products, acceptable residue limits in pork vary among countries, and the withdrawals included on the label may only be valid for the country in which the drug label was approved. As a consequence, some harvest plants have extended withdrawal periods for specific drugs to prevent residues using the limits of the destination country. It is important to accommodate the requirements of the destination market with the lowest residue limit. In most cases, the destination harvest plant will know the potential destinations for pork products they export and will have withdrawal suggestions. Dialogue with the harvest plant is imperative to create treatment withdrawals that protect a producer’s ability to market at the desired plant.

**Limiting Development of Resistance.** Numerous reports have recommended that all stakeholders concerned with the use of antimicrobials in both food animals and humans must be involved in an overarching global strategy to contain resistance (e.g., World Health Organization 2000a) and have recommended steps to enhance the prudent use of antimicrobials in animals, including the removal of growth promoters if these drugs are important in human medicine (e.g., World Health Organization 2000b). It is critical to note that resistance mechanisms are not limited to antimicrobials but have been reported for copper and zinc (Fard et al. 2011). Furthermore, as mentioned previously, direct evidence that even subtherapeutic levels of antimicrobials reduce the shedding of human pathogens by swine (Cornick 2010) must be considered in the formulation of risk mitigation regulations at the national level. At the international level, the World Organization for Animal Health, also known as the Office International des Epizooties, continues to formulate recommendations and options for risk management relating to antimicrobial use in animals. Outside the European Union (EU), other countries are in the process of assessing or starting to reassess the use of
antimicrobial drugs in food animals based on the importance of the drug in human medicine and the likelihood of exposure of humans to resistant bacteria or resistance genes arising from animals (e.g., Center for Veterinary Medicine, US FDA 2004; Health Canada 2002).

There are opportunities to apply innovation to reduce the amount of antimicrobials used in production. While the inclusion of antimicrobials in semen extenders does not represent a significant portion of the total use in swine, a recent study demonstrated the potential for innovation to limit antimicrobial use. In this study, Morrell and Wallgren (2011) used single-layer centrifugation to separate sperm from bacteria to create semen doses free of bacterial contamination.

In recent years, many countries have started to monitor resistance in both important pathogens (e.g., *Campylobacter jejuni*, *Salmonella*) as well as “indicator” commensal bacteria (e.g., *Enterococcus* species) isolated from animals, foodstuffs, and humans. For example, in the United States, the National Antimicrobial Resistance Monitoring System (NARMS) established in 1996 is designed to document emerging resistance problems, as well as to provide data on which public health policy decisions can be made for the use of antimicrobial drugs in food animals. In Canada, the Canadian Integrated Program for Antimicrobial Resistance Surveillance (CIPARS) has taken a similar approach to NARMS. In 2010, CIPARS reported (Deckert et al. 2010) that in sentinel grower-finishers, antimicrobials considered very important to human health were not frequently used. Additionally, resistance as determined by culture and sensitivity of samples obtained on farms was characterized as low.

One emerging resistance problem that will likely receive greater scrutiny and attention in the future is extended-spectrum cephalosporin resistance in multidrug-resistant *E. coli* and *Salmonella* serovars (Winokur et al. 2001; Zhao et al. 2003), in which the cmy-2 gene encoding expanded-spectrum cephalosporin resistance may be found on several different plasmids that can readily be transferred through bacterial conjugation (Carattoli et al. 2002). However, as deftly pointed out by Davies (2010), “Genes encoding for antimicrobial resistance are not foodborne pathogens.”

**Prudent Use Guidelines.** The widespread concern about antimicrobial resistance and the animal–human resistance link has led most major national veterinary organizations to improve antimicrobial drug use by development of prudent use guidelines. Such guidelines represent first steps in the more judicious use of antimicrobial drugs that may become considerably more complex over time if they address antimicrobial drug choice for particular diseases. An example of such guidelines, that of the American Association of Swine Veterinarians, is shown in Table 9.4.

### Table 9.4. American Association of Swine Veterinarians (AASV) Basic Guidelines of Judicious Therapeutic Use of Antimicrobials in Pork Production

<p>| | |</p>
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<tbody>
<tr>
<td>1.</td>
<td>Preventive strategies, such as appropriate husbandry and hygiene, routine health monitoring, and immunization, should be emphasized.</td>
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<tr>
<td>2.</td>
<td>Other therapeutic options should be considered prior to or in conjunction with antimicrobial therapy.</td>
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<tr>
<td>3.</td>
<td>Judicious use of antimicrobials, when under the direction of a veterinarian, should meet all requirements of a veterinarian–client–patient relationship.</td>
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<tr>
<td>5.</td>
<td>Extra-label antimicrobial therapy must be prescribed only in accordance with the Animal Medicinal Drug Use Clarification Act amendments to the Food, Drug, and Cosmetic Act and its regulations.</td>
</tr>
<tr>
<td>6.</td>
<td>Veterinarians should work with those responsible for the care of animals to use antimicrobials judiciously regardless of the distribution system through which the antimicrobial was obtained.</td>
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<tr>
<td>7.</td>
<td>Regimens for therapeutic antimicrobial use should be optimized using current pharmacological information and principles.</td>
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<tr>
<td>8.</td>
<td>Antimicrobials considered important in treating refractory infections in human or veterinary medicine should be used in animals only after careful review and reasonable justification. Consider using other antimicrobials for initial therapy.</td>
</tr>
<tr>
<td>9.</td>
<td>Utilize culture and susceptibility results to aid in the selection of antimicrobials when clinically relevant.</td>
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<tr>
<td>10.</td>
<td>Therapeutic antimicrobial use should be confined to appropriate clinical indications.</td>
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<tr>
<td>11.</td>
<td>Therapeutic exposure to antimicrobials should be minimized by treating only for as long as needed for the desired clinical response.</td>
</tr>
<tr>
<td>12.</td>
<td>Limit therapeutic antimicrobial treatment to ill or at risk animals, treating the fewest animals indicated.</td>
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<tr>
<td>13.</td>
<td>Minimize environmental contamination with antimicrobials whenever possible.</td>
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<tr>
<td>14.</td>
<td>Accurate records of treatment and outcome should be used to evaluate therapeutic regimens.</td>
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Note: The AASV website elaborates on these basic guidelines listed above (www.aasp.org/aasv/jug.html).

### Assessing Outcomes

**Treatment Failure.** Treatment failure has many causes. The antimicrobial selected may be inappropriate because of misdiagnosis, inactivity at the site of infection, failure to culture infections, inaccurate or inapplicable laboratory results, resistance of pathogens, chronic nature of the infection (which may affect metabolic state of the pathogen), or errors in sampling. These factors are more likely to cause failure than inadequate dosage although this may also be important. It is important that producers comply with dosing instructions. When failure occurs, diagnosis must be reassessed and samples are collected for laboratory analysis.
Evaluation of Clinical Trials. The best method for evaluating animal health interventions and to guide clinical decision-making is to conduct on-farm clinical trials (Dohoo et al. 2003). In a clinical trial, exposure to disease occurs naturally and the pigs are housed and managed under normal farm conditions, but the treatment is randomly assigned with a second group used as a control population. Clinical trials are difficult to conduct, and there is considerable potential for errors in design and misinterpretation of findings. The consequences of these failings may be inappropriate therapeutic recommendations and overall lack of success in treatment programs. Practitioners need to be aware of proper methodology as it relates to design and interpretation in order to evaluate therapeutics either by conducting a trial on a client’s farm, or in interpreting claims for a new drug as presented by a pharmaceutical company.

First, a study should have a limited number of objectives, generally one primary and possibly two or three secondary objectives, and these must be clearly stated (Dewey 1999). For example, a trial examining the use of a drug to control pneumonia in a finishing unit might have decreased mortality as its primary objective, and as secondary objectives, the researchers may be interested in improved growth rate and reduced weight variation. The design of the trial would likely be different if the primary objective was reduced weight variation.

Other important elements of a clinical trial include a defined study population, random allocation of subjects, masking or blinding of the observer, thorough follow-up, and appropriate analysis (Dewey 1999). A common error in the design of a trial is to base the statistical analysis on individual pigs although treatments were assigned on the basis of pen or even barn. Statistical analysis should be conducted at the smallest level at which the treatment can be applied. Therefore, in a feed trial where all the pigs in a pen are assigned one feed and all the pigs in the next pen are given a second feed, the pen is the unit of analysis. The number of animals or pens or barns required to assess whether a drug is beneficial or not can be calculated using formulas that can be found in standard statistics textbooks. The number of units will depend on the expected variation and the magnitude of the difference considered important. For example, if one assumes a coefficient of variation of 7% for growth rate, one would need approximately 43 pens per treatment to detect a difference in average daily gain of 5%. Whereas it would require only 12 pens per treatment to detect a difference in average daily gain of 10%. Typically, the confidence interval is chosen to be 95%, implying that the probability that the results were real and not due to chance alone is 95%. The P-value or level of significance is the opposite (i.e., P = 0.05 means there is a 5% chance that the results are due to chance alone).

Statistical power is typically set at 80%, implying that there is an 80% probability that we will find a difference when a difference truly exists. Therefore, 20% of the time such a trial will not distinguish a difference between treatment and control when there really is a difference. Statistical power can be increased by increasing sample size but higher power can be logistically and financially prohibited.

It is important that bias is minimized wherever possible. Therefore, subjects need to be assigned to a treatment group in a truly random manner, and if this is not possible, an alternative systematic assignment may be used. The intervention given to the control group needs to be similar to the treatment group. For example, if the treatment group needs to be restrained and injected with a product, the controls need to be handled in a similar manner and given a placebo. Ideally, the animal caregivers and the clinical observers should be kept blind to which animals are in the treatment group and which are in the control group.

Even when animals are assigned in a random manner and trials are carefully designed, confounding factors and other sources of error can be introduced in a farm setting. A great deal of care is needed in assessing the information gained from a clinical trial, but a well-executed field trial may be superior to judge efficacy of therapeutic measures relative to in vitro studies.

Enhancement of Management and Biosecurity. Modern housing and husbandry methods that tend to segregate age groups, allow for cleaning of the environment between production groups, and minimize the risk of disease introduction through strict biosecurity measures, are the most important methods of reducing the total use of antimicrobials and other therapeutic products as well as improving the efficacy of those treatments used. Immune system stimulation results in decreased feed efficiency and growth rate whether or not clinical disease occurs. The value of in-feed antibiotics is questionable when high-health status grower-finisher pigs are raised in a clean, biosecure environment (Van Lunen 2003). However, even under ideal management, there are circumstances when treatment is required and success of therapy depends on the diligence of stock people to identify clinical signs of illness early, to treat appropriately, and to provide an environment for the sick pig that promotes healing.

Vaccines. Vaccines are extensively used in swine production as primary preventive measures and as elements of more holistic programs designed to eradicate pathogens. Reports of the eradication of *M. hyopneumoniae* from continuous flow grower-finisher populations describe the concurrent strategic use of vaccinations and antimicrobials (Heinonen et al. 2011). The usefulness of vaccination varies among diseases and even from herd to herd. Despite rapid advances in the fields of immunology and molecular biology, there
are still diseases for which vaccines and biosecurity have only moderate to poor efficacy (Haesebrouck et al. 2004), and treatment or prophylaxis of clinical disease is required.

There are a number of important considerations that a veterinarian needs to evaluate in order to include a vaccine in the treatment program for a particular herd. The cost–benefit of vaccination needs to be considered, and this includes estimating the cost of the program, including labor to administer the vaccine. An estimate of the improvement one would expect from the vaccination program, which requires knowledge of vaccine efficacy and an understanding of the disease costs present in the herd, is required, and the relative value of alternative control measures should be considered. In addition, the veterinarian needs to be aware of possible negative effects, such as a potential tissue reaction that might lead to trimming losses or a transient loss of appetite that could cause a reduction in growth.

The decision to institute a vaccination program is complex, and unfortunately, there is a scarcity of unbiased data regarding the efficacy of vaccines used under practical farm conditions (Moon and Bunn 1993). There are examples of vaccines that have worked well in controlled experimental infection models but are of limited value in the field. Many of the important clinical diseases of swine are a complex of one or more infectious agents and of host, environmental, and management factors.

Swine practitioners are sometimes faced with an unexpected vaccination failure in a situation when using a product that has worked well under similar circumstances in the past. Possible causes of a failure include improper storage and handling of the vaccine, such as failure to refrigerate or protect from light; incorrect administration, such as subcutaneous injection when an IM injection is required; or possibly omitting to vaccinate whole groups of animals. In the case of vaccines administered via the drinking water, there are a number of concerns but possibly the most important is chlorine present in the water, which may kill live attenuated bacteria in vaccines (Kolb 1996).

Optimizing the timing of a vaccination program is often a problem. In order to maximize compliance and minimize labor, the swine industry prefers to use combination vaccines that require a single injection to be given at a time when animals are ordinarily handled (such as at weaning). Among the problems associated with this approach is the concern that for newly weaned pigs, there may still be high levels of passive immunity present to interfere with the stimulation of immunity from vaccination. Therefore, the consequences of vaccinating at a time of greatest convenience versus the extra labor costs and stress to the animals of vaccinating at the most appropriate time to ensure vaccine efficacy must be considered.

**Manipulating Passive Immunity.** Spray-dried animal plasma has been widely used in diets for newly weaned pigs and is associated with increased growth rates in the order of 27% (van Dijk et al. 2001). The mode of action of spray-dried animal plasma is not fully understood but is assumed to be at least partly due to the presence of immunoglobulins, which may provide a certain level of protection to the newly weaned piglet at a time when the supply of immunoglobulins from sow’s milk has ceased. The ability of plasma proteins to neutralize the effect of specific organisms is dependent on the immunizations and disease history of the pigs from which the blood is collected.

Specific antibodies from chicken egg yolk have been examined as a source of passive immunity for newly weaned pigs as well. Laying hens are vaccinated against specific pig pathogens such as _E. coli_. Antibodies are secreted into the yolk of the egg (IgY) in large quantities (up to 200 mg/egg) (Marquardt and Li 2001), and dried yolk is incorporated in nursery pig rations. Trials using specific egg-yolk products to prevent postweaning _E. coli_ diarrhea have produced inconsistent results (Chernysheva et al. 2004). Stability of the product during feed processing and passage through the pig’s gastrointestinal system are major concerns.

**OTHER THERAPEUTICS**

**Direct-Fed Microbials (Probiotic)**

Probiotics are defined as live microbials provided in the feed in an attempt to encourage proliferation in the intestine of the specific microorganism fed with the objective of providing health benefits to the host animals (Fuller 1989). The most commonly used probiotics include species of _Lactobacillus, Enterococcus, Bifidobacterium_, and _Saccharomyces_ (Alvarez-Olmos and Oberhelman 2001; Holzapfel et al. 2001; Rolfe 2000). Most studies involving probiotics have concentrated on improving intestinal health, particularly during the weaning period when the pig gut microflora undergoes dramatic change.

It is generally accepted that with careful attention to the criteria used to select the particular probiotic strain, there may be a place for probiotics in prevention of enteric disease, but results to date are inconsistent. There are a number of criteria that potential probiotic strains must meet in order to be considered for use as a probiotic, including the ability to demonstrate predictable and measurable health benefits. The screening and selection of a probiotic includes testing in vitro or in vivo of the following criteria:

- It must be nonpathogenic and proven safe.
- It must have stability in an acid environment, in the presence of bile, and resistant to degradation by digestive enzymes.
It must adhere to gut epithelial tissue and be able to persist in the gastrointestinal tract of the host.

In addition, the microbials used as probiotics must retain viability and stability during commercial production, feed processing, storage, and delivery, and must be cost-effective.

The main mechanisms whereby probiotics exert protective or therapeutic effects are not fully understood, but several ways have been postulated. Probiotics produce antimicrobial substances such as organic acids, fatty free acids, ammonia, hydrogen peroxide, and bacteriocins (Alvarez-Olmos and Oberhelman 2001). In addition, probiotics may enhance specific and non-specific host immunity (Kailasapathy and Chin 2000), and probiotics may prevent colonization of pathogenic microorganisms by competitive inhibition for microbial adhesion sites.

Inconsistent findings have been observed when probiotics have been used in trials to control pig disease or improve growth performance (Conway 1999). It is unlikely they will be capable of replacing antibiotics in the control of disease, but they may have a place alongside other techniques for improving the health of the gut microflora and reducing the shedding of pathogens such as Salmonella.

There is considerable interest in the use of fermented liquid feed, and there appears to be an association between its use and a reduction in Salmonella prevalence (van der Wolf et al. 2001). A possible explanation for the beneficial effect of fermented liquid feed is that the reduced pH of the diet and the presence of large numbers of organic acid-producing bacteria in the feed have a positive effect on the gut microflora and create an environment unsuitable for Salmonella and other coliform bacteria.

**Bacteriophages**

Bacteriophages or phages are bacterial viruses that invade bacterial cells and, in the case of lytic phages, disrupt bacterial metabolism and cause the bacterium to lyse (Sulakvelidze et al. 2001). From a clinical standpoint, phages appear to be innocuous, do not attack normal gut flora, and are extremely common in the environment. In spite of all the positive properties of lytic phages, they are not commonly used prophylactically or therapeutically. Recently, naturally occurring phages with activity against Salmonella species and E. coli were isolated from swine feces and this may facilitate the development of a direct application to enhance food safety (Callaway et al. 2011).

**Nutrients**

There is a plentiful supply of physiologically active feed ingredients that can improve pig performance and health by modifying the environment of the digestive tract (Pettigrew 2003). Zinc oxide added to nursery rations at a level of 2500 ppm for 2 weeks will result in increased growth rate and reduced prevalence of diarrhea (Jensen-Waern et al. 1998). In vitro studies have shown that zinc has antimicrobial effects, but in vivo studies show no reduction in E. coli numbers and no change in function of circulating neutrophils. There are concerns that high levels of zinc oxide will cause liver toxicity if fed longer than 3–4 weeks.

Likewise, copper sulfate at levels of up to 250 ppm has been added to pig feed to promote growth. However, the combination of zinc and copper does not result in an additive growth response (Hill et al. 2000). In the case of both copper and zinc, there are environmental concerns regarding their use because of the accumulations of these minerals in manure. The development of resistance is not specific to antimicrobials, and a transferrable copper resistance gene has been demonstrated in the fecal enterococci of swine in the United States.

The quest for alternatives to antibiotics in pig feed has caused interest in natural remedies, including herbs, spices, botanicals, and essential oils. These products may improve performance by improving feed palatability and by exerting antibacterial effects, but there needs to be further evidence of their effectiveness (Pettigrew 2003).

Organic acids (fumaric, formic, and lactic) are commonly added to feed or water in order to improve growth and reduce diarrhea during the postweaning period (Tsiloyiannis et al. 2001). Modes of action claimed for the growth-promoting effect of organic acids include decreased gastric pH, reduced coliform population, stimulated pancreatic exocrine secretion, increased pepsin activation, altered gut morphology, and improved intake and digestibility (Partanen 2001).

Response to acidification has been variable and may be attributed to feed and animal factors as well as differences in the properties of the various organic acids. Two problems that are associated with the use of high levels of organic acids are the acids may have a negative effect on palatability and the feed is corrosive to cement and steel in swine housing (Canibe et al. 2001).

An alternative approach to altering the gut microflora is to feed nondigestible material that provides a substrate for beneficial bacteria such as lactic acid-producing bacteria. These products are often referred to as prebiotics. In order for a feed to be classified as a prebiotic, it must be neither hydrolyzed nor absorbed in the upper part of the gastrointestinal tract, be a selective substrate for one or a limited number of potentially beneficial commensal bacteria, and induce luminal or systemic effects that are beneficial to the host’s health (Roberfroid 2001). Nondigestible oligosaccharides are the most common type of prebiotics, including fructo-oligosaccharides and mannan-oligosaccharides. In general, prebiotics are considered...
to provide small but positive improvements in growth rate. However, their role in providing health benefits, such as reducing *Salmonella* shedding, needs to be clarified.

Enzymes added to feed to encourage improved feed efficiency and, in some cases, potential health benefits are used widely. For example, feed can be supplemented with phytase to allow swine to digest plant phosphorus that is in the form of phytate. It has been hypothesized that the use of enzymes may allow the industry to utilize coarse feed particle size as a means of reducing gastric ulcers and the prevalence of *Salmonella* while still maintaining acceptable feed conversion. There is a possibility that in-feed antimicrobials for growth promotion could be reduced through a combination of feeding manipulations, such as the use of various combinations of liquid feeds or coarse particle size, enzymes, probiotics, prebiotics, and acidifiers.

**Antiparasitics**

In modern confinement operations, there are few parasitic problems (Roepstorff and Jorsal 1989). Under conditions of good hygiene and management, the regular application of anthelmintics may be of little or no benefit (Roepstorff 1997). Ascariasis is generally the main concern, and strategic medication with a wide range of effective products can easily control this parasite.

External parasitism caused by mange mites and lice no longer needs to be a significant problem because of good husbandry practices and effective drugs, particularly the avermectins. Failure to control sarcotic mange or lice infestation is generally due to a poor understanding of the epidemiology of the organisms and apathy on the part of the herdsman (Cargill et al. 1997). Antiparasitic products and their application are presented in Table 9.5.

**Hormones**

Oxytocin is widely used as an aid in stimulating parturition and milk letdown. Prostaglandin F₂α, or a synthetic analog can be used to induce parturition. Puberty can be induced in gilts by treatment with a single injection of 200 IU of human chorionic gonadotropin and 400 IU of equine chorionic gonadotropin. The injection of follicular stimulating hormone at weaning and an injection of luteinizing hormone approximately 72–80 hours later have been shown to induce a predictable ovulation (Barnabe et al. 2002) and can be used in artificial insemination programs where these hormones are licensed for this purpose. Estrus can be synchronized by administering a progestin for 14–18 days. The progestin inhibits follicular maturation until the progestin is withdrawn. Further details are presented in Chapter 20 “Reproductive System.”

Hormones are also used in certain countries for growth manipulation. Daily injection of porcine somatotropin (PST) strongly influences feed efficiency, growth, and carcass composition. Ractopamine, a phenethanolamine or β-agonist, is used as a feed additive in several countries. Its function is as a repartitioning agent causing improved feed efficiency and a lean carcass at slaughter.

**Anti-inflammatory Drugs**

Anti-inflammatory drugs including ketoprofen, flunixin meglumine, dexamethasone, indomethacin, and meloxicam have been evaluated in pigs as interventions for various clinical conditions with variable results that mostly suggest more investigation is needed. Meloxicam (Georgoulakis et al. 2006) was demonstrated to reduce the prevalence of clinical signs and the need for retreatment in pigs with respiratory disease when used as an adjunct to antimicrobial treatment. Additionally, flunixin has an approved label indication for control of pyrexia associated with swine respiratory disease.

**REFERENCES**


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**Table 9.5.** Common swine anthelmintics and doses

<table>
<thead>
<tr>
<th>Product</th>
<th>Dosage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dichlorvos</td>
<td>11.2–21.6 mg/kg body weight in feed with 1/3 of regular ration</td>
</tr>
<tr>
<td>Doramectin</td>
<td>300 μg/kg body weight IM</td>
</tr>
<tr>
<td>Fenbendazole</td>
<td>9 mg/kg body weight over 3–12 days via feed</td>
</tr>
<tr>
<td>Ivermectin</td>
<td>300 μg/kg SQ or 100 μg/kg body weight for 7 days via feed</td>
</tr>
<tr>
<td>Piperazine</td>
<td>275–440 mg/kg body weight in feed or water</td>
</tr>
<tr>
<td>Pyrantel tartrate</td>
<td>22 mg/kg of body weight as 1-day treatment or 96 g/t of feed as prophylactic dose</td>
</tr>
</tbody>
</table>
Scrutiny regarding the humane care of livestock raised for food and other commercial uses has increased dramatically during the past decade. This particularly pertains to procedures that inflict pain or distress. Surgical procedures performed on pigs are especially sensitive to the public because these procedures inherently cause stimuli that can be painful or distressful. Although the general public does not share the same values as animal rights groups, the consumer does share a desire that animals used for the production of food and other products are raised without undue stress and using humane practices. Thus, the swine surgeon must promote “best practices” in order to ensure that consumer confidence is maintained.

Surgical procedures increasingly conflict with economic goals. The veterinarian must critically evaluate economic benefit versus cost and prognosis of surgery. In most scenarios, services offered by the veterinarian are directed to the need of the enterprise. Surgery on an individual pig is not always cost-efficient. However, pigs selected for genetic improvement, show pigs, and pet pigs as well as others, may have significant individual value, and surgery may be performed with little regard to costs. Some spontaneous conditions such as inguinal and umbilical hernias, rectal prolapse, dystocia, and rectal stricture that can occur in large numbers of animals can be very costly and need to be investigated so that treatment and prevention solutions can be applied. In a commercial swine operation, the veterinarian is often a teacher, showing the manager and experienced personnel how to perform some minor surgical procedures during baby pig processing (castration, ear notching, teeth clipping, tail amputation) in a cost-effective fashion. It is the veterinarian’s role to make sure that these procedures are done properly and humanely.

Among purebred pigs, pet pigs, or pigs used as animal models in biomedical research, the individual animal may be of high perceived value, and surgery under conditions of idealized anesthesia and analgesia may be required. A veterinarian who is able to offer excellent surgical service to swine producers may have greater credibility as a herd consultant. The purpose of this chapter is to describe clinical swine anesthesia, routine surgical procedures done during baby pig processing, hernia repair, and some common surgical procedures of the digestive, urogenital, and musculoskeletal systems in swine.

**ANESTHESIA**

Minor surgical procedures (ear notching, teeth clipping, tail amputation, castration) in young swine are often performed without anesthetizing the animal. Performed skillfully, these surgical procedures are tolerated well by young pigs without an anesthetic. However, public scrutiny of management practices increasingly demands attention to pain and prevention of unnecessary distress. Management of swine anesthesia presents some difficulties. Swine resist mechanical restraint more and are usually more difficult to hold than other species. When possible, adult swine should be held off feed for 24 hours before general anesthesia, whereas piglets, which are prone to hypoglycemia, should be held from suckling for only 1–2 hours before anesthetic induction.

Malignant hyperthermia or porcine stress syndrome is a heritable condition in swine that can be triggered...
in a susceptible pig by any stress and many injectable (acepromazine, ketamine, succinyl choline) and inhalant anesthetics. Susceptible pigs are extremely muscular and usually have reduced subcutaneous fat. Halothane traditionally has been incriminated, but delayed onset of malignant hyperthermia can occur with exposure to isoflurane (Wedel et al. 1993). The clinical signs of malignant hyperthermia can be any of the following: a severe increase in body temperature, muscle rigidity, tachycardia, tachypnea, hypoxemia, cardiac arrhythmias, unstable blood pressure, and myoglobinuria. Death of an affected animal appears to be the result of peripheral circulatory changes that are produced by severe acidosis, vasoconstriction, hyperkalemia, decreased cardiac output, and hypotension. This susceptibility to malignant hyperthermia is due to an autosomal recessive defect in the gene that codes for the ryanodine receptor calcium channel in skeletal muscle (Rosenberg and Fletcher 1994). In response to a trigger, intracellular calcium rises abruptly, which causes muscle contracture and release of heat. Treatment of malignant hyperthermia is largely symptomatic. Early recognition is the key to successful treatment. Whenever malignant hyperthermia is suspected, volatile anesthetics should be discontinued. Aggressive cooling should be instituted using iced packs and alcohol baths. Dantrolene sodium (Dantrium) is effective in the treatment of a malignant hyperthermia or as a prophylaxis when given before the anticipated trigger. Suggested doses for swine are 1–3 mg/kg IV for treatment and 5 mg/kg orally given prophylactically.

Anticholinergics (atropine and glycopyrrolate) are recommended before sedation and anesthetic techniques in pigs. Atropine sulfate (0.04 mg/kg IM) or glycopyrrolate (0.02 mg/kg IM) will decrease the risk of bradycardia, excessive salivation, bronchoconstriction, and excessive airway secretion. In healthy swine receiving light surgical anesthesia, administration of supportive fluids is not considered necessary, unless the animal was off feed and water for more than 24 hours. However, long general anesthesia is best managed with intravenous fluid support. In swine that are hypovolemic or showing other signs of shock, fluid therapy is essential preferably before or during anesthesia. Fluid therapy is best administered in the ear vein (Figure 10.1).

In the authors’ experience, fluid therapy using balanced electrolyte solution before and during anesthesia is essential during cesarean section with dead piglets in utero. Anesthetic considerations for swine cesarean are important. One needs to remember that sow–fetal drug distribution is quite complete. Therefore, it must be assumed that any drug in the maternal circulation reaches the fetus rapidly in relatively high concentration. Because the fetal blood–brain barrier is extremely permeable, these drugs exert a profound anesthetic effect on the fetus. Also, anesthesia tends to persist after delivery because of the neonate’s poorly developed liver enzymes and renal function. Anesthetic agents should be chosen that will minimize fetal depression. General anesthesia will induce greater neonatal depression than regional anesthesia.

**Injectable Anesthetics**

Injectable agents are most appropriate for field use. With injectable agents, a minimum of equipment is needed. The drugs can be transported easily to the animal, compared with inhalation anesthetics, which are more expensive and can be difficult to transport to a field situation. Pigs have few superficial veins and arteries suitable for catheter placement and intravenous drug administration (Sakaguchi et al. 1996). Also, variation in accessibility of these vessels exists among swine breeds. The auricular (ear) vein is the safest and most accessible vein. It is usually located along the caudal margin of the ear. To inject or place a catheter in the ear vein, the pig can be restrained. The vein is held at the base of the ear by the fingers, forceps, or a rubber band to distend it. Rubbing the ear with alcohol and vigorous massage creates better visualization for needle insertion. Inserting a small catheter (20 gauge) will allow the administration of fluid or injection of additional anesthetic solution intravenously. The medial saphenous vein can be catheterized easily in the anesthetized or well-restrained pig (Figure 10.2). Anesthesia can be induced using intramuscular drug protocols or gas anesthetics via face mask followed by
hepatic enzymatic capacity of adults, pentobarbital should not be used as a general anesthetic in young piglets. In adults, an intravenous dose of 10–30 mg/kg will provide 20–30 minutes of relatively safe anesthesia, provided a patent airway is established and maintained until swallowing and other airway protective reflexes have returned. Preanesthetic medication (e.g., diazepam, acepromazine, xylazine) will decrease the amount of pentobarbital required for surgical anesthesia (Table 10.1). Recovery from pentobarbital administration is often prolonged and requires close patient surveillance. For intratesticular injections, 45 mg/kg body weight of a 30% solution is injected below the tail of the epididymis in the upper one-third of each testicle. The maximum volume is 20 mL per testicle, the onset of anesthesia is in 10 minutes, and recovery takes 20–40 minutes. Castration must be performed as quickly as possible to prevent continued absorption of the drug and a potentially lethal overdose (Henry 1968). The testes must be disposed of in a safe place. There are reports of fatal poisoning of dogs as a result of eating testes containing the residual drug. Blood on the floor is also dangerous (Henry 1968). The shorter-acting barbiturate thiopental (10–20 mg/kg) is used sometimes for induction before inhalation anesthesia or for very short procedures. A 2.5–5% solution should be used, and one-half of the calculated dose should be quickly injected. When the pig lies down, incremental amounts are injected until the desired plane of anesthesia is achieved. Apnea is often observed, and means of assisting ventilation should be readily available.

Acepromazine. Acepromazine decrease spontaneous motor activity. Used alone, it usually provides only slight inconsistent sedation in swine. Acepromazine will also predispose to hypotension and hypothermia and should not be used in debilitated pigs. The maximum dose recommended is a total of 10 mg. Acepromazine has been reported to decrease the incidence of malignant hyperthermia (Moon and Smith 1996). It is useful when combined with ketamine or tiletamine–zolazepam (Table 10.1).

Benzodiazepines (Diazepam and Midazolam). Diazepam (1–2 mg/kg IM) can be used in combination with ketamine or xylazine (Table 10.1). Midazolam can be absorbed more rapidly and completely than diazepam, because it is water soluble. They both ensure a smooth recovery and have a longer effect than xylazine. The expense associated with these drugs may make them impractical in some swine commercial operations.

Azaperone. Azaperone is a neuroleptic agent and can be given to tranquilize or immobilize swine (Table 10.1). The degree of sedation is dose dependent and should not exceed 1 mg/kg in large boars, because priapism has been reported (Moon and Smith 1996).
Azaperone must be given intramuscularly, because intravenous injection often results in excitation. Excessive salivation, hypothermia, sensitivity to noise, and hypotension have been seen in pigs receiving azaperone (Greene 1979). Azaperone is not an analgesic and is often used in combination with other drugs for surgical procedure. Deep tranquilization from azaperone should be obtained before ketamine is given. If azaperone is used alone for surgical procedures, local or regional anesthesia should be administered.

**Alpha-2 Receptor Agonists.** Swine are fairly resistant to xylazine compared with other meat-producing animals (Table 10.1). Sedation will result, but animals are aroused easily. Xylazine is usually used in combination with other drugs to produce good muscle relaxation and a smooth recovery. Vomiting has been seen following the use of xylazine in pigs with digestive disturbances. Medetomidine is a more potent alpha-2 agonist than is xylazine (Sakaguchi et al. 1992). Medetomidine in combination with atropine induces deeper sedation than does xylazine and its effects are enhanced by butorphanol. The anesthetic state is characterized by profound somatic analgesia, but visceral analgesia is poor. Medetomidine, butorphanol, and ketamine induce excellent surgical anesthesia in pigs (Table 10.1). This anesthetic regimen can be reversed by atipamezole (240 $\mu$g/kg), a selective and potent alpha-2 antagonist.

**Table 10.1. Injectable anesthetic agents for swine**

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose</th>
<th>Route</th>
<th>Onset (minute)</th>
<th>Duration (minute)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pentobarbital</td>
<td>10–30 mg/kg</td>
<td>IV</td>
<td>1–10</td>
<td>15–45</td>
</tr>
<tr>
<td></td>
<td>45 mg/kg</td>
<td>Each testicle</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Thiopental</td>
<td>10–20 mg/kg</td>
<td>IV</td>
<td>Immediate</td>
<td>2–10</td>
</tr>
<tr>
<td>Acepromazine</td>
<td>0.1–0.5 mg/kg</td>
<td>IM</td>
<td>20–30</td>
<td>30–60</td>
</tr>
<tr>
<td>Acepromazine and Ketamine</td>
<td>0.4 mg/kg</td>
<td>IM</td>
<td>5</td>
<td>15–30</td>
</tr>
<tr>
<td>Acepromazine and Ketamine</td>
<td>15 mg/kg</td>
<td>IM</td>
<td>2–4</td>
<td>40–50</td>
</tr>
<tr>
<td>and Telazol</td>
<td>0.03 mg/kg</td>
<td>IM</td>
<td>2</td>
<td>40–50</td>
</tr>
<tr>
<td></td>
<td>2.2 mg/kg</td>
<td>IM</td>
<td>7–10</td>
<td>20–40</td>
</tr>
<tr>
<td>Diamorphine and Ketamine</td>
<td>1–2 mg/kg</td>
<td>IM</td>
<td>10</td>
<td>20–40</td>
</tr>
<tr>
<td>Midazolam and Ketamine</td>
<td>0.1–0.5 mg/kg</td>
<td>IM</td>
<td>5–10</td>
<td>20–40</td>
</tr>
<tr>
<td>Xylazine</td>
<td>2–8 mg/kg</td>
<td>IM</td>
<td>5–15</td>
<td>60–120</td>
</tr>
<tr>
<td>Xylazine and Ketamine</td>
<td>0.5–3 mg/kg</td>
<td>IM</td>
<td>5</td>
<td>10</td>
</tr>
<tr>
<td>Xylazine and Ketamine and Telazol</td>
<td>2 mg/kg</td>
<td>IM</td>
<td>7–10</td>
<td>30–60</td>
</tr>
<tr>
<td>Xylazine and Ketamine and Tramadol</td>
<td>25 mg/kg</td>
<td>IM</td>
<td>7–10</td>
<td>30–60</td>
</tr>
<tr>
<td>Xylazine and Ketamine</td>
<td>4.4 mg/kg</td>
<td>IM</td>
<td>1–2</td>
<td>60</td>
</tr>
<tr>
<td>Xylazine and Ketamine and Telazol</td>
<td>2.2 mg/kg</td>
<td>IM</td>
<td>5–10</td>
<td>70–100</td>
</tr>
<tr>
<td>Xylazine and Ketamine and Midazolam</td>
<td>0.25 mg/kg</td>
<td>IM</td>
<td>5–10</td>
<td>70–100</td>
</tr>
<tr>
<td>Medetomidine and Butorphanol and Ketamine</td>
<td>80 $\mu$g/kg</td>
<td>IM</td>
<td>1–5</td>
<td>60–120</td>
</tr>
<tr>
<td>Medetomidine and Butorphanol and Ketamine</td>
<td>200 $\mu$g/kg</td>
<td>IM</td>
<td>1–5</td>
<td>75–120</td>
</tr>
<tr>
<td>Xylazine and Butorphenol and Ketamine</td>
<td>200 $\mu$g/kg</td>
<td>IM</td>
<td>1–5</td>
<td>60–120</td>
</tr>
<tr>
<td>Xylazine and Butorphenol and Ketamine</td>
<td>2 mg/kg</td>
<td>IM</td>
<td>1–5</td>
<td>60–120</td>
</tr>
<tr>
<td>Propofol and Fentanyl</td>
<td>11 mg/kg/h</td>
<td>IV</td>
<td>Immediate</td>
<td>Continuous infusion</td>
</tr>
<tr>
<td></td>
<td>2.5 mg/kg</td>
<td>IV</td>
<td>q30</td>
<td></td>
</tr>
</tbody>
</table>

Ketamine. Ketamine induces rapid onset of unconsciousness (Thurmon 1986). The anesthetic state is characterized by profound somatic analgesia, but visceral analgesia is poor. Ketamine has been used as a major component of many regimens to induce chemical restraint or anesthesia in pigs. Ketamine can be given intramuscularly, intravenously, or intratesticularly. Intratesticularly, a combination of ketamine (6 mg/kg) and xylazine (2 mg/kg) has been used successfully for castration (Thurmon 1986). When ketamine is used alone, it exerts some undesirable effects, such as poor muscle...
relaxation and analgesia emergence delirium, tachycardia, and hypertension. Ketamine is commonly combined with a muscle relaxant or sedative such as acepromazine, diazepam, xylazine, or droperidol.

**Tiletamine–Zolazepam (Telazol).** Telazol provides some muscle relaxation and sedation and also immobilizes swine (Moon and Smith 1996). Telazol requires a smaller volume of injectable compared with ketamine. Telazol is frequently combined with xylazine or acepromazine to provide better muscle relaxation and an easier recovery.

**Propofol.** Propofol has recently been used for intravenous anesthesia in pigs (Martin-Cancho et al. 2004). One dosage regimen reported was 11 mg/kg body weight/h for abdominal surgery. This was done in combination with fentanyl (2.5 mg/kg IV q30 min). Compared with pigs anesthetized with isoflurane, propofol-anesthetized pigs require a significantly longer time to recover consciousness.

**Guaifenesin.** Guaifenesin is a centrally acting muscle relaxant. Because it produces little analgesia, it should not be used alone. Intravenous infusion of guaifenesin combined with thiobarbiturates and with ketamine and xylazine has been used for induction and maintenance of anesthesia (Thurmon 1986). The authors recommend adding 500 mg of ketamine and 500 mg of xylazine to each 500 mL of 5% guaifenesin in 5% dextrose in water. The mixture is given rapidly in a catheter in the ear vein at a dose of 0.5–1 mL/kg for induction. Anesthesia is maintained by continuous infusion at a rate of 2.2 mg/kg/h. At the end of the surgery, recovery time may be hastened by administration of yohimbine (0.125 mg/kg) or tolazoline (2.5–5 mg/kg) to reverse the effect of xylazine (Thurmon 1986).

**Tramadol.** Tramadol has recently been reported as a beneficial drug for combination anesthesia in pigs (Table 10.1). Tramadol is an analog of codeine and has activity with opioid receptors. Tramadol acts as an agonist on μ-opioid receptors as well as stimulates the release of serotonin. As such, it is useful in mitigating moderate to severe pain. In combination with xylazine and ketamine in young piglets, tramadol increased the pain threshold of anesthetized piglets without having adverse effects on the duration of anesthesia or physiological parameters (Ajadi et al. 2009).

**Combinations of Injectable Anesthesia.** Combinations of injectable drugs have been used to increase quality, duration, and analgesia of anesthesia when they can be administered safely. A recent study compared three regimens for maintaining anesthesia in pigs induced using azaperone (1 mg/kg IM) and ketamine (2.5 mg/kg IM). These pigs were divided into three groups: (1) etomidate (200 µg/kg IV) and midazolam (100 µg/kg IV); (2) ketamine (2 mg/kg IV) and medazolam (100 µg/kg IV); and (3) pentobarbitone (15–20 mg/kg IV) (Clutton et al. 1997). Pentobarbitone provided the least satisfactory anesthesia because of profound respiratory depression, difficulty completing orotracheal intubation, and prolonged time to standing. Another study compared metedomidine–butorphenol–ketamine (MBK) (80 µg/kg–200 µg/kg–10 mg/kg, respectively, all intramuscularly) with xylazine–butorphenol–ketamine (X BK) (2 mg/kg–200 µg/kg–10 mg/kg, respectively, all intramuscularly) (Sakaguchi et al. 1996). MBK was found to provide longer and more satisfactory anesthesia as compared with XBK.

**Reversal Agents.** On occasion, reversal of anesthesia may be necessary. Yohimbine is an alpha-receptor antagonist that is commonly used to reverse the effects of xylazine. However, the selective nature of this drug in the reversal of alpha-agonists can be difficult to apply when combination anesthesia has been employed. In a study on the effectiveness of yohimbine to speed recovery of pigs anesthetized using tiletamine + zolazepam + xylazine, pigs were shown to recover more quickly when yohimbine was administered (Kim et al. 2007). In that study, pigs were given a combination of either xylazine + zolazepam or xylazine + tiletamine. Pigs attained sternal recumbency in significantly less time (52 minutes) when yohimbine was administered compared with pigs that did not receive yohimbine (76 minutes).

**Inhalation Anesthetics**

For debilitated swine, surgical procedures lasting more than 30 minutes, difficult procedures, or for valuable swine, an inhalation anesthetic provides a more controlled plane of anesthesia. For economic reasons, halothane is the most commonly used inhalation anesthetic in swine. Halothane possesses physical properties and potency consistent with rapid induction, alteration of anesthetic depth, and recovery from anesthesia. Inhalation anesthetics can be administered to small pigs by an open or semiopen method; in larger swine, the semiclosed or closed system is preferred. Swine weighing up to 140 kg can be anesthetized with an anesthetic machine designed for small animals (Tranquilli 1986).

Selection of induction technique and anesthetic protocol depends on the size and special needs of the swine, preference of the veterinarians, and availability of drugs and equipment. Sedation is desirable to reduce the stress of physical restraint before induction of anesthesia (Table 10.1). Swine up to 100 kg can be restrained in a webbed stanchion. Large swine can be restrained in a crate with a head catch or by a snare. Induction of anesthesia can be accomplished with a rapid bolus of injectable agent (barbiturate), by rapid infusions of a combination of drugs (Table 10.1), or by using a face mask.
mask delivering a high concentration of anesthetic agent (5% halothane). Tracheal intubation assures a patent airway for delivery of anesthetic and protects the airway from aspiration pneumonia. In the authors’ experience, facial, pharyngeal, and laryngeal anatomy of the pig makes endotracheal intubation more challenging than in other meat-producing species. It is often difficult to open the jaws wide enough for good laryngeal exposure, and the pig has a small narrow larynx that deviates ventrally, creating a sharp angle from the pharynx to the tracheal opening. Also, laryngeal spasms occur frequently and are induced easily. Occurrence of spasms can be reduced by achieving a sufficient depth of anesthesia prior to intubation or by spraying the larynx with lidocaine.

Following induction, the pig should be placed in sternal recumbency, and the jaws should be held open with small rope. Orotracheal intubation has been shown to be significantly easier when pigs are in sternal recumbency as compared with dorsal recumbency (Theisen et al. 2009). Pigs in sternal recumbency were intubated by experienced personnel in a mean of 17 seconds as compared with 58 seconds for pigs in dorsal recumbency. The tongue is pulled forward by an assistant. A laryngoscope and blades of different lengths are needed. For adult swine, the blade length must be at least 25 cm. The blade of the laryngoscope is placed at the base of the tongue, and downward pressure is applied until an unobstructed view of the larynx is provided. Endotracheal tubes should be available in sizes from 3 to 20 mm outside diameter and lengths of 25–50 cm. A malleable metal rod with the first 5 cm bent at a 30° angle is placed inside the endotracheal tube to act as a guide. With the laryngeal opening visualized, the endotracheal tube with the stylet extending slightly beyond the tip is placed into the laryngeal opening. The endotracheal tube is pushed over the tip of the stylet and with a twisting motion is passed through the larynx into the trachea. The tracheal diameter is surprisingly small in the pig. A 50-kg pig often requires only a 7- to 9-mm tube, and a 10- to 14-mm tube is often adequate for adult sows (Tranquilli 1986). In some cases, orotracheal intubation may not be possible. The authors’ have used face masks as the sole means of administration of oxygen and gas anesthesia for various surgical procedures when tracheal intubation could not be achieved (Figure 10.1).

This practice is not encouraged because of the inability to protect the airways and the possibility of respiratory distress or obstruction if dorsal displacement of the soft palate occurs. However, face mask can be effective for short procedures of less than 60 minutes.

Safe maintenance of inhalation anesthesia requires knowledge of the signs associated with anesthetic depth and continual monitoring of the patient and anesthetic equipment. Routinely monitored signs should include pulse quality and rate, respiratory rate, color of mucous membranes, capillary refill time, blood pressure, and electrocardiogram. The body temperature should be evaluated regularly, and appropriate padding should be placed. The pulse can be palpated over the median auricular artery. Direct auscultation of the heart should also be done. The normal heart rate in swine ranges from 60 to 90 beats per minute and may vary greatly during inhalation anesthesia. During recovery from inhalation anesthesia, frequent and careful monitoring is necessary, because life-threatening complications can occur (Moon and Smith 1996). Recovery should be in a quiet place, and the pig should be placed in sternal recumbency as soon as possible. The endotracheal tube should be maintained until the pig is spontaneously moving its head or will not tolerate the tube. The pig should not be returned to the herd until it is fully awake.

**Local Anesthesia**

The use of local anesthesia without additional chemical restraint is limited in swine. Pigs, even in the absence of pain, will resist physical restraint by continuing to struggle. In addition to chemical restraint, infiltration of 2% lidocaine around the surgical site will facilitate surgery involving the skin and superficial underlying tissues. Local infiltration of lidocaine is used commonly for surgical repair of umbilical and inguinal hernias and scirrhous cord removal.

**Epidural Regional Anesthesia**

Lumbosacral epidural anesthesia is the most commonly used form of regional analgesia in swine (Figure 10.3) (Skarda 1996).

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**Figure 10.3.** The injection site (needle placement) for a lumbosacral anesthesia in swine. L6 is the sixth lumbar vertebra and S1 is the first sacral vertebra.
Minimal equipment and expense are necessary to perform the procedure during epidural anesthesia. Compared with general anesthesia, the pig is in an awake state so the risk of aspiration pneumonia is minimal. Local infiltration of lidocaine compared with epidural anesthesia has several disadvantages. Infiltration requires a larger amount of lidocaine and can retard wound healing and muscle relaxation, and analgesia is not as profound. Lumbosacral epidural anesthesia is relatively easy to perform and greatly facilitates cesarean section; repair of rectal, uterine, or vaginal prolapses; repair of hernia; and surgery of the prepuce and penis or rear limbs (Skarda 1996). Lumbosacral epidural anesthesia should be avoided in patients that are in shock or toxemic because of sympathetic blockade and consequent depression of blood pressure (Skarda 1996). General anesthesia may be more appropriate than regional anesthesia when the sow is very aggressive. Complications that may result from faulty techniques during lumbosacral epidural injection include cardiovascular and respiratory collapse after overdose or subarachnoid injection, meningitis associated with septic technique and tremor, and vomiting and convulsions after injection of the analgesic into the vertebral venous sinus.

The block can best be administered while the animal is standing and restrained with a hog snare, lariat, or head catch. Large hogs can be restrained by placing their heads in the head catch of a cattle chute. The site for injection for epidural anesthesia in the pig is the lumbosacral space. The conus medullaris of the cauda equina of the pig terminates in the region of the first or second sacral vertebra. The filum terminale terminates at the sixth or seventh coccygeal vertebra. Although the meninges extend beyond the lumbosacral articulation, there is only a very slight probability of entering the subarachnoid space. The lumbosacral space is on the midline and identified by drawing a line across the animal's back from tuber coxa to tuber coxa. This line will be just cranial to the point of the stifle joint (Skarda 1996). The line passes usually through the spinous process of the last lumbar vertebra. The injection site is usually 1–2 in. caudal to this transverse line (Figure 10.3). The site of injection is prepared by clipping or shaving the hair, thoroughly scrubbing the site with a surgical soap, and applying a skin antiseptic. The location is infiltrated with a local anesthetic agent prior to needle insertion. A 6- to 8-cm-long, 20-gauge needle is used for pigs up to 30 kg. A 10-cm-long, 18-gauge needle is used for pigs between 35 and 90 kg, and a 12- to 16-cm-long, 16-gauge needle for pigs over 90 kg. The needle is inserted with the bevel directed cranially and at an angle of 10° caudal to perpendicular between the last lumbar and first sacral vertebrae. The needle penetrates the skin, back fat, muscle, and then the fibrous interarticular spinous ligament. The needle passes through a definite area of resistance as it encounters the ligament and a slight pop is felt as the needle passes into the epidural space and drops to the floor of the spinal canal. The lumbosacral space is large in the pig (1.5 × 2.5 cm) and allows for a relatively large margin of error (Skarda 1996). Aspiration should be attempted before injection of the anesthetic to ensure that the subarachnoid space or a blood vessel has not been entered. Little resistance to injection will be encountered if the needle is located properly in the epidural space.

The anesthetic agent most commonly used is 2% lidocaine. The dose is calculated by either weight or length of the pig (Skarda 1996). Generally, a dose of 1 mL/9 kg body weight is adequate. Analgesia should be present within 10 minutes and last approximately 2 hours. A total dose of 20 mL must not be exceeded regardless of the weight. Four milliliters per 100 kg and 6 mL/200 kg body weight are sufficient for standing castrations (Skarda 1996). Ten milliliters per 100 kg, 15 mL/200 kg, and 20 mL/300 kg have given satisfactory results for cesarean section (Skarda 1996). If the pig is restrained in lateral recumbency, it is important that the head not be placed in extension. In swine with the head extended, the soft palate can occlude the airway, and the patient can suffocate (Benson 1986).

In 28- to 35-kg pigs, epidural injections of xylazine (2 mg/kg diluted in 5 mL of 0.9% NaCl solutions) at the lumbosacral intervertebral space induce immobilization, mild sedation, and regional anesthesia that extends from the anus to the umbilicus within 10 minutes and lasts at least 2 hours (Ko et al. 1992). The injection of a xylazine (1 mg/kg 10% solution) and lidocaine (10 mL 2% solution) combination into the lumbosacral epidural space has produced excellent anesthesia for cesarean in large sows (Ko et al. 1993). The forequarters in these sows were immobilized by intravenous injection of 0.003 mL/kg body weight of a mixture containing 50 mg of Telazol/mL, 50 mg of ketamine/mL, and 50 mg of xylazine/mL/kg body weight (Ko et al. 1993). In preparing the Telazol, ketamine, and xylazine combination, 2.5 mL of 10% ketamine (250 mg) and 2.5 mL of 10% xylazine (250 mg) were used as the diluent instead of sterile water. During the described surgical procedure, sows were bloused with 0.1–0.5 mL as necessary. A mean of 3 mL of this combination was given per sow in total, and the sows were quiet and immobilized for an average of 105 minutes (Ko et al. 1993). The sows were able to walk 12 hours after surgery, and the piglets were without signs of sedation or tranquilization. Intravenous tolazoline (2.2 mg/kg) partially reversed the Telazol-, ketamine-, and xylazine-induced sedation after surgery but did not antagonize the xylazine–lidocaine epidural effect (Ko et al. 1993). Epidural analgesia used in combination with general anesthesia allows a light plane of anesthesia with good muscle relaxation distal to the midthoracic region. Medetomidine (0.5 mg/kg diluted
in 5 mL of 0.9% NaCl solution) injected epidurally in the lumbosacral space of swine produced sedation but minimal analgesia caudal to the umbilicus. The onset of these effects occurred in 10 minutes, and they lasted for less than 30 minutes. Intravenous administration of atipamezole (0.2 mg/kg body weight) reversed epidurally administered detomidine-induced sedation and immobilization (Ko et al. 1992). Atipamezole had no effect on epidurally administered xylazine-induced sedation and immobilization (Ko et al. 1992).

PAIN MANAGEMENT

Pain management is often discussed in the context of surgical procedures that are commonly done without anesthesia. These are primarily done on piglets and involve castration, tail docking, and ear notching. In a survey of Canadian veterinarians’ practices regarding the use of analgesics in livestock, piglets received analgesics for castration in <0.001% of procedures as compared with 6.9% for beef calves and 18.7% of dairy calves <6 months old (Hewson et al. 2007). Pain management for performance of on-farm surgical procedures needs more education and research to define optimal guidelines for veterinarians and producers to follow. For an extensive discussion of pain detection and mitigation, the reader is referred to Chapter 3 “Behavior and Welfare” of this book.

GENITOURINARY SURGERY

Castration

Castration of male pigs is routinely performed with the intention to improve performance, feed conversion, and carcass traits (Kiley 1976). Also, management of castrated pigs through to finishing may be easier than for intact male pigs. With the onset of puberty, boar meat becomes tainted with an unpleasant odor and taste. We recommend that baby pigs be castrated at 2 weeks old or younger to minimize the stress of castration and maximize performance to weaning. Castration of 14-day-old pigs is done by suspending the pig by the hind limbs while laying the pig across a smooth rail. The surgical site is wiped clean with alcohol or 2% tincture of iodine. If used, lidocaine anesthetic is injected subcutaneously (0.5 mL per site), overlying each testicle, and over each spermatic cord (0.5 mL per site) in the inguinal canal. A 1-cm-long incision is made over each testicle and the testicles pulled from the scrotum. Hemorrhage is minimal at this age. Transfixation ligation of the spermatic cord is recommended for older pigs (see castration of older pigs). Topic anti-septic ointment or spray may be applied at this time. Subcutaneous tissues may be sutured with no. 0 chromic gut in simple continuous pattern to reduce dead space and minimize postoperative swelling. Skin sutures are placed in a Mayo (Ford) interlocking pattern. We prefer to administer antibiotics for 3 days, beginning the day of surgery, to reduce the incidence of postoperative infection. Also, the barrow should be kept in a clean, dry stall during this period.

Complications of Castration. The most common complications following castration of pigs are hemorrhage,
abscess, scirrhus cord, inguinal hernia, and seroma or hematoma formation. Fatal hemorrhagic shock has been reported after castration of 7-week-old pigs by a lay person (Libke 1967). The testicles had been pulled through a 10-cm incision and cut using a knife. Fatal hemorrhage occurred into the pelvic canal and abdomen; thus, the cause of death was not recognized until necropsy. This report emphasizes the need for routine necropsy examination of all deaths for which the cause is not apparent. Meat inspection of 131 pigs with postcastration abscesses revealed that Actinomyces pyogenes, alpha-hemolytic streptococci, Streptococcus viridans, Staphylococcus aureus, and Pasteurella multocida were the most common bacteria isolated (Százados 1985). Approximately 65% of the abscesses were monomicrobial and 35% were polymicrobial infections. Evidence for bacteremia and septicemia was found in 28% and 11%, respectively. Of the 131 pigs inspected, 11% were judged to be unfit for human consumption. Bilateral hydronephrosis has also been reported as a complication of castration in a Hampshire pig castrated at 8 weeks old (McGavin and Schoneweis 1972). A ventral midline incision was used to remove both testicles and tincture of iodine applied after castration. Infection of the soft tissues occurred and the ensuing infection resulted in progressive occlusion of urethra at the level of the sigmoid flexure. Chronic resistance to urine outflow caused hydronephrosis, and the pig died 4 weeks after castration. This case illustrates the importance of adequate ventral drainage after castration.

Unilateral Castration. Indications for removal of only one testicle include testicular trauma, hematoma, seroma, and orchitis or periorchitis (Becker 1986). The damaged testicle may cause enough swelling, heat, and pressure to reduce fertility. The boar is placed under general anesthesia, a 6-cm incision is made over the testicle starting at the most ventral aspect of the scrotum, and the testicle is removed by transection ligation and excision. The wound may be left open or closed. Strict asepsis and clean housing are required for closure of the wound to prevent abscess formation. We prefer to leave the wound open for second intention healing. Antiseptic ointment is placed in the defect, antibiotics are administered for 5–7 days, and daily hydrotherapy is used to minimize postoperative swelling. Affected boars may return to productive service 30–60 days after surgery.

Testicular Abnormality (Cryptorchidism, Testicular Atrophy, and Ectopic Testicle). Veterinarians may be presented with barrows demonstrating “boar-like” traits for removal of retained testicular tissues. The testicles of swine descend in the last 30 days of gestation and should be palpable at birth (Van Straaten et al. 1979). True cryptorchidism (testicle not descended at birth) is a common congenital defect in swine. A homozygous recessive trait involving two gene loci has been postulated based on a breeding trial of cryptorchid Duroc swine (Rothschild et al. 1988). Interestingly, the presence of cryptorchid piglets in utero has been associated with litter size and mortality (Dolf et al. 2008). In that study, litter size was found to increase with increasing number of cryptorchid piglets and the number of stillborn piglets was greater in litters having a cryptorchid pig present. A progeny study of Lancombe and Yorkshire true cryptorchid boars resulted in 10.9% and 31.4% of male progeny being cryptorchid. Of boars with “late-onset” cryptorchidism (normal at birth but having only one testicle at 42 days old), 3.8% of male progenies were cryptorchid. Cryptorchid testicles are usually intra-abdominal and are usually found midway between the ipsilateral kidney and the inguinal ring (Thornton 1972). However, the affected testicle may be located within the inguinal canal and not readily palpable from either the inguinal region or the peritoneal cavity (Lachmayr 1966). Previous removal of the descended testis makes surgical removal of the retained testis more difficult because the incision is best made over the affected inguinal ring. Often, determination of which testicle has been removed is difficult. Manual restraint and local anesthesia may be adequate for pigs less than 50 kg, but we prefer to perform cryotricorchid surgery with the pig under general anesthesia. A 6-cm incision is made over the appropriate inguinal ring. Laparotomy may be performed by making a 4-cm incision 1–2 cm medial to the inguinal canal (parainguinal incision), or the inguinal ring may be enlarged by starting the incision at the cranial commissure of the external inguinal ring. The fingers of one hand are used to perform an exploration of the abdominal cavity starting at the pelvic brim and searching along the dorsal and lateral abdominal wall until the kidneys are encountered. For show pigs, we prefer to perform laparoscopic exploration and removal of abdominal testes because better cosmesis, fewer incisional complications, and more rapid incisional healing are achieved. True cryptorchidism should be differentiated from testicular atrophy or degeneration (“late-onset” cryptorchidism) and ectopic testicular tissue. Pigs affected by testicular atrophy are reported to have palpably normal testicles at birth and weaning, but having only one testicle present at 42 days old. Of 122 cryptorchid studied, 21 had “late-onset” cryptorchidism. At slaughter, only one testicle can be found and, occasionally, a small mass of lymphoid tissue or epididymis is identified. Ectopic testicular tissue has been observed in numerous pigs at the time of slaughter (Todd et al. 1968). These tissues occur as smooth, pink or tan nodules on the surface of the liver, spleen, mesentery, and other abdominal viscera. Initially, these masses may be interpreted as metastatic neoplasia, but histology reveals the presence of convoluted seminiferous tubules and interstitial cells. No evidence for neoplasia
is seen. Ectopic testicular tissues may be found in castrated or intact male pigs.

**Prolapsed Penis**

Penile and preputial prolapse have been seen after administration of neuroleptic drugs, but may also occur as a result of trauma to the penis or congenital defect (Figure 10.5).

While prolapsed, the penis is at great risk of further injury. The penis and prepuce must be returned to their normal position as soon as possible after prolapse. Treatment of penile prolapse usually requires that the boar be placed under general anesthesia. The penis is thoroughly cleaned with cold water and a topical antiseptic ointment applied to the surface of the penis. If a penile wound is present, debridement may be done. Penile wounds are typically not sutured closed unless they have occurred recently (within 2–4 hours) because of the likelihood of formation of an abscess. The penis and prepuce are gently massaged until reduction into the sheath is completed. Use of hydroscopic agents such as anhydrous glycerine may help reduce the swelling by resolving edema. After the penis and prepuce have been repositioned, a purse-string suture may be used to prevent recurrence of the prolapse. The purse string should be removed in 5–7 days. If wounds or abrasions are present, daily preputial lavage or administration of systemic antibiotics and anti-inflammatory drugs is indicated. If wounds are not present, sexual rest should be enforced for at least 14 days. If wounds requiring treatment are present, sexual rest should be enforced for 30–60 days, depending on the severity of the wound. Reevaluation of the penile injury is advisable prior to use for mating.

**Preputial Diverticulum**

Abnormalities of the preputial diverticulum may cause reproductive unsoundness. Preputial diverticulitis, diverticular ulcers, diverticular stones, urine retention, and penile deviation into the diverticulum may be found (Dutton et al. 1997; Tyler et al. 2000; Wieringa and Mouwen 1983). Preputial diverticulectomy may restore breeding soundness to affected boars. The boar is placed under general anesthesia and prepared for surgery. Any of three procedures for diverticulectomy may be performed:

1. **Trans-preputial orifice method:** Preputial diverticulectomy via the preputial orifice is done by passing forceps through the preputial orifice, into one lobe of the bilobate diverticulum, gently evert ing the lobe out through the orifice, and repeating this procedure for the remaining lobe (Figures 10.6–10.8). After both lobes of the preputial diverticulum are everted, the diverticulum is excised. Suturing is not required for young boars, but the opening to the diverticulum may be sutured closed in adults.

2. **Combined trans-cutaneous and trans-preputial orifice method:** A 6-cm incision is made overlying the lateral aspect of one lobe of the preputial diverticulum. The diverticulum is everted through the preputial orifice, excised, and sutured closed.

3. **Trans-cutaneous lateral approach:** A 6-cm incision is made as above, but the diverticulum is dissected free from the surrounding soft tissues, excised, and sutured closed (Figure 10.9).
reduced by application of hydroscopic agents (anhydrous glycerin, saturated magnesium sulfate solution). A preputial retaining tube, constructed from rubber or polyurethane tubing, may be placed into the preputial space to prevent prolapse but allow exit of urine. Stay sutures are placed through the tubing and attached to the skin at the preputial orifice. Four sutures are placed 90° from each other suture to maintain the proper position of the tube. Alternatively, a 1.25-cm-diameter Penrose drain may be sutured to the tip of the penis (no. 2-0 chromic gut suture) to ensure urine outflow. Often, the prolapsed prepuce has been traumatized and surgical removal of the affected tissues is indicated. Preputial amputation may be performed, but the opening to the urethral diverticulum must be maintained. Alternatively, the preputial diverticulum may be removed at the time of surgery. The boar is placed under general anesthesia, the prepuce is pulled cranially until normal preputial epithelium is exposed, and stay sutures or crossed pins (7.6-cm, 18-gauge needles) are placed through the exposed prepuce to prevent premature retraction into the sheath. The damaged tissues are amputated, and the two layers of the prepuce are sutured closed using an interrupted suture pattern (no. 2-0 chromic gut, polydioxanone (PDS), or polyglycolic acid suture). After anastomosis, antiseptic ointment is placed on the prepuce and it is replaced into the sheath. A purse-string suture is placed at the preputial orifice for 7–10 days, and sexual rest is enforced for 30–60 days. Systemic antibiotics should be administered perioperatively.

**Vasectomy or Epididymectomy**

Vasectomy or epididymectomy is done to produce teaser boars used for heat detection in sows for artificial insemination. For methods 2 and 3 above, extreme care must be taken not to perforate the diverticulum prior to removal because contamination will result in incisional infection. Flushing of the preputial diverticulum with antiseptic solutions before surgery is recommended to reduce this possibility. Also, filling the diverticulum with antiseptic solution or gauze pads before surgery makes identification of the diverticulum easier at the time of surgery.

**Preputial Prolapse**

Prolapse of the prepuce may occur with penile prolapse or may result from preputial injury and swelling. If wounds to the prepuce are not present, the prepuce may be repositioned within the sheath, as described for penile prolapse, and a purse-string suture used to maintain the reduction (Schoneweis 1971). Careful evaluation of the preputial swelling should be done to ensure that urination is possible. Preputial edema may be
insemination or breeding to valuable boars, or to promote onset of cyclicity in confined gilts (Becker 1986; Godke et al. 1979). For vasectomy, the boar is placed in dorsal recumbency under general anesthesia, and a 4-cm incision is made over each spermatic cord approximately 6 cm cranial to the ventral aspect of the scrotum. Each spermatic cord is elevated and incised, and the vas deferens isolated. The vas deferens lies next to the spermatic artery, is firm and pale, and an arterial pulse is not present. A 3- to 4-cm segment of the vas deferens is excised, and each end is ligated. The incision through the tunica is sutured with no. 2-0 PDS or polyglycolic acid, and the skin is sutured with no. 0 polymerized caprolactam in a Mayo (Ford) interlocking suture pattern. An alternative technique for vasectomizing boars enables the surgery to be done with the boar in lateral recumbency (Althouse and Evans 1997b).

Epididymectomy may be done more quickly, more easily, and with similar results as vasectomy in boars (Althouse and Evans 1997a; Arkins et al. 1989). Epididymectomy is done by making a 2-cm incision in the scrotum overlying the tail of the epididymis. The tail and 1 cm of the body of the epididymis is isolated. Ligatures are placed between the testicle and the tail of the epididymis and around the exposed portion of the body of the epididymis. The epididymis is excised between these two ligatures. The skin is closed with no. 0 polymerized caprolactam in an interrupted pattern.

Persistent Frenulum
The epithelial attachment of the penis and prepuce atrophies and these tissues separate between 4- and 6-month-old boars. Sexual maturity is achieved by 7–8 months old. Persistence of the frenulum attachment between the penis and prepuce beyond sexual maturity causes failure of breeding soundness and has been observed in boars (Roberts 1986). Surgical removal of the persistent frenulum is performed with the boar under general anesthesia or during a hand mating exercise. Resection of the tissue may be performed with scissors. Ligation is not required in most cases and minimal bleeding is observed after excision. Sexual rest should be enforced for 7–10 days after surgery.

Vaginal Prolapse
Vaginal prolapse can occur as a prepartum event but is uncommonly reported in pigs (Peek 1985). The cause of vaginal prolapse is unknown, but straining to urinate or defecate may be involved. Sows with lateral deviation of the bladder and difficulty urinating or with inflammation associated with cystitis and urethritis may develop vaginal prolapse because of straining. When the cause can be found, treatment should be aimed at resolving the initial lesion. The vagina is cleaned with cold water, hydroscopic agents (anhydrous glycerine, sugar, etc.) are applied, a towel is wrapped around the prolapsed portion, and constant gentle pressure is applied to reduce the edema and swelling. The prolapse can usually be reduced in 15–20 minutes. The vagina should be cleansed and topical antibiotic or antiseptic ointments are used to reduce the secondary bacterial vaginitis that invariably occurs. Administration of anti-inflammatory drugs may reduce swelling, shorten convalescence. A Buhner suture is placed around the vagina to prevent recurrence of the prolapse. The sow should be closely monitored, and the Buhner suture should be removed at the first indication of farrowing. If excessive swelling of the soft tissues in the pelvic canal has occurred, a cesarean section is indicated and should be performed early in the process of farrowing.

Bladder Displacement (Lateroflexed)
Displacement of the bladder occurs in multiparous sows in the latter stages of gestation (Greenwood 1989; Scott 1977). The bladder is displaced laterally and, occasionally, may become displaced caudally. When this occurs, urination is difficult. The displaced bladder may give the appearance of a vaginal prolapse when the sow is lying down. Affected sows may be seen straining because of the difficulty urinating, and this may lead to true vaginal prolapse. Decompression of the urinary bladder by cystocentesis or catheterization may allow permanent replacement of the bladder. When displacement recurs, an indwelling urinary catheter may be used to allow urination until after parturition. Ascending bacterial cystitis is a complication of the indwelling urinary catheter.

Urethral Obstruction
Urethral obstruction has been observed in miniature pigs most commonly. Clinical signs include signs of abdominal pain, tail flagging, straining to urinate, blood in urine, decreased activity or restlessness, decreased appetite, and teeth grinding. Potential causes of urethral obstruction to be considered include urolithiasis, urethral polyps, and urethral stricture or trauma. Retrograde catheterization of the pelvic urethra and bladder is difficult because of the urethral recess and mucosal flap that prevent passage of the catheter. Tube cystostomy with positive contrast urethrography was reported to be useful for diagnosis of obstruction of the distal urethra (Palmer et al. 1998). Urethral polyps were reported as a cause of urethral outflow obstruction in Vietnamese potbellied pigs (Helman et al. 1996). Surgical management of urethral outflow obstruction includes urethrotomy, cystotomy with normograde flushing, tube cystostomy, perineal urethrostomy, prepubic urethrostomy, and prepubic cystostomy. Prepubic cystostomy was successful in two Vietnamese pigs with urethral injury from castration or urethral stricture (Leon et al. 1997). In these two pigs, the pelvic urethral was exteriorized cranial to the brim of the pelvis and the urethral spatulated and the mucosa
sutured to the skin. Both pigs maintained urinary continence. Urethroscopy with laser lithotripsy was used successfully in two potbellied pigs to alleviate urethral obstruction caused by uroliths (Halland et al. 2002).

**Tube Cystostomy Procedure.** Patients are placed in dorsal recumbency under general anesthesia. An 8- to 10-cm paramedian approach is made 4 cm lateral to the prepuce and extending from immediately caudal to the preputial orifice to 4 cm cranial to the brim of the pelvis. The bladder is exteriorized and two stay sutures placed near the apex. A cystotomy is performed near the apex, and suction and lavage with saline were used to evacuate urine and debris from the bladder. A gallstone scoop may be used to facilitate removal of stones if present. Particular attention is paid to suctioning the region of the trigone and urethral origin to remove debris, which may have migrated into the urethra during positioning for surgery. Normograde flushing is attempted to clear the urethra of debris. The cystotomy incision is then closed in two inverting layers using 0 or 2-0 absorbable monofilament suture material. A purse-string suture is then preplaced in the ventrolateral aspect of the bladder, near the apex on the same side as the celiotomy incision. A Foley catheter of size appropriate to the animal (range, 12–18F) is placed through a stab incision lateral to the paramedian body wall incision to enter the abdominal cavity. The end of the Foley catheter is then placed through a stab incision in the bladder within the purse-string suture. The purse-string suture is then tied tightly, and the catheter balloon is inflated with saline. The bladder is pulled close to the body wall using tension on the Foley catheter, which was secured to the skin with a trapping suture pattern. The celiotomy incision is closed routinely using various suture materials, depending on surgeon preference.

**Oophorectomy**

Removal of the ovaries is rarely indicated in swine. However, oophorectomy may be requested to facilitate research or for pet pigs. For pet pigs, removal of the ovaries is easier, faster, and has less risk of fatal hemorrhage than ovariohysterectomy (OVX). The blood vessels of the broad ligaments of the uterus are extensive and require ligation when OVX is chosen. Both ovaries may be removed from a paralumbar (flank), ventrolateral, paramedian, or ventral midline incision. We prefer to perform ovariectomy via a flank or ventral midline incision. Access to the abdomen is excellent with these incisions, and, in our experience, the risk of postoperative complications (incisional infection, hernia) is less. In either case, we prefer to use general anesthesia while performing the surgery. For ventral midline approach, the incision may be started immediately caudal to the umbilicus and extended caudally. For a paralumbar approach, the incision is started ventral to the transverse processes of the lumbar vertebrae, midway between the tuber coxae and the last rib. Each ovary is elevated through the incision, two hemostatic forceps are placed on the ovarian pedicle, two ligatures (no. 2-0 polyglactin 910) are placed proximal to the first hemostat, the pedicle is cut between the two hemostats, and the ovary is removed. Each ovarian artery must be observed for hemorrhage prior to closure. Closure of the ventral midline is done using no. 1 PDS or polyglactin 910 in an interrupted suture pattern. Chromic gut suture should not be used in the linea alba because of the increased risk of postoperative incisional hernia. The skin is closed with no. 2 polymerized caprolactam in a Mayo (Ford) interlocking suture pattern. Paralumbar incisions are closed in three layers (transversus abdominis + peritoneum, internal + external abdominal oblique, skin). Ovariectomy, alone, may be performed in pet pigs that have not begun normal estrus cycles. Uterine atrophy is expected to occur after ovariectomy. We recommend OVX in sexually mature pigs because of the potential risk for pyometra in a uterus where the cervix has been open.

**SURGERY OF THE UTERUS**

**Hysterectomy**

Hysterectomy may be performed as part of a cesarean section and is discussed below. Elective hysterectomy is rarely done in swine. Hysterectomy has been reported for pet pigs having uterine neoplasia (Preissel et al. 2009). The authors have also performed hysterectomies on pet pigs because of chronic endometriosis. However, hysterectomy may be requested for research purposes or for pet pigs (Figure 10.10).

When hysterectomy is performed on pet pigs, the ovaries are also removed. General anesthesia should be used during hysterectomy. The uterus may be removed

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**Figure 10.10.** Uterus and ovaries exteriorized for ovariohysterectomy in a 4-month-old pet pig.
via a flank, ventrolateral, paramedian, or ventral midline incision. We prefer to perform hysterectomy via either flank or ventral midline incision. The uterus is elevated through the incision, the ovaries are removed as described above, the broad ligament of the uterus is ligated using two to four overlapping simple interrupted sutures for mass ligation of the blood vessels, and transfixation ligatures are placed in the uterine body immediately cranial to the internal os of the cervix. The uterus and ovaries are removed and the incision is closed as described above. All sutured pedicles should be checked for adequate hemostasis prior to closure.

Cesarean Section: Indication and Decision Analysis

Cesarean section is required when transcervical extraction of pigs from the uterus is not possible (complicated dystocia) and to obtain gnotobiotic or specific pathogen-free (SPF) pigs. Cesarean section for gnotobiotic pigs is usually performed with the sow under general anesthesia and is discussed below. The most common reported causes of dystocia in swine are uterine inertia, small pelvic size, inadequate dilation of the birth canal, fetal to maternal disproportion, fetal malpresentation, and abnormalities of the birth canal (Titze 1977). Cesarean section for dystocia is usually chosen as a “last-resort” procedure for fetal extraction because of economic pressures. Therefore, the mortality rate among sows and gilts having cesarean section is expected to be higher than for other species. This is not surprising because affected swine suffer extreme physical exhaustion, stress, and shock by the time the decision for cesarean section is made. Interestingly, multiparous sows had a higher frequency of collapse (25.8%) prior to cesarean section compared with primiparous pigs (16.4%) (Dimigen 1972). Owners and veterinarians may become reluctant to perform cesarean section because of expense, previous experiences with fatalities, and the high rate of dead pigs delivered. It is our opinion that unnecessary delays in the decision for surgery is the principal cause of sow and baby pig mortality associated with cesarean section. When the veterinarian is presented with a sow in dystocia, the decision as to whether the owner is willing to incur the costs of cesarean section should be ascertained as early as possible during the initial examination. Other factors influencing the decision for cesarean section includes the cause of dystocia, how long the sow has been in labor, how long the owner has tried to manually extract the pigs, and how swollen or traumatized the sow’s pelvic canal has become. Many owners are adept at extracting pigs, and their failure to successfully remove pigs may justify immediate cesarean if the cause of dystocia is not apparent. The most common indications for cesarean section in pigs are undersized pelvic canal, inadequate cervical and soft tissue dilation, prolonged labor (including uterine inertia), fetal to maternal disproportion, and trauma to the birth canal (Titze 1977). In our experience, cesarean section performed at the earliest indication has a high success rate for survival of the sow and a higher rate of live pigs obtained.

Swine that are physically exhausted, stressed, or in shock must be stabilized prior to cesarean section. Thirteen percent of sows in labor for less than 18 hours died or were salvaged compared with 30% of sows in labor for more than 18 hours (Dimigen 1972). Among sows necropsied after sudden death, retained fetuses and toxemia were found in approximately 10% (Sanford et al. 1994). Stabilization of the sow is often simple and readily achieved. We routinely place a 16- or 18-gauge, 2-in. intravenous catheter in the ear vein. This catheter is sutured or glued in place, and intravenous fluids (0.9% saline or lactated Ringer’s solution) are administered rapidly (initially 20–40 mL/kg body weight/h, then 4 mL/kg/h once stabilized) and continued for the duration of the surgery. The authors prefer to add dextrose (1.25% final solution) and calcium (1 mL/kg) to intravenous fluids after the patient has been stabilized. Furthermore, the shock status of the sow may be improved by administration of dexamethasone (0.5–1.0 mg/kg IV) or flunixin meglumine (1 mg/kg IV). Because extensive manipulation of the intrauterine environment prior to cesarean section increases the risk for postoperative septic peritonitis, we prefer to administer preoperative antibiotics (procaine penicillin G, 10,000 IU/kg IM; ceftiofur HCl, 3–5 mg/kg IM; or oxytetracycline, 5–10 mg/kg IM). In severely compromised sows, sedation (see the “Anesthesia” section) and local or regional anesthesia may be adequate for surgery. Intravenous fluids should be administered continuously. Epidural anesthesia (lumbosacral level) may also be useful. Overall, we prefer to perform general anesthesia rather than attempt cesarean using physical restraint and epidural anesthesia. In our experience, this causes the least stress on the patient, surgeon, and assistants. The respiratory rate and heart rate should be monitored, and supportive therapy should be adjusted appropriately.

Surgical Approach for Cesarean Section. Multiple surgical approaches have been described for cesarean section. Selection of the surgical approach depends on the preference of the surgeon, the condition of the patient, and means of restraint and anesthesia used for surgery. The most common approaches are paralumbar fossa, ventrolateral (horizontal low flank), ventral midline, paramedian, or paramammary (Mather 1966; Turner and McIlwraith 1989).

With a ventral or paramedian approach, movement by the sow must be prevented because of the risk of contamination of the incision. Also, the mammary veins must be carefully avoided or ligated to prevent...
pattern for closure of the uterus. Some veterinarians have advocated hysterectomy when performing cesarean section (Schoneweis 1971). This practice allows rapid removal of all pigs soon after entering the abdomen, ensures culling of the sow after the pigs have been weaned, and minimizes surgery time because removal of individual pigs is done by an assistant after removal of the uterus. The uterine arteries are ligated with #0 chromic gut, the broad ligaments are divided along the axis of the uterine horns, and the uterine body ligated using rubber tubing. The rubber tubing may be secured to the uterine body using #1 chromic gut suture. Alternatively, sterile 1-cm cotton tape (umbilical tape) suture may be used to perform transfixation ligation of the uterine body. Then, the gravid uterus is removed. We urge caution with hysterectomy that precise hemostasis must be achieved prior to removal of the uterus. Ligation of abdominal bleeders after removal of the uterus is difficult, and life-threatening hemorrhage may occur if the uterine arteries are inadequately ligated. We close the transversus abdominis muscle and peritoneum, together, and the external abdominal oblique and internal abdominal oblique muscles, together, using #2 chromic gut or #1 PDS or polyglycolic acid placed in simple continuous pattern. For closure of ventral midline or paramedian incisions, we do not recommend the use of chromic gut because of the higher rate of postoperative hernia formation. We use #1 PDS or polyglycolic acid suture placed in simple interrupted or interrupted cruciate pattern. Skin is closed using #0 polymerized caprolactam in a Mayo (Ford) interlocking pattern. Alternatively, a subcuticular suture pattern may be placed to oppose the skin edges (Figure 10.10). This suture pattern eliminates the need for skin suture removal at a later date. The sow should remain confined for a minimum of 14 days after surgery.

Uterine Prolapse

Prolapse of the uterus is occasionally seen in sows during or up to several days after parturition. Excessive straining because of fetal malpositioning, fetal/maternal disproportion, or trauma with swelling and inflammation in the birth canal is thought to cause uterine prolapse. Prolapse of the entire uterus has the greatest potential for a life-threatening crisis because of profuse hemorrhage, but partial prolapse may also occur. The sow must be stabilized prior to attempts to replace the uterus into its normal position. If hemorrhage, hypovolemia, or shock (tachycardia, peripheral cyanosis) is present, the sow should be placed into a warm environment, intravenous catheter is placed into an auricular vein, and intravenous fluids are administered. Hypotonic saline may be administered rapidly (5–7 mL/kg body weight IV over 5–10 minutes) followed by isotonic crystalline fluids (5–10 mL/kg/h). For replacement of the prolapse, the sow may be placed on an inclined

10.11. Location and subcuticular closure of the skin of a paramammary incision in an adult sow. This closure pattern eliminates the need for skin suture removal (photo courtesy of Matt Miesner).
floor or platform in sternal recumbency with the hind-quarters elevated. Epidural anesthesia (administered at the lumbar space), sedation, or general anesthesia may be required to eliminate struggling, straining, and agitation of the sow. The uterus is thoroughly cleaned with cold water and assessed for the presence of lacerations and necrosis. Small lacerations may be cleaned, superficially debrided, and sutured closed (#0 chromic gut, simple continuous pattern). Hemorrhage may be stopped by ligating affected vessels or by performing en bloc tissue imbrication. Sutures may be placed over stents to increase the region of pressure to control hemorrhage. Then, hydroscopic agents (anhydrous glycerine, sugar, etc.) may be applied to the uterus to assist in reducing edema. The uterus is wrapped into a towel and gentle pressure is applied, starting from the tip of the uterine horn and working toward the body of the uterus. After approximately 15 minutes, the edema should be sufficiently reduced to allow manipulation of the uterine horns. Each horn should be inverted starting with the tip and gradually reduced until the uterine body has been reached. Often, progress is impeded because of the extensive edema and swelling of the soft tissues of the pelvic canal. When this occurs, left paralumbar fossa laparotomy is indicated (Raleigh 1977). After appropriate preparation of the surgical site and surgeon, a 10-cm-long, vertically oriented incision is made in the middle of the left paralumbar fossae. The left arm is passed into the peritoneal cavity and into the everted uterus. One of the uterine horns is grasped and pulled back into the peritoneal cavity. The right arm or an assistant helps by applying gentle pressure on the everted horn from the exterior. After the uterus has been repositioned, all remaining fetuses should be removed. The laparotomy incision should be closed in three layers (transversus abdominis + peritoneum, internal + external abdominal oblique, skin). Chromic gut (no. 3) or a synthetic absorbable suture (polydioxinone, polyglactin 910, polyglycolic acid) is placed in simple continuous suture patterns in the muscle layers. Polyglycolic caprolactam (no. 2 braunamide) is placed in a Mayo (Ford) interlocking pattern in the skin. Antimicrobial and anti-inflammatory medications are desirable, but strict attention should be paid to drug residues in the meat prior to slaughter. Finally, a Bühner suture should be placed around the vulva to prevent recurrence of the prolapse. The Bühner suture (6.4-mm-wide sterile cotton tape) should be deeply placed at the junction of the labia and the skin of the perineum to recreate the function of the vestibular sphincter muscle. The Bühner suture may be removed in 7–10 days with minimal risk of prolapse. Oxytocin (20 units) is routinely administered to facilitate contraction and involution of the uterus and cervix. If prolapse reduction using laparotomy is not used as a “last-resort” treatment, sows should survive partial prolapse of the uterus (>75%), but complete prolapse carries a guarded prognosis (<50%).

Amputation of the Uterus

Amputation of the uterus is indicated when excessive bleeding, extensive laceration, trauma, or necrosis of a uterine prolapse is found. Prior to amputation, the uterus should be closely inspected to ensure that the bladder or small intestines are not entrapped. Hypovolemic or hemorrhagic shock may be present and should be addressed during the course of treatment. If the uterus is swollen, it should be elevated above the pig to encourage drainage of venous congestion. We recommend placing towels around the uterus so that pressure may be applied without further trauma to the wall of the uterus. Hydroscopic agents (anhydrous glycerine, granular sugar, etc.) may be used to help resolve edema of the uterine tissues. After venous congestion has been reduced, amputation is more easily performed. Transfixation ligatures are placed around the circumference of the uterus. Heavy suture material (0.5-cm sterile cotton tape, #3 polymerized caprolactam) is used because the thickness of the uterus requires extreme tension to completely occlude the uterine arteries. Stay sutures or cross pins (using 15-cm-long, 18-gauge needles) are placed in the vital uterus, and the prolapsed portion is amputated. Then, any bleeders are ligated with #1 chromic gut before the remaining tissues are released and placed back into the pelvic canal. A Bühner suture or purse-string suture should be placed into the labia at the level of the vestibular sphincter to prevent prolapse of the remaining tissues. Affected sows are salvaged as soon as possible or after weaning of the litter.

Mastectomy

Mastitis caused by Actinomyces suis may cause formation of abscesses, granulomas, and mammary fistulas. Surgical removal of the mammae is indicated for return of the sow to production soundness. Sows with at least 12 intact mammary glands and that are not in the first week or last 4 weeks of gestation are suitable candidates for surgery. The sow is placed under general anesthesia, and the affected mammary gland is prepared for surgery. An elliptical incision is made approximately 1 cm from the base of the swelling so that enough tissue remains to allow closure of the tissues with minimal tension. A combination of sharp and blunt dissection is used to extirpate the gland, granuloma, and abscesses. The cranial superficial epigastric vein (subcutaneous abdominal vein) should not be compromised, but hemostasis is essential. Hemostasis is ensured by using 2-0 chromic gut ligature of transected blood vessels. The wound is closed in three layers: deep subcutaneous, superficial subcutaneous, and skin. Each subcutaneous tissue layer is sutured with a simple continuous suture pattern (no. 0 chromic gut, no. 2-0 PDS, or poly-
before evisceration or intestinal strangulation or fistula occurs. A case of intestinal umbilical fistula has been described in a 30kg castrated male pig (Lewis 1973). The risk of intestinal incarceration and strangulation is more frequent with an umbilical hernia of small dimension, that is, hernia, ring smaller than 8cm. However, a pig with an umbilical hernia will often be discounted when it goes to slaughter.

Herniorrhaphy should be performed early in life. Following anesthesia, the pig is restrained in dorsal recumbency in a V-shaped trough. The surgical area is then cleaned and prepared for surgery. If surgical correction is performed on a male, the prepuce, preputial diverticulum, and penis should be reflected posteriorly or to one side. The hernia sac is then isolated, and dissection is performed to the hernia ring. The hernia sac with an abscess, if present, should be removed, and the edges of the ring should be freshened. If intestinal contents are adhered to the hernia sac, the adhesions are separated, bowel viability is assessed, and if judged acceptable, the bowel is replaced in the abdomen. If intestinal viability is compromised, resection and anastomosis of viable intestine should be performed. If no infection is present, the hernia sac can also be inverted into the abdomen. The abdominal defect is then closed using an overlapping or simple continuous pattern. The prepuce, preputial diverticulum, and penis are then repositioned and sutured to the abdominal muscle with absorbable suture material. The skin is then sutured using a simple interrupted pattern of nonabsorbable suture material. For surgical correction of umbilical hernia in the female, an elliptical incision is made around the hernia sac, and the excess skin is discarded. With a combination of sharp and blunt dissection, the hernia sac is then cut and removed, and the abdominal muscle is closed as in the male. The subcutaneous tissue and skin are then closed. Systemic antibiotic should be administered for 5 days, and the skin suture should be removed in 10 days.

Inguinal or Scrotal Hernia
Inguinal hernia and scrotal hernia are variants of a defect in which intestines or other abdominal organs pass into the inguinal canal. The hernia develops when there is an abnormally large and patent vaginal orifice through which the vaginal process and peritoneal cavity communicate. Scrotal hernia is the more exaggerated form of the defect in that the organs protrude into the scrotum (Vogt and Ellersieck 1990). These hernias are common in swine and have been the most common defect observed in swine (Vogt and Ellersieck 1990). The frequency of scrotal hernia among the porcine population varied between 0% and 15.7% with a realistic number of about 1% (Vogt and Ellersieck 1990). The development of these hernias seems to be genetically influenced (Ding et al. 2009). One study indicated that the variation associated with anatomical

![Image](68x564 to 297x721)

**10.12. Surgical repair of an umbilical hernia in a female. An elliptical incision is made around the hernia sac, and the excess skin is discarded.**

glycolic acid). Each suture is anchored to the deeper tissue layer in an attempt to close all dead space, thus minimizing the formation of postoperative seroma, hematoma, and abscess. Administration of perioperative antibiotics is indicated.

**ABDOMINAL SURGERY**

**Umbilical Hernia**
Umbilical hernia is a development defect of pigs that has recently been found to have a genetic cause (Ding et al. 2009). An umbilical hernia is a discontinuity of the abdominal wall at the umbilicus with protrusion of abdominal content into a hernia sac formed by the skin and surrounding connective tissue (Figure 10.12).

In swine herds, the frequency of umbilical hernias ranges from 0.4% to 1.2% and varies with breed and sex (Searcy-Bernal et al. 1994). In addition to heredity, the etiology of umbilical hernia may be navel infection and umbilical abscess. After the umbilical cord is cut at birth, iodine should be applied to decrease the likelihood of infection. Pigs with umbilical hernias may suffer from growth retardation and may die from intestinal strangulation. In one study, pigs sired by American Spotted and Duroc boars were more likely to develop hernia than those sired by Yorkshire, and umbilical hernias were often detected in pigs between 9 and 14 weeks of age (Searcy-Bernal et al. 1994). One possible reason for the recognition of the condition at that age may be the rapid growth of pigs, combined with increased weight of the abdominal contents, leading to a hernia of significant size. Females were at an increased risk of developing umbilical hernia. As with many other swine surgical conditions, the cost of treatment may preclude surgical correction. In that case, pigs should be consigned to an early slaughter soon (within 1 month) after detection of the hernia, before evisceration or intestinal strangulation or fistula occurs. A case of intestinal umbilical fistula has been described in a 30kg castrated male pig (Lewis 1973). The risk of intestinal incarceration and strangulation is more frequent with an umbilical hernia of small dimension, that is, hernia, ring smaller than 8cm. However, a pig with an umbilical hernia will often be discounted when it goes to slaughter.

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structures relevant to scrotal hernia is influenced polygenically. In that study, the heritabilities of susceptibility to scrotal hernia development were estimated to be 0.29, 0.34, and 0.34 in Duroc-, Landrace-, and Yorkshire-sired pig groups, respectively (Vogt and Ellersieck 1990). Inguinal and scrotal hernias need to be differentiated from hydrocele, scirrhous cord, and hematoma of the testicle. Taking a good history (e.g., a pig that has been castrated before is more likely to have a scirrhous cord) and direct manipulation will often give the diagnosis. If necessary, ultrasonography and needle aspiration can be used. Inguinal hernias are often encountered at the time of castration. Some of these hernias will reduce spontaneously but recur later. With chronic inguinal hernia, intestinal incarceration and strangulation may be observed.

Surgical repair of an inguinal or scrotal hernia is easier if done before the pig is castrated. With the pig restrained in dorsal recumbency and its rear quarters elevated, the inguinal and scrotal area is thoroughly cleaned and prepared for surgery. An oblique incision is made over the affected external inguinal ring. Once through the skin, the subcutaneous tissue is dissected bluntly. Also by blunt dissection, the tunica vaginalis is isolated. The tunica vaginalis should be kept intact, because this will keep the intestine contained. While external pressure is put on the scrotum, the tunics are gently pulled free from their scrotal attachment. The entire hernia sac is removed through the inguinal incision. The tunic and testicle are then twisted to force the intestines into the peritoneal cavity. The tunics and spermatic cord are transfixated as close to the inguinal ring as possible. The tunic and cord are then cut, and the inguinal ring is closed with interrupted or horizontal mattress suture. The herniorrhaphy is then checked by applying external pressure on the abdomen. The skin is then closed using absorbable sutures. The authors always recommend checking the other side for possible bilateral herniation before performing a castration. If the surgery was done to repair a large hernia in which marked serum accumulation in the scrotum is expected, an incision in the most ventral aspect of the scrotum should be performed via the inguinal incision before suturing to provide ventral drainage. If intestinal adhesion and incarceration are observed during surgical correction of a scrotal hernia, the vaginal tunic should be opened and the intestine is dissected free or an intestinal resection and end-to-end anastomosis are performed. If an inguinal hernia occurs after castration, one needs to clean and lavage the herniated bowel, enlarge the inguinal ring and replace the prolapsed intestine (if it is judged to still be viable), and then suture the inguinal ring.

**Intestinal Obstruction**

In swine with intestinal obstructions from intussusceptions or foreign bodies, clinical signs observed may include depression, vomiting, abdominal distension, and decrease in the amount of feces sometimes with blood and mucus in it. These two conditions are diagnosed rarely in live animals. If the condition is diagnosed early, a ventral midline celiotomy and an enterotomy are performed for the foreign body obstruction, and an intestinal resection and anastomosis are done for the intussusception. Spiral colon obstruction was diagnosed in an 8-year-old potbellied pig with depression, inappetance, and abdominal distension (Gallardo et al. 2003). Exploratory laparotomy found a stricture at the proximal centripetal loop of the spiral colon and megacolon proximal to the stricture. A side-to-side colonic anastomosis was performed and the pig returned to normal after surgery. A 7-month-old female potbellied pig was diagnosed with idiopathic megacolon based on abdominal palpation, abdominal radiographs, and exploratory surgery (Bassett et al. 1999). A subtotal colectomy was performed, and an ileocolonic anastomosis was performed. The pig survived and, after a period of diarrhea, returned to normal stool.

**ATRESIA ANI AND RECTAL STRICTURE**

Atresia ani occurs more frequently in the pig than any other species and is possibly the most important cause of intestinal obstruction. This congenital defect is transmitted genetically, but may occur spontaneously as well. Recently, atresia ani was reported in a piglet that was a clone derived by somatic cell nuclear transfer from a miniature pig that did not have atresia ani (Lee et al. 2005). The diagnosis is made by an absence of anal opening, abdominal distension, slower growth rate, and vomiting (Figure 10.13).
Because the pigs vomit, the diagnosis of atresia ani is sometimes not made until 3–4 weeks of age. In the female piglet, a fistula may occur between the rectum and vagina, so that the feces may be voided through the vulva. Surgical treatment of atresia ani is necessary for survival. Following anesthesia, a circular piece of skin is excised below the tail over the bulging rectum. Feces are usually discharged immediately. If the rectum is not present at the skin opening, pelvic dissection may be necessary. Depending on the location of the rectum, or if atresia of the rectum is also present, surgical correction may not be possible. Pigs with rectal stricture often show similar clinical signs as pigs with atresia ani, except that they have an anus and are generally older. Most cases of rectal stricture are the result of a rectal prolapse that has constricted after repair caused by gentle massage and retention by application of water shortage, certain antibiotics, zearalenone toxicity, being male, diarrhea, coughing, short tails, autumn and winter piling as a consequence of chilling, chronic water shortage, certain antibiotics, zearalenone toxicity, and a diet containing excess lysine (20% more than required) (Amass et al. 1995). Diagnosis of rectal prolapse is not difficult, but care should be taken that the prolapse does not contain other organs.

The simplest procedure for rectal prolapse is reduction by gentle massage and retention by application of a purse-string suture pattern using umbilical tape (Borobia-Belsue 2006). The suture is passed in and out through the skin around the anal opening at a distance of 1 cm from the anus. A one-finger opening should be left when tying the purse string. The suture is usually left in place for 5 days. This should be done only if the rectal mucosa is viable and no laceration is present on close inspection. If the mucosa is too necrotic to replace, correction of the prolapse can be approached in different ways (Vonderfecht 1978). One technique is a surgical amputation. For this surgery, required instruments are hemostats, blade, scissors, thumb forceps, two 18-gauge needles 3 or 4 in. long, suture material, and a small-diameter rubber tube. Following anesthesia, the tube is inserted in the rectum until 2 or 3 in. protrude. The tube is fixed in the rectum by inserting the two needles through the rectum at right angles to each other so that they pass through the rectum and tube and emerge from the opposite side. The dissection is started about a centimeter from the mucocutaneous border where the mucosa is still healthy, and the entire circumference of the exposed mucosa of the rectum is cut down to the serosa of the inner wall. Hemorrhaging is usually minor and controlled with gauze until all the layers have been dissected and the dorsal artery of the rectum is cut. Once the dissection is completed around the prolapse, the rectum is held in place because it is attached to the rubber tube with needles. Instead of using tubing and needles, one could use forceps applied at two or three places as the prolapsed rectum is amputated to prevent telescoping into the animal’s body (Kjar 1976).

To suture the ends of the rectum together, the authors suggest using size 0 absorbable suture material in an inside-out continuous pattern. After the simple continuous pattern has been placed around the rectum, the needles are then pulled from the tube, and the tube is removed from the rectum. The rectum then automatically retracts into place. An alternative method of rectal amputation is to use a prolapse ring, polyvinyl chloride (PVC) tubing, syringe case, or corrugated tube (Douglas 1985). The ring or tubing is placed in the rectum, and the halfway point on the tube needs to be inserted as far as the anal sphincter. A ligature or rubber band is then applied over the prolapse as near as possible to the anus. The ligature or rubber band must be tight enough to disrupt blood supply to the prolapse. Feces may go through the tube or may block the tube. Usually, the necrotic prolapse falls in 5–7 days with the implant in place, and then fecal production returns to normal. Three possible complications seen with rectal prolapse are bladder retroversion, eversion of the small intestine, and rectal stricture (Peyton et al. 1980).

**MUSCULOSKELETAL SURGERY**

Musculoskeletal injuries, particularly lameness, are increasingly recognized in swine. Lameness represents a significant animal welfare issue and is justification for concentrated research into cause and prevention of these disorders. A recent Bayesian analysis of leg disorders in finisher herds identified the need for accurate data collection and analysis (Jensen et al. 2009). In this study, three classes of leg abnormalities were examined: physical injury, inherited defects, and infectious agents. In a survey of musculoskeletal disorders in dead sows in Denmark, 72% of sows had a classification of locomotor abnormality (Kirk et al. 2005). In this study, the most common locomotor disorders were septic arthritis (24%) most commonly caused by Arcanobacterium pyogenes and fractures (16%) most commonly of the proximal humerus or femur. Interestingly, the study also revealed that disorders of the hoof and sole are common. Risk factor analysis is critical in the
prevention of limb injuries. The following focuses on treatment of pigs having such injuries.

**Septic Arthritis**

Septic arthritis may be caused by bacteremia, direct inoculation of bacteria into the joint, or extension of a local infection into the joint. Septic arthritis caused by direct inoculation or local extension is treated by wound management, joint lavage, and systemic antibiotic therapy. Infected joints may require daily, or every other day, lavage for 7–10 days or until granulation tissue has covered the wound. Sterile isotonic electrolyte solutions (0.9% saline, lactated Ringer’s solution) are lavaged through the joint by inserting an 18- or 14-gauge needle into the joint, injecting the solution under pressure into the joint, and inserting a second needle into the joint with as much separation as possible between the two needles. Approximately 500 mL of the solution is flushed through the joint. After lavage, antibiotics may be instilled directly into the joint to achieve maximal local antibiotic concentration. Success of treatment is assessed by improvement in lameness and wound appearance.

**Digit Amputation**

Digit amputation is indicated when severe foot abscesses or septic arthritis of the interphalangeal joints have caused unmanageable damage to a single digit. These injuries are most commonly caused by wounds from trauma on concrete flooring or metal side panels. The decision for amputation should not be delayed. If the infection has extended to the fetlock or more proximal on the limb, digit amputation will not be curative. Also, the soundness of the opposite digit should be assessed to determine if the pig will be able to ambulate on the remaining digit after amputation.

After induction of general anesthesia, the affected pig is placed proximal to the surgery site to prevent extensive hemorrhage during surgery. A circumferential incision is made through the skin and soft tissues at a 45° angle to the coronary band starting at the axial aspect of the digit and continuing proximally to the abaxial surface. The skin is reflected proximal to the site for amputation, and a sterile obstetrical wire is used to amputate the digit. The third phalanx and a portion of the second phalanx are removed by this procedure. The remainder of the second phalanx should also be removed. The remaining tissues are debrided and cleaned thoroughly, and the skin is opposed over the wound. A sufficient opening is left to allow drainage or a Penrose drain is placed into the wound. The foot is placed in a padded bandage for 7–10 days. Then, the foot is cleaned daily with water until the wound is healed. Perioperative antibiotics and anti-inflammatory drugs are indicated.

**Ankylosis of the Proximal or Distal Interphalangeal Joint**

Septic arthritis of the proximal or distal interphalangeal joint is an indication for digit amputation. However, the lateral claw of the hind limb is important to normal ambulation and breeding activity. Salvage of the digit by facilitated ankylosis is an option to preserve normal ambulation. The affected pig is placed under general anesthesia, and the digit is prepared for surgery. A 1-cm incision is made into the affected joint. The distal interphalangeal joint is approached by placing a 3.75-cm-long needle into the joint by inserting the needle immediately proximal to the coronary band and inserting the needle distally. The proximal interphalangeal joint may be located by palpation or insertion of the needle in the midpaster region. After the arthrotomy has been made, a 4- or 6-mm-diameter drill bit is used to destroy the articular surfaces of the joint. Currettes are used to debride the joint and remove all infected subchondral bone. A distinct difference in texture and hardness will be noted between the necrotic (gritty and irregular) and healthy (smooth and hard) bone. Thorough curettage of all infected bone is critical to establishing effective joint ankylosis. The tissues are extensively lavaged with normal saline, and antibiotics are administered for 10–14 days. Strict confinement for 6–8 weeks is needed for ankylosis to occur. A cast extending from the ground to the carpus or hock will hasten convalescence.

**Fracture Repair**

Swine with fracture of long bones are often salvaged because economic considerations preclude treatment. However, veterinarians may be asked to treat fractures in swine of potential value for genetic improvement. Treatment of fractures can be rewarding and Vaughan (1966) reported clinical experiences with fracture fixation in commercial swine. Fractures were associated with breeding injury (two pigs), injury on concrete flooring (three pigs), fighting injury (one pig), and were of unknown cause in five pigs. The most common fractures treated were tibia and fibula (five pigs), femur (three pigs), humerus (two pigs), and tibiotarsal joint luxation with fracture of the fibula (two pigs). Affected pigs weighed between 64 and 168 kg and were 6 months to 2 years old. Fracture of the tibia and fibula were treated by open reduction and internal fixation using a bone plate and full limb cast (three pigs) or by using a full limb cast, alone (two pigs). Fracture of the femur was treated by application of a bone plate (three pigs). Humerus fractures were treated by confinement (one pig) or by application of a bone plate (one pig). Tibiotarsal joint luxation with fracture of the fibula was treated by application of a bone plate and use of a full limb cast (two pigs). Of these 12 pigs, 10 returned to normal production use and 2 were salvaged; one pig with tibiotarsal joint luxation developed Escherichia
trauma injuries and fractures associated with nutritional deficiency are poor candidates for surgical repair.

Fracture of the greater trochanter of the femur has also been identified as a cause of lameness in pigs (Blowey 1992, 1994). A simple, oblique fracture of the mid-diaphysis of the femur was successfully repaired in a 10-month-old, 150 kg Berkshire boar using a bone plate (Grisel and Huber 1996). The boar returned to normal breeding 190 days after surgery.

**Canine Tooth (Tusk) Removal and Resection**

Removal of the canine teeth of adult boars is challenging because of the long dental root embedded in the mandible. Canine tooth extraction is done with the boar under general anesthesia. The gingiva and periosteum of the mandible are reflected laterally and ventrally using a periosteal elevator. Then the lateral alveolar plate of the tooth alveolus is resected following the course of the tooth root. After the periapical region of the alveolus has been reached, a periodontal elevator is used to disrupt the periodontal membrane around the circumference of the tooth and the tooth is removed. The alveolus is debrided, rinsed, and sutured closed. Alternatively, the alveolus can be left open to heal by second intention healing.

Resection of the mandibular canine teeth is an easy and rapid method of preventing tusk injuries to personnel and other pigs. The boar is placed under general anesthesia, and obstetrical wire is placed around the tooth. The wire is used to saw through the tooth approximately 3 mm above the gingival margin to prevent exposure of the pulp cavity. This procedure is repeated every 6–12 months as needed to restrict growth of the tusks.

**REFERENCES**


INTRODUCTION
Preventing transmission of disease and its corollary, biosecurity, are the cornerstones of modern veterinary service in the pig industry. Eradication of diseases from pig farms remains an important goal, though it is one rarely achieved. Fortunately, through the process of attempting to eliminate diseases, transmission of diseases and hence their frequency or occurrence (and often their severity) are reduced. This chapter describes a framework for understanding an infectious disease through knowledge of the pathogen’s interaction with the host, the production setting, and its broader environmental landscape; a framework described as “disease ecology.” Using an ecological framework for categorizing specific pathogens in the context of their mechanism for perpetuating themselves between the times they leave one host and find a new susceptible host, provides one with an informed approach to disease control and eradication. Once understood, unifying principles based on scientific evidence can be generated to establish logical and predictably successful biosecurity programs.

DISEASE TRANSMISSION
Disease transmission includes any mechanism by which an infectious agent is spread from an infected host, animate or inanimate vector, or environmental reservoir to a susceptible host. Implicit in a transmission event is the requirement that a new host becomes infected. This situation is in contrast to an exposure event whereby an infectious agent is presented to a potential host with transmission as one possible outcome. The possibility of transmission occurring can be stated as a probability of success representing the combined likelihood of several serially occurring events, each with its own conditional probability of success. Transmission requires that a pathogen successfully exits an infected host, escapes potential threats to its existence in the environment, breaches the innate defense systems of a new and susceptible host, and then reaches an anatomical site suitable for further replication or perpetuation in that host (Zimmerman 2003).

Some authors have elaborated on the need to draw distinction between modes of transmission and routes of infection (Smith 2006). Modes of transmission can be classified as horizontal (between contemporaries or animals of the same generation) or vertical (between infected animals of one generation and uninfected animals of the succeeding generation, in utero or through colostrum). Within horizontal modes of transmission, further distinctions can be made to categorize transmission events that occur as a result of direct or indirect contact with an infected animal, or through exposure to an airborne pathogen.

Route of infection specifies the means by which a pathogen enters the host including the alimentary, respiratory, or urogenital tracts, skin, or conjunctiva. To gain a thorough appreciation of the determinants of disease transmission, one must resist the urge to systematically classify pathogens based on their most likely, prevalent, or important pathway of travel between infected and uninfected animals. The advent of molecular-based diagnostic tests and advanced epidemiological analyses (made possible through inexpensive computing and easy-to-use software) has enabled a more holistic understanding of the complex interactions between host, pathogen, and the environment.
An alternative approach to understanding disease transmission in the context of this new information is through a framework that attempts to explain where the pathogen hides or resides when it is not in the host animal. An important objective for veterinarians, animal scientists, and farmers is the prevention, control, and when possible, eradication of disease in commercial livestock. Fundamental to these objectives is knowledge of the habitat in which the pathogen can persist. “If we can combat the pathogen only when it has invaded our stock we shall never eradicate it; if it has any other habitat to hide in, we must find that habitat” (Halpin 1975).

**ECOLOGY OF DISEASE: HOST–PATHOGEN–ENVIRONMENT**

In broad terms, disease is either a physiological or psychological dysfunction and includes both cellular pathology and clinical signs; these may be a result of infection with a pathogenic agent or be associated with any number of noninfectious causes. For the sake of this chapter, disease will be taken to mean only those pathologies and clinical signs caused by infection with a pathogenic agent. Disease may persist long after the pathogen has been cleared from the host. Disease may be classified based on temporal staging (peracute, acute, subacute, chronic, and persistent/latent), severity (inapparent, mild, moderate, severe), or organ system(s) affected. Under the right circumstances, organisms from all taxonomic kingdoms are represented among the list of potentially infectious pathogens of the pig.

An understanding of why disease occurs and how it can be controlled requires some knowledge of contributions made by the environment in which the host and pathogen reside. A more apt term that incorporates the complex interactions between host and pathogen, including environmental variables, is “disease ecology.” After making its first appearance in the scientific literature nearly 50 years ago (Bejarano 1960), the term was used primarily in the discussion of arthropod-borne and wildlife diseases until the 2000s. However in recent years, use of the term has expanded into epidemiological studies of animal and human diseases as analytical techniques capable of simultaneously integrating spatial, climatological, temporal, and demographical data with traditional measures of disease occurrence have become more widely used. Ecology is the study of the distribution and abundance of organisms, and their interactions with the environment (Begon et al. 2006). Implicit in the term is a sense of balance, an imperative that an ecological system will evolve over time into a stable circumstance that reacts to external influences in such a way to maintain that stability. In the context of disease ecology, one could use as an example the relationship that evokes between *Mycoplasma hyopneumoniae* bacteria and young pigs in the environment of a conventional, continuous-flow growing–finishing barn. Over time, a high prevalence of infection will be established in the pig population but with few overt clinical signs (endemicity). The prevalence of infection, the “nature” of the bacterium (phenotypic and genotypic characteristics), systematic environmental variables (throughput, pig density, and pig husbandry and management), and the pig’s own evolutionary progression (body functions, body size, social behavior, and body defense mechanisms) will remain stable. In the absence of external stimuli, there is no obvious imperative for the host, the pathogen, or the environment to change. From a more philosophical perspective, one can even incorporate the influence of public policy (as an environmental contributor to pig disease) into the disease ecology paradigm. Law and Mol presented a compelling discussion on the “politics of boiling pigswill” in a 2008 paper that offered their reflections on the influence that the regulation of waste-food feeding to pigs had on the 2001 foot-and-mouth disease virus (FMDV) outbreak in the United Kingdom. They suggested that through regulation of the “mundane and material” practice of boiling pigswill, competing politico-environmental ethics were established whereby the rich were divided from the poor through separation of FMDV-free countries from those in which the disease was endemic. The authors stated, “To boil pigswill at Heddon on the Wall [the index farm] is to reproduce a distinction between a productive and a less productive agriculture. It is also a technique for making and maintaining a specific geographical distribution of that productivity.” We are no longer raising pigs in the isolation of our own farms; we are subject to the effects of the political winds of the day, and this should be considered in the modern framework of disease ecology.

A specialized discipline within disease ecology has evolved to deal with the unique mode of disease transmission carried out through arthropod vectors, that being “landscape epidemiology.” Like disease ecology, landscape epidemiology had its origins in the scientific literature of the 1960s but experienced resurgence in use during the 2000s. Landscape epidemiology has a spatially defined focus with the intent of measuring changes in the dynamics of host, vector, and pathogen interactions and their association with the risk of disease transmission to the host species (Reisen 2010). Geographical information systems in combination with analytical techniques accessible to a broader group of scientists have allowed those working with vector-borne diseases (specifically arthropod-borne pathogens) to describe nidalities of infection. Nidalities are pathogen specific and are fundamentally determined by the concurrent spatial overlap of landscapes that offer the requisite combination of vegetative cover, elevation, latitude, and microclimate capable of supporting populations of competent and infectious vectors, competent vertebrate reservoir species, and
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Important to these measures is defining the number of animals in the population that are at risk of becoming infected. Commonly used measures of disease occurrence are shown in Table 11.1. Two important distinctions should be recognized when interpreting prevalence and incidence: (1) prevalence measures the occurrence of infection at a single point in time while incidence incorporates the frequency at which new infections occur over a defined period; and (2) prevalence assumes all individuals in the population are (or were) at risk of becoming infected some time before being diagnosed, while incidence considers only those animals susceptible to infection at the beginning of a defined time period.

Incidence measures tend to be of more use than prevalence in the study of disease transmission as calculations of both incidence risk and incidence rate consider only those animals known to be susceptible to infection at the beginning of the time period. Incidence measures inform one of the “force of infection” or the rate at which new cases occur over time, a particularly useful determinant in predicting the course of a disease epidemic. In calculating incidence rate, the cumulative time at risk for individuals in the population is recorded in order to precisely account for the time that has elapsed prior to an animal becoming infected. Figure 11.1 shows an example of how prevalence risk, incidence risk, and incidence rate differ for a population of pigs actively

Table 11.1. Commonly used measures of disease frequency

<table>
<thead>
<tr>
<th>Accepted Name</th>
<th>Count (Numerator)</th>
<th>Population at Risk (Denominator)</th>
<th>Calculation (Measure)</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prevalence risk</td>
<td>Number of infected animals</td>
<td>All animals in the population assumed to be at risk of becoming infected</td>
<td>Number of infected animals</td>
<td>Number of animals in the population</td>
</tr>
<tr>
<td>Incidence risk</td>
<td>Cumulative number of newly infected animals that occurred during the defined time period</td>
<td>Only those animals known to be susceptible to infection at the beginning of the time period</td>
<td>Number of infections that occurred during the period</td>
<td>Number of susceptible animals at the beginning of the period</td>
</tr>
<tr>
<td>Incidence rate</td>
<td>Cumulative number of newly infected animals that occurred during the defined time period</td>
<td>The sum of each animal’s time at risk of becoming infected</td>
<td>Number of infections that occurred during the period</td>
<td>Cumulative time at risk during the period</td>
</tr>
</tbody>
</table>

susceptible host species. Japanese encephalitis virus (JEV) is a mosquito-borne Flavivirus and is the most frequent cause of arboviral-induced encephalitis of people around the world; domestic pigs are the most important reservoir for this virus. Recent work utilizing a landscape epidemiology approach has shown that the seasonal increase in the number of human infections in Southeast Asia and Oceania is now occurring up to 2 months earlier in the year, and persisting for up to 2 months longer than typically expected (Hsu et al. 2008). The explanation appears to be related to the local effects of climate change on ambient temperature and the increased frequency and intensity of rainfall, both of which have contributed to a change in the ecology of the mosquito vector. Similar findings have been reported for West Nile virus and bluetongue virus (Weaver and Reisen 2010); evidence of both infections can be found sporadically in domestic pigs though their role in further transmission is unlikely (Sugiyama et al. 2009; Teehee et al. 2005). A landscape epidemiology approach enabled researchers to identify these important public health trends that have the potential for substantially impacting the pig industries in affected regions.

MEASURING THE OCCURRENCE OF DISEASE

There are several ways to measure disease occurrence depending on the question one is trying to answer.
infected with Aujeszky’s disease virus (ADV; pseudorabies virus) and includes an appropriate interpretation of each of the measures.

For relatively stable populations in which the incidence rate of a disease is constant (rare for most infectious diseases in commercial pig production), the relationship among prevalence, incidence rate, and disease duration can be represented by the following formula (Dohoo 2003): prevalence risk = \( \frac{\text{incidence rate} \times \text{average duration of disease}}{\text{incidence rate} \times \text{average duration of disease} + 1} \). A cascade of successful events must occur in order for disease transmission to take place starting with exit of the pathogen from an infected host and ending with the pathogen finding a suitable habitat or cell to occupy in a susceptible host. Each step in this cascade has an associated probability of success, sometimes conditional or dependent on event(s) that occurred in a previous step. The combination of these probabilities results in a determination of the likelihood that the new host becomes infected and can be expressed qualitatively (low, medium, or high likelihood) or quantitatively (10%, 50%, or 90% likelihood). In common terms, a pathogen might simply be described using descriptors such as “highly contagious” or “very infectious,” for example, when it is perceived that transmission between pigs or farms is inevitable. Given that current laboratory techniques allow precise enumeration of the number of pathogens shed by an infected host and determination of the minimum dose required for transmission to occur, some clarification of terms related to the likelihood of successful transmission is useful. “Infectious(ness)” describes those diseases (as distinct from pathogens) that are capable of being transmitted between individuals, or can be alternatively described as the inverse of those diseases having a noninfectious cause. For example, neoplasia (perhaps excepting those with suspected viral origins) is considered noninfectious while most conventional diseases of pigs have an infectious cause (bacteria, viruses, internal and external parasites, and fungi/yeasts). “Contagious(ness),” by contrast, is a qualitative descriptor synonymous with the combined likelihood of success of all the events necessary for transmission to occur. The combination of host, pathogen, and environmental variables that would make transmission from an infected host to a susceptible host very likely to occur would also promote rapid spread between individuals; these types of diseases would be considered very contagious. By these definitions, a pig in the chronic stages of a resolving pneumonia that was initiated by infection with influenza virus 2 weeks previously would be considered to have an infectious disease (the pathology was initiated by influenza virus and the lungs continues to harbor transmissible secondary bacterial pathogens) but is currently in an ecological state whereby the disease is not very contagious (influenza virus is no longer present and the secondary bacteria are unlikely to be transmitted to an otherwise healthy pig).

The number of pathogens that must be delivered to the host in order to successfully establish infection is referred to as the infective dose. Recognize that this does not represent the number of pathogens that must be delivered to the site of replication in the host, as this number would theoretically be the same for a given pathogen, regardless of the route of exposure. As all infectious pathogens (and the pig host) are subject to normal biological variation (related to virulence attributes of the pathogens, and active or innate immune effectiveness for the pig), one should anticipate that there will be variation in the infective dose associated with a given pathogen. To standardize calculation and
reporting of this information, infectivity of a pathogen is generally stated as an “infective dose 50 or ID50.” The ID50 of a pathogen represents the dose level that will result in successful infection of 50% of those hosts that were exposed (to that dose level). Using porcine reproductive and respiratory syndrome virus (PRRSV) as an example, the ID50 for oral, intranasal, parenteral routes of exposure were reported to be 10^{2.2}, 10^{4.0}, and 10^{2.2} tissue culture infective dose 50 (TCID\text{50}, interpreted similarly to ID50), respectively (Hermann et al. 2005). In this case, oral exposure required a 1263-fold higher dose as compared with parenteral exposure reflecting the additional steps involved in the transmission pathway for PRRSV to reach its site of replication through oral exposure; presumably the same number of virus particles reached the site of replication in both instances.

**PATTERNS OF DISEASE OCCURRENCE**

Understanding the pattern of disease occurrence at a population level is an intuitively important consideration when hypothesizing the cause of a disease and how it might best be prevented, controlled, or eradicated. Critical to determining the pattern of disease occurrence is establishment of a case definition. This may be as simple as accepting a positive diagnostic test result but more often includes multiple criteria such as the presence of one or more clinical signs, the presence of a characteristic histological lesion, or perhaps even the lack of a confirmed diagnosis of a known pathogen. Once a case definition is established (recognizing the definition may need to be refined over time), one can proceed in establishing the disease’s orientation in space and time. Examination of the course of disease events over time is important in establishing associations between cause and effect. This is particularly important considering the difficulty in establishing the incidence rate of the disease when pigs are raised in “open populations” characterized by the continuous movement of pigs into and out of the population over time (e.g., continuous-flow growing–finishing facilities).

From a temporal perspective, disease occurrence is often described as sporadic, endemic, or epidemic. Sporadic disease occurrence refers to episodes of disease that occur randomly over time; cases are clustered in time but with variable intervals of time between the clusters. This may occur when opportunities for contact between a pathogen and a susceptible host are infrequent or when the presence of multiple factors (or agents) necessary for disease to occur are absent. Endemic disease generally refers to a situation whereby disease occurs at a stable prevalence with a predictable and invariable incidence rate; cases occur at an “expected rate.” While endemic is often thought to indicate a disease of low prevalence, *M. hyopneumoniae*-induced pneumonia of growing pigs presents an obvious example of an endemic disease that can establish at a high prevalence. Seasonal fluctuations do not preclude the use of the term endemic as seasonal effects tend to be reasonably predictable. Endemic disease occurrence often suggests long-term adaptation by the host and pathogen, usually accompanied by little or no mortality. Epidemic disease occurs when incidence rate increases to the extent that prevalence rises “above an expected level.” The interval between epidemics can be variable or invariable based on the specific ecology of the disease condition. Epidemics are often a result of an imbalance between host and agent due to a change in virulence, a change in the disease ecology, or introduction of a novel agent.

Epidemic curves are a useful graphical means of determining the pattern of disease occurrence; examples of sporadic, endemic, and epidemic occurrence are shown in Figure 11.2.

Examination of an epidemic curve can provide insight as to the type of pathogen causing the disease and the stage of the epidemic (early, peak, or declining). A steep ascending curve suggests a highly contagious cause and/or a short incubation time (e.g., a point-source outbreak of influenza virus via introduction of an acutely infected pig). Furthermore, this same shape might provide evidence of a common-source disease introduction (e.g., introduction of *Salmonella dublin* into a growing–finishing farm through a contaminated feed source) whereby exposure of the entire population occurs simultaneously or over a very short period of time. The duration of the “plateau” around the peak occurrence of cases and the slope of the descending curve are functions of the availability of susceptible animals. In contrast to point- or common-source epidemics, propagating epidemics occur when the first infected case (the index case) replicates the pathogen then transmits it to a second wave of susceptible individual(s). This second wave of individuals then repeats the activity setting off a series of propagating waves, the interval between each wave approximately equaling the incubation period of the pathogen. For highly contagious, short incubation period pathogens (as might be expected with the influenza virus example above), the waves may occur so quickly that the epidemic curve takes on the appearance of a point-source introduction. However, pathogens with longer incubation times or that are less contagious (e.g., sartocptic mange of pigs) would be expected to generate an epidemic curve that shows a series of progressively higher peaks, the peak of each spaced approximately one incubation period apart. Commonly, epidemics (for very sensible reasons related to disease intervention, quarantine of cases, or removal of a common source) rarely proceed completely through their natural evolution. Thus, examination of epidemic curves is often of most value for identification or confirmation
**11.2.** Epidemic curves representing sporadic (A; ADV outbreaks from 1975 to 2006 in Minnesota [Anderson et al. 2008]), endemic (B; weekly preweaning mortality rate on a typical U.S. farm [Gillespie 2009]), and epidemic (C; weekly mortality before and during postweaning multisystemic wasting syndrome [Martelli et al. 2000]) disease occurrences.
that an epidemic has indeed occurred, monitoring the progression of the outbreak, and for monitoring the effect of interventions.

In describing epidemics, it is useful to classify animals in the population according to a simple system: susceptible, infectious, or recovered. Of course, pathogenesis varies among different infectious organisms such that other disease states may also need to be represented: incubating (infected but not yet infectious to others), latent (infected but not continuously infectious to others), recovered (but not resistant to reinfection), and others. To put these terms in perspective, Kennedy published the following description using a grass fire as an analogy for the evolution of an epidemic disease outbreak (Kennedy and Roe 1987):

An infectious epidemic behaves very much like a grass fire. Whether an epidemic (grass fire) develops or not will depend on current conditions, the “pressure of infection” (amount of sparks/flames), a threshold proportion of susceptibles (dry grass) in the population, and the size and density of the susceptible population. The more resistant individuals (green grass) in a population, the more the epidemic is impeded. However, if the pressure of infection (fire) is high enough, even the resistant individuals may succumb (burn). Human intervention may also affect the course of the epidemic. Susceptibles may be immunized (back-burning) and cases may be treated (sprayed). Also, the infective and susceptibles may be isolated and quarantined (fire-breaks). Eventually, an epidemic may burn itself out as the number of susceptibles falls below the threshold, or may smolder on at endemic level until conditions are again conducive to another epidemic.

There exist several motivations for the development of tools that can describe or predict the nature of disease transmission events within animal populations. One obvious reason is to facilitate exotic disease response planning but other less obvious reasons include animal welfare (minimizing the number of animals affected by disease), economic evaluation of disease management strategies, and to support public policy around international trade of animals and animal products. Software tools utilizing either mathematically based modeling techniques or outbreak simulations have been developed to provide information on the likelihood, extent, and time course of disease epidemics.

The mathematically based tools generally utilize a state-transition approach whereby all individuals are assumed to exist in one of the three states: susceptible (S), infectious (I), or recovered (R). Assumptions are made about the contagiousness of the disease and the duration for which an individual remains contagious to others, then a series of differential equations are solved to transition individuals from one disease state to another at the predicted rates (Anderson 1982). The model assumes that every individual in the population can only be in one state at a given time, that susceptible (S) individuals become infectious (I) immediately after sufficient contact with an infectious individual, that an individual will infect others at a rate that is a function of the pathogen’s inherent contagiousness, and that infectious individuals remain infectious for a fixed, known time period. At the end of this infectious state, an individual is assumed to become resistant (R) for life. This simplistic approach ignores the latent state when individuals are neither susceptible nor infectious; it assumes that protective immunity is established immediately after but not before the end of the infectious state, and that immunity will be maintained for life. It also assumes that the process is irreversible; hence, an infectious or resistant individual cannot become susceptible again. Of course, SIR models can be varied to accommodate reversible and/or additional pathways when required. SIR models are usually based on daily state transitions though are flexible enough to use whatever time interval is appropriate for a given disease.

In its simplest form, an SIR model can be run with only two parameters: the transmission coefficient $\beta$ (beta) and the recovery coefficient $\alpha$ (alpha). The transmission coefficient represents the rate at which new infections occur given the number of individuals (or proportion) of S and I on the given day of the outbreak; the transmission coefficient is very closely associated with the “reproduction ratio,” which is defined as the number of successful transmission events produced by a single infected individual during one infectious period. At the beginning of a disease outbreak when almost all individuals are susceptible, the basic reproduction ratio “$R_0$” is used to describe the force of infection. If $R_0$ is greater than 1, an epidemic is likely to occur, if $R_0$ is equal to 0 an epidemic is unlikely to occur, and if $R_0$ is less than 1, infection dies out and an epidemic will not occur. The recovery parameter represents the rate at which individuals leave the infectious state, and is essentially related to the length of the shedding period of a pathogen or the time period for which a pathogen remains infectious in the environment. Output from a simple SIR model is shown in Figure 11.3 and illustrates the effect of varying contagiousness on the speed and magnitude of an epidemic.

While SIR models may seem overly simplistic, they are particularly useful in evaluating the effect of potential interventions, when attempting to identify the start of an outbreak (predicting backward in time to identify an index case or time), or predicting when the peak of an epidemic is expected to occur. For complex diseases, SIR models can be expanded to incorporate the effect of open populations (introduction of new susceptible individuals), latency, or the potential for reinfection of recovered individuals.
The pattern of disease occurrence is related to spatial factors as well as time. An important assumption underlying SIR modeling is that all individuals in the susceptible population have an equal probability of becoming infected. In recent years, spatially relevant modeling procedures have become available as an alternative to, or enhancement of, traditional transmission modeling methods (Pfeiffer 2008). Advancement in computer programming and the wide availability of vector-based geographical information systems now makes spatial modeling of transmission accessible to most epidemiologists. By combining spatial and temporal information about animal populations, the extent and frequency of contact between individuals, and the relevant ecological factors in the environment, accurate assessments of the likelihood of disease transmission in a real-life setting are possible. Recent work done in the United States utilized these methods as part of a regional PRRSV elimination program whereby clusters of farms related by geographical distance and by movement of pigs were identified (Mondaca-Fernandez and Morrison 2007); knowledge of this relationship between farms is critical to successful implementation of control programs for highly contagious diseases. Similar techniques are used in landscape epidemiology when knowledge of landforms, climate, spatial position, and animal demographics is required to understand risk related to insect vectors.

MECHANISMS OF DISEASE PERSISTENCE

The essence of veterinary medicine lies in control of animal diseases. Stopping transmission of pathogens between infected and susceptible hosts is a prerequisite for disease control as the economic, moral, and animal-welfare implications of disease control programs that rely entirely on treatment postinfection are substantial. There exist numerous modes of transmission including aerial spread, direct contact with an infected host, or indirect contact with an infected host through an animate or inanimate vector. Many pathogens take advantage of more than one of these modes in order to gain entry into the susceptible host through the alimentary tract, reproductive organs, respiratory system, across the integument, or by inoculation (iatrogenic or wounds). Despite our best efforts, management of these modes of transmission or routes of entry does not reliably result in eradication of disease unless one also considers the mechanism by which a pathogen is able to persist in the absence of a suitable host. For a pathogen to perpetuate, a mechanism must be available for it to survive in nature. Thorough study of the mechanisms by which pathogens are able to perpetuate themselves will also have the advantage of giving insight into potential reservoirs of emerging agents. With this information, we may be better equipped to anticipate the impact of the changing ecology of traditionally important pig pathogens, as production settings are altered to meet the challenges of global food distributors, exacting consumers, and environmental mandates.

Classification of pathogens based on their mechanism for perpetuation leads to sensible guidance on the complexity of control and eradication programs that are required for their management. Through modification of early published insight on the topic of pathogen perpetuation (Matumoto 1969), five categories are proposed and described in order of the least to the most complexity required for pathogen eradication: vector-borne pathogens, short-cycle pathogens, long-cycle pathogens, resistant pathogens, and commensal pathogens.

Vector-borne Pathogens

For the current discussion, pathogens that absolutely require a vector for successful transmission between infected and susceptible hosts will be considered vector-borne pathogens. Two features of this criterion should be clarified, namely that the vector is animate and that the vector is required for transmission to occur. Inani-
mate vectors (e.g., boots contaminated with pig feces containing *Salmonella*) are essentially an extension of the infected pig itself and suggesting that there is any particular role the infected boot is playing in the transmission pathway is nonsensical. Also, for the sake of clarification, pathogens such as African swine fever virus (ASFV) or PRRSV that are known to utilize insect vectors in their transmission but are not limited only to arthropod-borne spread will not be considered here. As an example, ASFV has three distinct transmission pathways: namely a sylvatic cycle involving *Ornithodoros* ticks and wild suids, an *Ornithodoros* tick and domestic pig cycle, and a direct pig-to-pig transmission cycle, suggesting that simply controlling the tick vector will not ensure eradication of the disease from a population of infected commercial pigs (Arzt et al. 2010). The essence of a vector-borne disease in the context of disease eradication is that control of the vector will ensure eradication of the disease.

At least two important infectious agents of pigs should be considered as exclusively vector-borne pathogens: JEV and vesicular stomatitis virus (VSV). Both agents are relevant to this discussion but for distinctly different reasons. Though the clinical disease due to JEV can be mild in pigs, pigs do serve as an important reservoir of infection for maintaining a population of infected mosquitoes in a geographical region. Japanese encephalitis is a globally important pathogen of humans for which pigs play an important role in the disease's landscape epidemiology. Similar to JEV, VSV is also transmitted between pigs by an arthropod vector (the virus has been recovered from several different genera of mosquitoes, flies, and midges). However, the disease occurs relatively infrequently though the New Jersey serotype of VSV is particularly well adapted to pigs (Martinez et al. 2003). The significance of the disease, at least among FMDV-free countries, is that VSV produces vesicular lesions in pigs that are grossly indistinguishable from other notable vesicular diseases including those produced by FMDV, vesicular exanthema virus (VEV), and swine vesicular disease virus (SVDV). Clinical outbreaks of VSV in pigs will produce predictably significant short-term effects on international trade until other exotic pathogens are ruled out.

For these examples, introduction of the pathogen into a population of pigs, maintenance of the pathogen in the pig population, and transmission of the pathogen between pigs in the population can be absolutely controlled by preventing contact with their respective arthropod vectors. This makes their control and eradication a straightforward, though practically, very difficult process.

**Short-cycle Pathogens**

Short-cycle pathogens include those agents whose greatest imperative is to quickly be transmitted to the next susceptible host as they generally have a very limited ability to exist outside the host. At first, one might assume this category would include all viruses as viruses are absolutely unable to replicate outside their host. However, there are at least a few notorious viruses and many bacteria that are perfectly content to bide their time (waiting, though not increasing in number) in a suitable environment until the right combination of ecological factors occurs that facilitates their entry into a new host where replication can begin again. Characteristics of short-cycle pathogens include rapid inactivation outside the host, short incubation periods, commencement of shedding shortly after infection becomes established (but without persisting for an extended time), rapid development of protective immunity, and a requirement for access to large populations of susceptible hosts in order for continuous transmission to occur.

Both bacterial and viral agents are represented in the short-cycle pathogen category with swine influenza virus (SIV) being an obvious member. SIV enters the respiratory tract as part of an oronasal or aerosol transmission event. Clinical signs of the disease become apparent in a matter of 3–5 days concurrent with establishment of high viral loads in lung tissue. High levels of virus are expelled in sputum or droplet nuclei over a relatively short period (less than 1 week) at which point clinical signs rapidly abate and solid immunity is conferred. Dense populations of susceptible individuals are necessary for the virus to propagate into an epidemic form. Influenza virus epidemics are seasonally apparent in people but are less apparent in modern pig production as a result of the establishment of large production cohorts (in pig-dense regions) on a continual basis, thus meeting the requirement for access to large populations of susceptible hosts. The virus has evolved in a way that ensures an adequate size population of susceptible pigs (or a population of cohorts of pigs) remains available; SIV has the propensity to genetically drift and recombine at a rate faster than immunity from a prior infection is able to persist. Though having a different pulmonary cell tropism, porcine respiratory coronavirus follows a pattern similar to that of SIV and thus is also classified as a short-cycle pathogen.

Several enteric agents can also be considered short-cycle pathogens. Transmissible gastroenteritis virus (TGEV) and a related coronavirus, porcine epidemic diarrhea virus (PEDV), share similar features of oral exposure, rapid incubation (hours), high levels of virus shedding over a short period of time, and generation of a robust immune response.

Given their need for large populations of susceptible hosts, their infected host's short duration of contagiousness, and their limited ability to remain viable outside the host, control and even eradication are achievable goals for short-cycle pathogens. The fundamental element in eradication plans for these
Long-cycle Pathogens

Long-cycle pathogens are those characterized by the ability to establish an infection in such a manner that the host remains contagious for an extended period of time. While these pathogens may share some attributes of short-cycle pathogens such as a rapid incubation phase, their hallmark feature is the ability to find an ecological niche in the host that will allow them to resist clearance by the normal immune mechanisms of the pig. Congenital and other forms of vertical transmission are also common features among long-cycle pathogens. Because of their propensity to establish a long-term contagious state in the host, these pathogens are not dependent on having access to large or dense populations of susceptible hosts; their ability to persist is largely independent of population size.

Several noteworthy viruses fall into the category of long-cycle pathogens as do a number of rather unique bacterial species. *Actinobacillus pleuropneumoniae*, *M. hyopneumoniae*, and *Mycobacterium* species (especially *Mycobacterium avium*) are respiratory bacterial pathogens characterized by their ability to induce chronic, smoldering infections in the lungs. While sporadic outbreaks do occur, particularly with *A. pleuropneumoniae*, all three establish long-term residency in their own unique biological niche (pulmonary abscesses for *A. pleuropneumoniae*, the extracellular surface of the bronchial epithelium for *M. hyopneumoniae*, and lymph node or pulmonary abscesses for *M. avium*) that keeps them well isolated from the direct effects of the pig's immune system and promotes an extended period of contagiousness. Similarly, long-cycle viral pathogens exist that have a pulmonary tropism including ADV and PRRSV. Despite both demonstrating the short-cycle attribute of rapid incubation period (and creating explosive epidemics as a result), their most significant epidemiological feature is the ability to establish a persistent infection. As a member of Herpesviridae, ADV creates a classic latent infection through invasion and residency in the trigeminal nerve where it remains ready to be reactivated to systemic sites (in preparation for an impending transmission event) when biological stress is experienced by the pig. Neurons are a site preferentially sequestered from most of the usual pathogen clearance mechanisms of the pig as most of these mechanisms are lethal to the host cell that is harboring the pathogen; with little redundancy in the nervous system, any immune response that results in destruction of neurons is likely to have a profoundly negative effect on the host. While much of the pathogenesis related to PRRSV infection has been reported, the exact means by which it escapes clearance by the immune system remains unclear. The virus has a strong preference for long-term residency in various immune cells of monocytic lineage and evasion mechanisms including production of quasi species, presentation of decoy epitopes, and induced dysregulation of various cytokines and other immune messengers have been hypothesized though not fully explained (Mateu and Diaz 2007).

Long-cycle pathogens also occur in other body systems beyond the respiratory system. Various serovars of *Leptospira interrogans* occur in pigs and establish long-term infections in the kidney and urinary tract, *Mycoplasma suis* resides in the blood and hematopoietic system for extended periods, and *Brucella suis* establishes persistent infection of male reproductive tissues. The enteric system is home to several unique long-cycle pathogens. *Lawsonia intracellularis* presents in several different clinical forms, but all share the unique histological feature of chronic inflammation of the small intestine caused by persistent intracellular infection of enterocytes and lamina propria cells by the bacterium. Spirochetal diseases of the colon (*Brachyspira hyodysenteriae* and *Brachyspira pilosicoli*) can create clinical signs reminiscent of the diarrhea associated with *L. intracellularis* but are generally more severe. Intracellular residency in the epithelial cells of the colon again provides refuge from the immune system as it attempts to resolve the ongoing infection. *Salmonella* species, particularly *Salmonella choleraesuis* var. *kunzendorf*, also take advantage of the intracellular niche to avoid detection by the immune system. Erysipelas is another disease caused by a bacterial pathogen that finds a location preferentially sequestered from the immune system. Often as sequelae to the acute stages of the systemic illness (producing the unique erythematous dermatitis lesions that are the hallmark of erysipelas), the bacterium will often lodge in joint fluid, synovial membrane, and surrounding tissues to cause chronic arthritis. The acute bacteremia apparently violates the integrity of the joint-blood barrier allowing *Erysipelothrix rhusiopathiae* to enter the joint space; after resolving the acute infection, the integrity of the barrier is restored, effectively separating the bacterium from the full range of immune clearance mechanisms.

The significance of long-cycle pathogens is the difficulty in which successful eradication programs can be established (excepting of course, through complete depopulation of herds). Similar to short-cycle pathogens, most long-cycle pathogens are not particularly well equipped to thrive outside the living host for extended periods. What they are well equipped for is thriving inside the host for extended periods. Few reliable protocols for eradication of these pathogens exist
at the herd level, and even fewer at an industry level. Quality diagnostic tests become the critical arbiter for most eradication programs designed for these pathogens. Aujeszky’s disease stands out as a uniquely eradicable long-cycle pathogen given the combination of a highly efficacious vaccine, the availability of diagnostic tests that can differentiate infected from vaccinated animals (DIVA), and accessibility to a wide array of highly accurate, sensitive, and specific diagnostic tests for both ruling-in and ruling-out infection with ADV. For the foreseeable future, efforts in managing most of the long-cycle diseases will be through control plans rather than eradication efforts. However, over time and with good compliance, effective control plans can lead to pathogen elimination but with whole-herd test and removal procedures often required to identify the last of the persistently infected pigs.

**Resistant Pathogens**

Some pathogens adopt a perpetuation strategy that involves creation of a stable form resistant to environmental degradation or inactivation. Whether this strategy is a result of evolutionary selective pressure or simply in response to the failed ability to transmit between hosts at short intervals is unknown but regardless of the reason, it has developed into a highly successful strategy by a few bacteria and viruses, and also by most of the pig nematodes. The characteristic feature of resistant pathogens is simply their resistance to inactivation in the environment (many remaining infective over months to years); resistant pathogens have no reliance on access to large or dense populations of susceptible animals. An additional common, though not requisite feature of many long-cycle pathogens, is their ability to indiscriminately affect different host species.

Two important bacteria will serve as fitting examples of resistant pathogens. The first bacterium is *Bacillus anthracis* or simply “anthrax.” *Bacillus anthracis* can produce various lesions and clinical signs in pigs ranging from acute sudden death to less dramatic syndromes that induce multifocal lymphadenitis. It is a historically important disease (though rarely seen anymore in modern pig industries) but remains an important zoonotic risk for abattoir workers, particularly in those plants processing grass-fed ruminants. *Bacillus anthracis* is a large gram-positive bacillus that when shed into the environment (often as a result of the death of an acutely affected animal), is signaled through exposure to oxygen to form a capsular spore that makes the organism highly resistant to inactivation by heat, desiccation, ultraviolet light, and disinfectant chemicals. Contaminated environments (notably, burial pits established as part of cleanup efforts after large outbreaks of anthrax) can remain a hazard for susceptible hosts for years. *Bacillus anthracis* is able to infect nearly any mammalian species. The second bacterium that will be used as an example of a long-cycle pathogen actually represents most of an entire genus of bacteria: *Clostridia* spp. *Clostridium perfringens*, given its high prevalence in many commercial pig populations, will serve as a convenient example of the genus though a similar case could also be made for *Clostridium novyi*, *Clostridium botulinum*, *Clostridium chauvoei*, or other clostridial species. *Clostridium perfringens*, like *B. anthracis*, is a relatively non-species-specific, gram-positive bacillus, proficient at spore formation under appropriate environmental conditions. Infection of young pigs by this particular species of *Clostridium* generally results in neonatal diarrhea with high within-litter prevalence. Farrowing sows that are housed outdoors often have a higher prevalence of clostridial diarrhea in their nursing piglets compared with their peers that farrow indoors, likely due to the simple fact that routine and periodic cleaning/disinfection of indoor farrowing facilities reduces (though does not eliminate) the infectious dose of bacteria presented to the neonatal pig. Genotyping of *C. perfringens* occurs as part of diagnostic investigation on some farms in order to support appropriate choice of prefarrowing vaccinations, but once diagnosed on a farm, the farmers tend to manage the disease rather than make any attempt to eliminate the organism. *Clostridium perfringens*, though potentially zoonotic, is rarely transmitted directly from livestock to people. It is, however, commonly found in commercial bovine, sheep, and goats with no or minor genetic changes relative to those isolates recovered from pigs.

Internal parasites comprise most of the membership in the group of resistant pathogens. For most of the pig nematodes of which the reader will already be familiar, the adult form of the parasite is relatively fragile in an environment outside the host. It is the parasite’s ova that present the long-cycle perpetuation opportunity for the pathogen. Life cycles vary extensively between *Ascaris*, *Ostertagia*, *Trichuris*, and other significant pig nematodes, but the parasites do share some common features: They have long and complex prepatent periods (“incubation periods” relative to bacteria and viruses), require sexual reproduction, and produce ova capable of lasting years or decades in typical pig-raising environments. These ova can successfully resist desiccation, chemical inactivation, and, in some cases (*Ascaris suum*), even physical removal from the environmental surface itself. Notable microscopic parasites that share some of these same features include *Trichinella spiralis* and *Toxoplasma gondii*; while previously mentioned nematodes such as *A. suum* do periodically infect man, *T. spiralis* and *T. gondii* have the well-deserved reputation of being substantial risks to public health in some countries.

In the context of disease transmission, control, and eradication, pathogens that perpetuate through use of environmentally resistant life stages present both opportunities and challenges. The bacteria used as
 examples demonstrate the difficulty that would be faced in attempting to establish eradication programs as the life span of the resistant organism likely exceeds the life span of the pig and the farmer! If one chooses to raise pigs in an environment that was used by a pig in a former time, there can be reasonable surety that the resistant pathogens have remained behind. Often, only low infective doses are required to establish infection with resistant pathogens, and once the first successful transmission event occurs, the infected individual can effectively recontaminate the environment with a sufficient number of the agent to repeat the cycle again in a week, a month, a year, or a decade later. Similarly, the internal parasites described above can be difficult to eradicate depending on the environment in which one chooses to raise the pigs. Over time, contaminated indoor facilities can be rid of parasite ova with fastidious hygiene, persistent use of anthelmintics, and screening of new stock to avoid reintroduction of the pathogen. In an outdoor environment that has previously housed pigs, eradication is likely an unachievable goal. Conceptually, for control of resistant pathogens, all one has to do is avoid contact between the susceptible host and the agent. Practically, that is also the biggest problem. “Where once a pig has lived, the roundworm will remain.”

**COMMENSAL PATHOGENS**

Commensal, derived from the Latin word *commensalis* (com- meaning together and -mensalis indicating a feature of the table), was originally cast to describe those infectious organisms habituating the alimentary tract but causing no apparent harm to their host. The term has come to more broadly indicate the coassociation of any two organisms that are neither dependent nor parasitic on each other. Using this definition, one is provided with an apt descriptor for a class of pathogens that seem to be found in association with otherwise healthy pigs but have the notorious reputation for periodically transforming into a virulent state with expression of fulminant clinical disease. Mechanisms involved in this periodic occurrence are poorly understood for most of the pathogens in this group, though concurrent or recent infectious conditions, the presence of an external stressor causing a disruption in homeostatic mechanisms, or diminished barrier function have been implicated as contributing factors in humans (Tkaskalova-Hogenova et al. 2004).

Multifactorial causation is another term for the requirement that specific cofactors must be present in order for a commensal pathogen to cause clinical disease. Perhaps the prototypical pig diseases in this respect are the porcine circovirus-associated diseases (PCVADs); the causative agent, porcine circovirus type 2 (PCV2) only recently emerged in pigs (Firth et al. 2009). Its associated clinical expressions remain a significant challenge to reproduce experimentally despite its widespread occurrence across pig farms around the world (Madec et al. 2008). Its classical presentation as a cause of nonresponsive wasting in juvenile pigs cannot be reliably reproduced in an experimental setting even when using low-passage field virus from affected farms. Characteristic tissue lesions can be generated in this setting but only rarely to the degree of severity and prevalence common in field outbreaks. Infection in the laboratory or in a field setting results in high levels of viral shedding with subsequently high levels of transmission occurring. However, the overt clinical signs described for PCVAD only seem to occur when the virus is present in combination with other infectious and noninfectious cofactors. Vaccination of pigs infected or expected to become infected with PCV2 reliably prevents the occurrence of clinical signs of PCVAD in affected farms (Kixmoller et al. 2008; Neumann et al. 2009) and even more interestingly, anecdotal evidence is accumulating that suggests vaccination against PCV2 can improve the performance of pigs on farms with known presence of the virus but without any clinical signs of PCVAD (Agten et al. 2010; Brons et al. 2010; Luppi et al. 2010; Sidler et al. 2010).

Commensal pathogens that appear to require the presence of an external stressor in order to be induced into causing clinical disease include *Haemophilus parasuis* and *Streptococcus suis*. Vertical and horizontal transmission pathways have been described for both bacteria; indeed, it is very likely that no pig farm in the world is free of either of the pathogens. While genetically distinct strains of each organism are known to occur and these strains possess different combinations of identified virulence factors, it is rare that the presence of one of these virulence factors in a given strain is enough to simply explain the occurrence of clinical disease. The anecdotal literature presents examples of farms that operate with little or no clinical disease related to either pathogen for extended periods of time until without clear explanation, a spontaneous outbreak occurs (MacInnes and Desrosiers 1999; Tokach 1993). External stimuli proposed to induce outbreaks of clinical disease for *H. parasuis* or *S. suis* include temperature changes, feed outages, and coinfection with other agents (Drum and Hoffman 1998; Oliveira and Pijoan 2004; Villani 2003). Another streptococcal bacteria, *Staphylococcus hyicus* is a common inhabitant of the pig and its environment and in most instances, appears to simply exist as part of the external skin flora. However, the agent is implicated as the causative agent of exudative epidermitis or “greasy pig disease.” Both the anecdotal and peer-reviewed literature reports that the disease appears both sporadically and epidemically on farms often without explanation; hypothesized cofactors for expression of the disease include low herd-level parity, hygiene, and overall poor quality of farm management and pig husbandry (Clark 2002;
Colibacillosis, presenting both as neonatal diarrhea (primarily *Escherichia coli* possessing the fimbrial antigens F4, F5, F6, and F41) and postweaning diarrhea (*E. coli* F41 or F18), presents an interesting situation with regard to diseases that are caused by commensal pathogens. Numerous members of Enterobacteriaceae are considered commensal organisms, generally categorized as such by the absence of one of these recognized fimbrial types. However, reports describing typical clinical cases of neonatal colibacillosis or postweaning diarrhea, but associated with nontypical or untypeable *E. coli* are becoming more common (Harel et al. 1991). The extent to which an *E. coli* is either “pathogenic” or “commensal,” or whether a given pathovar can move between these states has not been fully resolved. Genetic mechanisms (horizontal gene transfer [HGT]) for exchange of virulence factors between pathovars of *E. coli*, which allow for rapid shifts in the severity of the infection, have been described in human infections (Croxen and Finlay 2010). In pigs, virulence factors similar or identical to those described for human isolates of the bacterium have been described, and it is likely that the same genetic mechanisms for their exchange occur (Wu et al. 2007; Zhang et al. 2007). Data from field studies of pig farms have shown that at least on a population basis, numerous fimbrial types of *E. coli* are present in pigs from shortly after birth (including those presumed to be pathogenic) and that the relative contribution of each fimbrial type changes with pig age (Katouli et al. 1995). The F4 fimbrial type has been studied extensively in this regard, and a particular pathovar expressing the O149 somatic antigen has been isolated from both healthy and diarrheic pigs (Amezcua et al. 2002, 2008; Melin et al. 2004). An explanation for its apparent presence both as a commensal and a pathogen on a single farm remains unexplained but may involve contributions from the host, the environment, and additional virulence factors that are expressed or acquired in response to these contributing factors. The extent to which these genetically based changes are random or occur as a result of selective pressure is not entirely known.

The significance of recognizing the existence of a commensal pathogen type is that one has to recognize the fact that some pathogens are extraordinarily unlikely to be eradicable. Management of commensal pathogens will rely on establishing control measures (vaccination, treatment, and husbandry) that temporarily shift the balance of power into the hands of farmers and veterinarians and away from the pathogens.

**CAUSATION**

Any discussion of disease transmission would be lacking without remarking on our own evolving view of disease causation. The early days of infectious disease research brought forth rapid and substantial leaps forward in our understanding of disease causation. However, it soon became clear that the ideal model of single-agent diseases was inadequate to explain the occurrence of the range of maladies presented on livestock farms. Schwabe (1982) published an essay on the five stages of evolution of the world’s understanding of animal disease control, starting with man’s dependency on subsistence agriculture through to the epidemiological revolution that started in the 1960s. Table 11.2 presents a summary of his essay and goes on to propose a sixth stage in the evolution that attempts to incorporate our current understanding of animal disease causation and management from an ecological perspective.

Formal postulates for determining disease causation had their beginnings during the mid-19th century and were most famously incorporated into an essay written by Jakob Henle (1838). His postulates were subsequently revised by one of his former pupils, Robert Koch, and presented in their more commonly accepted form in 1890: The agent must be present in every case of the disease under appropriate circumstances, the agent should occur in no other disease as a fortuitous and nonpathogenic agent, and the agent must be isolated from the body in pure culture, repeatedly passed, and induce disease when reintroduced to the host. The inadequacy of these postulates in covering all the potential relationships between pathogen and host became apparent within 10 years of their publication but nonetheless, remain today as a useful framework for establishing infectious disease causality. Because of limitations introduced by strict interpretation of the Henle–Koch postulates, authors have attempted to generate other lists of causal criteria that incorporate an epidemiologically sensitive perspective of the agent–host–environment relationship embodied in disease ecology. A set of criteria for determining disease causation was published by Hill (1965) that seems to have captured the essence of an epidemiological diagnosis:

- **Strength of association**—There is a strong association between the putative factor and disease (as measured by relative risk, odds ratio, and others).
- **Consistency**—The association should be repeatedly observed by different persons, and across different places, circumstances, and times.
- **Specificity**—The occurrence of one (or a group of) causal factor(s) should lead to only one disease, and the disease should result only from that cause.
- **Temporality**—Cause must precede effect; the putative factor must precede the occurrence of the disease.
- **Biological gradient**—When exposure to the factor falls along a gradient, higher exposure should result in more severe (or frequent) disease; a dose–response effect should exist.
Table 11.2.  Evolution of the science of animal disease management

<table>
<thead>
<tr>
<th>Strategic Approach to Healing</th>
<th>Theory of Disease Causation</th>
<th>Tactical Approach to Disease Problems</th>
<th>Typical Signal Indicating Disease Had Occurred</th>
<th>Crisis Propelling an Evolutionary Leap</th>
<th>Response to Crisis</th>
</tr>
</thead>
<tbody>
<tr>
<td>None (early farming systems and subsistence)</td>
<td>The supernatural</td>
<td>Prayer, exorcism, divination, sacrifice</td>
<td>Death of draft animals (oxen, asses)</td>
<td>Urbanization required more intensive production than provided by subsistence</td>
<td>Emergence of veterinary “healers”</td>
</tr>
<tr>
<td>Local veterinary healers (up to first century AD)</td>
<td>Natural environmental factors, for example, miasmas</td>
<td>Recognition and treatment of clinical signs, for example, laying on of hands Quarantine Slaughter of sick animals</td>
<td>None (early farming systems and subsistence)</td>
<td>Overdependency of large states on horses for conquest, transport, and communication</td>
<td>Development of military veterinary organizations</td>
</tr>
<tr>
<td>Military power and authority (first century AD to 1762)</td>
<td>Humoral imbalances</td>
<td>Clinical diagnosis of diseases</td>
<td>Rinderpest; death of civilians, multipurpose animals</td>
<td>Increased density of human populations made the economy economically sensitive to animal plagues Repeat of the European experience in the United States</td>
<td>The “great sanitary awakening”; creation of veterinary schools and organized medicine</td>
</tr>
<tr>
<td>Veterinary sanitary authorities (1762–1884)</td>
<td>Man-created environmental factors, for example, “filth”</td>
<td>Farm hygiene Slaughter regulation Appearance of clinics</td>
<td>Significant animal plagues continue in Europe, zoonoses recognized</td>
<td>The “microbiological revolution”; creation of specialized veterinary laboratories</td>
<td>Creation of specialized veterinary epidemiological services</td>
</tr>
<tr>
<td>Area/mass actions (1884–1960)</td>
<td>Specific etiological agents; infectious agents</td>
<td>Mass testing Laboratory diagnosis Vector control Mass immunization Mass treatment Applied ecology Education</td>
<td>Recognition of nonhost specificity, nonfulfillment of Henle–Koch postulates, appreciation of “disease complexes”</td>
<td>Demand for economic justification, occurrence of insidious “production-type” diseases, recognize special needs created by intensive farming practices</td>
<td></td>
</tr>
<tr>
<td>Surveillance and selective actions (1960–2010)</td>
<td>Interaction of agent–host–environment</td>
<td>Epidemiological diagnosis Surveillance Qualitative and quantitative data analysis</td>
<td>Occurrence of animal plagues even in well-managed national herds, concurrent disease in people and livestock</td>
<td>Emergence of novel viruses, wildlife pathogens leaping to humans and livestock, global climate change</td>
<td>Techniques for analysis of spatial factors and molecular evolution</td>
</tr>
<tr>
<td>Disease ecology and landscape epidemiology (2010)</td>
<td>Disease ecology and landscape epidemiology</td>
<td>Risk management Process monitoring and statistical process control Molecular diagnostics</td>
<td>Occurrence of animal plagues even in well-managed national herds, concurrent disease in people and livestock</td>
<td>Emergence of novel viruses, wildlife pathogens leaping to humans and livestock, global climate change</td>
<td>Techniques for analysis of spatial factors and molecular evolution</td>
</tr>
</tbody>
</table>

*Table reproduced with minor variations from Schwabe (1982). Grayed areas added by the author.

Plausibility—The association should be plausible with respect to biological knowledge, recognizing that our knowledge is limited to facts known at the time.

Coherence—The cause-and-effect interpretation of the association should not seriously conflict with the generally known facts about the natural history and biology of the disease.

Experiment—it may be possible to generate prospective experimental evidence whereby removal of the putative factor results in less severe (or frequent) disease.

Analogy—Similar known examples of cause and effect can support the existence of a causal association.

Reading these criteria points out the need to understand the distinction between necessary and sufficient causes of disease. Multifactorial diseases are those created by exposure to a combination of several independent factors. A necessary cause is considered to be an exposure (to an agent or risk factor) without which the disease cannot occur. Implicit in this definition is
that this exposure, when occurring in isolation, may not cause disease in all individuals. Other risk factors (or agents) may need to be present simultaneously in order for the disease to occur. When in combination, exposure to a defined group of factors results in disease in all the individuals, this group of factors is considered to be a sufficient cause. Necessary and sufficient cause provides a flexible framework for undertaking disease outbreak investigations in which the true cause of the outbreak is unknown. A cautionary note is however required. When sorting through the myriad potential causal factors associated with occurrence of a disease, it is inevitable (particularly in the early phases of an outbreak investigation) that factors will be identified that have the appearance of being causally related to the disease but in truth are not causal. These factors are termed confounding variables or “confounders.” By definition, a variable is a confounder if it is associated both with the true causal exposure variable and with occurrence of the disease; however, the confounding variable must not be caused by the disease. A simple example illustrates the concept: As facts, we know that (1) smoking is strongly associated with the occurrence of lung cancer; (2) people who smoke tend to have yellow staining on the skin of their forefingers; and (3) only people who smoke have yellow staining on their fingers. If an early researcher was investigating the cause of lung cancer and did not consider the impact of smoking on the occurrence of lung cancer, he or she might erroneously report yellow fingers as the cause of lung cancer (i.e., strong evidence of a causal relationship as described above by Hill’s criteria)! Unfortunately, most real-life situations are not this clear as we may have very little prior knowledge about risk factors and their relationship to a disease outcome. In order to organize one’s thought processes during disease investigation activities, path diagrams or “webs of causation” can be created to evaluate the potential role of confounding variables in the outbreak. A causal web illustrating the association of various risk factors with respiratory disease in pigs is shown in Figure 11.4 as an example. Analytical methods are available for assessing the certainty, strength, and interaction of risk factors and disease outcome when presented as causal webs (Stage et al. 2004).

### BIOSECURITY

Biosecurity plans at their most rudimentary level attempt to manage the risk of introducing new pathogens into farms, livestock industries, or countries and to minimize the transmission of endemic diseases between farms within these levels. These goals are accomplished through segregation of uninfected from infected animals (or the pathogen itself), thorough cleaning of livestock premises and facilities, and appropriately managed disinfection of the same (Madec et al. 2010). There is, however, a greater imperative at stake when considering biosecurity than just the health of livestock species. We have an opportunity and perhaps
even an obligation to develop a more holistic approach to biosecurity that appreciates agriculture’s contribution to zoonotic disease transmission, farmed animals as a source of human food-borne pathogens, and our broader impact on the environment and biodiversity. In the text that follows, principles involved in establishing biosecurity plans at local (farm), national, and international levels will be described.

Biosecurity is fundamentally about managing two sources of special cause variation on farms: pathogens and people. Sources of variation in the biological or financial performance of a farm can be attributed to either normal (or random) variation or special cause variation. Normal variation, be it related to a pig’s genetic potential for growth, the lysine content of a soybean, or the average ambient temperature in Ames, IA, during December, is by definition predictable given some current and historical knowledge of the biology, chemistry, or physical properties of that particular variable. By contrast, special cause variation describes those fluctuations in the value of a variable that exceed the boundaries of what we consider to be “normal”; this exceptional fluctuation may be defined as one that falls beyond plus or minus three standard deviations from the mean value, contributes more to the risk of a particular outcome than what would normally be expected, or any other measure one may determine is appropriate for the purpose. Systematic fluctuations due to normal variation in livestock production systems cannot be eliminated, though one strives to reduce the range of normal variation. The occurrence (and magnitude or frequency) of special cause variation in production systems, however, can absolutely be managed with the ultimate goal being to eliminate it entirely.

Biosecurity as a discipline began making an appearance in the livestock-related literature during the 1990s with several authors presenting work aimed at creating consensus around the definition of the term and the scope of activities that it might encompass (England 2002; Pyburn 2001). A review of relevant literature on the subject around the same time (Amass and Clark 1999) confirmed a paucity of facts that could scientifically support the recommendations being made by veterinarians to their clients about biosecurity measures. The same author then embarked on a series of innovative studies to debunk biosecurity myths (Amass et al. 2000), to establish the efficacy around commonly used disinfectants and their appropriate usage (Amass 2004), and to understand the role of people and inanimate objects in disease transmission (Amass et al. 2003a–c).

With the advent of real and perceived global worry over terrorism, the occurrence of large-scale outbreaks of animal disease, including the emergence of novel zoonotic pathogens (Table 11.3), a plethora of organizations began publication of biosecurity guidelines for use by livestock producers. A group of authors surveyed

Table 11.3. Two decades of pig disease; emergence or reemergence of pig pathogens (1990–2010)

<table>
<thead>
<tr>
<th>Year</th>
<th>Disease</th>
<th>Location/Emergence</th>
<th>Species</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1999</td>
<td>West Nile virus</td>
<td>United States</td>
<td>Humans</td>
<td>Promed-Mail: Archive Number 19990925.1708</td>
</tr>
<tr>
<td>2000</td>
<td>Classical swine fever virus</td>
<td>United Kingdom</td>
<td>Pigs</td>
<td>Promed-Mail: Archive Number 20000809.1331</td>
</tr>
<tr>
<td>2001</td>
<td>Foot-and-mouth disease virus</td>
<td>United Kingdom</td>
<td>Multiple</td>
<td>Promed-Mail: Archive Number 20020825.5147</td>
</tr>
<tr>
<td>2004</td>
<td>Methicillin-resistant Staphylococcus aureus (MRSA)</td>
<td>United States</td>
<td>Humans, pigs</td>
<td>Promed-Mail: Archive Number 20050819.2436</td>
</tr>
<tr>
<td>2005</td>
<td>Streptococcus suis</td>
<td>China</td>
<td>Humans, pigs</td>
<td>Promed-Mail: Archive Number 20060412.1087</td>
</tr>
<tr>
<td>2006</td>
<td>Porcine high-fever disease</td>
<td>China</td>
<td>Pigs</td>
<td>Promed-Mail: Archive Number 20070607.1845</td>
</tr>
<tr>
<td>2007</td>
<td>African swine fever virus</td>
<td>Georgia</td>
<td>Pigs</td>
<td>Promed-Mail: Archive Number 20081211.3896</td>
</tr>
<tr>
<td>2009</td>
<td>Pandemic H1N1 influenza virus</td>
<td>Worldwide</td>
<td>Humans, pigs</td>
<td>Promed-Mail: Archive Number 20100420.1284</td>
</tr>
<tr>
<td>2010</td>
<td>Foot-and-mouth disease virus</td>
<td>Japan</td>
<td>Multiple</td>
<td>Promed-Mail: Archive Number 20100420.1284</td>
</tr>
</tbody>
</table>
the literature and the Internet for farmer-targeted publications on biosecurity in the United States and found 111 publicly available sources representing all the major livestock species (Moore et al. 2008).

Despite a similarity in recommendations made across many of the publications, the authors speculated that the sheer mass of information available likely contributed to poor biosecurity compliance due to confusion over “which guidelines do I use?” Indeed, several authors have reported poor farm-worker compliance with biosecurity recommendations (Nespeca et al. 1997; Vaillancourt 2005) with at least one study suggesting that farmers could not perceive which of the biosecurity practices (that they were asked to perform) were most useful or important (Casal et al. 2007).

**Biosecurity Planning Within Farm (Herd Level).** Biological risk management (BRM) has been suggested as a term for use of management tools that help to identify infectious hazards to a farm (or veterinary practice, other livestock premises, etc.), to assess the risk presented by each hazard, then to develop plans for managing each of the hazards (Bickett-Weddle 2005). Based on traditional thinking, a farm’s biosecurity plan would be considered a failure at the moment a disease was introduced to that farm. However, a more modern approach to biosecurity planning such as that embodied in BRM planning recognizes that disease risk cannot be completely eliminated, but only managed. Also, because few farms are constructed or operated in identical manner, it would be illogical to think one biosecurity plan could meet the needs of all farms. Several risk-based methodologies have been established for the development of biosecurity plans including an online tool for veterinarians called Infection Control (Center for Food Safety and Public Health, Iowa State University, Ames, IA), an audit-based approach developed specifically for use on pig farms called the Production Animal Disease Risk Assessment Program (www.padrap.org; American Association of Swine Veterinarians, Perry, IA), and an Australian swine-specific biosecurity assurance program that has its basis in hazard analysis and critical control point (HACCP) methodology (Animal Health Australia, Deakin, ACT, Australia).

HACCP methodology is a useful tool in constructing BRM plans. HACCP methodology had its origin in the U.S. National Aeronautics and Space Administration (NASA) in the 1960s. Logistical planners involved in the design of systems that would support astronauts while they were in space recognized a need for systematic methods of quality assurance around food products that would accompany the astronauts. Recognizing the inadequacies of a quality system that relied on end-product testing of food (accuracy and reliability of testing, determination of which hazards should be tested, high cost and time factors related to testing), NASA began working with process engineers at Pillsbury to develop a method that would ensure the creation of safe food products through quality monitoring of the production process, rather than the production output. The full history and development of HACCP is reported elsewhere, and the reader is encouraged to read further for more detail (Dunkelberger 1995; Sperder and Stier 2009). Process monitoring rather than end-product testing is a useful paradigm for establishing BRM plans.

HACCP methods are based on seven principles: (1) identification of potential hazards; (2) determination of critical control points (CCPs) at which a process can be established to mitigate any hazard that is introduced; (3) establishment of critical limits around each CCP; (4) creation of a process to monitor each CCP; (5) establishment of corrective actions to be taken in the event a critical limit is exceeded; (6) institution of record-keeping procedures that can document CCP monitoring activity; and (7) verification of HACCP system performance through periodic quality testing of the end product. As described above, pathogens and people present the two most important sources of special cause variation. The fact that both are animate variables (as opposed to a kernel of corn, a concrete floor, or a mechanical fan) gives them the unique ability to respond to feedback from the farm operator, and they are thus likely to generate special cause variation. Pathogens respond to this feedback through evolutionary mechanisms such as acquiring resistance to antibacterials (Aarestrup et al. 2008), modifying their epitopic presentation to a pig’s immune system (Ostrowski et al. 2002), acquiring virulence mechanisms (Villa and Carattoli 2005), or simply by emerging from nature as a novel pathogen (Kirkland et al. 2007). People respond to feedback through all of the predictable behaviors that typify the human condition: avoidance of disliked tasks, poor compliance with procedures having little direct or short-term benefits to themselves, reluctance to seek clarification when requested assignments are poorly understood, and the “creative will” or tendency to modify established processes for their own best fit. Using HACCP methods as a basis for the development of BRM plans, especially when they are developed in conjunction with the farmworkers expected to comply with the BRM plan, provides a platform whereby biosecurity compliance can become the norm, rather than the exception. HACCP helps us to separate special cause variation from normal variation, identify the cause of the special variation, then remediate the process.

A simple example using HACCP to manage the hazard of disease introduction (e.g., leptospirosis, toxoplasmosis, swine dysentery, others) through exposure of pigs to rodents will illustrate the process. Step one involves identification of hazards around the process. For this example, step one or hazard identification is...
straightforward: lack of an effective rodent control program will increase the likelihood of contact between pigs and the disease vector. Step two is identification of appropriate CCPs; this step may be assisted through construction of process flow diagrams or causal webs as has been described above. Logical CCPs in this rodent control process might include placement and management of baiting stations, and environmental management around the periphery of buildings (avoiding accumulation of materials that may be used by rodents as nesting/hiding spaces, controlling vegetation, and avoiding feed spills). Establishment and monitoring of critical limits around these CCPs are straightforward and will fulfill the third and fourth steps in HACCP. For example, the expected rate of bait usage can be estimated with assistance from a pest management company, and monitoring the usage rate of the farm can be as simple as tracking the history of bait purchase and ensuring the appropriate amount is being used each month. Establishing and monitoring critical limits around the environmental management CCP require a bit of creative thinking but little hard work: Grass and weeds should be kept mowed to a height less than 10 cm, a walking space the width of a person’s outreached arms should be maintained clear of debris around the exterior of all buildings, and all feed spills should be cleaned up on a daily basis (effectively, zero tolerance). Step five or establishment of corrective actions is again straightforward in this example but has to be considered in two phases. Phase one is giving immediate attention to the CCP that is out of control, for example, “go mow the grass” or “go clean up the feed spill.” Phase two of the corrective action is as important as phase one but is often forgotten, that being the need to remediate any product (sic pigs) that may have been negatively affected during the period the CCP was out of compliance. In our example, it may be the case that the farm was participating in a Leptospira-free production scheme and that the potential exposure to rodents may require a self-imposed quarantine period, a “no-ship” period, temporary use of vaccines or medication, or undertaking targeted surveillance in order to reenter the scheme. Step six in HACCP is creation of a system of written documentation that provides evidence of all management activities related to each CCP. This can seem an onerous task to the farmer but actually provides a value-added opportunity for the willing veterinarian, as well as providing information useful when working backward in time to determine the cause of a disease outbreak or production loss. Periodically, HACCP-monitored processes require validation to ensure they are actually doing the job for which they were designed. Validation is the essence of the seventh and final step in HACCP. Returning to the rodent control example, validation steps might include an inspection of the building spaces for evidence of rodents (feces, holes in walls, bedding material), a nighttime visit to maximize the chance of visualizing rodents, rodent trapping, and serological disease testing of the pigs.

Establishing a thorough understanding of a risk-based process for developing a BRM plan is critical for both the farmer and the veterinarian. It offers an intuitive process for engaging the farmer and the staff; it provides a logical framework for organizing one’s ideas about the specific biosecurity needs of a given farm; it forces one to methodically analyze effectiveness of a BRM plan; and it creates a clear understanding about the consequences of noncompliance with the plan.

The specific elements to be included in a BRM plan can vary extensively between farms and geographical regions. Resource documents are widely available from producer and veterinary organizations (Moore et al. 2008), state and national animal health departments, and international organizations such as the World Organization for Animal Health (OIE) and the Food and Agriculture Organization of the United Nations (FAO). The reader is encouraged to familiarize himself or herself with the resources available, much of which can be freely accessed via the Internet. A recent and comprehensive guide that defined a number of good practices for pig farm biosecurity was produced by FAO in 2010 (Madec et al. 2010). Given the global audience of FAO, the guide necessarily discusses the unique issues presented by several types of pig industries including village-based, low-intensity pig scavenging production; small-scale but confined pig production; and large-scale intensively managed production. The guide avoids an explicit discussion of risk-based BRM plan development but instead, provides a succinct discussion of the major causal pathways for disease introduction related to a failure in biosecurity; this is a fitting addition to the early planning stages of risk-based BRM plan development in modern intensive production systems.

Between Farms (National Level). Biosecurity planning does not stop at the farm gate and in many respects, is not confined to one’s own sector of the agricultural industry. National-level biosecurity planning has achieved a much higher profile than even 10 years ago for reasons including real and perceived anxiety over national security; terrorism; negative responses to large-scale euthanasia campaigns associated with animal plagues; and emergence of novel human, animal, and zoonotic pathogens. These factors when combined with increasing public worry over our care of the environment, concerns about global climate change, growing sensitivity to animal use and well-being, and the recent occurrence of several unusual food-borne disease outbreaks have created a paradigm shift among national policy makers. This paradigm shift is all about harmonization.
In a key guidance document published by FAO in 2007, the following definition of biosecurity was proposed: “a strategic and integrated approach to analyzing and managing relevant risks to human, animal and plant life and health, and associated risks to the environment” (Hathaway et al. 2007). Through use of this definition, the authors developed arguments that supported the development of harmonized national biosecurity policies that could take advantage of synergies that may exist across different economic sectors, and between industries within a single economic sector. Intuitively, there would seem to be an advantage that could be gained from establishing a single authority that could manage all the biosecurity risks across primary production (animal feed and livestock), food processing (slaughtering and processing), and the environment (water and air quality) as the activities involved in all these economic sectors are interdependent. This national biosecurity model was proposed by authors of the FAO report a priori, meaning there existed little prior evidence at the time to establish that the harmonized approach would, as a matter of fact, be an improvement over the traditional approach to biosecurity whereby primary production, food processing, and the environment are managed under separate authorities. At the same time, we do have compelling evidence that inadequacies do exist in traditional national biosecurity models, so the current momentum to shift toward a more modern approach will likely continue with some advantages and disadvantages yet to be realized.

Risk-based biosecurity planning remains important at the national level just as it did at the local farm level described above. While the seven steps in HACCP allow one to work at a relatively high level of detail for planning that is customized to the specific needs of a farm, a modification of the approach is required for use at the national level. The HACCP model places particular emphasis on hazard identification, recognition of special cause variation, then remediation of the process or product after an out-of-control event has been detected. The modifications to HACCP that have been made in order for it to function at a national level shift the emphasis to gaining a better understanding of the nature of a particular identified hazard: What is the probability that a recognized hazard will occur, what are the consequences if the hazard does occur, and what is the cost-to-benefit ratio of mitigation strategies that might be employed to prevent the hazard from occurring?

The OIE pioneered some of the early efforts to establish a harmonized framework for assessing risk around national biosecurity and animal health, and the framework is widely used as the basis for many types of risk analysis today (Anonymous 2010). The OIE risk-analysis process includes four steps: hazard identification, risk assessment, risk management, and risk communication (Figure 11.5).

11.5. OIE risk analysis framework.

The OIE hazard identification step is identical to step one in HACCP and attempts to answer the question “What can go wrong?” and, depending on the reason for conducting the risk analysis, may be answered simply through creation of a list of unwanted livestock pathogens or diseases, or may be more complex including identification of hazards that cross species or economic sectors. Step two of the OIE risk analysis is the actual risk assessment itself. The distinction between “risk analysis” and “risk assessment” is important. Risk analysis reflects the entire process from hazard identification through the communication plan around the process and its outcomes. Risk assessment, however, refers specifically to the characterization of the risk associated with each identified hazard and includes an assessment of the likelihood of occurrence and the likely severity of an occurrence. In the context of a risk assessment conducted to inform policy around proposed changes to importation of a risk good (animals, animal products, semen, etc.), OIE specifies four tasks that should be completed as part of a complete risk assessment. First, a release assessment describing the biological pathway(s) necessary for an importation event that would result in the introduction of the pathogenic agent into a country should be prepared; this should include an estimate of the probability of those events occurring. Second, an exposure assessment should be conducted to describe the pathways and associated probability of a domestic animal (or human, or farm) becoming exposed to an imported pathogen that was able to successfully negotiate the barriers designed to prevent its release. The third task that should be carried out is a consequence assessment that explores the consequences (production losses, welfare implications, financial impact) of a successful exposure event. This necessarily includes determination of the farm, national, and cross sector consequences of a successful exposure. At the conclusion of these three tasks, a fourth task called the risk estimation is undertaken. The risk estimation combines the probabilities of all the events occurring, from hazard identification through the consequence assessment, to arrive at an overall estimate of the risk associated with the proposed activity; the risk estimation may be quantitative, qualitative, or descriptive depending on the quality of data inputs and the needs of the relevant...
stakeholders. After completion of hazard identification and risk assessment, the risk management step is initiated. Risk management, as the name implies, explores the possible mitigation strategies that will be considered to manage any residual risk that was identified during the risk estimation step. In a thorough risk analysis, the risk management proposals will be analyzed using a process similar to the process used for risk assessment and will yield three important risk estimates for comparison: the baseline likelihood of entry (the status quo risk that existed prior to the proposed activity being undertaken); the unrestricted likelihood of entry (the expected post facto risk if the proposed activity is undertaken, but no risk mitigation[s] are implemented); and the restricted likelihood of entry (the expected post facto risk that remains if the proposed activity is undertaken and the recommended risk mitigation[s] are implemented). These three estimates of risk are compared in light of what is considered an acceptable level of risk (ALOP) and may include a macroeconomic cost-to-benefit analysis as well. The fourth and final step in the OIE risk analysis framework is referred to as risk communication. Ideally, conducting risk analyses should be a participatory process (consultation with stakeholders at each step in the analysis), iterative, transparent, and public (inform stakeholders of the conclusions that were reached).

A successful paradigm shift to a more holistic and harmonized national biosecurity system will likely require a concerted and sustained effort. Breeze (2006) discussed the topic at length in a 2006 paper whereby he graphically (Figure 11.6) presented the necessary frame shift that would be required by scientists, policy makers, and the animal industry stakeholders for successful transition to occur.

The evolution to a cross sectoral integrated national biosecurity mindset requires rethinking some of the tenets upon which traditional national biosecurity strategies have been based. Transboundary diseases are no longer a product nor the sole responsibility of the agricultural sector. The globalization of trade, a country’s commitment to meet obligations of international treaties and agreements, and national security matters related to the threat of purposefully introduced pathogens require that multiple branches of government engage with industry in establishing national policy on biosecurity. Given the urbanization of most countries, the general population is more disconnected from
primary production and food processing than ever before, making the job of biosecurity planners more difficult and more necessary. Making consumers and citizens aware of national biosecurity programs presents a double-edged sword: education in context is a good thing; education out of context introduces yet another share of challenges. For example, trying to convince the average person of the wisdom in using mass slaughter as the cornerstone policy for exotic disease response will be an increasingly difficult task—even when it is the right response.

Introduction of an exotic disease into a country is only rarely a random occurrence. It is unlikely that people frequently, or flagrantly, disregard long-held tenets of border security such as not carrying unprocessed pork salami into their home country after purchasing it in a part of the world endemically infected with ASFV (then casually tossing the remnants of it into the pen of the local backyard pig owner). However, in the context of more than 2 billion international air travelers per year (Fact Sheet: Industry Statistics, June 2010; International Air Transport Association, Montreal, Quebec, Canada), every year since 2005, the occurrence of even highly unlikely events is nearly certain.

Across Borders (International Level). Methodology for management of transboundary animal diseases at an international level is similar to that described for national policy on biosecurity. The additional influence presented by the current global political situation, the global economy, and international treaties and agreements to which a country is bound bears mentioning. Regulation of international biosecurity is only complicated if a country wishes to conduct trade with another country. In the absence of trade, there is no mandate to follow any other country’s recommendations or requirements. However, most countries do not have the ability (or desire) to remain independent from international trade of goods and nearly always, some give and take is required in order for two countries to agree on the terms of trade.

International standard setting organizations such as the Codex Alimentarius Commission (CAC), the OIE, and the World Trade Organization (WTO) develop standards that can serve as a unifying framework, if not obligatory standard, by which countries can reach consensus around their differing trade requirements. The CAC is a body that was created in 1963 by FAO and the World Health Organization (WHO) for the purpose of establishing standards related to foods, food production, and food safety. The OIE was established through an international agreement in 1924 (in response to an outbreak of Rinderpest in Belgium in 1920); the organization is responsible for improving animal health around the world and currently has 176 member countries and territories. The WHO is an agency within the United Nations that has a role similar to that of OIE but focused primarily on public health rather than animal health; WHO was established in 1948. The WTO is responsible for the Agreement on the Application of Sanitary and Phytosanitary Measures (SPS Agreement). The SPS Agreement is an international treaty managed by the WTO since 1995. Under the SPS Agreement, guidelines relating to the safe importation of food, animal, and plant products are negotiated and maintained.

Rushton in 2006 presented four principle threats that could contribute to the occurrence of a transboundary biological emergency (Rushton and Upton 2006): (1) emergence or occurrence of transmissible disease from a local wildlife population, (2) disease relocation as a result of movement of migratory wildlife or birds, (3) disease introduction through legal or illegal exportation of wildlife and livestock products, and (4) emergence of a disease or difficulty related to changes in the way we raise livestock. Rushton presented examples of recent historical disease outbreaks (occurring in the previous two decades) that fit into these threats and effectively validated his hypothesis. Of more significance, during the 4-year period since his 2006 publication, notable outbreaks fitting into each of the categories have occurred again (promedmail.org; accessed September 1, 2010): (1) a multispecies outbreak of FMDV in captive wildlife at a city zoo in India in 2007; (2) repetitive incursions of FMDV and anthrax into domestic cattle in Mongolia during the 2000s and likely related to migration of gazelle; (3) ASFV introduction into pigs in Georgia in 2007 (with subsequent spread to other countries), which was likely a result of illegal importation or feeding of contaminated food waste from infected countries in Africa; and (4) widespread pig exposure to Reston-Ebola virus in The Philippines in 2008 likely due to spatial or ecological overlap in commercial pig housing and fruit bat habitat. One cannot produce any compelling evidence to suggest that the ongoing occurrence of these epidemiologically interesting disease outbreaks will end any time soon.

Other factors of unknown significance might also be added to the list of global contributors to animal, human, and zoonotic diseases. It seems the extensive interconnectivity of modern society has created numerous complex systems, defined by nonlinear flow processes that by their very nature tend to be unstable, unpredictable, interdependent, and that when fail produce sequelae that cannot be anticipated. Who could have anticipated the chain of events that would be required to link purposeful melamine contamination of wheat gluten by a Chinese supplier, to contaminated pet food in California, to melamine exposure in pigs in various regions of the United States (and the subsequent worry over human exposure through consumption of tainted pork) (Baynes et al. 2008; Burns 2007)?
Plant pests, and biosecurity plans to manage them, have their own historical narrative, but a theme that runs through the commercial agronomy sector (but with application to the commercial livestock sector) is the risk presented by monoculture of particularly desirable crop species. Given the introduction of a suitable pest, monoculture plantings create a bounty of susceptible hosts of which a pest can take advantage; the introduction of soybean rust (*Phakopsora pachyrhizi*) into the midwestern United States (Li et al. 2008) and the epidemic infestation of pine bark beetles (*Dendroctonus* spp.) in forests and timber plantations on North America’s Pacific coast (Flint et al. 2009) are classic examples of the vast biological niches available to an appropriately positioned pest in today’s modern agriculture. Do commercial pigs essentially represent a mammalian monoculture? It is a challenging task to qualify what differentiates one pig breed from another in modern commercial pig genetics, but at least at a phenotypic level, there exists a huge similarity between pigs across the world, a population ready to be exploited by a fortuitous or cunning pathogen.

The significance of the two most important sources of special cause variation, pathogens and pigs, cannot be underestimated. The role of pathogens has been described in the preceding paragraphs. However, the role of people deserves another special mention, that being the risk of intentional introduction of infectious diseases to livestock populations. Agricultural bioterrorism has been an increasingly worrisome topic for national biosecurity managers and policy makers alike. Though one author has identified the occurrence of only two intentional biological attacks on North American agriculture (purposeful iatrogenic contamination of veterinary supplies with anthrax and Glanders around the time of the first World War; and contamination of dead stock that was destined for processing into animal feed, with pesticides in 1997) (Ackerman and Giroux 2006), the world investment in modification of infectious agents as weapons (for people or animals) is extensive (Szinicz 2005). Indeed, our ability to create life from the pool of available molecules and laboratory accessories appears to become more proficient each day (Gibson et al. 2010).

Distinguishing special cause from normal variation (or, signal vs. noise) will be an important requisite skill for risk analysts, biosecurity managers, and veterinarians in the future. It presents the evidence for distinguishing risk from reality in the spectrum of livestock disease.

**PRINCIPLES OF EVIDENCE-BASED BIOSECURITY**

All the features of size, production flow, commercial imperative, geographical location, local industry density, connectedness to other farms, and physical building characteristics that describe a farm are the same features that make it impossible to develop a one-size-fits-all BRM plan suitable for use across the pig industry. As an alternative, the following 10 principles are proposed as fundamental criteria that must be fulfilled when developing biosecurity plans for pig farms. Scientific evidence is available to support the application of each principle that is appropriate for most farm and disease settings that will be encountered. Readers are encouraged to develop their own studies that can further our knowledge of these topics for use by an even broader cross section of the international industry. The principles are not complicated, though they can be difficult to institute effectively and with adequate levels of compliance.

A distinct boundary must exist between “clean” and “dirty” areas of the farm. These boundaries may exist virtually or physically, and they must be readily identifiable.

Cleaning must precede disinfection. The effectiveness of all disinfectants is reduced considerably in the presence of organic matter.

Sterility is a myth; the objective of biosecurity is to reduce the pig’s level of exposure to a pathogen. Reducing the pig’s exposure is a function of both exposure time and pathogen density.

Unidirectional flow of both people and pigs is imperative. The direction of flow should be away from the customer, away from the population least easily recovered from a disease introduction, and away from the most disease-susceptible population, in that priority order.

Categorical descriptors of the health status of a farm (e.g., “high health,” “conventional health”) are meaningless. Considering farms to be of “comparable” or “compatible” health status is only marginally more useful. Ad hoc and routine submission of samples to a veterinary diagnostic laboratory is a required part of a BRM plan.

Isolation and acclimatization procedures are mandatory prior to introduction of genetic stock into farms. A herd’s health status is only as good as the last time it was tested. Imperfect diagnostic test sensitivity and specificity need to be considered when determining required sample sizes for establishing the disease status of a population.

Purchase of pigs never comes with a guarantee of their health status. Health status is not static nor is it a deterministic variable. Disease risk is managed, not contractually obligated.

Health status of all farms will decline over time. Biosecurity planning should consider payoff schedules that inform one of the “cost-to-benefit ratio” of BRM processes and procedures.

Procedures that are established as part of a BRM plan are meant to be followed by everyone, especially veterinarians.
REFERENCES

INTRODUCTION

The ultimate purpose of rearing domestic swine is to supply wholesome and nutritious protein to humans. Although this high-quality protein has a positive impact on human nutrition, there is some risk for negative human health impacts. These impacts can occur via consumption of pork products, occupational exposure to swine, or through environmental exposures. In this chapter, we give an overview of potential negative human health risks associated with food-borne, direct contact, or environmental exposures associated with pigs.

FOOD-BORNE DISEASE RISKS ASSOCIATED WITH PORK

Physical Hazards

Physical food-borne hazards are foreign objects that can cause injury to those that consume food products. Physical hazards associated with pork originate from two primary sources: preharvest practices on the farm, primarily from the use of hypodermic needles; and postharvest hazards associated with processing and packaging environments. Reports in the literature are scarce regarding the frequency and health implications of physical hazards. The scope of the problem can be estimated from records of meat and poultry recalls and reports from passive surveillance based on the United States Department of Agriculture Food Safety Inspection Service (USDA FSIS) consumer complaint reporting systems. A recent review of recalls from 1994 to 2002 indicates that physical hazards accounted for 15% of total meat and poultry recalls (Teratanavat and Hooker 2004). In 2006, there were 982 consumer complaints. Of these, the majority, 499 (51%) were related to foreign object complaints, which was reported as a rate that was similar across the 5-year period. These reports are not specific to pork products but represent the proportion of undetected hazards that make it to the retail or consumer level. Based on data from the U.S. Food and Drug Administration (FDA) complaint recording system, the most common human illnesses/injuries associated with physical hazards is laceration or abrasion of the perioral area, gastrointestinal distress, and damage to teeth or dental prosthetics (Anonymous 2008b).

Preharvest Control of Physical Hazards. The primary concern for physical hazard risk preharvest in swine is the risk of broken hypodermic needles and needle fragments in carcasses. Many factors contribute to the risk of broken hypodermic needles (Hoff and Sundberg 1999). The strength of a hypodermic needle contributes to the risk of breakage. Strength is determined by the length, gauge, hub material, and manufacturer. Studies indicate that hypodermic needles are very resilient to breakage under conditions of static load. Needles that were bent and restraightened were at greater risk for breakage. Needles that were reshaped twice post-bending event had a 96.7% failure rate. Simulations of animal movement during injection impacted needle failure rates. Additionally, the location of the injection as well as animal movement impacts the needle puncture strength.

Appropriate restraint, needle selection, and injection techniques are critical to avoiding broken needle events in swine. Producer and worker education is a key intervention for prevention, and development of standard
operating procedures for when a broken needle event occurs (including permanent identification of suspect animals and communication with the processor) is a best practice for control of physical hazards in pork.

Detection during processing is a second line of defense to prevent physical hazards in pork. Common methods for detecting/removing physical hazards during processing include physical separation using filters or sieves and magnets (not efficacious for whole meat products) and detection using X-ray. Needles have varying ability for detection by magnets and X-ray during processing depending on the metal alloys used as well as the needle orientation while being screened by the X-ray.

One way to decrease the risk of hypodermic needles as physical hazards is to remove them from the production system environment entirely. Needleless injection systems are increasingly being used in swine for both control of physical hazards as well as indications of improved immune response from intradermal injections (Chase et al. 2008).

Chemical Hazards
Chemical hazards are toxic substances and any other compounds that may render food unsafe for human consumption. At the preharvest level, primary concerns for control of chemical hazards involve veterinary drugs, pesticides, and environmental contaminants. Other potential preharvest chemical risks involve adulteration of animal feed for the purposes of economic fraud or intentional disruption of the food supply.

Codex Alimentarius represents the global food standard setting body, which establishes standards for residue limits in food, including meats. In the United States, the FDA is responsible for the approval of all drugs for use in animals and animal feeds. FDA sets the standards for tolerance determination of chemical hazards, while the USDA FSIS conducts the surveillance and detection of chemical residues in meats. The FDA is in charge of enforcement regarding chemical residues.

Control of chemical residues is managed by prohibition of use or, for chemicals approved for use in animals, determination of maximum residue limits (MRLs), which are managed via withdrawal time periods prior to harvest. Codex Alimentarius standards may differ from those of their domestic regulations regarding chemical residues. This has significant implications for the international trade of pork, a critical component for economic success of pork production, as pork destined to be exported to other countries may need to adopt different formularies and withdrawal periods in order to meet these standards of the importing country. It is important to be aware of these differences and implement on-farm practices that will meet export requirements. In the United States, label indications do not indicate these withdrawal differences. These differing withdrawal periods can be accessed via consultation with pharmaceutical manufacturers.

Extra-label Drug Use. In the United States, the Animal Medicinal Drug Use Clarification Act of 1994 (AMDUCA) allows for veterinarians to prescribe some veterinary drugs, under specific clinical conditions, in ways that are not compliant with the label indication (extra-label drug use, ELFU). The availability of ELDU is essential for animal health and welfare. From a chemical hazard safety standpoint, this contributes challenges to establishment of appropriate withdrawal periods to avoid violative residues. In this case, the sole burden of avoiding residue violations rests with the prescribing veterinarian. AMDUCA compliance guide suggests that veterinarians establish extended withdrawal periods when ELDU occurs. Resources for a decision-based algorithm for ELFU and determination for the duration of the withdrawal period for ELDU can be accessed via the Food Animal Residue Avoidance and Depletion Program (www.farad.org/).

Veterinary Drug Residues in Pork. The USDA FSIS conducts two types of sampling in domestic animals for chemical residues (Anonymous 2008a): (1) scheduled sampling, which consists of the random sampling of tissue from healthy-appearing food animals for the purpose of surveillance; and (2) inspector-initiated sampling, which is conducted by in-plant public health veterinarians for detecting residues in animals determined to be of high risk. Scheduled sampling is used for exposure assessment and for exploratory purposes. Exposure assessments are used to evaluate human exposure to chemical residues from food and to determine regulatory actions when violative residues occur. Exploratory sampling is also scheduled to reinvestigate animal populations when violation rates are at 1% or greater, to investigate animal populations when the compounds have no established tolerances, and to respond to intelligence reports from the field.

In the United States, swine residue violation rates are very rare (Anonymous 2008a). Among swine production classes in the United States (market pigs, boars/stags, sows, and roaster pigs) in 2008, the proportion of violative residues from scheduled sampling for exposure assessment was 0.20%, 0.37%, 0.15%, and 0.28%, respectively. Violative residue rates for those samples initiated by public health veterinarians were 0.04%, 0.0%, 0.03%, and 0.0% for market pigs, boars/stags, sows, and roaster pigs, respectively. For both exposure assessment and inspector-generated sampling, the veterinary drug compounds for which residues were violative were gentamycin sulfate, sulfamethazine, carboxodax, and avermectins. Two occurrences of residues identified in the boar/stag class were not veterinary drugs, but chlorinated hydrocarbons. These data suggest that at least in the United States, veterinary drug residues
are well controlled in swine production. Continued vigilance on the part of veterinarians and producers to continue to ensure this level of food safety is necessary to protect public health.

**Dioxins.** Polychlorinated dibenzo-\(p\)-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), and polychlorinated biphenyls (PCBs) are collectively referred to as dioxins due to their similar structures, actions in biological systems, and chemical properties. They are ubiquitous environmental contaminants that bioaccumulate in the food chain. They are often considered an industrial contaminant since they are formed as by-products of chlorine-containing manufacturing, but incineration and forest fires are significant contributors to dioxin loads. The majority of human exposure to dioxins is estimated to be from consumption of animal and fish products, especially fats (Liem et al. 2000). Animals are exposed via air deposition of dioxin on forage and, to a lesser extent, soils (Huwe 2002).

Dioxin contamination of animal feeds has occurred sporadically around the globe including feed components contaminated by pentachlorophenol-treated wood and bakery waste contaminated during the drying process in Europe (Huwe et al. 2009). In the United States, ball clay used as an anticaking agent in poultry and catfish feed, and mineral mix formulated with a smelting by-product used in swine feeds have also been identified as dioxin-containing feed contaminants (Huwe et al. 2009). Dioxin contamination of swine feed in Ireland prompted the recall of 2 months of production of pork products (Dixon 2009) and euthanasia of animals.

A single toxic equivalency value (TEQ), which is the sum of individual dioxin-like compounds, is used for intake assessments or residue determinations. The USDA has conducted surveys of commodities since the mid-1990s. There has been a decreasing trend in TEQs from the mid-1990s to 2008, although the significant decrease for pork was from the mid-1990s through 2002, and TEQs were steady from 2002 to 2008 (Huwe et al. 2009). The levels were similar to levels published from surveys in Europe and Asia (Huwe et al. 2009), and fall below the European Union regulation for accepted limits of dioxins in meats of 1–3 pg of TEQ per gram of lipid (Huwe 2002).

**Biological Hazards**

Biological hazards are those food-borne hazards caused by bacteria, viruses, parasites, and other infectious agents. The following sections represent the most common biological hazards associated with pork consumption.

**Salmonella.** Salmonellosis is a major food-borne disease threat to public health in the United States and around the world. During 1998–2002, *Salmonella* represented the most commonly reported bacterial cause of food-borne outbreaks and illnesses, as well as the second largest etiological cause of death among bacterial food-borne pathogens in the United States (Anonymous 2009a). *Salmonella* in humans most commonly results in gastrointestinal symptoms, such as diarrhea, abdominal cramping, and vomiting. The immunocompromised, young, and elderly are at greater risk for severe disease.

It has long been recognized that swine as well as many other species can be subclinical carriers of *Salmonella*. In essence, all vertebrates and many invertebrates have been identified to be able to be colonized with *Salmonella*. *Salmonella* is considered to be ubiquitous in the environment and can survive indefinitely, particularly in farm environments (McLaren and Wray 1991; Plym-Forshell and Eskebo 1996). Its wide host distribution and indefinite environmental survival provides significant challenges for on-farm control. In the United States, the National Animal Health Monitoring Service (NAHMS) reported that in its Swine 2006 survey (Anonymous 2009b), the prevalence of *Salmonella*-positive production sites was 52.6% and the prevalence of positive pigs was 7.2%.

*Salmonella* is the only food-borne pathogen currently utilized by the USDA FSIS for process control during swine harvest. The passage of the Hazard Analysis Critical Control Point (HACCP)/Pathogen Reduction Act (Anonymous 1996) mandated the monitoring of contamination of carcasses for “generic” *Escherichia coli* as an indicator of contamination with gastrointestinal contents as well as testing for *Salmonella*. Recent data from FSIS monitoring indicate that the proportion of positive swine carcasses that are *Salmonella* positive was 2.3% in 2009, as compared with 8.7% in 1988, prior to implementation of the HACCP/Pathogen Reduction Act (Anonymous 2010). This indicates significant improvements in control of contamination at harvest and processing.

In the United States, control of *Salmonella* is primarily focused at the processing phase. Sweden since 1961 (Anonymous 1995) and Denmark as of 1993 (Mousing et al. 1997) have established *Salmonella* monitoring and control plans. These plans include routine herd surveillance as well as interventions at processing. There are mandatory intervention requirements on farms if they are identified as having high *Salmonella* prevalence. These interventions can include changes in hygienic practices, feeding protocols, and pig flow (i.e., moving from continuous to all-in/all-out pig flow). Additionally, farms identified as high prevalence are penalized at harvest with decreased sale values, and product may be required to go into cooked final products. Additionally, high prevalence herds may be harvested at different facilities or as the final groups harvested at the end of the day or shift to prevent cross contamination.
Despite a multitude of investigations, there is a lack of strong evidence for cost-effective preharvest (on-farm) interventions for *Salmonella* in swine. Review of the literature (Funk and Gebreyes 2004) suggests that a multitude of risk factors are associated with the *Salmonella* status of farms, including hygiene practices, production flows, feed form, and season. There is evidence that efforts at the farm level may be lost through reinfection during lairage at harvest (Hurd et al. 2002). There is an absence of studies that have evaluated *Salmonella* control measures in controlled on-farm trials. Recent systematic reviews of the literature suggest that vaccination (Denagamage et al. 2007) and feed form (O’Connor et al. 2008) may be important for *Salmonella* control. Unfortunately, both studies concluded that the quality of the reports was insufficient to strongly support these interventions. Mathematical models of *Salmonella* control strategies suggest that the greatest impact on *Salmonella* contamination of carcasses comes from interventions during harvest and processing (Alban and Stärk 2005; Goldbach and Alban 2006). In order to understand the role and efficacy of preharvest *Salmonella* interventions, on-farm controlled clinical trials are needed to evaluate these proposed interventions.

**Campylobacter.** Campylobacteriosis is considered one of the most common bacterial causes of food-borne disease in the United States (Mead et al. 1999) and in the developed world. Clinical symptoms in humans include gastrointestinal-related symptoms: bloody diarrhea, nausea, fever, headache, and abdominal cramps. Most human cases of campylobacteriosis are self-limiting, therefore requiring no antimicrobial treatment. Chronic sequelae are also possible, most notably Guillain–Barré syndrome. In the United States, most human clinical disease is caused by *Campylobacter jejuni*, although a small percentage is attributed to *Campylobacter coli* (Horrocks et al. 2009).

*Campylobacter* have been found in the intestinal tract of domestic and wild mammals, poultry, wild birds, and in untreated water (Horrocks et al. 2009). Swine are most commonly infected with *C. coli* (Horrocks et al. 2009). Most infected swine are subclinical. Pigs are thought to be colonized soon after birth, and the prevalence of *Campylobacter* reported in pigs is high. A recent review reported farm prevalence of 100% and individual pig prevalence ranging from 25% to 100%.

*Campylobacter* contamination of retail pork products has been reported to be low. The FDA conducts a survey of retail meats and reported in 2008 that 0.3% of pork chops were *Campylobacter* positive (Anonymous 2008c).

Control strategies to prevent *Campylobacter* contamination of pork products focus on minimizing fecal contamination of carcasses during slaughter. Blast chilling of carcasses has been shown to reduce *Campylobacter* on swine carcasses better than traditional carcass cooling (Thakur and Gebreyes 2005).

**Yersinia enterocolitica.** The public health burden of *Y. enterocolitica* is estimated at 96,368 annually in the United States (Mead et al. 1999). It is believed that most *Y. enterocolitica* infections are asymptomatic, or at least mild enough to go unreported. The main clinical symptoms are self-limiting fever with diarrhea and/or vomiting.

*Yersinia enterocolitica* clinical illness can also mimic appendicitis, which has resulted in unnecessary surgical interventions.

*Yersinia enterocolitica* has been recovered from several livestock species, but swine are the only species that have been consistently linked to human infections and are considered the primary reservoir for pathogenic *Y. enterocolitica* (Christensen 1980; Schiemann 1989; Toma and Deidrick 1975).

There have been few studies in the U.S. swine population regarding *Y. enterocolitica*. Estimates of *Y. enterocolitica* prevalence in pigs at harvest are approximately 25%. The farm-level prevalence in the United States is likely high. Funk et al. (1998) found 92% of market lots had at least one pig contaminated with *Y. enterocolitica*. In a study by Bowman et al. (2007), of the 2349 pigs sampled on eight U.S. swine farms, 120 (5.1%) tested positive for *Y. enterocolitica*, and of those, 42.5% harbored a virulence gene associated with the ability to cause human illness. At least one positive animal was found on seven of the eight farms. On all positive farms, there was a consistent trend of increasing prevalence as pigs mature, with market-ready pigs and sows having the highest prevalence.

*Yersinia enterocolitica* is a cold-loving (psychrotrophic) organism that can grow at refrigeration temperatures. It has the highest prevalence in swine during cooler seasons in temperate regions (Fukushima et al. 1983; Toma and Deidrick 1975; Tsubokura et al. 1976) and is more prevalent in regions with cooler climates (Fredrikkson-Ahomaa and Korkeala 2003; Smego et al. 1999). Human disease is also more common during cooler months, but whether this is a factor of its psychrotrophic nature or human behaviors are not known (Ray et al. 2004).

Specific control measures for *Y. enterocolitica* have been primarily directed at control of contamination of carcasses with fecal contamination. The use of a plastic bag to seal the rectum during slaughter greatly reduced *Y. enterocolitica* contamination of carcasses (Nesbakken et al. 1994).

**Shiga-toxin-producing *Escherichia coli.*** Shiga-toxin producing *E. coli* (STEC)-associated food-borne illnesses represent an estimated incidence of 4.57/100,000 (Anonymous 2009b) in the United States. Of these, 1.12/100,000 are caused by serogroup O157. Acute
symptoms of STEC include severe stomach cramps, diarrhea (sometimes bloody), and vomiting. Of those diagnosed with STEC infection, 5–10% develop a potentially life-threatening complication known as hemolytic uremic syndrome (HUS). There has been a predominant focus on O157 serogroups in the United States as a result of large-scale outbreaks. Non-O157 STEC illnesses are likely highly underrepresented, as few diagnostic laboratories conduct assays for non-O157 STEC (Brooks et al. 2005).

Recent reports suggest that non-O157 STEC may contribute to a large proportion of the disease burden, upward of 50% domestically (Johnson et al. 2006; Manning et al. 2007; Phan et al. 2007). The concerns regarding non-O157 STEC are important for pork production as current evidence suggests that pigs are more likely to be infected with non-O157 STEC as compared with O157 serogroup.

STEC (stx2e-toxin-producing) have long been known to be the etiological agent associated with edema disease in swine which cause gastrointestinal and central nervous signs in pigs postweaning. There is a lack of clarity in the literature regarding the role of stx2e STEC strains from swine for human clinical disease (Beutin et al. 2008; Sonntag et al. 2005; Werber et al. 2008). Recent cross-sectional studies in the U.S. swine herd have isolated STEC strains that have the potential to cause human illness (Fratamico et al. 2004). In the study by Fratamico et al. (2004), 31.8% of pigs were STEC positive. Of particular interest was the fact that all isolates were non-O157 serotypes. In fact, a total of 29 STEC serotypes harboring stx1, stx2, or stx2e were identified.

Epidemiologically, pork products are infrequently associated with food-borne illness as compared with food products contaminated with ruminant feces (Bettelheim 2007). Nonetheless, outbreaks and illnesses have been associated with pork products (Bettelheim 2007; Conedera et al. 2007). Further research is needed to assess pork’s contribution to the burden of food-borne illness from STEC.

Trichinella spiralis. Trichinella spiralis is a nematode parasite that encysts in the tissues of a broad range of wild and domestic animals. Transmission is via the consumption of these tissue cysts in raw or undercooked meat products derived from infected animals. While there are eight species of Trichinella, only T. spiralis is considered important in domestic livestock. Pigs have traditionally been considered the domestic livestock most likely to be infected with Trichinella due to past practices such as feeding of uncooked meat containing waste products and outdoor access to wildlife carcasses. These risk factors are largely eliminated in modern confinement production systems.

Symptoms and clinical signs of trichinellosis in humans include eosinophilia, abdominal pain, fever, periorbital edema, myalgia, and, rarely, death (Kennedy et al. 2009). In the United States, reported trichinellosis cases have fallen from 393 during 1947–1952 to 11 annual case compatible reports in the period from 2002 to 2007 (Kennedy et al. 2009). This decline in human cases has mirrored the decline in prevalence of Trichinella in commercial pork products that has resulted from the move to confinement rearing of pigs (Gamble and Bush 1999). The majority of trichinellosis cases in the United States are currently linked to consumption of wild game, especially bear (Kennedy et al. 2009; Roy et al. 2003).

Cooking, curing, and freezing are used to inactivate T. spiralis in pork. Trichinella larvae can be killed at temperatures as low as 55°C; however, uneven heat distribution should be taken into account during the cooking process to assure that all portions of the meat reach the proper temperature. Freezing remains effective in killing Trichinella in pork since freeze-resistant species of Trichinella in the United States have low infectivity and persistence in pigs (Kapel and Gamble 2000).

In the United States, the NAHMS conducts a statistically based survey of pork production every 5–6 years. Since 1990, serology for Trichinella has been conducted on a portion of animals in the survey. The seroprevalence has fallen from 0.16% in 1990 to 0.013% in 1995, and no positive samples were identified in the 2000 and 2006 surveys. This virtual elimination of Trichinella in the commercial pig industry has been accompanied by an increase in the number of production sites reporting adherence to good production practices that reduce the risk of Trichinella infection (D. E. Hill, personal communication). However, the move to nonconfinelement production systems may signal a potential increasing risk for human cases of trichinellosis. Gebreyes et al. (2008) reported two positive samples from 324 animals tested in niche market, outdoor, antibiotic-free herds compared with 0 positive samples from 292 animals sampled in conventional U.S. herds.

Toxoplasma gondii. While felines are the definitive host of the protozoan parasite T. gondii, a wide range of species have been shown to be susceptible to infection by T. gondii. Humans can become infected with T. gondii through three potential routes: exposure to oocysts shed in cat feces, consumption of under- or uncooked meat from infected animals, or congenitally when infection occurs in a susceptible pregnant woman. Toxoplasma gondii is a public health concern, and in the United States, it is estimated that 500–5000 newborns are born congenitally infected each year and that 1.26 million people are affected by ocular toxoplasmosis (Jones et al. 2009). Mead et al. (1999) estimated that T. gondii was the third leading cause of food-borne illness deaths in the United States largely due to reactivation of bradyzoites in immunocompromised patients.
Pigs become infected with *T. gondii* when they consume feedstuffs or other substances contaminated with cat feces containing infective oocysts. They can also be infected by consuming carcasses of rodents and other wildlife that have *T. gondii* muscle cysts or through cannibalism of infected swine carcasses. Traditionally, pork has been considered a source of human infection due to waste feeding and other dietary habits of swine that are largely eliminated by modern pork production methods.

Nationwide surveys of the U.S. swine herd are conducted every 5–6 years by the USDA as part of NAHMS. In 1990, only sows were tested and 20% were seropositive; in 1995, 15% of sows and 3.2% of finishers were seropositive; in 2000, 6% of sows and 0.9% of finishers were seropositive; and in 2006, when only finishers were sampled, 2.6% were seropositive (Hill et al. 2009). It should be noted that the serological method changed in 2006 from the microscopic agglutination test (MAT) to an enzyme-linked immunosorbent assay (ELISA) that makes direct comparison of these results difficult. The NAHMS 2000 results are similar to what was observed by McKean et al. (2003) in 2002 who found a 0.75% seroprevalence in midwestern market swine and Gebreyes et al. (2008) who found a 1.1% seroprevalence in confined pigs in three states.

The NAHMS surveys also gathered information on production practices that were considered to be potential risk factors for *Toxoplasma*, and analysis of that data found that farms where pigs were not housed in total confinement were 7.7 times more likely to be positive than total confinement farms (Hill et al. 2009). Additional risk factors included not using baits/poisons/traps for rodent control and certain carcass disposal techniques (Hill et al. 2009). Similar findings were reported by Gebreyes et al. (2008) with extensively raised pigs almost seven times more likely to be seropositive than pigs in confined herds.

It is uncertain what percentage of human infection is due to exposure to cat feces versus consumption of infected meat. A cross-sectional seroprevalence study compared Seventh-Day Adventist (nonmeat eaters) to a control group (meat eaters) in one region of Maryland and found that seroprevalence was approximately 50% less for the non-meat-eating group (Roghmann et al. 1999). However, concerns with that study include that the seroprevalence of the control group (31%) was approximately twice the national average seroprevalence in the United States, while the non-meat-eating group had a similar seroprevalence to the national average (Jones et al. 2003). Additionally, the control group was recruited from commercial fishermen, seafood processing plant workers, and visitors to a seafood festival. This is important since bivalves such as oysters and clams can concentrate oocysts, and consumption of raw oysters, clams, or mussels has been shown to be a risk factor for *T. gondii* seropositivity (Jones et al. 2009).

Retail meat sampled from 28 major geographical areas across the United States found viable tissue cysts were isolated from 0.38% of pork samples and 0.57% of pork samples had antibodies to *T. gondii*. A case-control study of U.S. patients with recent *T. gondii* infections identified eating raw ground beef, rare lamb, and locally produced cured dried or smoked meat; working with meat; drinking unpasteurized goat's milk; and having three or more kittens as significant risk factors (Jones et al. 2009). Consumption of pork was not a significant risk factor in that study; however, the move to niche pork from nonconfined production systems may increase the risk of exposure to *T. gondii* in the United States.

**Taenia solium.** *Taenia solium* is commonly called the pork tapeworm, although the definitive host is actually humans. Swine are the natural intermediate host for the parasite. Humans acquire taeniosis by eating undercooked pork that contains cysticerci, the larval form of *T. solium*. The adult worm is found in the intestine of infected humans, and infective eggs are regularly shed in the feces. In endemic areas, inadequate sanitation allows pigs to access human feces containing infective *Taenia* eggs. Upon ingestion, the eggs hatch in the pig's intestine and migrate throughout the body preferentially encysting in the striated or cardiac muscle (Acha and Szyfres 2003a).

The main public health concern arises when humans ingest the eggs shed in the feces of infected humans. This can occur through fecal contamination of water or food or autoinoculation. In this case, the *Taenia* larva migrates throughout the human as it would in the pig and forms cysticerci. In humans, the principal site of migration is the central nervous system resulting in neurocysticercosis (NCC) (Carabin et al. 2005a).

NCC is reported to be one of the main causes of preventable epilepsy in low-income countries. Carabin et al. (2005b) estimated that the costs for medical care and lost productivity due to NCC in India were $634 million annually. It should be noted that more than 90% of Indians are either Muslim or Hindu and do not consume pork. However, the inadequate sanitation results in person-to-person transmission and allows for access of pigs to human feces, completing the parasite lifecycle.

In the United States, cysticerci are identified in very few hog carcasses. In 1998, only one carcass of more than 75 million inspected by the USDA was condemned due to visible cysticerci. Although infection in pigs is rare, there is some increase in human cases in the United States, often resulting from either travel to endemic countries or exposure to persons infected while in endemic countries (Sorvillo et al. 2007).
NON-FOOD-BORNE ZOONOSES

Many zoonotic diseases are a risk from direct contact with swine. Although many of the previously discussed food-borne diseases can be transmitted via direct contact, the following are those that are primarily transmitted via methods that do not include a food vector.

**Methicillin-resistant *Staphylococcus Aureus***

*Staphylococcus aureus* is a common organism found on the skin and mucous membranes of animals and humans. It can function as an opportunistic pathogen causing superficial and/or invasive infections. A subset of *S. aureus* are resistant to methicillin with this resistant encoded by the *mecA* gene, which codes for penicillin-binding protein 2a that confers resistance to all beta-lactam antimicrobials (Kluytmans 2010). Estimates of human prevalence in the United States are that 31.6% are colonized with *S. aureus* and 0.84% with methicillin-resistant *S. aureus* (MRSA) (Graham et al. 2006).

MRSA emerged as a health-care-associated infection in the 1960s, and in the late 1990s, community-acquired MRSA was recognized (Naimi et al. 2003). In 2004, a new MRSA strain was identified in Holland that was resistant to digestion with the restriction endonuclease *Sma*I when typing with pulsed-field gel electrophoresis (PFGE) was attempted and was associated with contact with pigs (Voss et al. 2005). This strain belongs to the multilocus sequence type ST398. MRSA ST398 is usually resistant to tetracycline and is generally resistant to a narrower range of other antimicrobials than most health-care-associated MRSA strains. MRSA ST398 has been referred to as the livestock-associated strain and has been found in several other food and companion animal species. Since the discovery of MRSA in Dutch swine herds, numerous studies have been conducted, and the presence of MRSA in pigs, pork producers, and veterinarians has been reported globally.

The European Food Safety Agency (EFSA) conducted a Europe-wide survey of swine farms in 2008, and 17 of 24 member states identified positive farms with a prevalence of 22.8% (European Food Safety Authority 2009). Positive farms that raised breeding stock were identified in 12 of 24 member states with a prevalence of 14% (range 0–46%), and 16 of 24 member states had MRSA-positive farms raising market hogs with a prevalence of 26.9% (range 0–51%) (European Food Safety Authority 2009). ST398 was the predominant type isolated with a mean of 1.4% of isolates being non-ST398 (European Food Safety Authority 2009). The first North American reports of MRSA in pigs were in 2008 when Khanna et al. (2008) reported that 9 of 20 farms and 24.9% of pigs were positive with the predominant spa-type fitting into the clonal complex 398. In the United States, one published paper reported high levels of colonization in pigs (70%) within one production system, while another production system was negative for MRSA ST398 (Smith et al. 2009c). A somewhat larger study of 312 pigs on 13 farms in the United States found 13% prevalence across all farm types and 23% in confinement swine (Harper et al. 2009). MRSA ST398 is the predominant type found in pigs and swine workers globally, although as more studies are conducted, a wider range of types is being identified.

Risk factors for MRSA-positive status of swine/swine farms include animal source, herd size, age/time in herd, transport and lairage, and perhaps others (Broens et al. 2009, 2010). The role of antimicrobial use in MRSA status is uncertain, with some studies suggesting an association and others not demonstrating a significant association (Broens et al. 2010). It has been suggested that due to the tetracycline resistance observed in ST398 that tetracycline use contributes to its presence in swine herds. Aarestrup et al. (2009) analyzed methicillin-sensitive *S. aureus* (MSSA) ST398 and MRSA ST398 isolates from Danish swine. They found that similar percentages of both MRSA and MSSA were resistant to tetracycline; however, 74% of MRSA ST398 isolates were resistant to zinc chloride while none of the MSSA ST398 showed zinc resistance. They concluded that the use of nonantibiotic zinc compounds to prevent postweaning diarrhea in piglets may contribute to the emergence of MRSA in Danish swine herds.

Swine workers were sampled in several studies, and those working in facilities with positive pigs have higher than expected rates of nasal colonization, although there is little evidence to indicate that they have higher than expected rates of MRSA clinical disease. Veterinarians who work with all species of animals have also been reported to have higher rates of colonization with MRSA than the general public. Invasive illness with ST398 MRSA has been reported, albeit rarely, and published studies often make it difficult to determine if human cases are nasal carriers or indeed have clinical infection. It has been concluded in Europe that the potential for humans colonized with ST398 to contribute to the spread of MRSA in hospitals is less than for the health-care-associated MRSA strains.

MRSA ST398, as well as other strains of MRSA, have been isolated from pork and other meats globally (de Boer et al. 2009; Kluytmans 2010; van Loo et al. 2007; Weese et al. 2010a). Public health agencies in the United States and Europe have not found evidence that contaminated meats contribute to an increased risk of MRSA (Centers for Disease Control and Prevention 2008). Weese et al. (2010a) quantified the amount of bacteria in positive samples purchased at retail stores throughout Canada and found that 37% of positive samples were below the detection threshold for quantification, and <100 CFU/g was found in most quantifiable samples.
**Streptococcus Suis**

*Streptococcus suis* is common in domestic swine worldwide. Clinical signs in pigs include central nervous system signs (head tilt and paddling), arthritis, and polyserositis. Since it was first reported in 1978, until 2005 human cases of *S. suis* were rarely reported with about 200 reports globally. Clinical signs most commonly observed in humans include meningitis and/or septicemia. During 2006, the first human case of *S. suis* was reported in the United States (Willenburg et al. 2006), although it has likely been underdiagnosed or unreported.

In 2005, over 200 human cases of *S. suis* were reported in China. Human cases were largely associated with butchering and handling uncooked meat products from clinically ill animals (Gottschalk and Segura 2007; Yu et al. 2006). Twenty-eight percent of the Chinese cases of 2005 suffered a toxic-shock-like syndrome (Yu et al. 2006). Investigation of the Chinese outbreak revealed that most cases had close contact with sick or dead pigs, and many were involved in the slaughter or butchering of the animals (Gottschalk and Segura 2007; Yu et al. 2006). Furthermore, the investigation also identified that the human cases were caused by one clone of *S. suis* (Gottschalk and Segura 2007). A pathogenicity island has been found in the genome of isolates from the Chinese cases, but its exact role and function in *S. suis* virulence are poorly understood (Chen et al. 2007).

**Clostridium Difficile**

The role of *C. difficile* as a zoonotic agent from pigs is unknown. *Clostridium difficile* is a gram-positive spore-forming anaerobe that is an important cause of enteric disease in humans. It is one of the most commonly diagnosed causes of hospital- and antibiotic-associated diarrhea in people and is emerging as a community-associated pathogen. *Clostridium difficile* has also been isolated from numerous animal species (Gottschalk and Limbago 2010; Jhung et al. 2008); meats (Norman et al. 2009; Songer et al. 2007; Weese et al. 2009); vegetables (Bakri 2009); hospital, barn, and household environments (Baverud et al. 2003; Weese 2010b); water (Al Saif and Brazier 1996); pets (Borriello et al. 1983; O’Neill et al. 1993; Weese et al. 2010b); and healthy humans (McDonald et al. 2007; Rupnik 2010).

There are various schemes utilized to type *C. difficile* including polymerase chain reaction (PCR) ribotyping, PFGE, and toxinotyping, and strains are often described using classifications from more than one of the different methods to improve discrimination. There are two strains of *C. difficile* that are of special interest in the apparently changing epidemiology of *C. difficile* in humans: a toxinoype III strain designated 027/NAP1 (NAP1) and a toxinoype V strain designated ribotype O78. NAP1 has emerged as an important hospital-acquired infection with increased incidence and severity that is thought to be influenced by the use of fluoroquinolone antimicrobials (McDonald et al. 2005). The second strain, ribotype 078, is an important strain associated with the increased recognition of community-associated *C. difficile* infection. Both of these strains, as well as others, have also been isolated from animal and food sources. The overlap of strain types among humans, animals, and meat has led some to hypothesize that animals may be an important source of *C. difficile* human infections. However, there are three possible explanations for this overlap that must be considered: (1) a common source causing infections in both humans and animals, (2) human-to-animal transmission, or (3) animal-to-human transmission.

*Clostridium difficile* colitis is not uncommonly diagnosed in neonatal pigs (Songer 2004), although diagnosis is complicated as results of commercial toxin tests in feces often poorly correlate with culture results (Rupnik 2010; Songer 2004). Several studies have examined the prevalence of *C. difficile* shedding among pigs in North America and globally. Methodological differences in sampling and isolation make comparison of apparent prevalence between studies impractical. If fecal shedding is considered as a source for contamination of pork that could potentially serve as a public health threat, it is important to note that prevalence of fecal shedding in piglets is higher than in adult swine. Weese et al. (2010c) noted that there was a significant decrease in colonization over time with 74% of piglets on day 2, 56% on day 7, 40% on day 30, 23% on day 44, and 3.7% shedding on day 62. This is similar to what Norman et al. (2009) observed with 50% of nursing pigs and only 3.9% of grower–finisher pigs with positive fecal cultures, as well as Gebreyes (2009) who found 74.5% of preweaned piglets, 0.45% of nursery pigs, and 0% of finishing pigs to be shedding. The majority of the isolates in these studies were toxinoype V, although other types were also isolated.

Studies of retail meats, including pork, have demonstrated the presence of *C. difficile*. Techniques vary between studies so it is difficult to compare the prevalence between studies. Most of the studies also used enhancement steps that make enumeration of *C. difficile* spores in these products impossible. Weese et al. (2009) found that 71% of 14 ground pork samples were positive by enrichment culture only. Of the four that were positive by direct culture, 20 spores/g were present in three while 60 spores/g were present in one. This would indicate that while *C. difficile* contamination of meat is not uncommon, levels of contamination are low. The infectious dose of *C. difficile* in either healthy or compromised humans is not known.

**Influenza A Viruses**

Influenza A virus is an RNA virus and its genome is composed of eight separate segments that encode up to 11 proteins. Influenza is a common pathogen in
numerous species, and interspecies transmission has been documented. Interspecies transmission provides the virus an opportunity to reassort, or exchange gene segments, when two or more strains infect a cell at the same time. The 2009 pandemic H1N1 virus has been called a “quadruple-reassortant” virus because it contains neuraminidase (NA) and matrix (M) gene segments from Eurasian swine influenza viruses combined with triple-reassortant proteins of North American swine influenza viruses human-origin polymerase B1 (PB1), avian-origin polymerase B2 (PB2), and polymerase A (PA); and classical swine-origin hemagglutinin (HA), nucleoprotein (NP), and nonstructural (NS) genes. The evolutionary analysis of the 2009 pandemic H1N1 shows that the generation of this strain was not likely a recent event. In fact, in order to facilitate human-to-human spread, it probably adapted to the human host through secondary reassortments in humans (Ding et al. 2009). Smith et al. (2009b) raised the possibility that the 1918, 1957, and 1968 pandemic strains were generated through a series of multiple reassortment events and emerged over a period of years prior to the pandemic recognition. They also noted that each of these strains was produced by reassortment of a previously circulating human virus and at least one virus of animal origin (avian or mammalian) (Smith et al. 2009a).

There have been a total of 37 civilian human cases of zoonotic swine influenza virus (SIV) infection reported between 1958 and 2005 (Meyers et al. 2007; Van Reeth 2007). A majority of these infections were with classical swine H1N1 viruses, and the total does not include the Fort Dix cases in 1976 that resulted in one death and up to 230 infected soldiers (Meyers et al. 2007; Van Reeth 2007). Since that time, 11 cases of triple-reassortant H1 infections in humans have been reported in the United States including a report of pig-to-human transmission with a strain currently circulating in the U.S. pig population at an Ohio County Fair in 2007 (Shinde et al. 2009; Vincent et al. 2009). Persons identified as infected with the triple-reassortant H1 SIV typically had close contact with pigs, and 8 of 11 cases had contact with clinically ill pigs (Shinde et al. 2009). Antibodies to swine influenza may be present in up to 23% of humans with occupational exposure to pigs, although presence of antibodies is also associated with other factors such as age and previous influenza vaccination (Van Reeth 2007). It is not clear if the seropositivity of this population relates to exposure or clinical illness.

**Hepatitis E Virus**

Hepatitis E virus (HEV) is the leading cause of enterically transmitted sporadic non-A and non-B hepatitis in tropical and subtropical countries. Inadequate sanitation had led waterborne HEV epidemics in these countries (Meng 2000). Mortality due to HEV infection is usually low (<1%), except in pregnant women where mortality in Asia and Africa approached 20%. While human cases of HEV are only sporadically reported in the United States and other industrialized countries, serosurveys indicate a larger proportion of the population has been exposed to the virus (Meng et al. 2002). Swine workers in the United States and Taiwan have been shown to be at increased risk of seroconversion, but history of clinical disease among these workers is largely lacking (Hsieh et al. 1999; Meng et al. 2002; Withers et al. 2002).

Meng et al. (1997) first reported an HEV from swine that was closely related to, but distinct from, human HEV isolates. An HEV was subsequently identified in swine in Taiwan that was distinct from the U.S. swine strain but similar to the human strains in Taiwan (Hsieh et al. 1999). Generally, HEV strains identified are genetically related, and molecular studies of human and swine HEV isolates globally found that swine and human isolates from the same geographical region are more similar to each other than they are to swine or human HEV isolates from other regions (Clemente-Casares et al. 2003; Meng 2003). Swine HEV appears to be ubiquitous with incidence of swine HEV antibodies in swine herds globally varying by geographical region and age of animals sampled, ranging from 4.1% to 79% (Smith 2001). Anti-HEV antibodies have also been identified in rodents, chickens, dogs, cattle, sheep, and goats (Meng 2000).

In 2003 in Japan, the first direct evidence of zoonotic transmission of HEV was documented in two separate incidents involving human consumption of undercooked pork liver and raw sika deer meat (Yazaki et al. 2003). HEV-RNA was identified in 1.9% of raw pork livers in Japanese grocery stores and in 14 of 127 pork livers in U.S. grocery stores (Feagins et al. 2007; Yazaki et al. 2003). While sporadic cases such as those in Japan provide strong evidence that HEV is zoonotic, it is uncertain what the contribution of swine HEV is to the human burden of illness due to HEV. Meng et al. (1999) surveyed swine in four countries, two with a high prevalence of human HEV cases and two with a low prevalence of human cases. They found that swine HEV was endemic in all four countries regardless of the prevalence of human cases. Human exposure (as measured by antibody response) to HEV in both developed and developing countries is not uncommon, while clinical illness is rare in developed countries.

**Japanese Encephalitis**

Even though Japanese encephalitis virus (JEV) in humans usually results in unapparent infections, it is the most frequent cause of mosquito-borne encephalitis globally (Oya and Kurane 2007; van den Hurk et al. 2009; Weaver and Reisen 2010). The distribution of JEV has expanded throughout East, Southeast, and South Asia and into the South Pacific (Oya and Kurane 2007;
van den Hurk et al. 2009) and has been associated with increases in human population, irrigated rice production, and pig production.

Wading birds are considered the primary endemic hosts of JEV; however, pigs are important in the JEV transmission cycle as they are the only known mammals to fulfill the criteria as an amplifying host for the virus (van den Hurk et al. 2009). High levels of JEV infection in pigs often precede human epidemics (Acha and Szyfres 2003b), and sentinel pigs provide a useful system for estimating JEV risk in humans (Oya and Kurane 2007). In some regions of Asia, increasing pig populations have been linked to increasing human infections, but in Japan, that trend was reversed. During the last 40 years, the number of pigs produced in Japan has increased while the number of farms has decreased. As more pigs are raised in modern facilities that are segregated from residential areas, human cases of JEV have declined, demonstrating that pig farming can be maintained without increasing the transmission of JEV to humans (Oya and Kurane 2007).

**Nipah Virus**

Nipah virus (NV) was first identified in 1999 in Malaysia during an outbreak of respiratory illness and encephalitis in pigs accompanied by an often fatal encephalitis in people with close contact with pigs as well as abattoir workers (Teng et al. 2009) and military personnel involved in culling infected herds (Ali et al. 2001). There were also reports of human-to-human transmission of NV among health-care workers (Teng et al. 2009). More recently, NV has been diagnosed as the cause of fatal encephalitis cases in people without close contact with pigs in Bangladesh during the winters of 2001, 2003, and 2004 (Bellini et al. 2005).

NV belongs to the genus *Henipavirus*, in the family *Paramyxoviridae*, and is closely related to the Hendra virus of equines (Bellini et al. 2005). Fruit-eating bats (*Pteropus* and *Chiropotes* species) are considered the natural reservoir for NV, and humans are infected through either contact with an intermediate host such as pigs or exposure to infected bats, material/foods contaminated by bats, or direct human-to-human transmission (Bellini et al. 2005; Teng et al. 2009). The implicated host species of bats is found across South-east and South Asia (Teng et al. 2009). Outbreaks in pigs have not been observed since the 1998–1999 outbreak in Malaysia.

**Reston-Ebola Virus**

Reston-Ebola virus (REBOV) is the only member of the filovirus family that is thought to be pathogenic in Asian monkeys but not in African monkeys or humans (Morikawa et al. 2007). Other filoviruses are associated with acute fatal hemorrhagic disease of humans or nonhuman primates.

In July 2008, REBOV was identified in swine diagnostic samples from The Philippines that were being tested at the USDA Foreign Animal Disease Diagnostic Laboratory. These samples were associated with clinically affected animals thought to be affected with highly pathogenic porcine reproductive and respiratory syndrome virus (PRRSV). REBOV was only found in samples that were also positive for PRRSV. Six humans of 141 with swine exposure in The Philippines had positive immunoglobulin G (IgG) titers to REBOV, but no human illness was detected (Barrette et al. 2009).

**ANTIMICROBIAL RESISTANCE**

Antimicrobial resistance (AR) in bacterial pathogens is a global public health concern. It is clear that human consumption of antimicrobials provides selection pressure for AR infections in humans. The scientific and political debate is focused on the contribution of animal antimicrobial use to the burden of AR infections in humans.

Evidence of the contribution of antimicrobial use in animals to AR infections in humans include food-borne pathogens (e.g., multidrug resistant [MDR] *Salmonella newport* and fluoroquinolone-resistant *C. jejuni*) as well as emergence of community-acquired commensal infections associated with poultry and swine (e.g., vancomycin-resistant *Enterococcus* spp.) (Aarestrup et al. 2000). Because AR genes can be horizontally transmitted between bacteria, nonpathogenic bacteria can serve as a reservoir of AR. AR of bacterial zoonoses have led to the ban of subtherapeutic antimicrobial use in the European Union (Casewell et al. 2003) and the removal of specific uses of fluoroquinolones in chickens in the United States (Department of Health and Human Services, US Food and Drug Administration 2005).

The evidence regarding the impact of antimicrobial use in animals and the ultimate impact on human health is mixed. For example, in Denmark, the removal of avoparcin as a growth promotant for poultry and swine has decreased the prevalence of vancomycin-resistant *Enterococcus* (Anonymous 2009c). Yet, there is increased use of antimicrobials for therapeutic purposes in swine since the ban and certainly mixed results regarding AR changes in some pathogens as a result (e.g., *Salmonella*). A recent systematic review by Young et al. (2009) compared organic production with antimicrobial-using production systems and found that there was an association with antimicrobial use and fluoroquinolone resistance in *Campylobacter* from poultry, and generally increased AR in bacteria isolated from other species, yet AR bacteria were isolated from organically produced animals and animal products.
Unintended consequences to food safety as a result of impaired animal health from subtherapeutic antimicrobial removal have been demonstrated (Singer et al. 2007). The use of macrolides in food animals has been suggested to present an extremely low probability of human treatment failure (Hurd et al. 2004).

A best practice for control of emergence of AR bacteria for both human and animal health is the judicious use of antimicrobials. Judicious use guidelines for antimicrobials have been developed (Anonymous 2009d).

CERTIFICATION PROGRAMS

There are several certification programs within the United States that focus on production practices that will help to assure safe and wholesome pork products. All of these programs have some level of third-party oversight that certifies the education of, or adherence to, the production practices.

Pork Quality Assurance Plus

The Pork Quality Assurance (PQA) program was originally introduced in 1989 to address concerns over violative drug residues in pork. Subsequent revisions widened the scope of the program to address a broader range of physical, chemical, and microbiological hazards. The PQA Plus, an expanded version of the original PQA was introduced by the Pork Checkoff in 2007. PQA Plus was developed as a continuous improvement program, retaining the original focus with added emphasis on the responsible use of antimicrobials in pork production, animal caretaker training, and animal care and well-being. PQA Plus has been well accepted by the U.S. meat packers, and most of them require either certification or site assessment status of the producers who supply them with pigs.

There are three distinct aspects of certification and verification of the PQA Plus program: PQA Plus certification, PQA Plus site assessment, and a PQA Plus survey. PQA Plus certification entails training of the producer by a trained PQA Plus advisor. The certification is of education and does not include an assessment of compliance to the PQA Plus Good Production Practices. Specific PQA Plus Youth Certification is available for youth 8–19 years of age. Certification must be renewed every 3 years. The PQA Plus site assessment is conducted by a PQA Plus advisor and assesses the producer’s site against a series of criteria. Most of these criteria focus on animal care and well-being, but there are food safety criteria including demonstration of a veterinary/client/patient relationship and adequate medication and treatment records. Finally, the PQA Plus survey, conducted by third-party evaluators, will evaluate the implementation of PQA Plus in the industry and serve to identify opportunities for improvement.

Trichinae Herd Certification

The USDA and pork industry groups (National Pork Board, National Pork Producers Council, and the American Association of Swine Veterinarians) have worked cooperatively to develop the U.S. Trichinae Certification Program. This program is consistent with the on-farm control recommendations of the International Commission on Trichinellosis (ICT) Recommendations on Methods for the Control of Trichinella in Domestic and Wild Animals Intended for Human Consumption (Gamble et al. 2000). Under this program, a qualified accredited veterinarian (QAV) audits a production facility for compliance with production practices that address known risk factors for Trichinella infection of swine farms. Audits are conducted on a regular schedule as established by the Trichinae Certification Program Standards. Program effectiveness is verified by testing a statistically valid sample of market swine from Trichinae Certified production sites. The ICT recommendations state that under such a control program, individual carcass testing could be eliminated. The European Commission has also developed regulations (European Community 2005) that lay out requirements for certifying pigs from individual farms or from categories of farms. In addition, it provides requirements for exemption from Trichinella testing in areas officially considered to have negligible risk of Trichinella in domestic pigs.

Safe Feed/Safe Food

The American Feed Industry Association (AFIA) established the Safe Feed/Safe Food Certification Program, which now operates independently of AFIA. The Safe Feed/Safe Food Certification Program is a voluntary, third-party-certified initiative aimed at feed mills and feed- and ingredient-related facilities in the United States and Canada. The program has established standards addressing documentation, training, facility planning and control, manufacturing and processing, monitoring devices, infrastructure, ingredient purchasing, traceability, and control of nonconforming products. A producer-oriented introduction to the Safe Feed/Safe Food quality assurance processes has been developed by AFIA and the National Pork Board.

REFERENCES

Special Considerations for Show and Pet Pigs

Amy L. Woods and Valarie V. Tynes

SHOW PIGS

Introduction to the Show Pig Industry

The show pig industry is often overlooked by many in the swine industry since it represents a significantly smaller number of animals than the commercial swine production industry. However, the show pig industry is a significant business when the number of people and amount of money invested are considered. It is difficult to estimate the exact size and monetary value of the U.S. show pig industry. Based on pig show registrations and sale numbers, it is estimated that approximately 1 million pigs enter the food chain through pig shows and exhibitions each year. Another 1–2 million head are born and raised as show pigs and enter the food chain through other venues. These may be littermates that are not selected for show, pigs serving as backup show pigs or those that do not meet the weight criteria for a given show, and so on.

With the growth of the show pig industry, a considerable “niche” market has developed for veterinary products and services. Because of the unique nature of show pigs, an awareness of some of the special considerations of the industry will allow practitioners to better address the needs of the pigs and the client. There are different types of show pig clients—those that breed and farrow to produce their own pigs and pigs for sale, and those that purchase young pigs and only raise grow–finish pigs. Some clients may be casual show pig producers with a goal of just having a project to show at a local fair. Others are extremely serious about the show pig industry and spend considerable time, money, and attention on their show pigs. Many boar studs market semen specifically to the show pig customer.

There are several characteristics that distinguish show pigs from commercial pigs. It is important to realize that individual show pigs or pets can be extremely valuable—up to tens of thousands of dollars. These animals are bred and raised primarily for exhibition, not necessarily for pork production. The same pigs may frequently be shown at various locations during a show season. The show season will vary depending on the geographical area of the United States but typically lasts around 3 months. Many of the more serious show pig clients will participate in a jackpot show circuit, in which the pigs earn points and money for the placing they receive at numerous shows. These pigs may be traveling to as many as four shows per month throughout the show season. At the culmination of the show season, pigs may be exhibited at a terminal show and go directly to harvest. Alternatively, many animals (particularly breeding animals) return home after the show season. This show schedule creates unique biosecurity and health concerns.

Biosecurity

When selecting a show pig to purchase, often health status or source is not considered. Pigs are purchased according to their pedigree and appearance. Veterinarians need to advise clients on proper biosecurity measures and the risks involved when mixing pigs from various sources. Treatment may also be indicated when purchasing these pigs and mixing them together for the first time. Veterinarians must also educate clients about taking proper biosecurity measures when bringing animals back home after a show. A survey conducted after the 2002 Indiana State Fair (at the end of the Indiana show season) indicated that nearly half of the pigs exhibited went back home or to another farm
after the fair (Amass et al. 2004). Often, there are no isolation or testing procedures in place for these animals returning from a show.

Veterinarians must be aware of the state and federal regulations regarding movement, identification, and exhibition of swine in their area since show pigs travel frequently, often crossing state lines. Some states also have regulations regarding isolation periods and testing procedures before and/or after movement or exhibition. Veterinarians may also be hired by specific pig sales and shows to ensure compliance of all participants with any state and federal regulations regarding identification, health certificates, and animal health. Since show pigs are primarily identified by ear notches, veterinarians must be proficient in reading standard ear notches in order to meet these regulations.

Zoonotic Implications
Zoonotic disease potential is important in commercial pork production, but with show pigs and pet pigs, there is increased risk due to the closer interaction between show pigs and their caretakers. Additionally, members of the general public can closely interface with animals at local fairs and exhibitions. Swine influenza virus (SIV) infections have been documented in people with no swine exposure except for visiting a local fair in Wisconsin (Wells et al. 1991) and Ohio (Vincent et al. 2009), and Shiga-toxigenic Escherichia coli O157:H7 has been demonstrated to be present in cattle, pig, sheep, and goat feces at public fairs (Keen et al. 2006). Veterinarians must be available to quickly identify and treat and/or recommend removal of sick animals from a fair in order to minimize the zoonotic disease transmission risk. Veterinarians should also educate clients as well as the public about these zoonotic risks and may be expected to advise fair officials in the event of a zoonotic disease.

General Health Issues
Vaccination and Health Protocols. Veterinarians need to be involved with formulating a vaccination protocol for show pig clients. The typical approach used with commercial herds using incidence of clinical signs, serological testing, or postmortem diagnostics of mortalities is not practical when working with show pigs due to the small herd size. Some established show pig herds may have historical disease information to use when deriving a vaccination protocol. In addition, some pig sales and shows will require specific vaccinations.

Show pig health protocols must also include regular treatments for both internal and external parasites, as both are extremely common in show pigs. The risk of infestation with mange is higher in show pig herds due to the wide exposure to other animals at sales and shows. Show pigs are often housed and/or exercised on dirt lots or older barns where pigs have been present for many years, increasing the exposure risk for internal parasites. It is common to utilize a rotation of antiparasitic products, such as fenbendazole, dichlorvos, and ivermectin, throughout the growing period. It should also be ensured that all show pig breeding herds are routinely treated with antiparasitic products.

Individual Animal Medicine. Unlike commercial herds, where veterinarians are focused on population medicine, show pig veterinarians often focus on individual animal problems. Clients may call their veterinarian to examine a sick, injured, or lame animal. Common problems include pneumonia or scours and should be treated the same as these issues are treated in a commercial farm. Lameness in show pigs is extremely common since they are exercised vigorously and are often very heavily muscled. Common lameness problems result from osteochondrosis (OCD) and osteoarthritis (OA), joint infections, hoof cracks, pad bruising, and traumatic injuries. Infectious causes of lameness in show pigs can often be related to Mycoplasma hyosynoviae or erysipelas. Lameness that would be of no consequence to a commercial pig can easily render a show pig unfit for the intended use. Most show pig clients are willing to invest in the best possible treatment as a first choice since they often have a significant financial, time, and emotional investment in the animal. Oral joint supplements containing glucosamine and chondroitin are used extensively in show pigs to promote joint health and minimize problems from OCD and OA, although there is no efficacy data available for this practice. These supplements are often fed for several weeks leading up to a show or when an animal may start to show clinical signs of joint stiffness.

A common problem for outdoor show pig operations is sunburn. If burnt severely enough, these pigs will have scabs and scarring across the back. In the acute stages, they will appear to have a neurological deficit, exhibiting an unusual gait in the rear legs induced by the pain across the back. Sunburns are treated with over-the-counter aloe products and addition of shade in the pen. Many show pig producers prevent sunburn by applying a sunscreen to light-colored pigs before taking them outside.

Veterinarians are also asked to perform a variety of surgical procedures on show pigs. These procedures are not economically justifiable on most commercial farms. Examples of commonly requested surgical procedures for show pigs include the following:

1. late-age castration—many show pigs remain boar prospects until an older age and require anesthesia for castration;
2. scrotal rupture repair;
3. abdominal hernia repair—may often also have abscess present;
4. cryptorchidectomies—may be requested by breeder before castration if only one testicle is descended or requested by exhibitor after their pig starts exhibiting boar behaviors;
5. abscess removal—as a consequence of castration, due to wounds, or at injection sites;
6. scirrhous cord removal—due to castration site infection and scarring;
7. preputial diverticulectomies—performed on boars or barrows to eliminate urine pooling, as many clients prefer not to manually express the contents routinely; also performed on boars to eliminate masturbation into the diverticulum;
8. penis problems—examination and repair of a persistent frenulum or trauma; it is helpful to use 10-in. curved Bozeman uterine forceps to exteriorize the penis of an anesthetized boar;
9. extra dewclaw amputation—some breeds will not allow animals with extra dewclaws to be registered as purebreds, so the decision to perform this surgery may have ethical implications;
10. rectal prolapse repair—often subsequent to cool environmental conditions and piling of pigs, coughing, or diarrhea after a feed change.

Anesthesia options and surgical techniques are detailed in Chapter 10 of this book.

Breeding Management
Veterinarians are often consulted for assistance and advice on artificial insemination techniques and estrous management in show pig herds. Unlike commercial farms, show sows and gilts are typically bred to a specific boar based on the compatibility of phenotypes, or predicted phenotypic outcome of the individual cross. Semen is purchased from boar studs that specialize in show pig semen, and doses can be expensive. Due to the timing of the show season, show pig producers desire to breed animals to farrow at very specific times. Veterinarians may be expected to develop and implement estrus synchronization options. In addition, many show pig herds are small and do not have a boar present to assist in heat detection and stimulation during breeding.

Farrowing Management
Obstetrical difficulties are often more common in show animals as well. Show pig producers tend to farrow more gilts in order to keep up with the most current genetic trends in the show industry. There is often a difference between the phenotypes of gilts that are successful in the show ring and the breeding herd. Birth weights may be heavier due to lower litter size and/or the genetic type. Show herd sows are often overconditioned. The combination of these factors results in an increased rate of dystocia. Since many show pig producers are not as experienced at assisting sows with difficult dystocias as commercial producers, and the animals have greater monetary value, veterinarians are more likely to be called to provide obstetrical care. In extreme dystocia cases, cesarean section may be indicated.

Porcine Stress Syndrome
Even though the commercial swine industry has mostly eliminated porcine stress syndrome (PSS) from the pig population, it is maintained in the show pig industry. Since PSS is associated with an increase in muscling and leanness, the show pig industry has inadvertently selected for this gene. Many breed associations are encouraging producers to eliminate PSS by not allowing animals that are heterozygous (carrier) or homozygous recessive (positive for the trait) to be registered. For example, Yorkshires, Hampshires, Durocs, and Landrace cannot be registered with the National Swine Registry unless they are homozygous dominant (negative). Veterinarians may be asked to provide assistance on testing animals to verify their PSS status. This is typically performed via a blood test at a private lab. Some producers are proficient in collecting samples for testing animals themselves with a small blood sample from an auricular vein placed on a blotter card. Veterinarians may observe more clinical PSS in show pigs and therefore need to be aware of how to recognize and avoid it.

Nutrition
Nutritional aspects of the show pig industry are much different than the commercial industry. Show pigs are typically fed much higher protein diets than comparable commercial pigs. A wide variety of supplements and feedstuffs are utilized in show pig diets. Some of these include human foods or feedstuffs typically utilized by other species. Efficacy and safety of these supplements are generally not available. The desired appearance of successful show pigs tends to change every few years, and subsequently, the composition of feedstuffs and supplements also changes to produce the desired appearance. One common supplement that deserves special mention is ractopamine HCL. Veterinarians can play an important role in educating clients on feeding the proper rate of this supplement in small batches of feed.

Veterinarians must be aware of some of these non-traditional feedstuffs and supplements, since there may occasionally be some health issues related to their use. For example, the increased fat in show pig diets has been known to result in steatitis in the inguinal and abdominal area (R. Bush, personal communication). The desire to enter a pig in a specific weight class may result in withholding water or use of a diuretic, which can cause dehydration.
Education
The show pig veterinarian must be heavily involved in the education of clients. Most show pig producers lack the experience and knowledge of those producers in the commercial industry. Veterinarians must be diligent in educating these clients about proper pharmaceutical use, administration, and withdrawal times since many pharmaceutical products are used in show pigs. In addition to reaching individual clients, veterinarians are also asked to speak to 4-H or FFA groups about general pig care, health, and disease prevention. There are also opportunities to be involved in formal educational programs for show pig producers, such as the National Pork Board’s Pork Quality Assurance Plus (PQA+) or Youth PQA+ training sessions.

Ethics
Any veterinarian working with show pig producers must determine their own set of ethical standards concerning the procedures and recommendations they provide. As with many competitive industries, there are clients who will do anything to win and may ask their veterinarian to provide a service that is contradictory to good judgment or ethics. Practitioners must also be mindful of how their recommendations might affect all pork producers and consumers. Some categories of ethical dilemmas that veterinarians may face include altering animal identification, illegal drug use, and surgical procedures that alter conformation, among many others.

In addition to the obvious ethical issues that arise, there are also instances in which routine veterinary care and treatments are banned by particular shows. For example, many shows prohibit the use of any “performance-enhancing product,” such as nonsteroidal anti-inflammatory drugs (NSAIDs), since it may mask a lameness problem. Even when these products are used according to label directions and meet legal withdraw times, pigs may be disqualified from shows for their use. Most major shows will drug test pigs for these types of substances. Veterinarians working with show pigs must be mindful that show ring rules may not always correspond to the legal standards that apply to commercial animals.

Acknowledgments
The author greatly appreciates the input and assistance of all those who contributed to the show pig section of this chapter: Drs. Max Rodibaugh, Dave Farnum, Jodi Sterle, Keith Adams, Mr. Ryan Harrell, and Mr. Alan Duttlinger.

MINIATURE PET PIGS
While their diminutive size makes them more appropriate as pets, owners often need to be reminded that miniature pigs are still members of the family Suidae and share similar behaviors, environmental needs, diseases, and parasites. They are anatomically and physiologically much like the larger commercial varieties of swine, and with a few exceptions, treatment will be similar.

Behavior and Training
Aggression in the pet pig is a significant concern and results in the abandonment or rehoming of many pigs (Lord and Wittum 1997; Tynes et al. 2007). For that reason, pet owners should be counseled about the potential for problems as soon as possible after they acquire the pig. No aggression directed toward humans should be ignored in the hope that the problem will get better on its own. As is the case with most behavior problems, aggression rarely decreases without treatment, and it usually worsens with time if left untreated. Pet pigs usually begin displaying aggressive behavior as they mature socially (6 months–3 years), and typically, the first victims of aggression will be visitors to the home (Tynes et al. 2007). This is believed to be a normal response to an unfamiliar individual, similar to how pigs behave when meeting unfamiliar conspecifics. Aggression toward familiar people within the home also commonly begins around the time of social maturity and appears to be a form of dominance aggression. While many responses to this behavior have been described by pet owners and trainers, it is recommended that pig aggression be treated similarly to dog aggression; the animal should be taught that humans are the leaders of the group. This is safest and easiest to accomplish by conditioning the pig to a harness and leash at a very early age; harnesses made specifically for miniature pigs are recommended and will fit better and be easier to use than harnesses intended for dogs. The pig should then be taught to respond to a simple command, such as “sit,” before being given anything that it desires. It is relatively simple to teach the pig to sit using a food lure, just as you would a dog. It should then be asked to sit before being fed, brushed, petted, invited onto furniture, allowed outside, and so on.

The pig should always be wearing its harness when visitors are expected, and a responsible adult should have control of the leash whenever visitors arrive at the home. Once a visitor has been in the home for a few minutes, they can be allowed (if they desire to interact with the pig) to ask the pig to sit and reward it with small pieces of fruit, vegetables, dry cereals, or other food treats, when it complies. Alternatively, the owners can simply be advised to separate their pig from all visitors by confining it to another room or pen whenever visitors arrive at the home.

Restraint
Physical. Just as with larger swine, miniature pigs resist firm restraint by struggling and vocalizing. Superficial examination and vaccination are possible using
physical restraint, but for more thorough examinations, tusk trimming, and nail (hoof) trimming, chemical restraint is often necessary. Most owners of miniature pigs consider their pigs a pet and will be unhappy if their pig is treated as a farm animal. For that reason, most will prefer to pay for chemical restraint rather than see (or hear) their pigs restrained while struggling and squealing. In addition, miniature pigs have a smaller, less stable cardiovascular system than large swine and can be stressed to the point of collapse. Due to their propensity to joint dislocation, they should never be lifted by their legs. Nose snares are rarely useful as the miniature pig may simply panic and thrash about; they do not reliably back away from the snare and injuries have been reported with snare use.

To lift the small- or medium-sized pet pig, place one arm in front of the front limbs and the other arm behind the rear limbs. Hold the pig firmly to your chest and move it as quickly and smoothly as possible to an examination table. Once the pig is on the table, do not try to firmly restrict its movements; it should simply be corralled with the arms. A rubber mat or other nonslip surface will greatly decrease the pig’s distress and help keep it calm. If the person restraining the pig will scratch it firmly on the neck, flank, belly, or inner thigh, this will also help to keep the pig quiet and calm. Continuously feeding the pig small bites of food (if anesthesia is not planned) will typically keep it occupied and allow for examination and vaccination.

Larger pigs can be lifted by two people using a version of the “fireman’s carry”; approaching the pig from each side, each individual places one arm in front of the pig’s forelegs and the other arm behind the pig’s rear legs. The two people then grasp elbows and lift the pig, squeezing it between them. The pig can then quickly be placed on an examination table.

Hammock-like slings with a hole cut for each of the pig’s feet have also been used for pet pigs. The sling can be very useful for aiding restraint in some larger and less docile pigs. Many pigs will allow nail and teeth trimming while sitting in the sling.

**Chemical.** Achieving predictable results with injectable agents can be challenging in the miniature pig due to their thick layer of body fat. A 1.5- or 2-in. needle may be required to deposit the injection in the muscle of adult pigs. The preferred site for injection of anesthetic agents is the semimembranosus or semitendinosus muscles. Injection into the gluteal muscles may lead to lameness. The commonly used dissociative anesthetic agents have been associated with prolonged and violent recovery and bizarre behavioral changes after recovery. However, when inhalation anesthesia is not available, dexmedetomidine (40mcg/kg), butorphanol (0.3mg/kg), and midazolam (0.3mg/kg) combined in one syringe and administered intramuscularly has been found to be safe and reliable while offering a smooth recovery. In addition, the effects of the dexmedetomidine can be reversed with atipamezole to speed recovery.

Inhalation anesthesia has also been shown to be safe and effective, and offers a quick and relatively predictable recovery. Most pigs can be easily masked down by including nitrous oxide with the oxygen for 30–60 seconds initially, then very slowly turning on the anesthetic gas (isoflurane). Once the pig visibly relaxes, the nitrous should be discontinued.

Malignant hyperthermia has been rarely documented in the miniature pig but appears to be uncommon. The prevalence of the PSS gene that predisposes a pig to malignant hyperthermia is currently unknown.

Miniature pigs should be monitored carefully during recovery from anesthesia as complications such as laryngeal edema, hypothermia, and cardiovascular compromise are not uncommon.

**Vaccinations**

In spite of the fact that many pet pigs will never be exposed to other swine, some vaccinations are still important to ensure their continued good health as well as prevent the potential spread of zoonotic diseases and diseases of economic interest to the commercial swine industry. No single vaccination protocol will be best for all pet pigs, but all of them should be routinely vaccinated against erysipelas. Other vaccinations will depend on the pig’s age, prior vaccination status, and the environment and geographical area in which it resides as well as potential exposure to other pigs.

**Parasites**

Pet pigs are subject to the same parasites that can infect larger swine. Sarcoptic mange (Sarcoptes scabiei) is one of the more commonly seen problems. Miniature pigs often exhibit abundant dark crusty exudate in their external ear canals, but this appears to be normal, and ear mites (Otodectes cynotis) are rarely, if ever, found within the discharge. However, S. scabiei mites are often present in ear swabs of infested pigs.

Internal parasites, such as roundworms, whipworms, and threadworms, are less common in pigs acquired from reputable breeders, and large worm burdens are rare, as is clinical illness associated with endoparasites. All pet pigs should nevertheless have fecal flotations performed at 8–12 weeks of age. After reaching adulthood, fecal exams are less critical if the pig is maintained in a clean environment and is not exposed to environments where other swine have been. If parasites are indentified on a fecal exam, the pet pig can be treated with the same anthelmintics at the same dosages as larger swine.

**Teeth Trimming**

All miniature pigs have four permanent canine teeth that erupt at about 5–7 months and, in males, grow
continuously. Even the tusks of gonadectomized males can reach a dangerous length, although they usually grow more slowly than in intact males. The best-behaved pigs can do damage to furnishings and accidentally harm humans with these long, sharp teeth, so regular trimming is recommended. Anesthesia makes the procedure safer and easier for all involved. Obstetrical wire, high-speed dental tools, and dremel tools have all been used successfully. Crushing instruments should be avoided as they can fracture the tooth longitudinally causing pain and possibly infection. The teeth should be cut as short as possible while avoiding the pulp cavity, the length of which can vary between individuals. A sanding blade on a dremel tool can be used to smooth the tooth so that no sharp edges remain.

Removal of the canine teeth is not recommended unless necessary due to fracture or infection.

Hoof Trimming
Pet pigs that live in the home and get minimal exercise on rough surfaces usually have hooves that require regular trimming. Overgrown hooves hyperextend the joints of the legs and, along with obesity, are a common cause of lameness in pet pigs. Routine trimming is usually required and will be most easily performed when the pig is anesthetized. However, many pigs will tolerate the procedure while being restrained in a sling or held on their backs. Trimming is easily accomplished using trimmers and rasps as you might use for goats or horses. In some cases, especially where the hooves have become severely overgrown, the sensitive laminar tissue (the “quick”) will be extended. Cutting deeply into the quick can cause lameness that lasts for several days and should be avoided when possible.

Common Surgical Procedures
All female pigs not intended for breeding should be ovariohysterectomized. A high rate of reproductive tract neoplasia has been noted (Mozzachio et al. 2004) in pet pigs over 5 years of age. While these tumors are not usually malignant, they can reach extreme sizes, leading to discomfort, occasional colic-like signs, cardiovascular compromise, and death. Removal of the tract after the tumors have become very large is more difficult and places the patient at higher risk.

Ovariohysterectomy is easier for the surgeon and less stressful for the pig if performed prior to the pig reaching a weight of about 25 lb. In a smaller animal, the immature reproductive tract can be more difficult to access, and in a heavier patient, the larger amount of intra-abdominal body fat may make accessing the tract difficult, result in excessive bleeding, and increase the risk of dehiscence. Obese patients are at a greater anesthetic risk as well. A ventral midline approach is recommended. The use of a spay hook should be avoided as it can be damaging, leading to excessive bleeding within the fat, and is generally unnecessary.

Closure of the skin using an absorbable suture and a subcuticular pattern and burying the knot alleviates the necessity of a later struggle to remove sutures.

All boars intended to be kept as pets should be castrated, preferably before 12 weeks of age. Intact boars are less likely to make good pets due to their unpredictable behavior and strong, objectionable odor. While early castration greatly reduces odor and leads to a decrease in size of the preputial diverticulum, odor and drainage can still occur in some pigs. Preputial diverticulectomy as described by Lawhorn et al. (1994) greatly increases pet owner satisfaction in the author’s experience. All pet pigs neutered later than 2–3 years of age are likely to be more satisfactory as pets if the preputial diverticulectomy is also performed at the time of castration. Castration should be performed through a prescrotal incision using a technique similar to that used in the dog. The vas deferens and associated blood vessels should be ligated and excised. Removing the cremaster muscle, tunic, and extraneous subcutaneous tissues decreases dead space and reduces the chance of seroma formation. Closing the skin with absorbable sutures using a subcuticular pattern eliminates the need for the later restraint and struggle associated with suture removal.

Cryptorchidism and inguinal hernias are commonly seen in miniature pigs. Inguinal rings should be examined at the time of castration and surgically closed if patent.

Common Disease Problems and Therapeutics
Physical exam on the pet pig should be conducted similarly to a physical exam on any animal. One of the noted differences between the miniature pigs and the larger commercial breeds of swine is that their normal resting rectal body temperature is likely to be lower with temperatures as low as 37.6°C (99.7°F) reported in one study (Lord et al. 1999).

While subject to all of the same diseases that occur in other swine, illness is uncommon in the pet pig that is properly vaccinated, fed, and housed. However, health problems may include the following:

1. Obesity—Obesity is common in the pet due to lack of exercise and improper feeding. Many pet owners think obesity is normal; they are unaware of the numerous health problems associated with obesity in the pig. Obesity contributes to chronic lameness and blindness secondary to excessive fat accumulation around the eyes, and places strain on the heart and lungs. Pet owners must be instructed as to the importance of feeding pet pigs a commercial diet made especially for miniature pigs. In addition, placing food in food-dispensing toys (hollow balls or plastic jugs with holes cut in them), or simply broadcasting the ration over a clean, grassy area of
the yard, requires that the pig expend more calories acquiring its food.

2. Arthritis—Miniature pigs have been reported to live 15–18 years. One of the more prevalent health problems associated with aging in the pig is OA. This is often secondary to chronic obesity and/or overgrown hooves. Ongoing lameness that eventually becomes nonresponsive to anti-inflammatories and pain killers is a common cause for euthanasia in the geriatric pig.

3. Dental disease—While pigs develop impressive amounts of dental tartar as they age, severe periodontal disease is uncommon. Regular teeth cleaning similar to that for a dog may be beneficial for some individuals. The most common dental problem seen in geriatric pigs is tusk root abscessation in males. This may present as chin or jaw abscesses that recur after initial treatment. Radiographs are diagnostic, often revealing extensive bone lysis. Successful treatment will require tusk removal.

4. Uterine neoplasia—see the “Common Surgical Procedures” section.

Regulatory Issues
Many pigs have been abandoned by their owners after they discover that zoning regulations prohibit the keeping of a pig in their community. In the eyes of the government and many individuals, a miniature pig is a farm animal and subject to the same federal rules and regulations governing the maintenance and movement of other swine. While most owners will deny the possibility of their pet pig ever entering the food chain, it has been reported (Lord and Wittum 1997), and for this reason caution must be used when administering or prescribing medications for the pet pig. Drugs illegal for use in food animals must be avoided, and discharge instructions should include mention of drug withdrawal times.

ACKNOWLEDGMENTS
The author gratefully acknowledges the assistance of Kristie Mozzachio, DVM, DACVP, and Bruce Lawhorn, DVM, in the preparation of the pet pig section of this chapter.

REFERENCES
14  Cardiovascular and Hematopoietic Systems
15  Digestive System
16  Immune System
17  Integumentary System: Skin, Hoof, and Claw
18  Mammary System
19  Nervous and Locomotor Systems
20  Diseases of the Reproductive System
21  Respiratory System
22  Urinary System
The cardiovascular and hematopoietic systems are essential components that conjointly aid in the distribution of oxygen, nutrients, minerals, proteins, and cellular constituents to the body systems. Disease or functional disruption of either system can have detrimental effects. This chapter will briefly highlight normal porcine anatomy and physiology, and the pathology of frequently encountered porcine cardiovascular and hematopoietic diseases.

**ANATOMY AND PHYSIOLOGY**

A thorough understanding of anatomy and physiology is essential for the appropriate detection and evaluation of porcine cardiovascular abnormalities. Further review of veterinary references, such as the *Textbook of Veterinary Anatomy* (Dyce et al. 2010) and *Duke's Physiology of Domestic Animals* (Reece 2004), may be beneficial.

**Hematopoietic System**

Blood is the medium through which essential life-sustaining elements are distributed throughout the body. There is approximately 56–69 mL of blood per kilogram of body weight in the pig (Fox et al. 1984). Blood is composed of both acellular and cellular components. Acellular constituents (water, minerals, electrolytes, gasses, acid–base regulatory ions, proteins, lipids, and carbohydrates) are regulated by other body systems and are introduced into the cardiovascular system for tissue distribution and excretion. Both direct and indirect alterations of acellular biomarkers can be important indicators of locomotor, digestive, respiratory, urogenital, endocrine, cardiovascular, or nervous disease. Reference intervals for porcine acellular biochemical analytes can be found in Table 14.1.

The hematopoietic system generates the cellular components of the blood, which include lymphoid (lymphocytes) and myeloid (nonlymphoid leukocytes and erythrocytes) elements. Hematopoiesis is primarily localized to the bone marrow of adult animals, but regularly occurs in extramedullary tissues in fetal and neonatal animals. Bone marrow is not frequently evaluated in swine, but myeloid to erythroid reference ratios of 1.77–2:1 have been reported (Jain 1986; Sanderson and Phillips 1981). Porcine hematological parameters fluctuate based on age, breed, sex, diet, stage of gestation, lactation, management practices, and season. As a result, normal complete blood count (CBC) reference intervals are quite broad (Table 14.2). Friendship et al. (1984) and *Schalm's Veterinary Hematology* (Thorn 2000) are useful citations for reference intervals based on age, sex, gender, and other confounding factors.

Porcine erythrocytes typically circulate for 86 days before removal from the circulatory system. Morphologically, porcine erythrocytes average 6 µm in diameter, lack basophilic stippling, and commonly exhibit poikilocytosis (Brockus and Andreasen 2003; Thrall 2004). Immature forms of red blood cells (RBCs), nucleated RBCs, and reticulocytes normally circulate in healthy pigs but are found in higher proportions in suckling animals (Brockus and Andreasen 2003).

The structure and function of porcine leukocytes and platelets are similar to those seen in other domestic mammals. *Schalm's Veterinary Hematology* (Thorn 2000) is a useful reference for morphological and functional characteristics of pig leukocytes.

**Cardiovascular System**

The porcine heart is charged with the responsibility of distributing blood throughout the body. Anatomically,
Table 14.1. Porcine clinical biochemistry reference intervals

<table>
<thead>
<tr>
<th>Biochemical Analyte</th>
<th>Reference Interval</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspartate aminotransferase (AST)</td>
<td>0–125</td>
<td>U/L</td>
</tr>
<tr>
<td>Alanine aminotransferase (ALT)</td>
<td>0–103</td>
<td>U/L</td>
</tr>
<tr>
<td>Alkaline phosphatase (ALP)</td>
<td>0–300</td>
<td>U/L</td>
</tr>
<tr>
<td>γ-Glutamyltransferase (GGT)</td>
<td>0–82</td>
<td>U/L</td>
</tr>
<tr>
<td>Glutamate dehydrogenase (GD)</td>
<td>0–8</td>
<td>U/L</td>
</tr>
<tr>
<td>Creatine kinase (CK)</td>
<td>0–10,101</td>
<td>U/L</td>
</tr>
<tr>
<td>Lactate dehydrogenase (LDH)</td>
<td>0–1,893</td>
<td>U/L</td>
</tr>
<tr>
<td>Total protein</td>
<td>49–67</td>
<td>g/L</td>
</tr>
<tr>
<td>Albumin</td>
<td>19–29</td>
<td>g/L</td>
</tr>
<tr>
<td>Globulins</td>
<td>28–41</td>
<td>g/L</td>
</tr>
<tr>
<td>Albumin/globulin ratio</td>
<td>0.52–0.95</td>
<td>–</td>
</tr>
<tr>
<td>Urea</td>
<td>1.7–4.5</td>
<td>mmol/L</td>
</tr>
<tr>
<td>Creatinine</td>
<td>88–130</td>
<td>μmol/L</td>
</tr>
<tr>
<td>Total bilirubin</td>
<td>0.0–1.0</td>
<td>μmol/L</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>2.0–4.2</td>
<td>mmol/L</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>0.3–2.7</td>
<td>mmol/L</td>
</tr>
<tr>
<td>Free fatty acids</td>
<td>0.0–1.0</td>
<td>mmol/L</td>
</tr>
<tr>
<td>Glucose</td>
<td>4.3–8.6</td>
<td>mmol/L</td>
</tr>
<tr>
<td>Inorganic phosphate</td>
<td>2.8–4.3</td>
<td>mmol/L</td>
</tr>
<tr>
<td>Calcium</td>
<td>2.5–3.1</td>
<td>mmol/L</td>
</tr>
<tr>
<td>Magnesium</td>
<td>0.9–1.2</td>
<td>mmol/L</td>
</tr>
<tr>
<td>Sodium</td>
<td>143.0–156.0</td>
<td>mmol/L</td>
</tr>
<tr>
<td>Potassium</td>
<td>4.8–7.8</td>
<td>mmol/L</td>
</tr>
<tr>
<td>Sodium/potassium ratio</td>
<td>19.4–28.8</td>
<td>–</td>
</tr>
<tr>
<td>Chloride</td>
<td>99.5–112.3</td>
<td>mmol/L</td>
</tr>
<tr>
<td>Iron</td>
<td>9–54</td>
<td>μmol/L</td>
</tr>
</tbody>
</table>

Source: Adapted from Klem et al. (2010).

Table 14.2. Porcine hematological reference intervals

<table>
<thead>
<tr>
<th>Hematology Analyte</th>
<th>Reference Interval</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red blood cells (RBCs)</td>
<td>6.4–8.4</td>
<td>×10¹²/L</td>
</tr>
<tr>
<td>Hemoglobin concentration (HGB)</td>
<td>105–135</td>
<td>g/L</td>
</tr>
<tr>
<td>Hematocrit (HCT)</td>
<td>0.34–0.44</td>
<td>L/L</td>
</tr>
<tr>
<td>Mean corpuscular volume (MCV)</td>
<td>49–59</td>
<td>fl</td>
</tr>
<tr>
<td>Mean corpuscular hemoglobin concentration (MCHC)</td>
<td>287–325</td>
<td>g/L</td>
</tr>
<tr>
<td>Erythrocyte distribution width (RDW)</td>
<td>15–24</td>
<td>%</td>
</tr>
<tr>
<td>Platelets</td>
<td>211–887</td>
<td>×10⁹/L</td>
</tr>
<tr>
<td>White blood cells (WBCs)</td>
<td>15.6–38.9</td>
<td>×10⁹/L</td>
</tr>
<tr>
<td>Neutrophils</td>
<td>3.0–17.4</td>
<td>×10⁹/L</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>7.7–20.4</td>
<td>×10⁹/L</td>
</tr>
<tr>
<td>Monocytes</td>
<td>0.6–3.4</td>
<td>×10⁹/L</td>
</tr>
<tr>
<td>Eosinophils</td>
<td>0.1–2.3</td>
<td>×10⁹/L</td>
</tr>
<tr>
<td>Basophils</td>
<td>0.1–0.3</td>
<td>×10⁹/L</td>
</tr>
<tr>
<td>Large unstained cells (LUCs)</td>
<td>0.1–1.4</td>
<td>×10⁹/L</td>
</tr>
</tbody>
</table>

Source: Adapted from Klem et al. (2010).

it is located in the mediastinum and extends from the second to fifth ribs. The heart is a two-pump system composed of four chambers: the right atrium, right ventricle, left atrium, and left ventricle. The right and left atria collect blood from the systemic and pulmonary venous systems, respectively. The right ventricle pumps deoxygenated blood to the lungs where it becomes oxygenated, and the left ventricle ejects highly pressurized blood to the arterial system. A series of heart valves work as one-way regulators to restrict the systolic backflow of blood from highly pressurized regions into the preceding chamber. Arteries and arterioles carry blood to the peripheral tissues where oxygen exchange occurs at the capillary level. Deoxygenated blood is then returned to the heart by a series of venules and veins. Fluid that escapes from the blood is returned to the vascular system by lymphatic vessels.

The pericardium is a fibroserous sac that surrounds the heart. It acts to reduce motion and inhibit cardiac overexpansion. One to 2 mL of clear serous fluid is located within the pericardium to reduce friction with the epicardium. The epicardium is a continuation of the pericardium and forms the outer fibrous layer of the heart. The muscular myocardium is located subjacent to the epicardium and is composed of specialized striated cardiac muscle, which generates the force necessary to pump blood. The endocardium lines the inner layer of the heart and valves, and is composed of vascular endothelium and supporting collagen, elastin, and small caliber blood vessels. Specialized electrical conduction fibers are distributed throughout the subepicardium, myocardium, and subendocardium. Conduction fibers coordinate the rhythmic depolarization of the cardiomyocytes, which results in muscular contraction. Cardiac electrical impulse is influenced by the autonomic nervous system and is generated by the pacemaker sinoatrial (SA) node. The SA node chronologically and subsequently transmits electrical impulse to the atroioventricular (AV) node, bundle of His, right and left bundle branches, and Purkinje fibers, which ultimately stimulate cardiomyocytes and result in the synchronized contraction of the heart.

**POSTMORTEM EXAMINATION OF THE HEART**

The heart should be visualized in a consistent and methodical manner so that the pericardium, myocardium, and valvular endocardium, and great vessels are examined. All lesions that are identified should be grossly described and placed in fixative for microscopic examination by a pathologist.

Following death, the thoracic cavity should be carefully opened so that the lungs and heart can be visualized. The pericardial sac should be examined and incised in situ before any organs are removed. Normally, the epicardium and pericardium should glisten due to the presence of a small amount of serous fluid. The pericardium should be observed for thickening, adhesions, presence of fibrin, and the volume and nature of the pericardial fluid. Swabs, fluid samples, and tissue samples should be taken if abnormalities are evident.
The heart should be removed from the pluck and examined for abnormal shape, size, and color. Rigor mortis occurs early in the heart, in comparison to skeletal muscle, and may give the heart a very firm rigid appearance. Conversely, the heart may appear dilated and flabby. Chambers may dilate antemortem due to myocardial disease or secondarily to postmortem autolysis. Evaluation of the endocardium can help determine antemortem from postmortem dilation. Heart chambers that dilate prior to death frequently have a thickened and fibrotic mural endocardium. Histopathology should be performed on these sections to definitively rule out underlying myocardial disease.

A common method used to ensure consistency when examining the heart is to view myocardial structures in the direction of normal blood flow. The right atrium should be opened from the caudal vena cava and followed along the right atrial wall, through the tricuspid valve, down along the septum to the apex of the right ventricle, and up along the septum of the attached free wall to the pulmonic valve and outflow tract. The right side of the left atrium should be vertically incised and the incision extended to the apex of the left ventricle. The aortic valve and outflow tract can be visualized after the mitral valve is incised. Clotted blood should be removed; the heart rinsed in water; and the endocardium observed for thickened regions, pallor, hemorrhage, and valvular changes. Incision through the ventricular free walls, atria, and septum can aid in visualizing myocardial lesions that do not extend to the epicardium or endocardium. Sampling of the papillary muscles, which are located at the base of the chordae tendineae, for histopathology is recommended as these muscles are highly active and susceptible to myocardial damage.

### CARDIAC PATHOLOGY

The heart functions to supply adequate amounts of blood to the peripheral organs. Cardiac output is the amount of blood expelled by the heart per unit time and is negatively influenced by conduction abnormalities; heart malformations; disease in noncardiovascular organs; and epicardial, myocardial, or endocardial lesions. The speed at which heart disease develops is dependent on the underlying etiology and location of the abnormality or diseased tissue.

### Congenital Anomalies

Porcine congenital cardiovascular malformations have been reported to occur at an incidence of 0.49–14.6% (Salsbury 1970; Wang 1978) in conventionally raised swine. Malformations may have minimal pathological affects or result in fatal cardiovascular compromise.

Hsu and Du (1982) identified 122 cardiac anomalies in a study of 1906 crossbred and purebred pigs. This study identified anomalies in pigs ranging from 1 day to 4 years of age, but found the highest incidence of malformations in animals 29–110 days old. Both single and multiple anomalies were frequently identified within individual pigs. Common porcine cardiovascular anomalies are listed in Table 14.3.

### Diseases of the Pericardium

Hemorrhage, transudate, modified transudate, or exudate can accumulate within the pericardium of swine. Increased pericardial fluid is capable of applying external pressure to the heart resulting in decreased diastolic filling of the chambers and venous congestion. Congestive heart failure may ensue if the material is not removed.

#### Hemopericardium

Hemopericardium is the expansion of the pericardial cavity with blood. This condition commonly develops from traumatic insult to the epicardium or major blood vessels within the pericardium. Idiopathic rupture of an atrium, coronary artery, or the aorta is occasionally diagnosed in swine. Similar cardiovascular ruptures have been reproduced in experimental models of porcine copper deficiency (Shields et al. 1962). These findings may suggest an underlying role for copper deficiency in the development of idiopathic ruptures.

#### Hydropericardium

Expansion of the pericardium with clear to yellowish transudate or modified transudate is termed hydropericardium. This condition most commonly develops due to nonspecific insult to the vascular endothelium with subsequent fluid and fibrin loss into the pericardial cavity. Hydropericardium, in swine, has been associated with edema disease, mulberry heart disease (MHD), cachexia, hypoalbuminemia, and congestive heart failure. Wispy fibrin strands in pericardial fluid can be from modified transudate and should not be overinterpreted as fibrinous pericarditis.
Table 14.4. Infectious etiologies of inflammatory heart disease

<table>
<thead>
<tr>
<th>Lesion</th>
<th>Reference Chapter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endocarditis</td>
<td></td>
</tr>
<tr>
<td>Bacterial</td>
<td></td>
</tr>
<tr>
<td>Arcanobacterium pyogenes</td>
<td>64</td>
</tr>
<tr>
<td>Erysipelothrix rhusiopathiae</td>
<td>54</td>
</tr>
<tr>
<td>Streptococcus sp.</td>
<td>62</td>
</tr>
<tr>
<td>Viral</td>
<td></td>
</tr>
<tr>
<td>Encephalomyocarditis virus</td>
<td>42</td>
</tr>
<tr>
<td>Foot-and-mouth disease virus</td>
<td>42</td>
</tr>
<tr>
<td>Porcine circovirus 2</td>
<td>26</td>
</tr>
<tr>
<td>Porcine reproductive and respiratory syndrome virus</td>
<td>31</td>
</tr>
<tr>
<td>Pseudorabies virus (porcine herpesvirus 1)</td>
<td>28</td>
</tr>
<tr>
<td>Swine vesicular disease virus</td>
<td>42</td>
</tr>
<tr>
<td>Myocarditis</td>
<td></td>
</tr>
<tr>
<td>Bacterial</td>
<td></td>
</tr>
<tr>
<td>Bacteria associated with septicemia</td>
<td>62</td>
</tr>
<tr>
<td>Streptococcus sp.</td>
<td>62</td>
</tr>
<tr>
<td>Viral</td>
<td></td>
</tr>
<tr>
<td>Encephalomyocarditis virus</td>
<td>42</td>
</tr>
<tr>
<td>Foot-and-mouth disease virus</td>
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<tr>
<td>Porcine circovirus 2</td>
<td>26</td>
</tr>
<tr>
<td>Porcine respiratory and reproductive syndrome virus</td>
<td>31</td>
</tr>
<tr>
<td>Pseudorabies virus (porcine herpesvirus 1)</td>
<td>28</td>
</tr>
<tr>
<td>Swine vesicular disease virus</td>
<td>42</td>
</tr>
<tr>
<td>Pericarditis and epicarditis</td>
<td></td>
</tr>
<tr>
<td>Bacterial</td>
<td></td>
</tr>
<tr>
<td>Actinobacillus sp.</td>
<td>48</td>
</tr>
<tr>
<td>Haemophilus parasuis</td>
<td>55</td>
</tr>
<tr>
<td>Mycoplasma sp.</td>
<td>57</td>
</tr>
<tr>
<td>Mannheimia hemolytica</td>
<td>64</td>
</tr>
<tr>
<td>Pasteurella multocida</td>
<td>58</td>
</tr>
<tr>
<td>Salmonella sp.</td>
<td>60</td>
</tr>
<tr>
<td>Staphylococcus sp.</td>
<td>61</td>
</tr>
<tr>
<td>Streptococcus sp.</td>
<td>62</td>
</tr>
</tbody>
</table>

Pericarditis. Pericarditis is the expansion of the pericardial cavity with inflammatory exudate and is one of the most common causes of pericardial disease in swine. Exudate can be fibrinous, purulent, or fibrinopurulent based on the amount of fibrin, number of inflammatory cells, and presence or absence of pyogenic bacteria. Pericarditis can arise from hematogenous dissemination of bacteria, or lymphatic extension of inflammation from adjacent tissues such as the lungs or pleura. Grossly, the pericardium is typically expanded with fibrin that gives the pericardial surface a roughened “shaggy” appearance. Granulation tissue will replace the fibrin if mesothelial damage is sufficient or the exudate is not promptly removed. Chronic constrictive pericarditis can lead to cardiac dysfunction and result in congestive heart failure. Causes of porcine epicarditis and pericarditis can be found in Table 14.4.

Diseases of the Myocardium

Myocarditis. Inflammation of the myocardium can develop from hematogenous distribution of pathogens or extension of inflammatory lesions from the adjacent pericardium or endocardium. Porcine myocarditis is frequently induced by a number of bacterial and viral etiologies (Table 14.4). Although less common, parasitic stages of Toxoplasma gondii, Trichinella spiralis, Taenia solium, and Taenia saginata may infest the myocardium and produce nodular or cystic lesions. Inflammation within the myocardium, regardless of cause, can alter cardiovascular function by inducing myocardial degeneration and necrosis, altering electrical conduction, and disrupting myocardial contraction. These factors can result in acute death associated with dysrhythmia or contribute to chronic heart failure.

Myocardial Degeneration and Necrosis. Porcine myocardial degeneration and necrosis can develop from a number of primary insults to the heart or secondarily from systemic disease. Primary causes in swine can include injectable iron toxicosis, ionophore and gossypol toxicosis, and nutritional cardiomyopathy. Myocardial necrosis may also develop secondarily from hypertrophic or dilatative cardiomyopathy, fever, anemia, disseminated intravascular coagulation (DIC), toxemia, neurological lesions (brain–heart syndrome), porcine stress syndrome, systemic infections, or myocardial inflammation. The wide array of potential causes of myocardial degeneration and necrosis frequently make identification of a definitive etiology difficult.

Mulberry Heart Disease. MHD is one of multiple diseases historically associated with vitamin E and selenium deficiency in swine. Affected animals are primarily found dead with hemorrhagic foci within the walls of the heart. The term MHD was coined based on the distinct transmural hemorrhage that appropriately resembles a mulberry.

MHD occurs sporadically in young rapidly growing swine. Disease incidence is generally low, although epidemics have been reported (Moir and Masters 1979). Clinical signs are uncommon as the disease primarily manifests as sudden death in well-conditioned animals. On rare occasion, affected animals may develop weakness, cyanosis, slight icterus, subcutaneous edema, tachycardia, and increased serum muscle enzymes (Gudmundson 1976). Death is thought to be associated with dysrhythmia that develops secondary to myocardial damage. Diagnosis of MHD must be confirmed by necropsy.

To date, the definitive pathophysiological mechanism behind MHD development remains elusive. Current theory suggests that disease development is associated with the lack of balance between free radical development and scavenging, that is, uncompensated oxidative metabolic stress. Free radicals are highly reactive molecules generated during normal oxidative metabolism. Cells ordinarily neutralize free radicals with antioxidant scavengers such as superoxide dismutase, glutathione peroxidase, and vitamins E and C. Selenium is historically included in this list due to its essential involvement in glutathione peroxidase activ-
ity. Unscavenged free radicals are capable of inducing cellular damage by reacting with cellular proteins, membrane lipids, and nucleic acids. Deficiency of free radical scavengers can result in severe cellular injury and death.

Traditionally, vitamin E and/or selenium deficiency were thought to be essential components of disease development. This theory was initially developed based on the experimental reproduction of MHD in swine deficient of one or both of these constituents (Grant 1961). This hypothesis has somewhat fallen from vogue due to inconsistencies in identifying deficient levels of vitamin E, selenium, or glutathione peroxidase activity in animals with MHD (Nielsen et al. 1989; Pallarés et al. 2002; Rice and Kennedy 1989). This has led many to believe that MHD is likely multifactorial and not solely related to vitamin E or selenium deficiency alone. Current hypotheses suggest that animals that die of MHD lack a sufficient balance between free radicals and free radical scavengers, which predispose animals to oxidative damage. Predisposing factors perhaps associated with increased oxidative damage include stress; increased iron tissue concentrations (Korpela 1990); increased calcium and decreased magnesium concentrations (Korpela 1991); and diets containing corn oil (Nolan et al. 1995), polyunsaturated fatty acids, aflatoxins, excess vitamin A, or dried distiller grains. It has also been hypothesized that individual animals may be genetically predisposed to free radical damage, have an altered vitamin E metabolism, or may have decreased vitamin E bioavailability.

Gross pathological findings typically include hydropericardium, pulmonary edema, and transmural cardiac hemorrhage. More specifically, the pericardium is often distended by a large amount of cloudy to straw-colored fluid that contains fibrin strands. Multifocal to coalescing ecchymotic hemorrhages are evident in the epicardium, myocardium, and occasionally extend to the endocardium. Regions of myocardial necrosis may or may not be grossly evident.

Histological hallmarks of MHD include interstitial hemorrhage, subserosal edema, and variable amounts of myofiber degeneration, necrosis, and mineralization. Histological lesions can vary based on chronicity of the lesions. In cases of acute death, hemorrhagic lesions may predominate, and degenerative and necrotic lesions may be minimal or absent. Hemorrhage with myocardial degeneration, necrosis, and mineralization is typically present in subacute cases. Microscopic lesions consistent with dietary microangiopathy may also be found in arterioles and capillaries of the heart, kidneys, liver, stomach, intestine, mesentery, skeletal muscle, and skin. These systemic vascular lesions can vary in severity from nonspecific endothelial hypertrophy, capillary microthrombosis, or fibrinoid necrosis. Animals that die of MHD may also have lesions consistent with centrilobular hepatocellular necrosis (hepato-

A diagnosis of MHD is typically made based on the presence of gross and microscopic lesions. Deficient tissue concentrations of vitamin E and/or selenium can support a diagnosis but are not always evident.

Treatment of affected animals is usually not plausible due to the lack of distinct clinical signs and rapidity of death. Van Vleet et al. (1973) suggested that parenteral injection of all pigs aged 1–4 months with commercially available selenium–vitamin E products may be beneficial in herds experiencing increased death losses due to MHD. Rations may also be supplemented with vitamin E and selenium, and evaluated for increased levels of polyunsaturated fatty acids. Suplemental vitamin E should be favored over supplemental selenium since the latter is more associated with hepatitis dietetica and can cause toxicity at relatively low doses.

Diseases of the Endocardium

A majority of endocardial lesions in swine involve the heart valves. Disruption of valvular function can negatively impact hemodynamics, decrease cardiac efficiency, and result in cardiac hypertrophy and/or chamber dilatation. Sequelae of valvular disease include chordae tendineae rupture, endocardial mural fibrosis, and thromboembolism.

Valvular endocarditis is a commonly acquired lesion of the porcine endocardium. Bacterial etiologies are often associated with these lesions (Table 14.4) with Streptococcus suis probably the most common and occasional epidemics with Erysipelothrix as well. Fungal and parasitic causes are rarely diagnosed. The mitral valve is most commonly affected followed in frequency by the aortic, tricuspid, and pulmonic valves. Regardless of the affected valve, postmortem findings typically consist of one or more irregular friable reddish-gray to yellow nodules that extend from the valve leaflets and may involve the adjacent mural endocardium. This lesion is typically referred to as “vegetative valvular endocarditis” based on the floret-like appearance of the inflammatory mass. Microscopically, acute lesions are composed of ulcerated valvular endothelium covered by concentric layers of fibrin, bacterial microcolonies, and variable numbers of inflammatory cells. Granulation tissue may cover the valves in chronic lesions. Valvular dysfunction can be auscultated as murmurs, and affected animals may die from heart failure. Tissue infarction can also result when vegetative nodules break free from the endocardium and form thromboemboli.

Valvular cysts can be found in swine and usually contain either blood (hemoctyst) or yellow serous fluid (serous cysts). Both types may be congenital or acquired, and have little clinical significance.
Diseases of the Conduction System

Diseases of the conduction system are sparsely reported in conventional swine. Abnormalities may result in myocardial dysrhythmia, which can alter the systemic contraction of the heart, disrupt circulatory hemodynamics, and result in acute death. A majority of conduction system diseases arise secondary to cardiac, central nervous system (CNS), or pulmonary disease; drugs (e.g., anesthetic agents); or systemic alterations such as hypo- and hyperthermia, anemia, shock, sepsis, excitement, and electrolyte imbalances (e.g., hyperkalemia, hypocalcemia). These diseases may induce ectopic impulses that result in premature complexes, tachycardia, flutter, and fibrillation, or result in conduction alterations such as nodal arrest, conduction blocks, and pre-excitation. Electrocardiography is required for the diagnosis of conduction system diseases, which may explain the lack of epidemiological data associated with these entities in swine.

Compensatory Mechanisms

The heart is incapable of generating new cardiac muscle to cope with increased workloads or repair damaged myofibers. Consequently, the cardiovascular system utilizes compensatory mechanisms to temporarily increase cardiac output and meet systemic metabolic demands. Cardiac dilation, myocardial hypertrophy, and tachycardia are compensatory changes of the heart that increase cardiac output. Neurohormonal mechanisms also modify the vascular system by increasing vascular resistance, promoting vasoconstriction, and increasing the systemic blood volume.

Heart Failure

Congestive heart failure ensues when the heart can no longer compensate to meet systemic metabolic needs. Mechanistically, heart failure can be characterized as a decrease in cardiac output and/or decrease in venous return of the blood. Heart failure may result from impaired myocardial function (cardiomyopathies, decreased myofiber contractility, lack of distensibility, and dyssrhythmia) or an increase in cardiac demand (cor pulmonale, pressure overload, and volume overload). Heart failure may be characterized as left or right sided dependent on the location of diseased tissue and underlying mechanism. Left-sided heart failure results in stasis of blood and fluid in the lungs. This pathologically manifests as pulmonary congestion and edema, and can become clinically evident as dyspnea and tussis. Pathological findings associated with right-sided heart failure include systemic congestion, edema, and tissue hypoxia.

VASCULAR PATHOLOGY

Vascular pathology can arise from a magnitude of different etiologies and result in abnormal hemodynamics, fluid loss from the vascular compartment, hemolytic anemia, and tissue ischemia.

Vascular Rupture and Aneurism

Iatrogenic laceration of large blood vessels is a common side effect associated with blood sampling in swine. Lacerations, as well as other causes of vascular rupture, can lead to hypovolemic shock and death.

Copper is an essential element utilized by lysyl oxidase to cross-link collagen and elastin in blood vessel walls. Copper-deficient swine are predisposed to arterial aneurisms due to the lack of sufficient vascular integrity (Coulson and Carnes 1963). Affected vessels may bulge, dilate, and rupture.

Vascular Degeneration and Necrosis

Degeneration. Atherosclerosis naturally occurs in aged pigs fed high-cholesterol diets. It develops slowly over time and commonly affects larger arteries. The disease is characterized by the plaque-like narrowing of vascular lumens, which alter hemodynamics, predispose to thrombus formation, and can result in vascular occlusion and tissue infarction. Microscopically, vessel walls are expanded by lipid deposited in smooth muscle cells, aggregates of lipid-laden macrophages, and fibrous connective tissue. Due to similarities in the mechanism and development of human atherosclerosis, the pig has become a useful model to study the disease in man.

Vascular mineralization due to vitamin D poisoning is occasionally observed in swine and usually associated with accidental feed overdoses. Vitamin D toxicosis results in hypercalcemia and/or hyperphosphatemia. Increased serum concentrations of calcium or phosphate predispose to vascular and soft tissue mineralization, which hinders vascular elasticity and organ function.

Necrosis. Fibrinoid necrosis develops secondary to endothelial damage with subsequent fibrin and serum protein deposition into vessel walls. Edema disease (Chapter 53), mercury toxicosis (Chapter 70), and dietary microangiopathy are three diseases associated with fibrinoid necrosis in swine.

Toxic ergot alkaloids (Chapter 69), produced by Claviceps purpurea, can also produce endothelial necrosis and vascular thrombosis by stimulating adrenergic nerves in the vascular smooth muscle. Stimulation results in marked vasoconstriction, endothelial damage, and thrombosis, which may provoke infarction of limbs and extremities.

Vascular Thrombosis

Thrombosis is characterized by intravascular coagulation and can be induced by endothelial damage, hypercoagulable disorders, and abnormal blood flow. If severe, it can completely occlude vascular lumens and result in tissue infarction. Arteritis, regardless of the
reduces intravascular colloidal osmotic pressure and leads to increased fluid transudation into the interstitium. Causes of hypoproteinemia can include liver disease, malnutrition, and protein-losing enteropathies and nephropathies.

Interstitial fluid is normally removed by regional lymphatic vessels. Lymphatic blockage by thrombosis, compression, or inflammation can reduce the effective drainage of the interstitium and result in localized edema.

Diseases of the Body Cavities
The pericardial, peritoneal, and pleural cavities normally contain small amounts of serous fluid. Normal cavity fluid should be colorless to yellow tinged, translucent to slightly turbid, and contain less than 2.5 g/dL of protein and 5000 nucleated cells/µL (Rakich and Latimer 2003).

Characterization of cavity fluids can aid in the identification of ongoing disease processes. An increase in fluid volume and turbidity is abnormal, and may result from increased cellularity, increased protein and lipid concentrations, fibrin, bacterial colonization, or ruptured organs. Abnormal fluid can be classified as transudate, modified transudate, or exudate. Characteristics of these fluids are presented in Table 14.6. Transudates commonly develop from decreased plasma osmotic pressure associated with hypoalbuminemia. Modified transudates are less diagnostic and can be associated with increased hydrostatic pressure, increased vascular permeability, or secondarily result from heart or liver disease. Exudates commonly result from increased vascular permeability associated with inflammatory processes.

Shock
Shock is a rapidly progressive disturbance of hemodynamics and cellular metabolism. It can be characterized as hypovolemic, cardiogenic, or vasogenic based on the underlying mechanism. Despite the mechanism, it concludes with vascular hypotension, tissue hypoperfusion, cellular hypoxia, and acidosis, and may lead to death.

Hypovolemic shock develops from a significant decrease in blood volume, which results in decreased

<table>
<thead>
<tr>
<th>Classification</th>
<th>Reference Chapter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacterial</td>
<td></td>
</tr>
<tr>
<td><em>Actinobacillus</em> sp.</td>
<td>48</td>
</tr>
<tr>
<td><em>Erysipelothrix rhusiopathiae</em></td>
<td>54</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>53</td>
</tr>
<tr>
<td><em>Haemophilus parasuis</em></td>
<td>55</td>
</tr>
<tr>
<td><em>Leptospira interrogans</em></td>
<td>56</td>
</tr>
<tr>
<td><em>Salmonella sp.</em></td>
<td>60</td>
</tr>
<tr>
<td><em>Streptococcus suis</em></td>
<td>62</td>
</tr>
<tr>
<td>Viral</td>
<td></td>
</tr>
<tr>
<td><em>African swine fever virus</em></td>
<td>25</td>
</tr>
<tr>
<td><em>Classical swine fever virus</em></td>
<td>38</td>
</tr>
<tr>
<td><em>Ovine herpesvirus 2</em></td>
<td>28</td>
</tr>
<tr>
<td><em>Porcine circovirus 2</em></td>
<td>26</td>
</tr>
<tr>
<td><em>Porcine reproductive and respiratory syndrome virus</em></td>
<td>31</td>
</tr>
</tbody>
</table>

**Table 14.6.** Body fluid classifications and parameters

<table>
<thead>
<tr>
<th>Fluid Type</th>
<th>Transudate</th>
<th>Modified Transudate</th>
<th>Exudate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cellularity (cells/µL)</td>
<td>&lt;1500</td>
<td>1000–7000</td>
<td>&gt;5000</td>
</tr>
<tr>
<td>Color</td>
<td>Colorless</td>
<td>Variable</td>
<td>Yellow to tan</td>
</tr>
<tr>
<td>Protein (g/dL)</td>
<td>&lt;2.5</td>
<td>2.5–5.0</td>
<td>&gt;2.5</td>
</tr>
<tr>
<td>Turbidity</td>
<td>Clear</td>
<td>Variable</td>
<td>Cloudy to opaque</td>
</tr>
</tbody>
</table>

**Table 14.5.** Infectious causes of porcine vasculitis

<table>
<thead>
<tr>
<th>Classification</th>
<th>Reference Chapter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacterial</td>
<td></td>
</tr>
<tr>
<td><em>Actinobacillus</em> sp.</td>
<td>48</td>
</tr>
<tr>
<td><em>Erysipelothrix rhusiopathiae</em></td>
<td>54</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>53</td>
</tr>
<tr>
<td><em>Haemophilus parasuis</em></td>
<td>55</td>
</tr>
<tr>
<td><em>Leptospira interrogans</em></td>
<td>56</td>
</tr>
<tr>
<td><em>Salmonella sp.</em></td>
<td>60</td>
</tr>
<tr>
<td><em>Streptococcus suis</em></td>
<td>62</td>
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<tr>
<td>Viral</td>
<td></td>
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<tr>
<td><em>African swine fever virus</em></td>
<td>25</td>
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<tr>
<td><em>Classical swine fever virus</em></td>
<td>38</td>
</tr>
<tr>
<td><em>Ovine herpesvirus 2</em></td>
<td>28</td>
</tr>
<tr>
<td><em>Porcine circovirus 2</em></td>
<td>26</td>
</tr>
<tr>
<td><em>Porcine reproductive and respiratory syndrome virus</em></td>
<td>31</td>
</tr>
</tbody>
</table>
vascular pressure. Blood volume can be decreased by the direct loss of whole blood (i.e., hemorrhage) or fluid components (i.e., dehydration) from the vascular system.

Cardiogenic shock is characterized by the heart’s inability to efficiently pump blood. Lesions within the endocardium, myocardium, or pericardium may hinder efficiency and result in shock by decreasing stroke volume and cardiac output.

Vasogenic shock arises from peripheral vasodilation. Inadequate vascular tone leads to pooling of blood, reduction of circulating blood, and tissue hypoperfusion. Vasogenic shock is commonly identified in pigs and can result from trauma, stress, anaphylaxis, sepsis, endotoxemia, or insult to the CNS.

**DISEASES OF THE HEMATOPOIETIC SYSTEM**

**Anemia**

Anemia is defined as a decrease in the hematocrit level, hemoglobin concentration, or RBC mass. A number of classification systems exist that categorize anemia based on physical properties (erythrocyte size and hemoglobin concentration), bone marrow response (regenerative vs. nonregenerative), or the underlying pathological processes (defective erythropoiesis, hemorrhage, and hemolysis). Clinical signs may be variable dependent on the severity of the anemia and the underlying mechanism. Anemia can clinically manifest as mucus membrane pallor, weakness, tachycardia, and/or tachypnea. Potential causes of porcine anemia are listed in Table 14.7.

**Defective Erythropoiesis.** Defective erythropoiesis is an important cause of porcine anemia and can develop due to nutritional deficiencies, chronic disease, or infectious agents. Suckling piglets are particularly susceptible to iron-deficiency anemia (IDA) due to rapid growth, a low iron storage capacity, and a lack of sufficient dietary intake. Piglets require approximately 7 mg of iron daily, and only obtain half of that requirement through milk. Modern indoor husbandry practices have reduced the amount of iron naturally obtained by piglets from the soil and have increased the need for neonatal iron supplementation. Affected piglets classically appear normal at birth but become unthrifty and pale, and develop edema of the lungs, muscles, and connective tissues at approximately 1–3 weeks of age. IDA is characterized by erythrocyte microcytosis and hypochromasia. Bone marrow may be dark red and hyperplastic, and exhibit an erythroid shift, but may become hypoplastic with chronic deficiency.

Copper promotes iron absorption and is essential for the incorporation of iron into hemoglobin (Lee et al. 1968). Copper deficiency thus can result in a lack of iron availability and predispose to IDA.

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**Table 14.7. Causes of porcine anemia**

<table>
<thead>
<tr>
<th>Classification</th>
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</thead>
<tbody>
<tr>
<td>Defective erythropoiesis</td>
</tr>
<tr>
<td>Anemia of chronic disease</td>
</tr>
<tr>
<td>Nutritional deficiencies</td>
</tr>
<tr>
<td>Iron</td>
</tr>
<tr>
<td>Copper</td>
</tr>
<tr>
<td>Viral infections</td>
</tr>
<tr>
<td>Porcine reproductive and respiratory syndrome virus</td>
</tr>
<tr>
<td>Hemolytic</td>
</tr>
<tr>
<td>Autoimmune</td>
</tr>
<tr>
<td>Neonatal isoerythrolysis</td>
</tr>
<tr>
<td>Erythrocyte fragmentation</td>
</tr>
<tr>
<td>Disseminated intravascular coagulation</td>
</tr>
<tr>
<td>Vasculitis</td>
</tr>
<tr>
<td>Erythrocyte parasitism</td>
</tr>
<tr>
<td>Mycoplasma suis (Eperythrozoon suis)</td>
</tr>
<tr>
<td>Hemorrhagic</td>
</tr>
<tr>
<td>Enterocolitis</td>
</tr>
<tr>
<td>Proliferative enteritis</td>
</tr>
<tr>
<td>Salmonellosis</td>
</tr>
<tr>
<td>Swine dysentery</td>
</tr>
<tr>
<td>Gastric ulceration</td>
</tr>
<tr>
<td>Hemorrhagic bowel syndrome</td>
</tr>
<tr>
<td>Parasitism</td>
</tr>
<tr>
<td>External</td>
</tr>
<tr>
<td>Lice (Haematopinus suis)</td>
</tr>
<tr>
<td>Internal</td>
</tr>
<tr>
<td>Thorny-headed worms (Macracanthorhynchus hirudinaceus)</td>
</tr>
<tr>
<td>Threadworms (Strongyloides ransomi)</td>
</tr>
<tr>
<td>Whipworms (Trichuris suis)</td>
</tr>
<tr>
<td>Navel bleeding</td>
</tr>
<tr>
<td>Thrombocytopenia</td>
</tr>
</tbody>
</table>

Anemia of chronic disease (ACD) can occur with multiple infectious, inflammatory, or neoplastic processes. Mechanistically, ACD develops secondarily to the impact of inflammatory cytokines on iron availability, erythropoietin production, and the bone marrow’s response to erythropoietin. Erythrocytes are typically normocytic and normochromic, but microcytosis and hypochromia may be evident. The primary disease process commonly dominates the clinical assessment, and anemia typically resolves once the primary disease has been alleviated.

Certain viruses also play a direct role in the development of anemia. Porcine reproductive and respiratory syndrome virus can experimentally induce anemia in pigs (Halbur et al. 2002). The exact mechanism of anemia is not known, but the virus has been postulated to directly or indirectly disrupt erythropoiesis.

**Hemolytic Anemia.** Hemolytic anemia (HA) develops from the premature destruction of erythrocytes. In swine, it has been associated with immune-mediated mechanisms, erythrocyte parasitism, and erythrocyte fragmentation. Clinically affected animals may develop icterus and hemoglobinuria.

Neonatal isoerythrolysis is an immune-mediated disease of pigs and other animals. HA develops through
the passive acquirement of maternal antibodies that have been primed against fetal erythrocytes. Once introduced into the neonatal circulatory system, maternal antibodies bind to piglet erythrocytes and activate the complement cascade. Complement lyases erythrocytes and releases hemoglobin into the plasma.

*Mycoplasma suis*, Chapter 57, is a hemotropic Mycoplasma sp. capable of attaching to and infecting porcine erythrocytes. Extravascular hemolysis develops secondary to the removal of infected erythrocytes from the circulatory system.

Microangiopathic anemia results from the removal of injured erythrocytes from the circulatory system. Erythrocyte membranes may become fragmented by fibrin when passing through damaged blood vessels. Vasculitis, disseminated intravascular coagulation, and vascular neoplasia are potential causes of microangiopathic anemia in swine.

**Hemorrhagic Anemia.** Hemorrhagic anemia (HeA) frequently develops secondarily to an acute or chronic blood-loss event. External evidence of hemorrhage is usually evident in cases of trauma or umbilical hemorrhage, but may be externally inconspicuous in cases of gastric ulceration, hemorrhagic bowel syndrome, enterocolitis, external or internal parasitism, and thrombocytopenia. Clinical signs may or may not be present based on the amount of blood loss, chronicity of disease, and distribution of lesions. A regenerative response is evident within days of blood loss, but may become nonregenerative in chronic cases due to iron deficiency.

**Hemoglobin Disorders**

Pathological alterations of hemoglobin reduce the erythrocyte’s ability to carry oxygen to the tissues. Carboxyhemoglobinemia and methemoglobinemia have been recognized as hemoglobin disorders in swine. Both conditions typically result in cyanosis and anoxia.

Carbon monoxide poisoning is a potentially fatal disease that manifests in pigs housed indoors with defective heaters and improper ventilation. Carbon monoxide is a colorless, odorless, and tasteless gas generated from the partial or incomplete oxidation of carbon-based fuels. Carbon monoxide has a higher binding affinity to hemoglobin, in comparison to oxygen, and blocks the oxygen-carrying capacity of hemoglobin. Affected animals have bright cherry red blood as a result of the formation of carboxyhemoglobin. Animals that do not die can develop CNS disorders as a result of anoxia.

Swine exposed to excessive amounts of oxidizing agents, such as nitrate and nitrite, are at increased risk of developing methemoglobinemia. Normally, the iron component of hemoglobin must be in the reduced ferrous state to effectively bind to oxygen and form oxyhemoglobin, which transports oxygen to the tissues.

Methemoglobinemia develops when ferrous hemoglobin is oxidized to methemoglobin and can no longer transport oxygen. Blood of affected animals classically turns a distinctive dark chocolate brown color.

**Coagulation Disorders**

Bleeding disorders are fairly uncommon in swine but can develop due to maternal isoimmunization (thrombocytopenic purpura) (Nordstoga 1965) or consumption of anticoagulant compounds such as warfarin. Pathological lesions are restricted to multiorgan hemorrhage. Diagnosis is by identifying sow-derived isoantibodies to piglet platelets or the presence of anticoagulants in body tissues.

**CARDIOVASCULAR AND HEMATOPOIETIC NEOPLASIA**

Naturally occurring neoplasia is infrequently diagnosed in swine due to the short life span of production animals. Porcine tumors do not frequently cause clinical signs and are typically diagnosed as incidental lesions in diagnostic specimens or in carcasses at slaughter. Tumors that are most frequently identified in the cardiovascular and hematopoietic systems of swine include lymphosarcoma, hemangiosarcoma, hemangioma, and cardiac rhabdomyoma.

Lymphosarcoma is the most commonly diagnosed and economically important neoplasia in swine. It develops from the neoplastic proliferation of T or B lymphocytes and most frequently develops in pigs less than 1 year of age. A number of factors, such as C-type viruses and hereditary genetic components, have been associated with the development of lymphosarcoma. Multicentric, thymic, and leukemic forms have been identified with the former being the most common. Porcine multicentric lymphosarcoma is frequently of B-cell origin and commonly occurs in the spleen, liver, kidney, intestine, and bone marrow. Conversely, thymic lymphosarcoma develops in the cranial mediastinum and is of T-cell origin. Leukemia is rarely identified in swine but can occur in late stages of either thymic or multicentric lymphosarcoma.

Tumors of the porcine cardiovascular system are rare. Over an 11-year period, Fisher and Olander (1978) identified five vascular tumors in tissues and necropsy samples submitted to Purdue University. These tumors were diagnosed as cutaneous and testicular hemangiosarcomas and cutaneous and meningeal hemangiomas, respectively.

Congenital cardiac rhabdomyoma is the only tumor regularly identified in the porcine heart. This tumor consists of non-neoplastic nodular proliferations of dysplastic myocardial fibers (Omar 1969). Affected hearts may have well-demarcated white myocardial nodules that impede into the heart chambers. Most of these tumors are identified as incidental lesions at
necropsy and are not thought to cause significant pathology.

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INTRODUCTION

Diseases of the gastrointestinal tract affect pigs from birth to market and significantly limit the efficiency and profitability of pig production globally. Advances in effective disease control measures, such as development of new vaccines and antimicrobial products, have provided new approaches to disease control. However, issues such as development of antimicrobial resistance, the limitations of the use of antimicrobial growth promoters, and the public expectation of food safety, particularly in relation to food-borne pathogens and food residues will need to be addressed. *Salmonella* surveillance and control continue to be major objectives in countries following the schemes initiated in Scandinavia. These types of issues demand new standards of hygiene and care involving a whole-chain approach to disease control. There is also increasing societal concerns over intensive farming systems, with a trend toward more natural, organic, or welfare-friendly methods of farming. Regulation and legislation have been introduced in some countries and is likely to increase, thus presenting new challenges for pig producers and veterinarians in relation to effective control of enteric diseases. There is a constant threat of new diseases or reemerging diseases, neither of which are uncommon events. Clearly, there will be changes in farming practices that will require alternative approaches to enteric disease prevention, control, or elimination.

In parallel with these practical issues, there has been substantial ongoing research into the enteric physiology, immunology, and nutrition interactions in the pig. This work provides the foundation for future practical advances in enteric disease diagnosis and control, and is briefly reviewed in the following sections. Thereafter, there is an overview of enteric diseases (many of which are covered in depth in other chapters).

ANATOMICAL AND HISTOLOGICAL FEATURES

The conformation and growth characteristics of swine have changed dramatically over the years as the result of genetic selection. However, the gut morphology is apparently unchanged and an area of little focus or attention. The exception is the development of swine genotypes resistant to F18 and F4 (K88) *Escherichia coli* infection where pigs do not possess the required receptor for these organisms to cause disease.

Gut maturation occurs rapidly after birth in response to factors such as oxygenation, enteral nutrient presentation, development of microflora, and hormones such as cortisol and epidermal growth factor. Neonatal hypoxia is associated with intestinal dysfunction and an increased incidence in neonatal enterocolitis (Cohen et al. 1991; Powell et al. 1999). At birth, the sudden increase in blood arterial oxygen is a vital factor in gut development. Common occurrences such as delayed or prolonged parturition and congenital or periparturient pneumonic infections could be initiating factors for neonatal diarrhea. After birth, the small intestine undergoes a rapid growth in response to enteral presentation of nutrients (Burrin et al. 2000). This is stimulated by a wide range of factors such as hormones and growth factors (Sangild 2001). Good intake of milk in the early stages of life is important for rapid gut growth as well as promoting piglet vigor and provision of colostral immunity.

Little attention has been paid to piglet dentition and the effects of tooth eruption on feeding behavior until...
fairly recently (Tucker et al. 2010a). Piglets are born with fully erupted “milk” or “needle” teeth. The majority of deciduous premolars erupt between the first and fifth week of life (Tucker and Widowski 2009) followed by other deciduous teeth that are replaced by permanent teeth over time. The age at which the premolars erupt was found to affect piglets in different ways. Piglets that were less than 17 days of age were inhibited from feeding, presumably due to the associated discomfort. Piglets that were 21 days of age or older were actually attracted to feed, possibly getting comfort from the mechanical effects of feed against the gums (Tucker et al. 2010a). The problems of weaning at very young ages could be compounded by anorexia associated with tooth eruption (Tucker et al. 2010b).

Digestive enzymes are produced primarily by the pancreas and enterocytes. The production of enzymes by the pancreas, such as amylases and lipases, is under nervous and hormonal control and increases substantially during the first 6 weeks after birth (Pluske 2001). During the first 3–4 weeks of life, fetal enterocytes that have high endocytotic activity are gradually replaced by adult-type enterocytes devoid of such activity. The process occurs in a proximal-to-distal direction in the intestine and is an important part of intestinal maturation (Baintner 1986). Changes in enterocyte generation influence the expression of brush border enzymes. Lactase activity is high in the newborn pig and steadily decreases to become minimal after weaning, whereas sucrase and maltase activities are low in the newborn but increase after weaning (Pluske et al. 1997). Intestinal maturation may be hastened in the unweaned pig, for example, by stimulating additional milk intake (Pluske et al. 1996a,b) or feeding kidney bean lectin (Phaseolus vulgaris) (Biertan et al. 2001; Rådberg et al. 2001). This dietary means of promoting rapid gut maturation could be of value in reducing or preventing postweaning diarrhea problems in pigs in the future.

Weaning is associated with adverse effects, such as abrupt withdrawal of sow’s milk, low and variable feed intake, growth stasis, and compromised integrity of the small intestinal epithelium. Changes in gut morphology at weaning include reduced villus height and increased crypt depth (Hampson 1986; Kelly et al. 1991). These adverse effects can be diet dependent and diet independent (Boudry et al. 2004; McCracken et al. 1995). Villus length reduces by 30–40% at days 4–7 postweaning but can be restored by 14 days postweaning (Verdonk et al. 2001a). Additionally, a reduction in the length of microvilli occurs by 3–7 days after weaning (Cera et al. 1988).

There are important impacts of voluntary feed intake and diet composition on mucosal architecture (Makkink et al. 1994; Pluske et al. 1996b). Low feed intakes or a period of starvation postweaning reduces the villus height throughout the small intestine particularly in the proximal jejunum. Moreover, feed ingredients that contain antinutritional factors such as antigenic proteins, protease inhibitors, some types of (harmful) lectins and tannins, or are poorly digestible can induce changes in gut morphology as well and can influence the rate of recovery. To minimize the changes, diets high in milk products, highly digestible ingredients, and low levels of antinutritional factors are utilized.

Raw cereals and legume seeds have a significant adverse effect on gut mucosa, especially in young piglets. Feeding properly heat-processed (e.g., cooking, extrusion, micronizing, pelleting) legume seeds and cereals can improve postweaning growth (Lawlor et al. 2001). The temperature of the feed when presented to the newly weaned pig can also influence feed consumption and growth performance. Significant benefits were recorded when mash was fed at a temperature of 34°C as compared with 14°C (Reiners et al. 2008).

Anorexia at weaning might contribute to local inflammation in the piglet’s small intestine (McCracken et al. 1999). With low nutrient intake, paracellular transport is higher, and this increases over the first 4 days after weaning (Verdonk et al. 2001b). Therefore, it would appear that the integrity of the gut mucosa is worse in piglets with low intake levels postweaning due to higher permeability of the tight junctions between cells (paracellular transport route), which can contribute to the appearance of dietary toxins and endotoxins in the bloodstream.

The dietary changes at weaning and the resultant villus atrophy and crypt hyperplasia leads to decreased digestive and absorptive capacities in the intestine (Pluske et al. 1997; Rådberg et al. 2001). If severe, this can result in a malabsorptive form of diarrhea due to electrolytes and nutrient solutes being retained in the lumen, as well as osmotically associated water. Other factors of importance include poor feed consumption, inflammation in response to bacterial metabolites, rotavirus, and hypersensitivity to antigenic components of the diet (Hampson and Kidder 1986; Kelly 1990; Kenworthy 1976). These physiological changes can result in alterations in the number and balance of the enteric bacterial flora often allowing enteropathogenic bacteria to proliferate, resulting in serious postweaning enteropathies such as colibacillosis. The morphological and functional changes in the small intestine of the newly weaned pig have been reviewed by Pluske (2001).

Transport-associated stress of pigs has been shown to lower the intestinal pH and result in increased intestinal permeability. Permeability is the highest immediately after transportation and decreases after 2–3 hours of rest (van der Meulen et al. 2001). Translocation of bacteria and/or endotoxins from the gut into the systemic circulation is possible due to increased permeability (Berg 1999; Zucker and Krüger 1998). This might explain the increase in disease problems seen after transportation (Berends et al. 1996).
Digestive Physiology

The intestinal mucosa receives nutrients from two sources—the diet (brush border membrane) and the systemic circulation (basolateral membrane). The gut tissues have their own particular nutrient requirements for growth and function. The gastrointestinal tissues in the very young pig utilize up to 50% of the dietary intake of key amino acid such as lysine, glutamine, leucine, and threonine (Burrin et al. 2001). A substantial proportion of amino acid and glucose needs are derived from the arterial circulation rather than from direct dietary sources, while some enteric fermentation products such as butyric and lactic acid serve as nutrients and metabolic regulators of gastrointestinal tissues as well (Burrin et al. 2001). The amino acids are utilized in many ways, such as the formation of secretory mucins (Stoll et al. 1998) and biosynthesis of other amino acids (Stoll et al. 1999), glutathione (Reeds et al. 1997), and nucleic acids (Perez and Reeds 1998).

Nutrient supply for the neonate is derived solely from colostrum and milk. Additionally, colostrum and milk contain a large number of biologically active peptides that have important functions in regulating growth and differentiation of intestinal tissues. Targeted expression of key genes for production of milk peptides holds potential for the future (Kelly and Coutts 1997). On low-protein diets, the amino acid requirements of the gut remain relatively high and are preferentially met, which limits the systemic availability of amino acids for lean tissue growth (Ebner et al. 1994). Exposure to microbial antigens (both pathogenic and nonpathogenic) stimulates a proinflammatory acute-phase response (Johnson 1997; MacRae 1993), which results in the loss of dietary amino acids and reduced availability of amino acids to support growth or reproduction. The use of in-feed antibiotic growth promoters enhances growth rates by limiting this process.

Dietary carbohydrates are comprised of sugars, oligosaccharides, starch, and nonstarch polysaccharides (NSPs), these being vital dietary components in addition to protein and fat. The enzyme activity responsible for carbohydrate degradation adapts according to the age of the pig and dietary composition. In the young pig, there is efficient preecal digestion of lactose during suckling, and sucrose and starch after weaning. Carbohydrates that do not get digested in the small intestine are fermented in the large intestine by a diverse population of anaerobic bacteria. These are mostly NSPs and, in some instances, starch from poorly digestible sources, especially in young piglets.

Increasing the amount of fermentable carbohydrates and straw in the diet increases the total gastrointestinal tract weight by 5% to as much as 25%. With fermentable carbohydrates, the main weight increase occurs in the colon, whereas with straw, it occurs in the stomach and colon (Rijnen et al. 2001).

Most of the starches used in pig diets are highly digestible, with up to 98% of digestion occurring in the small intestine of pigs with mature digestive systems (Bach Knudsen and Canibe 2000; Glitsø et al. 1998). NSPs (fiber) are present in cereals—such as barley, wheat, oats, and rye—and legume seeds—such as soybeans, peas, and fava beans. Although there is some digestion of NSPs in the small intestine, the major site of NSP degradation is in the large intestine. Passage of ingesta through the large intestine generally takes 20–40 hours, allowing time for bacterial degradation. The most common bacterial isolates are Streptococcus sp., Lactobacillus, Fusobacterium, Eubacterium, Bacteroides, and Peptostreptococcus (Moore et al. 1987). Fermentation of carbohydrates and NSPs in the large intestine results in the production of short-chain fatty acids, mainly acetate, propionate, and butyrate, and the gases H2, CO2, and CH4. Increasing the level of NSP entering the large intestine leads to higher activity of the microflora (Bach Knudsen et al. 1991; Jensen and Jørgensen 1994). It also increases the production of short-chain fatty acids (Giusi-Perier et al. 1989) and the production of gases (Jensen and Jørgensen 1994). Short-chain fatty acids are rapidly absorbed from the large intestine and may provide up to 24% of the maintenance energy requirements for growing pigs (Yen et al. 1991) and potentially even more for adults. The total tract digestibility of NSPs is influenced by a number of complex factors, such as the source of NSPs, the level of inclusion in the diet, the solubility, the degree of lignification, the age and weight of the animal, the transit time, and the microbial composition (Bach Knudsen and Jørgensen 2001).

Large volumes of fermentable substrate arriving in the large intestine can cause osmotic overload, with resulting diarrhea. This may result from excessive intake or malabsorption of carbohydrate in the small intestine. The increased volatile fatty acid (VFA) production can overwhelm the colonic buffering capacity causing reduced pH and increased numbers of lactic-acid-producing bacteria. As lactic acid is absorbed at a slower rate than VFAs, further acidification occurs, with the resulting influx of water and solute from tissue into the lumen. Diarrhea results from this process if acidification is excessive. Adult animals are more capable of degrading fiber than growing pigs due to the greater intestinal volume and slower transit times. Adaptation to dietary changes in terms of digestibility of NSP residues is considered to take 3–5 weeks (Longland et al. 1993).

In weaned pigs, the use of enzyme combinations has been found to increase the apparent ileal digestibility of feeds based on hullless barley, which have high beta-glucan concentrations (Yin et al. 2001). Due to the improved apparent ileal digestibility, there was also a reduction in hindgut fermentation. Similarly, the addition of certain enzymes to wheat-based diets of growing
pigs has been found to have beneficial effects (Hazzledine and Partridge 1996). There is increasing interest in the role of dietary factors, especially NSP and feed-processing methods in so-called nonspecific colitis of pigs (Strachan et al. 2002; Thomson et al. 2004). This condition is thought to be an important precursor to other forms of colitis in pigs, although the pathogenesis of this diet-associated form of colitis is poorly understood.

**IMMUNOLOGY**

For the first 24–48 hours of life, the pig intestine is capable of absorbing macromolecules, including immunoglobulins by pinocytosis, providing the neonate with passive immunity from colostrum (Westrom et al. 1984). Although this process commences prepartum, the major absorptive function occurs postnataally (Sangild et al. 1997). This is a specific maturational process that is timed to maximize immunoglobulin uptake shortly after birth. Piglets born prematurely have a lower capacity for protein absorption than piglets born at full term (Sangild et al. 1997); therefore, fetal maturity is an important factor in successful immunoglobulin uptake from the colostrum.

The intestinal immune system of the young pig is very immature, and its slow development may result in increased susceptibility to disease (Stokes et al. 2001). Intestinal lymphoid tissue is present in the form of mesenteric lymph nodes, intestinal Peyer’s patches, and lymphocytes distributed through the mucosal lamina propria and intraepithelial sites. In the jejunum, there are between 11 and 26 discrete Peyer’s patches containing multiple lymphoid follicles (B lymphocytes), separated by T cells. Plasma cells containing immunoglobulin M (IgM), immunoglobulin G (IgG), and immunoglobulin A (IgA) are present in the subepithelial lymphoid dome and between the follicles (Brown and Bourne 1976). The dome region contains dendritic-type cells that express high levels of major histocompatibility complex (MHC) II antigens. Microfold cells (M cells) that are thought to absorb luminal antigens occur in the overlying lymphoepithelium (Gebert et al. 1994).

In the mature pig, the intestinal lamina propria is heavily populated with lymphocytes. Plasma cells and B cells predominate in the crypt area, whereas T cells are found mainly in the villi, CD8+ cells occur in the subepithelial sites, and CD4+ occur in association with capillary plexuses in the lamina propria (Vega-Lopez et al. 1993). The majority of intraepithelial lymphocytes express CD2, but in mature pigs, a high proportion also express CD8 (Stokes et al. 2001). The general immune status of piglets can be influenced by factors such as weaning age and photoperiod allowed for piglets after weaning. Niekamp et al. (2007) found benefits to piglet immunity from weaning at 28 days as compared with 14 or 21 days, and from a daily photoperiod allowance of 16 hours as compared with 8 hours.

Functionally, the intestinal immune mechanisms play a complex role in preventing and controlling harmful intestinal infections while tolerating many dietary antigens and nonharmful antigens from the intestinal flora. The surface epithelium serves as an effective barrier, providing it remains intact. IgA antibodies play an important defensive role. Significant quantities of dietary protein are absorbed across the intestinal mucosa (Telemo et al. 1991; Wilson et al. 1989).

So-called intestinal tolerance to dietary proteins has been demonstrated in the pig, whereby immune responses to the dietary proteins are regulated to prevent inflammatory reaction and tissue damage associated with foreign protein absorption (Bailey et al. 1993). The interaction between the different components of the intestinal immune system is complex, and the basis of inflammation and apoptosis versus downregulation of immune responses is the subject of ongoing research.

Development of the intestinal immune system occurs in response to antigen exposure. Full development of lymphoid tissue can take 7–9 weeks and, as previously indicated, can be delayed by early weaning at 3–4 weeks of age, which is common in most modern pig-producing countries (Vega-Lopez et al. 1995). This, among many other factors, predisposes to postweaning diarrhea associated with enterotoxigenic *E. coli* (ETEC) or other pathogens (Wellock et al. 2007). There is increasing interest in the role of glutamine in intestinal immune function. Glutamine is an important nitrogen source for enterocytes and plays a key role in maintaining mucosal cell integrity and gut barrier function (den Hond et al. 1999). Key functions of lymphocytes are dependent on glutamine provision (Graham et al. 2000); glutamine depletion results in immunosuppression, whereas glutamine administration has been found to have significant beneficial effects on the gut mucosal structure and intestinal immune function of piglets after weaning (Pierzynowski et al 2001). Enhanced intestinal immune function of piglets at weaning by feeding nucleotides for 2–4 weeks after weaning was found to have an immune-enhancing effect on piglets through improving T-cell-mediated responses (Cameron et al. 2001).

The use of dietary immunomodulators including yeast extracts, plant extracts, and animal by-products in weaned pigs has been reviewed by Gallois and Oswald (2008). To date, the most promising results have been obtained with the use of spray-dried animal plasma, particularly porcine plasma. A number of studies in which pigs were orally challenged with pathogenic *E. coli* have shown that pigs fed spray-dried plasma had less adverse clinical signs and growth effects (Bosi et al. 2004; Niewold et al. 2007; Torrallardona...
et al. 2007; Yi et al. 2005). In addition to providing specific antibody protection, nonspecific binding of the plasma molecules to intestinal receptors for E. coli is also thought to occur in pigs fed spray-dried plasma. The role of conjugated linoleic acid (CLA) in the prevention of bacterial-induced colonic inflammation in the pig has been studied by using a swine dysentery challenge model (Hontecillas et al. 2002). Feeding CLA-supplemented diets for 7 or 10 weeks prechallenge was found to prevent clinical signs and lesions of swine dysentery. Despite being colonized by Brachyspira hyodysenteriae, the CLA-supplemented pigs maintained cytokine profiles and lymphocyte subset distributions in keeping with nonchallenged control pigs. This suggests that CLA modulates the pig’s immune effector mechanisms preventing the normal host response to infection, instead of targeting the bacterial agent. Finding cost-effective methods of enhancing immune responses that promote tolerance of intestinal pathogens in weaned and grower pigs under commercial conditions would bring significant benefits to the pig industry.

**GUT FLORA**

The intestinal flora of the pig is extremely complex and diverse, making it difficult to study quantitatively and qualitatively, but this is an area of increasing interest in relation to maintenance of gut health and optimal function. The intestinal microflora of the large intestine has been studied and reviewed by Robinson et al. (1981, 1984). More recently there has been increased focus on microflora in the upper gut (Richards et al. 2005). Changes to the balance of the large intestinal microbiota can disrupt normal bacterial fermentation and absorption of VFAs. The concurrent absorption of water is proportionately reduced, resulting in diarrhea in the absence of mucosal morphological abnormalities.

Marked changes occur in the gut environment (e.g., pH and organic acids) and microbial activity along the gastrointestinal tract of pigs (Bach Knudsen et al. 1991, 1993). Differences in the diet composition can impose further changes and could affect the diversity of the gut microflora. In experiments involving diets containing different levels of soluble and insoluble NSP, a medium to high content of NSP resulted in higher microbial diversity in the small intestine and rectum (Högberg et al. 2001, 2004). The high-NSP diets induced a higher proportion of propionic acid, and the low-NSP diets induced a higher proportion of acetic acid, suggesting that the balance of the microbial flora had been altered accordingly by differences in the dietary carbohydrate composition. The ratio of soluble and insoluble NSP also influenced the coliform diversity in the large intestine. A higher proportion of soluble NSP increased the coliform diversity reflecting a more balanced microbiota compared with that generated by the diets that were higher in insoluble NSP.

The quantity and quality of protein fed to pigs can also have an impact on the microbiota, particularly if there is an imbalance between fermentable carbohydrate and potentially fermentable protein in the large intestine (Bikker et al. 2006; Piva et al. 2006). Microbial protein digestion in the large intestine may result in increased levels of potentially toxic substances, for example, ammonia, amines, and phenols that can contribute to the adverse effects of enteropathogenic processes (Bikker et al. 2006; Nyachoti et al. 2006).

The potential value of prebiotics (compounds other than dietary nutrients that act as substrates for populations of beneficial microbial organisms in the gut) and probiotics (preparations containing beneficial bacteria) has been the subject of many studies in relation to gut health and prevention of enteric infections. Prebiotics achieve their beneficial effects in two ways. First, compounds such as fructo-oligosaccharides can be fermented by favorable bacteria (e.g., bifidobacteria and lactobacilli), giving them competitive advantage (Houdijk 1998; Nemcová et al. 1999). Second, mannose-containing compounds added to the diet result in binding with pathogenic bacteria such as E. coli and Salmonella that may have mannose-specific lectins in fimbriae. This reduces attachment of pathogenic bacteria to receptor sites on gut mucosal cells (McDonald et al. 2002). Inulin is a natural polymer of fructose extracted from chicory that is considered to have prebiotic properties (Gibson and Roberfroid 1995; Roberfroid et al. 1998). Digestion of inulin in weaned pigs is mainly microbial and takes place in the large intestine where it modifies the profile of fermentation metabolites, resulting in an increase in N-valerate and propionate and a decrease in acetate and ammonia (Rossi et al. 1997). The short-chain fatty acids including butyrate stimulate the growth of bifidobacteria and lactobacilli. Butyrate regulates processes associated with proliferation and differentiation of colonic enterocytes as well as apoptosis, thereby having a direct effect on colonic health (Tako et al. 2008). Using an in vitro adhesion assay for E. coli, 5% inulin was found to partially inhibit adhesion of F4-positive E. coli to the small intestinal villi. The study also suggested that inulin might have immunomodulatory effects by boosting IgA and IgM antibodies in pigs exposed to foreign proteins (Rossi et al. 2001). In newly weaned pigs, addition of fructo-oligosaccharide and/or sugar beet pulp to the diet tended to increase the number of intestinal Bifidobacterium species and reduce the level of E. coli, although there was little difference in the incidence of diarrhea (Kleingeibbinck et al. 2001). The population of Bifidobacterium species is variable in piglets and constitutes less than 1% of the total bacterial population (Mikkelsen and Jensen 2001). Certain plant metabolites may interact with short-chain fatty acids to create inhibitory

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conditions for pathogens such as *E. coli* O157 (Duncan et al. 1998). The use of prebiotic is likely to be an area of growing scientific interest in the future.

Probiotics work on the principle of competitive exclusion of pathogenic bacteria and, for example, have been used successfully to control Yersinia infection in pigs (Asplund et al. 1996). Feeding probiotic bacteria, especially *Lactobacillus* and *Bifidobacterium*, may help with controlling enteric infections postweaning when the resident microflora is not yet stable. Probiotics may also inhibit adherence of enteropathogenic *E. coli* and other gram-negative bacteria to enterocytes through occupying receptor sites (Mack et al. 1999; Spencer and Chesson 1994). This has potential for improved control of a wide range of enteric infections, particularly those of zoonotic importance such as *Salmonella* and *Campylobacter*. *Salmonella* infections in pigs are common, often resulting in clinical salmonellosis or subclinical disease (Lax et al. 1995). Up to 30% of finisher pigs might shed *Salmonella*, thereby presenting risk of carcass contamination at slaughter (Berends et al. 1996). *Campylobacter* is one of the most common causes of human enteric disease and has been isolated from a wide range of raw meats, including pork (Fricker and Park 1989; Stern et al. 1985; Zanetti et al. 1996). The predominant species in pigs is *Campylobacter coli* (Stern et al. 1985; Weijtens et al. 1993, 1997; Young et al. 2000); however, some pig farms can also have a high prevalence of *Campylobacter jejuni* (Harvey et al. 1999). Contact with the sow during the suckling period results in a high prevalence of *Campylobacter* in piglets, whereas piglets removed from the sow after 24 hours and reared in isolation units have significantly less *Campylobacter* (Harvey et al. 2001).

### DIETARY INTERVENTIONS

The mechanisms by which antimicrobial agents enhance growth and feed efficiency are poorly understood (Anderson et al. 1999; Commission on Antimicrobial Feed Additives 1997). Two of the suggested mechanisms are inhibition of subclinical bacterial infection and less translocation of pathogens, and changing the small intestinal mucosal structure, mainly by increasing villus height promoting uptake of nutrients from the digesta.

Following the European Union ban on the use of the majority of antibiotic growth promoters, alternative measures to control the number and activity of intestinal bacteria have been explored. These include changes in management, feeding, and hygiene practices; the use of products such as probiotics, prebiotics, enzymes, herbs and plant extracts, prefermented feeds, and organic acids (de Lange et al. 2010; Thomke and Elwinger 1998); selective breeding for resistance; improving the pig’s immune response through the use of vaccines, cytokines, and other immunomodulatory agents; organic acids; inorganic chemicals such as zinc oxide; and the use of specific bacteriophages or bacteriocins (de Lange et al. 2010; Gallois and Oswald 2008; Hampson et al. 2001).

Finding effective non-antimicrobial alternatives requires a good understanding of the mechanisms that promote the beneficial effects in different age groups of pigs.

### Dietary Fiber and Cereals

Different forms of fiber in the diet can influence the composition and metabolic activity of the large intestinal microflora in pigs (Bach Knudsen et al. 1991; Jensen and Jørgensen 1994; Reid and Hillman 1999; Varel 1985; Varel et al. 1982). Little is known of mechanisms by which the commensal colonic microflora interacts with pathogenic bacteria; therefore, the basis of dietary control of infectious enteric diseases is not understood.

Other ways in which diet could influence pathogenic enteric infections include changing the amount or balance of substrates available for the organism at particular sites, influencing the viscosity, accessibility to receptor sites, and/or intestinal motility. For example, different cereal types and particle sizes have been shown to alter epithelial cell proliferation and lectin-binding patterns of the epithelium of the large intestine of pigs (Brunsgaard 1998).

The diet can also influence intestinal function. Components in boiled rice inhibit secretion in the small intestine and therefore reduce the magnitude of secretory diarrhea due to pathogens such as ETEC (Mathews et al. 1999).

One of the best recognized examples of dietary effects on enteric pathology is gastric ulceration (complete discussion below), in which ulceration of the pars esophagea occurs, particularly in growing and finishing pigs. Such lesions can be associated with reduced growth rates (Ayles et al. 1996b) but more importantly can be a cause of gastric hemorrhage and perforation leading to acute illness and death (Friendship 1999). Many studies have demonstrated a strong association between finely ground high-wheat diet and gastric ulceration (Accioly et al. 1998). The role of bacteria in the pathogenesis is unclear. The spiral bacterium *Helicobacter suis* has been associated with gastric ulceration in some studies (Barbosa et al. 1995; Queiroz et al. 1996), whereas in other studies, the association was weak or equivocal (Phillips 1999). Experimental inoculation of gnotobiotic pigs with *H. helimannii* failed to produce lesions of gastric ulceration in pigs fed a carbohydrate-enriched liquid diet (Krakowka et al. 1998). However, inoculation of pigs with *Lactobacillus* sp. and *Bacillus* sp. when fed the same diet did produce ulcers, possibly due to fermentation by these bacteria in the presence of readily available dietary substrate. It is possible that production of short-chain fatty acids
produced through the fermentation process resulted in ulceration (Krakowka et al. 1998).

Early studies on the influence of diet in postweaning colibacillosis suggested that inclusion of fiber sources reduced the severity and incidence of diarrhea (Bertschinger et al. 1978; Bolduan et al. 1988). Later studies have compared the effects of feeding different post-weaning diets in experimental E. coli challenge models. Conclusions from research by different groups are inconsistent, revealing that this area is still poorly understood. For example, studies by McDonald et al. (1997, 1999, 2001) showed that diets supplemented with soluble NSPs increased the number of E. coli in piglet intestines and concluded that these diets were “provocative” in terms of causing diarrhea. Conversely, Wellock et al. (2008) found that inclusion of soluble NSP in the diet was beneficial to piglets challenged with ETEC. The occurrence of diarrhea was significantly reduced, and there was lower cecal digesta pH and increased Lactobacillus-to-coliform ratio when compared with pigs fed diets containing insoluble NSP. Clearly, this is a complex area in which the quantity, nature, and viscosity of the small intestine contents interrelates with the proliferation rate of intestinal microbes. This affects the rate of intestinal motility, fermentation processes, and ultimately the health of the newly weaned piglet.

The most work has been done on the influence of diet on swine dysentery. Whereas a cooked rice diet was found to be protective in some studies (Siba et al. 1996), it was not in others (Kirkwood et al. 2000; Lindecrona et al. 2003). Feeding the cooked rice diet to pigs already affected with swine dysentery did not reduce the duration or severity of disease (Durmic et al. 2000). In a study of different cereal types, feeding steam-flaked maize or sorghum reduced the incidence of the disease (Pluske et al. 1996a). Soluble NSP and resistant starch were identified as important factors promoting large intestinal fermentation and bacterial colonization, whereas with the addition of a source of mainly insoluble NSP (oat chaff), the diet remained protective (Pluske et al. 1998). Addition of enzymes to wheat-based diets and the use of heat extrusion to increase digestibility of starch in the small intestine were tested in terms of their potential protective effects in swine dysentery, but neither processes prevented colonization (Durmic et al. 2000). Likewise, the use of a sorghum-based diet was tested as sorghum is inherently low in soluble NSPs, but this diet was not protective against swine dysentery (Durmic 2000). However, the grind size of diets was important with significantly more pigs developing swine dysentery with coarsely ground wheat or sorghum than with these grains finely ground (Hampson et al. 2001). In a comparative study, using a barley/triticale-based diet supplemented with either rapeseed cake or dried chicory roots and sweet lupins, the latter diet was found to be completely protective when pigs were challenged with B. hyodysenteriae (Thomsen et al. 2007). The colonic microflora of those pigs had higher proportions of bifidobacteria and Megaspaphera species that might have inhibited colonization by B. hyodysenteriae (Mølbak et al. 2007). Conversely, studies carried out in Sweden have shown that high levels of soybean meal in the diet promote the onset of swine dysentery (Jacobson et al. 2004). In porcine colonic spirochetosis, pigs on the cooked rice diet developed infection later and to a lesser extent than pigs on wheat-based diet (Hampson et al. 2000; Lindecrona et al. 2004).

Carriage of Oesophagostomum dentatum in pigs is enhanced by diets rich in insoluble fiber (Petkevicius et al. 1997) whereas colonization by Trichurus suis did not appear to be significantly influenced by diets that differed in terms of their carbohydrate properties (Thomsen et al. 2006). Addition of chicory roots and sweet lupins to the diet did not prevent colonization by Trichurus suis in pigs that were challenged experimentally (Thomsen et al. 2007).

**Dietary Protein**

The protein levels in commercial rations for newly weaned pigs are usually high in order to promote optimal growth rates. However, in comparative studies using high (21%) and low (13%) protein diets, Prohaszka and Baron (1980) showed that the high-protein diet, especially when the protein is of reduced digestibility, predisposed to postweaning colibacillosis. These findings have been supported by a number of subsequent studies in piglets with naturally acquired infections and also in experimental challenge studies using ETEC (Nyachoti et al. 2006; Wellock et al. 2006, 2007). The differences in protein-associated effects were more marked in earlier-weaned piglets at 4 weeks of age as compared with those weaned at 6 weeks. The benefits of reducing protein level in weaners reared under large-scale commercial conditions require careful assessment. However, protein reduction could be beneficial in units with high infection pressure with ETEC after weaning, where repeated problems of postweaning diarrhea results in piglet losses.

**Organic Acids, Inorganic Compounds, and Fatty Acids**

Alternatives to antibiotic inclusion in weaner diets include the use of organic acids such as potassium diformate (Roth et al. 1998). Addition of 1.8% potassium diformate to a piglet starter ration decreased the onset of swine dysentery (Prachasvaha and Fatty Acids) 

Alternatives to antibiotic inclusion in weaner diets include the use of organic acids such as potassium diformate (Roth et al. 1998). Addition of 1.8% potassium diformate to a piglet starter ration decreased the counts of total anaerobic bacteria, lactic acid bacteria, yeasts, and coliforms in the digesta from the stomach, distal small intestine, cecum, and midcolon over a period of 4 weeks after weaning (Canibe et al. 2001). The apparent antimicrobial effects were attributed to the protons and anions formed from formic acid
passing through the bacterial cell wall. These have a disruptive effect on protein synthesis and inhibit bacterial enzymes, thereby reducing bacterial replication (Partanen and Mroz 1999). Other studies agree that coliform counts in the stomach and proximal colon decrease when levels of formic acid increase (Gabert et al. 1995; Kirchgessner et al. 1992), or when potassium diformate is used (Février et al. 2001).

Supplementation of weaner diets with organic acids or other salts have been shown to reduce the incidence of postweaning diarrhea and improve growth performance of piglets (Sutton et al. 1991). When the coliform inhibitory effects of various organic acids were compared, the results going from most effective to least effective were benzoic, fumaric, lactic, butyric, formic, and propionic acid. By far, the greatest increase in the use of organic acids in pig rations has occurred in response to *Salmonella* control programs in European countries. Clinical trials have shown that including formic acid or lactic acid in the rations of growing and finishing pigs significantly reduces the seroprevalence of *Salmonella* when tested by “meat-juice” enzyme-linked immunosorbent assay (ELISA) at slaughter age (Croez et al. 2007; Dahl 2008). A similar effect can be achieved by supplying organic acids to pigs via the drinking water (van der Wolf et al. 2001). The impact of these organic acids on other enteric organisms has not been closely studied. However, it seems probable that they lead to a general reduction in coliform numbers that could have additional benefits if other pathogens are prevalent.

Feed supplemented with 2500ppm zinc oxide has been shown to reduce postweaning diarrhea and has been widely used in commercial pig production although the mechanism of action is unclear (Holm 1998). In a study of the effects of zinc oxide on the enteric flora, no differences were recorded between the zinc-oxide-supplemented group and control group in the number of coliforms, enterococci, or *Clostridium perfringens* excreted per gram of feces. However, there was an overall reduction in the diversity of the fecal coliform flora in the zinc-oxide-treated pigs. In the second week, postweaning zinc-oxide-treated pigs showed significant increases in growth rate over controls (Melin et al. 2001). Some countries have a legislation that limits the maximum permissible level of zinc inclusion in pig diets.

**Essential Oils**

Some herbal products including essential oils have been shown to have antimicrobial properties in vitro. Generally, the phenolic components of these products tend to be most active and appear to act by increasing the permeability of bacterial membranes (Burt 2004). A variety of essential oils have been tested against *Salmonella typhimurium* and *E. coli* F4 (Si et al. 2006a), and although they retained their antibacterial activity in the presence of pig cecal content (Si et al. 2006b), pig challenge studies showed poor efficacy. One explanation for the lack of efficacy in vivo has been put forward by Michiels et al. (2008) who demonstrated that certain essential oils are absorbed in the stomach of pigs and may not be available to act on bacteria in the small intestine.

**Fermented Liquid Feeds**

Liquid feeding can improve the feed intake, growth, feed conversion, and health of weaned pigs (Brooks et al. 1996). However, steeping feed in water promotes bacterial proliferation in the feed, which reduces the quality of the feed and presents health risks. Feeding fermented liquid feed has been used as a means of controlling enteric infections through acidification. As part of the diet for newly weaned pigs, fermented liquid feed has beneficial effects on the villus height and ratio of villus height to crypt depth in the proximal jejunum (Scholten et al. 1999). The mechanism is uncertain but it could be due to a lower pH, increased levels of organic acids, and an altered microbiological status. The use of fermented liquid feed in newly weaned piglets caused a significant reduction in the coliform population in the terminal ileum, cecum, and colon compared with piglets fed dried feed (Jensen and Mikkelsen 1998; Moran et al. 2001). Feed was prepared by inoculating the diet with lactic acid bacteria (*Lactobacillus plantarum*) and steeping it for 5 days at 25°C before feeding. Fermentation prevents bacterial colonization and spoilage of the liquid diet by enteropathogens and other spoilage bacteria. The fermentation temperature is important in relation to bacterial survival. *Escherichia coli* was eliminated more effectively at 37°C than 20°C (Beal et al. 2001). There were also strain differences in terms of *E. coli* survival in fermented liquid feed, with F4 (K88) being the most resistant to killing by fermentation (Beal et al. 2001). The effect of temperature has implications for the management of fermented liquid feed systems. Cold-shock proteins help *E. coli* survive at lower temperature (Phadtare et al. 1999). Fermented diets not only lower the acidity of the diet but also reduce the soluble NSP content of the diet (Hampson et al. 2001).

Piglets showed a significant preference for freshly prepared liquid feed over fermented liquid feed when given the choice (Demeckova et al. 2001). To prevent spoilage, chlorine dioxide added at 300 ppm was found to eliminate coliforms from liquid feeds for young piglets without adversely affecting palatability or growth performance (Demeckova et al. 2001). Chlorine dioxide is a strong oxidizing agent with broad antimicrobial spectrum, being active against bacteria and viruses (Junli et al. 1997). Addition of chlorine dioxide to freshly prepared wet feed did not significantly alter the intake when compared with nonsanitized freshly prepared wet feed. Chlorine dioxide is reported to kill...
**E. coli** through loss of permeability control of the outer bacterial cell membrane (Berg et al. 1986).

**REHYDRATION OF DIARRHEIC PIGLETS**

Oral rehydration fluids are used in piglets with acute diarrhea especially during outbreaks of ETEC and rotavirus infections. Studies in rats and clinical studies in children have shown that reducing the osmolality of oral rehydration solutions has beneficial effects on the course of diarrhea and the clinical outcome (Thillainayagam et al. 1998). Using an experimental pig model, Kiers et al. (2001a) demonstrated that solutions with low osmolality promoted intestinal fluid absorption. However, ETEC infection resulted in a decrease in net fluid absorption independent of osmolality, as compared with that of unaffected tissue. In an experimental model, mold-fermented soybean products were found to be beneficial in maintaining fluid balance during postweaning ETEC infection through preventing fluid loss (Kiers et al. 2001b). The mechanism is uncertain. It might interfere with the attachment of **E. coli** to epithelial cells or modulate the effects of toxin in the intestine.

**REGIONAL DISEASES AND PATHOLOGY OF THE DIGESTIVE SYSTEM**

**The Oral Cavity**

There are several recognized congenital defects affecting the oral cavity. Cleft palate and palatoschisis are both multifactorial developmental abnormalities. Cleft palate in piglets has occurred with feeding poisonous plants such as poison hemlock (**Conium maculatum**) or wild tree tobacco (**Nicotiana glauca**) to sows or gilts in early pregnancy (Keeler and Crowe 1983; Panter et al. 1985). Likewise, accidental contamination of sow feed with seeds of **Crotalaria retusa** during pregnancy has resulted in palatoschisis in piglets (Hooper and Scanlan 1977). Brachygnathia superior (shortness of maxillae) is an inherited condition that is progressive and may be confused with progressive atrophic rhinitis. Hypertrophy of the tongue is a rare congenital anomaly in pigs that interferes with normal suckling behavior. Epitheliogenesis imperfecta affects the gingiva and tongue and can be seen as irregular, well-demarcated red areas that are devoid of epithelial tissue.

Oral lesions arising from traumatic damage are relatively common. Clipping the teeth of baby pigs exposes the dental pulp. If the dental pulp becomes infected, pulpitis, dental abscession, and osteomyelitis can ensue. Gingivitis and periodontal inflammation is usually associated with poor teeth clipping technique resulting in damage to the gingival epithelium. Stomatitis and tooth root abscesses may follow. **Fusobacterium necrophorum** is a common isolate from such lesions. Pigs have a diverticulum of the pharynx in the posterior wall immediately above the esophagus. Barley awns and other fibrous materials can lodge there and penetrate the pharynx causing pharyngeal cellulitis. This is usually seen only in young pigs. Stomatitis can also be caused by irritant chemicals, such as caustic or toxic compounds, and by physical burns. Blistering and erosion of the snout epithelium can arise due to sunburn. Oral “thrust” or candidiasis is occasionally seen in piglets, presenting as pale plaque-like lesions on the tongue, hard palate, or pharynx. In rare cases, they can extend further, affecting the esophagus and stomach. **Candida** species are isolated from the plaques. Factors such as frequent antibiotic administration or intercurrent diseases, for example, neonatal porcine reproductive and respiratory syndrome (PRRS) virus infection, can promote the onset of candidiasis.

A number of important infectious diseases show lesions on the snout and oral tissues. These are primarily the viral vesicular diseases, including foot-and-mouth disease, swine vesicular disease, and vesicular stomatitis. Lesions include blanching of the epithelium, vesicles, erosions, and epithelial flaps. Sunburn, and occasionally parvovirus infection, can cause lesions on the snout resembling vesicular diseases. With pseudorabies (Aujeszky’s disease) virus (PRV), vesicles and erosions can occur on the snout in addition to necrotizing tonsillitis and pneumonia that are classically associated with this infection.

Ulcerative glossitis and stomatitis have been reported in piglets with exudative epidermitis. Piglets may also develop ulcers on the dorsum of the tongue and occasionally on the hard palate associated with **Staphylococcus hyicus** infection (Andrews 1979). Oral erosions and ulcers can also be seen in piglets with congenital swine pox. **Actinobacillus lignieresi** can cause swelling and inflammation of the tongue, with nodule and ulcer formation. Soft tissues of the pharynx and neck can also be affected. Of the parasitic infections, cysticercosis and **Trichinella spiralis** can affect the tongue and muscles of mastication. **Gongylonema** species has been found in the mucosa of the tongue in grazing pigs where they cause mild, localized inflammation (Zinter and Migaki 1970).

The tonsils have a strategic role in immune surveillance of the oropharynx (Horton et al. 2003). A host of bacterial agents, including many types of **Streptococcus suis** and **Pasteurella**, are frequently carried in the tonsils (Torremorrell et al. 1998). Crypt inflammation and lymphoid hyperplasia are associated with bacterial infections. Necrotizing tonsillitis occurs with pseudorabies; the tonsils being the site of primary virus replication (Terpstra and Wensvoort 1988). Tonsillitis is also a feature of swine vesicular disease. Hemorrhagic necrotizing tonsillitis can occur in pigs with anthrax. In weaner pigs with postweaning multisystemic wasting syndrome (PMWS) due to porcine circovirus type 2 (PCV2) infections, the lymphoid tissue of the tonsils is...
involved (Chae 2004). The altered status of the tonsilar lymphoid tissue in particular is thought to facilitate bacterial infections of the tonsils, with an increased likelihood of bacteremia.

There are few reported problems concerning the salivary glands in pigs, but sialoadenitis occurs in vitamin A deficiency (Barker et al. 1993). The interlobular ducts of the salivary glands undergo squamous metaplasia leading to salivary stasis, secondary infection, and purulent inflammation. This results in pronounced swelling of salivary glands. Epithelial degeneration of salivary ducts is seen in swine vesicular disease.

**The Esophagus**

Conditions affecting the esophagus are uncommon but include hyperkeratosis, parakeratosis, mycotic infection, obstructions, and traumatic lesions. Hyperkeratosis and thickening of the epithelium are associated with vitamin A deficiency or chlorinated naphthalene toxicity. Parakeratosis of the esophagus occurs in pigs with cutaneous parakeratosis due to zinc deficiency. Parakeratotic thickening of the epithelium of the distal esophagus, with basal hyperplasia of the epithelium, is commonly seen in pigs with ulceration of the pars esophagea of the stomach. Reflux esophagitis is recognized in some pigs with ulceration of the pars esophagea. The gastric secretion has corrosive effects on the squamous epithelium resulting in mucosal erosion, ulceration, and inflammation. Some cases develop annular scarring as part of the healing process, leading to stenosis and muscular hypertrophy of the distal esophagus.

Mycotic esophagitis caused by Candida albicans can occur in suckling piglets and weaners that are immunocompromised and in piglets in which the mucosal flora has been significantly disrupted for some reason, as previously mentioned.

Obstruction and/or perforation of the esophagus are associated with ingestion of large objects such as stones, potatoes, apples, or corn cobs. Perforation can result from ingestion of sharp objects such as fence wire or nails. Inflammation and subsequent stricture are sequelae that affect the esophagus at the site of perforation. Such conditions lead to dysphagia and distension of the esophagus cranial to the site of obstruction or stricture. Encephalitis affecting the medulla oblongata and/or the nuclei or tracts of the cranial nerves involved in swallowing (V, IX, X, XII) also results in dysphagia. Such conditions are very rare in the pig. Nematode parasites (Gongylonema species) occasionally occur in the esophageal mucosa leaving serpentine-shaped tracts. However, these parasites do not appear to have any adverse effects in pigs.

**The Stomach: Gastric Ulceration**

Conditions affecting the stomach are mainly physical or functional in nature, the most important being gas-troesophageal ulceration affecting the pars esophagea. Abattoir surveys demonstrate that the prevalence of stomach lesions, including parakeratosis, erosions, and ulcers, often approaches 90%, depending on the feeding and husbandry practices (Driesen et al. 1987). There is great herd-to-herd variation in prevalence and severity. Ulceration of the pars esophagea can affect any age of pig, but the highest rate of ulceration occurs in pigs 3–6 months of age. Mortality due to gastric ulceration among grower–finisher pigs has been reported to be about 1–2% on some farms, with much higher levels occurring sporadically (Deen 1993; Melnichouk 2002). Sows, at the time of parturition, are also a relatively high-risk group. Examination of culled sows revealed 60% with stomach lesions and 10–15% with ulcerations (Hessing et al. 1992; O’Sullivan et al. 1996). Frequently, sow stomachs have extensive scar tissue, indicating previous severe ulcerative episodes. Gastric ulceration has been reported as a common cause of sow mortality (Chagnon et al. 1991; Sanford et al. 1994).

The presence of gastric ulcer cannot be ruled out without examination of the pars esophagea. Lesions associated with ulceration of the pars esophagea rarely extend into the contiguous esophagus or the glandular region of the stomach. Ulcerations and erosions of the pars esophagea may involve only a small portion or all of the gastric squamous mucosa. The most common site for ulceration is at the junction of the pars esophagea and the cardiac mucosa (Penny and Hill 1973). The normal epithelium of the pars esophagea is smooth, white, and glistening, and is easily distinguished from the surrounding glandular mucosa. It is believed that lesions usually progress from hyperkeratotic parakeratosis that causes a thickened, rough appearance to fissuring and peeling that result in erosion and eventually ulceration.

Frequently, the hyperplastic epithelium of affected pars esophagea is greenish yellow as a result of bile staining, particularly when the surface is rough and thickened due to parakeratosis. This type of corrugated surface may flake and peel off readily. When ulceration involves the entire pars esophagea, the lesion has a punched-out or crater-like appearance with elevated ridges at the margin of the circular ulcer. The floor of the ulcer may be so smooth that it is misinterpreted as normal tissue (Barker et al. 1993). The relationship between gross and microscopic lesions has been investigated by Embaye et al. (1990), who concluded that, generally, gross appearance was directly related to microscopic findings. However, these researchers found that in 155 apparently normal stomachs, 32% showed histological evidence of parakeratosis, 30% had minor erosive lesions, and microscopically 23% had severe ulceration.

Gross evaluation of stomach lesions is commonly incorporated into an abattoir surveillance program to monitor disease status. To examine the pars esophagea,
the stomach should be incised along the greater curvature and inverted. Emptying the contents and rinsing the stomach before examination will improve the accuracy of the inspection. Various classification schemes have been used to grade stomach lesions at slaughter (Ayles et al. 1996b; Christensen and Cullinane 1990).

Histologically, the lesion is the result of thickening and parakeratosis, with nucleated cells present on the mucosal surface. Rete pegs and proprial papillae are elongated, and neutrophils and eosinophils are often present at the tips of the proprial papillae. Epithelial separation and erosion usually occur beneath a band of cells with cytoplasmic pallor and nuclear degeneration (Embaye et al. 1990). Ulcers of the pars esophagea usually involve only the submucosa, but they may advance to the muscularis externa and occasionally to the serosa (Barker et al. 1993).

In the case of a pig that has died acutely of gastric ulceration, postmortem diagnosis is usually straightforward. The carcass is typically pale but in good body condition. The stomach may contain clotted and unclotted blood and fibrinous exudate enclosing a variable amount of food. Blood may be found in the intestine as well. A blood clot may be adhered to the surface of the ulcer, which is generally deep and extensive in the case of a fatal hemorrhagic episode.

Ulceration may occur rapidly, and the progression from normal pars esophagea to complete ulceration may take less than 24 hours. Clinical signs tend to reflect the degree of blood loss associated with the gastric lesion. Frequently, a pig that had appeared to be healthy only a few hours earlier is found dead, and the carcass is extremely pale. If blood loss occurs more slowly, signs associated with anemia will be apparent, such as paleness, lethargy, weakness, increased respiratory rate, and anorexia. In addition, black tarry feces may be noted, and some pigs show signs of abdominal pain by grinding their teeth and arching their backs. Vomiting may occur. Rectal temperatures of affected pigs are often below normal. It is important to remember that not all pigs that die from gastric ulcers will reveal others in the group that are showing signs of anemia. When ulceration occurs without significant blood loss, animals will generally appear to be in normal health. Evidence that subclinical ulceration reduces growth rate is inconsistent. Various researchers have attempted to correlate severity of stomach lesions at slaughter with growth performance during the grower–finisher phase. Certain studies have found no relationship (Backstrom et al. 1981; Pocock et al. 1969), whereas Elbers et al. (1995a) observed a 50–75 g/day decrease in growth rate for pigs with ulcers versus pigs with normal stomachs. This last result is in agreement with the findings of a trial that followed pigs using endoscopic examination to measure gastric lesions (Ayles et al. 1996b).

Ulcers can occur rapidly and heal quickly, making it difficult to relate lesions at slaughter with performance during the grower–finisher stage. Evidence of past ulceration can sometimes be seen as cicatrization and a reduction in size of the pars esophagea. In extreme cases, the pars esophagea is entirely destroyed, and stenosis of the esophagus at its entry to the stomach occurs. Pigs afflicted with stenosis are observed to vomit shortly after eating but are hungry and therefore will immediately resume eating. These pigs tend to grow more slowly than penmates despite maintaining good appetites.

The exact cause or causes of gastric ulceration is not completely understood, but many of the risk factors are well known. There is considerable interaction between many of the risk factors, particularly in their effect on the fluidity of the stomach content, the speed of passage of ingesta through the stomach, and whether or not the stomach contains feed. Generally, factors that increase the firmness of the stomach content help prevent gastric lesions and factors that cause increased fluidity of the contents increase the risk of ulcers (Nielsen and Ingvartsen 2000).

Fine particle size of feed has been shown to increase the prevalence of gastric lesions (Ayles et al. 1996b; Hedde et al. 1985; Mahan et al. 1966; Maxwell et al. 1970, 1972; Potkins and Lawrence 1989a; Reimann et al. 1968; Wondra et al. 1995a). In addition, pelleting of feed may also increase the likelihood of ulcers developing (Chamberlain et al. 1967; Potkins and Lawrence 1989b). The prevalence and severity of ulcers vary with the cereal component of the diet. Generally, oats and barley appear to have a sparing effect (Reese et al. 1966) and corn and wheat tend to be more ulcerogenic (Smith and Edwards 1996).

The method by which the grain is processed affects the prevalence of ulcers. Grain that is ground using a hammer mill tends to be more ulcerogenic than if a roller mill is used (Nielsen and Ingvartsen 2000; Wondra et al. 1995b). Feed particle size is affected by grain component, milling procedure, and processing. Grains such as wheat are more likely to shatter during grinding and result in finer particle size compared with oats or barley. If grain is processed using a roller mill, there is less chance of the grain kernel shattering and creating “fines.”

In addition, the pelleting process causes a further decrease in particle size. Nielsen and Ingvartsen (2000) showed that, in general, barley and rolling prevented stomach lesions while wheat grinding and pelleting
increased the prevalence and severity of ulcers. The overall effect of a feed with very fine particle size is that the stomach content is very fluid and the emptying time is relatively rapid (Regina et al. 1999), and as a result, the pH gradient between the neutral proximal part of the stomach and the acidic distal region is lost.

The method of feeding may be as important as feed processing and composition. A major risk factor of ulcer development is an interruption of feed intake (Henry 1996). Fasting of pigs has been a consistent method of experimentally producing gastric lesions (Lawrence et al. 1998; Pocock et al. 1968). Various workers have noted dramatic increases in ulcer severity and prevalence at slaughter in pigs examined after a 24-hour holdover period compared with pigs from the same herds slaughtered on the day of arrival at the abattoir (Chamberlain et al. 1967; Davies et al. 1994; Lawrence et al. 1998; Straw et al. 1992). There is at least one study that documented no association between a 24-hour feed withdrawal and an increased prevalence or severity of ulcers (Eisemann et al. 2002). Presumably, there is an interaction between factors affecting stomach emptying such as feed particle size and the effect of feed withdrawal that can explain the differences in findings between these studies.

Interruption of normal feed intake commonly occurs on almost all farms because of mechanical problems or human error. Periods of transition in feeding patterns, such as when gilts are transferred to the breeding herd from a finishing barn or when sows approach the time of parturition, should be viewed as high risk for ulcerogenic events (Henry 1996). Hot weather resulting in dramatic reduction in feed intake is associated with gastric ulcer outbreaks (Deen 1993).

It is likely that factors such as acute infectious disease and season influence ulcer development in a similar manner to management practices that interrupt feed intake. Acute respiratory disease is associated with an increased likelihood of gastric ulceration (Dionissopoulos et al. 2001). In addition to respiratory disease causing inappetence, it is possible that increased levels of histamine as a result of infection could play a role, in that histamine is a powerful stimulant of gastric acid secretion. Injection of histamine has been shown to experimentally produce ulceration of the pars esophagea (Hedde et al. 1985; Huber and Wallin 1965; Muggenburg et al. 1966). Experimental infection of gnotobiotic pigs with various viral pathogens, including PRRS virus, does not result in gastric ulceration; however, experimental infection of concurrent PRRS virus and PCV2 in cesarean-derived, colostrum-deprived pigs has caused gastric lesions (Harms et al. 2001).

Research clearly demonstrates that ulceration of the pars esophagea is not mediated by glucocorticoids (Zamora et al. 1980). Chronic elevation of corticosteroids in response to stressful conditions has been shown under controlled trials to not result in an increase in ulcer prevalence or severity (Jensen et al. 1996). Genetic susceptibility may play a role in ulcer development. Researchers have reported a link between fast growth rate and/or low back fat and a high prevalence of gastric ulcers (Berruecos and Robinson 1972). It has also been reported that injection of swine with porcine somatotropin causes an increase in prevalence and severity of ulcers (Smith and Kasson 1991).

There has been considerable interest in finding an infectious cause of porcine gastric ulcers similar to the situation in humans. Helicobacter-like organisms have been identified in the glandular region of the stomach (Mendes et al. 1990) and appear to be widespread in the pig population. More than 80% of market hogs may be infected (Hellemans et al. 2007; Szeredi et al. 2005). Isolates of these tightly coiled spiral bacteria have been successfully cultured, and the name Helicobacter suis sp. nov. has been proposed (Baele et al. 2008). Whereas some workers have observed a correlation between the presence of these bacteria in the pyloric region and the prevalence and severity of lesions in the pars esophagea (Barbosa et al. 1995; Queiroz et al. 1996), other researchers have not (Magras et al. 1999; Melnichouk et al. 1999; Szeredi et al. 2005). Bacterial colonization of the glandular stomach causing gastritis and the development of ulcers in the pars esophagea region have been observed in experimental challenge studies (Haesebrouck et al. 2009; Krakowka et al. 2005). It has been hypothesized that because the Helicobacter is found in the antrum in close contact to hydrochloric-acid-producing cells, gastritis due to Helicobacter infection may result in parietal cell stimulation causing hyperacidity and indirect damage to the unprotected epithelium of the pars esophagea. There are a number of disease conditions that have been shown to be associated with gastric ulceration presumably by causing anorexia. This might also be a possible mechanism by which Helicobacter gastritis is linked to gastric ulcers.

Many of the factors associated with an increased risk of ulcer development are closely tied to economic competitiveness such as the use of finely ground feed and fast-growing, lean genetics. Therefore, steps to reduce the prevalence of gastric ulcers need to be carefully balanced between economic considerations and welfare concerns. Treatment for the most part is prohibitively expensive, laborious, and often unsuccessful. In addition, early diagnosis of gastric ulcer disease is difficult. Therefore, prevention of stomach lesions is generally considered the most appropriate approach to handling this problem. Many causative factors and complex interactions of nutrition, environment, and management contribute to the expression of this disease, but a coordinated effort by feed providers, owners, production personnel, and herd veterinarians can result in feed preparation standards and management that limit gastric ulceration as a swine production problem.
without reducing animal performance (Henry 1996). The use of roller mills instead of grinding feed appears to be one of the best methods of reducing ulcers (Nielsen and Ingvartsen 2000). Feeding practices must be carefully monitored. An interruption of feed intake appears to be a major contributing factor to ulcer formation. Blocked feeders or waterers, heat stress, unpalatable feed, or the presence of vomitoxin in the feed can lead to ulcer problems. Good management practices can minimize the occurrence and influence of these factors.

Various attempts have been made to incorporate protective substances in swine feeds to prevent ulcer development. Increasing levels of antioxidants such as vitamin E and selenium beyond physiological requirements does not appear to be useful (Davies 1993). There is some evidence to suggest that the inclusion of vitamin U (methylmethionine sulfonium) reduces the prevalence and severity of ulcers (Elbers et al. 1995b; Hegedus et al. 1983). Alfalfa has been used at levels of up to 9%, because it is high in vitamins E and K, as well as a source of increased fiber. Alfalfa was not effective in reducing the level of severity of ulcers in pigs treated with porcine somatotropin (Baile et al. 1997). The addition of sunflower hulls to diets was shown to be effective in reducing lesions (Dirkzwager et al. 1998).

Products that reduce gastric emptying have been examined and found somewhat effective, at least in an experimental setting. Melatonin has been reported to slow gut motility and when included at levels as low as 2.5 g/t results in less ulcer development (Ayles et al. 1996a). Similarly, diets containing sodium polyacrylate are retained for longer periods of time in the stomach of swine, and gastric ulceration is reduced (Yamaguchi et al. 1981). There may be circumstances in which various therapeutic agents are useful for treatment or prevention, but because of the many contributing factors and the interactions of these factors, it is unlikely that a single pharmaceutical product or management technique will be found that completely prevents gastric ulceration caused by various combinations of factors in different herds.

The Stomach: Other Gastric Conditions

Gastric overdistension can occur in adult pigs, especially in sows, but the cause is uncertain. It is thought to be associated with excessive intake of finely ground grain and water, resulting in excessive fermentation and gaseous distension.

Gastric volvulus is thought to be due to rapid intake of large quantities of feed and water followed by physical activity in a competitive group situation. Clockwise torsion is apparently most common, although torsion can occur in either direction about the long axis of the stomach. The spleen is often involved and becomes extremely enlarged due to blood engorgement (Morin et al. 1984). The stomach becomes massively distended with gas and fluid, and intense mucosal congestion develops. This condition is rapidly fatal.

Gastric foreign bodies such as stones are frequently found in stomachs of outdoor sows that are maintained on stony ground. Stone chewing is a regular activity, and swallowing of the stones is thought to be accidental. Large quantities of gastric stones have been recorded in some sows, limiting the capacity of feed intake and resulting in loss of body condition.

Gastric venous infarction occurs in pigs, usually in association with bacteremia, such as salmonellosis, erysipelas, or Glässer’s disease, or with toxemia. The lesion can also be seen in classical swine fever (CSF) (Elbers et al. 2003). The mucosa in the fundic area becomes reddish black in color and caseous mucosal necrosis may be evident. Thrombosis of capillaries and venules in the mucosa and submucosa with fibrin plugging is the cause of infarction. Multifocal areas of gastric infarction have also been recorded in cases of porcine dermatitis and nephropathy syndrome (PDNS), due to fibrinoid vasculitis of capillaries.

Edema of the stomach wall is a characteristic change in edema disease caused by specific strains of E. coli. Edema affects the submucosa, particularly on the greater curvature of the stomach. Other conditions causing gastric edema include hypoproteinemia, arsenic toxicity, and portal hypertension. In these conditions, edematous changes are less pronounced than those of “edema disease.”

Gastritis in pigs is most commonly associated with ulceration of the pars esophagea and the inflammatory sequelae affecting tissue in the cardiac zone of the stomach, as previously mentioned. Candidiasis of the pars esophagea may occur in association with preulcerative epithelial hyperplasia and parakeratosis. Gastritis could occur due to accidental intake of toxic compounds—such as arsenic, thallium, formalin, bronopol, and phosphatic fertilizers—and by the toxic principles in bitterweed (Hymenoxon odorata) or the blister beetle (Epicanta sp.). In commercial farming, such conditions are very rare and should be easy to exclude. Mycotic gastritis is occasionally seen in piglets, usually in association with repeated use of antibiotics. Lesions present as multifocal yellowish plaques on the gastric mucosa, with intense congestion of the peripheral gastric mucosa. Fungal hyphae colonize the mucosa and invade tissue and capillaries resulting in thrombosis. Agents are usually zygomycetes such as Rhizopus, Absidia, or Mucor species. Aspergillus species involvement is rare (Mahanta and Chaudhury 1985).

Parasitic gastritis is now rare in large commercial pig farms but can be problematic in organic units, small holdings and backyard piggeries, in instances where anthelmintics are seldom used. Affected pigs show poor bodily condition due to chronic gastritis. Of the parasites that can cause gastritis, Hysteromyctylus rubidus is of most importance because it is associated with poor
growth rates in young stock or loss of body condition in adult pigs.

Other parasites that can cause gastritis in heavy infections include *Ascarops* sp. and *Physocyphalus* sp. These parasites are common in many parts of the world, in feral pigs and those kept under extensive conditions with access to grazing and foraging conditions. *Simondia* spp. are found in Europe, Asia, and Australia and are associated with nodular gastritis in pigs. The parasite * Gnathostoma* sp. occurs in Asia. It invades the mucosa and undergoes development within inflammatory cysts in the submucosa. In heavy infestations, this parasite can cause thickening of the stomach wall.

The Intestinal Tract

**Congenital Defects.** Atresia ani is the most common congenital defect of the intestinal tract of pigs, and it is believed to be hereditary (Norrish and Rennie 1968). It arises due to failure of perforation of the membrane separating endodermal hindgut from ectodermal anal membrane. Evident at birth, the defect can be corrected by minor surgery unless atresia of the rectum is also present. Persistent Meckel’s diverticulum is a rare anomaly in which there is persistence of the omphalomesenteric duct. It presents as a tube of intestinal tissue similar to the ileum that branches from the intestine to the umbilicus. Occasionally, it can be involved in abdominal catastrophes such as intestinal strangulation.

**Intestinal Displacements**

Intestinal displacement and obstruction are common in pigs, and a number of different conditions arise.

**Rectal Prolapse and Rectal Stricture.** Rectal prolapse is relatively common and an important condition affecting growing pigs and adults. The rectum is held in place by a complex matrix of fascia, collagen fibers, muscles, and ligaments, and rectal prolapse will occur if the support mechanism is either overcome by pressure or weakened for some reason (Smith and Straw 2006). Pressure on the support mechanism may be brought about by straining (proctitis, urethritis, constipation, coughing, and farrowing) or by physical pressure (excessive slope on the floor or increase in abdominal pressure for any reason). Brockman et al. (2004) produced rectal prolapse in ten 49–74 kg pigs by insufflating the abdominal cavity with water at pressures of 222–343 mmHg (mean 292 mmHg). Pigs of all ages can be affected by rectal prolapses, and outbreaks do occur and can be prolonged. The incidence has been reported as 1–10% (Kjar 1976), 0.7–4.7% (Garden 1988), and as high as 10–15% (Becker and Van der Leek 1988). Perfumo et al. (2002) found that rectal prolapse was responsible for 7.7% of deaths that occurred in pigs from weaning to market. Daniel (1975) noted that rectal prolapse in sows could occur in all sizes of units and that the incidence varied from 0.5% to 1%.

Rectal prolapse is sometimes associated with enterocolitis caused by viral, bacterial, parasitic, or mycotic infection (Pfeifer 1984; Straw 1987). In cases where the inflammation is severe and irritation of the rectum occurs, tenesmus results and rectal prolapse may be a sequel. In older swine, urethritis and vaginitis from any cause may lead to straining, which may in turn lead to prolapse of the rectum or vagina or both.

Sudden changes in the diet (e.g., from meal to whey) may lead to occasional cases of rectal prolapse. Constipation caused by chronic water shortage or low-fiber diet may result in straining and rectal prolapse. Other nutrition-related associations that have been reported include high lysine levels (Amass et al. 1995) and lupin bean meal toxicosis (Casper et al. 1991).

Injury to the rectum or urethra from service by the boar may also lead to tenesmus and prolapse. In addition, gradual weakening of the pelvic diaphragm may arise as sows age or during pregnancy as the abdominal contents become heaviest. Rupture of one or more of the supporting structures may then occur, with prolapse of either rectum or vagina or both as a sequel.

Guise and Penny (1990) noted that rectal prolapses occurred when pigs were transported at high stocking density. Rectal prolapse has been described in growing pigs fed therapeutic levels of tylosin in the diet. Likewise, rectal prolapse due to edema has been noted when pigs were medicated with lincomycin. This reaction is frequently observed when swine are first placed on the drug, but clinical signs usually subside within 72 hours (Kunesh 1981). Genetic factors have been suggested (Becker and Van der Leek 1988; Hindson 1958; Saunders 1974).

It is generally agreed that rectal prolapse occurs more commonly during winter months, and there is some evidence to support this (Gardner et al. 1988; Kjar 1976; Prange et al. 1987; Wilson 1984). It has been suggested that cold weather causes pigs to pile, thus increasing the likelihood of prolapses; no objective data have been produced to support this hypothesis.

Rectal prolapse is a common sequel to vulvovaginitis caused by mycotoxicosis (see Chapter 69).

Gardner et al. (1988) noted that pigs of low birth weight (less than 1000g) were more likely to suffer from rectal prolapse later in life. It was hypothesized that low-birth-weight pigs that have fewer muscle fibers at birth have an inherently weaker rectal support mechanism, which may fail when a period of rapid growth occurs. Muirhead (1989) noted that a behavioral aberration, anal nuzzling, in recently weaned pigs led to a rectal prolapse problem (4–6% incidence). Improvement to the climatic environment prevented further cases from arising. An outbreak of anal nuzzling was reported by van Sambraus (1979).
When pigs cough, the rectal mucosa often protrudes temporarily. As with piling, it has been suggested that coughing may precipitate rectal prolapse, but again there are no objective data to support this hypothesis. Indeed, Gardner et al. (1988) could find no relationship between coughing and the prevalence of rectal prolapse. In another study, the prevalence of rectal prolapse was dramatically reduced from 4.7% to 0.7% when weaners (30–35 kg) were placed in a strawed yard for 3 weeks between being held in the second-stage flat deck and the fully slatted finishing accommodation (Garden 1985).

Diarrhea is not a common precursor of rectal prolapse, and in one herd studied by Gardner et al. (1988), an outbreak of transmissible gastroenteritis (TGE) did not increase the prevalence.

Henry (1983) suggested that lack of anal sphincter control due to infection or trauma of pelvic nerve centers such as might occur after docking (especially short docking) or tail biting could lead to rectal prolapse.

Apart from treatment and noting any factors peculiar to each case, it is not considered worthwhile to implement any specific control or preventive measures for sporadic cases of rectal prolapse (Smith and Straw 2006). It is common practice to deal with rectal prolapse in feeder pigs by isolation only, where the prolapse is simply left to resolve naturally in 10–14 days. However, this practice is not good welfare, and a simple nonsurgical amputation technique such as described by Douglas (1985) should be considered. Many surgical procedures for treating rectal prolapses have been described (Chalmin 1960; Daniel 1975; Grosse-Beilage and Grosse-Beilage 1994; Hindson 1958; Ivascu et al. 1976; Kjar 1976; Kolden 1994; Moore 1989; Schon 1985; Vonderfecht 1978).

Some prolapses resolve spontaneously, but more commonly, the tissue becomes traumatized or removed by penmates followed by scar formation as part of the healing process. This can result in rectal stricture (Becker and Van der Leek 1988; Hâni and Scholl 1976; Jensen 1989; Prange et al. 1987; Saunders 1974; Van der Gaag and Meijer 1974; Von Muller et al. 1980) and progressive obstruction leading to marked distension of the colon. In a study of 25 pigs with rectal prolapses that were allowed to heal naturally without treatment, Smith (1980) noted that three developed complete rectal stricture and died; the remainder grew normally, but in every case, there was evidence of partial rectal stricture at slaughter. Rectal stricture can also be a sequel to infection. Wilcock and Olander (1977a) noted that many cases of rectal stricture were preceded by severe enteric disease. *Salmonella enterica typhimurium* was frequently isolated, and ulcerative proctitis, a possible precursor of rectal stricture, was also noted. In later studies, Wilcock and Olander (1977b) produced rectal strictures experimentally by injecting chlorpromazine into the cranial hemorrhoidal artery and suggested that rectal prolapses may be a sequel to ischemic proctitis induced by thrombosis associated with salmonellosis. In an outbreak investigation, Harkin et al. (1982) considered that a strong genetic component was implicated in the etiology of rectal stricture, after ruling-out infectious causes and a lack of a history of rectal prolapses in an outbreak investigation. Treatment of rectal stricture is rarely cost-effective, but a surgical technique has been described (Boyd et al. 1988).

Occasionally, severe intestinal prolapse can accompany vaginal prolapse, via laceration to the vaginal fornix.

Intestinal Obstruction, Impaction, and Hernia. Intestinal impaction and obstruction can occur in a variety of situations—for example, deaths have occurred in piglets maintained on wood shavings or other fibrous materials such as peat due to impaction of the ileum or colon with such materials (Figure 15.1). On occasions, heavy infestations of *Ascaris suum* have been found to cause small intestinal impaction in piglets. Herniation of the intestine is most commonly associated with a patent umbilicus. This occurs when weakened supporting muscles around the navel or umbilical stump impede umbilical closure. Genetic and environmental factors can contribute to this (Searcy-Bernard et al. 1994; Zhao et al. 2009). Suggested environmental factors include excessive stretching of the umbilical cord during farrowing, placing navel clips too close to the skin, and neonatal bacterial infections of the umbilicus. Close inspection often reveals evidence of chronic bacterial infection in growing pigs. Small lesions are of little consequence unless expanded by trauma or infections. The larger, pendulous lesions are often traumatized, with increased risk of intestinal strangulation.

15.1. Obstruction of the colon in a 10-day-old piglet due to impaction with wood shavings.
within the hernia unless the defect is corrected surgically.

Hernias via the inguinal and vaginal rings into the scrotal sac can also occur. Should the gut become incarcerated within the hernia site, life-threatening intestinal infarction occurs. The wisdom of keeping pigs with umbilical or scrotal hernias through to finishing is questionable for welfare and economic reasons. Though there is little specific data on this, one study showed a 15% mortality rate for affected pigs in the finisher and significantly lower growth rates than unaffected pigs (Straw et al. 2009). Up to 50% of the pigs that survive to reach slaughter weight can be condemned for peritonitis (Keenliside 2006).

**Intestinal Torsion and Hemorrhagic Bowel Syndromes.** Torsion of the long axis of the mesentery is a common condition in pigs and leads to rapid death. The torsion may involve the small intestine or both the small and large intestines (Figure 15.2). Rotation is usually counterclockwise when viewed from the ventrocaudal aspect. Torsion is associated with pigs making sudden unpredictable movements, such as sudden deceleration combined with abrupt changes in direction, particularly when the gut is filled—for example, after feeding or drinking a large volume of water, or when the intestines are gas filled due to pigs being on a highly fermentable ration. Once torsion occurs, pigs rapidly develop distension of the abdomen. The intestinal loops, apart from the first 20–30 cm of the duodenum, become very turgid and reddish black in color. The lumen is distended with gas and contains dark red, watery fluid. The mesenteric vasculature is extremely engorged due to obstruction to the venous return.

In pigs with “intestinal hemorrhage syndrome” (also called “hemorrhagic bowel syndrome,” “porcine intestinal distension syndrome,” or “bloody gut”), the intestines have a similar appearance to cases of intestinal torsion, but no obvious displacement of the intestines or mesenteric torsion is detected at necropsy. Typically, this occurs under similar circumstances to cases of intestinal torsion, where pigs receive a highly fermentable ration, particularly fed in liquid form. Feeding fresh whey ad libitum has been associated with significant problems of intestinal torsion and/or “hemorrhagic bowel syndrome,” such that the term “whey bloat” is given to the condition. The hypothesis that such pigs can die due to excessively high intra-abdominal pressure in the absence of intestinal torsion was supported by pressure measurements in the order of 40 mmHg being recorded in pigs immediately after death (Thomson et al. 2007). In biomedical research studies involving pigs, artificially induced pressures of this magnitude were found to cause occlusion of the mesenteric veins and obstruction to venous return with fatal consequences (Gudmundsson et al. 2001). Prevention of “whey bloat” can be achieved by allowing the whey to ferment before feeding, limiting intake to no more than 20% of the ration and feeding it as part of a complete diet to avoid preferential ingestion of excessive quantities. In instances where nonwhey rations are associated with the problem, efforts to reduce the rapidly fermentable properties of the diet or use of “little and often” feeding strategies helps to reduce the incidence of cases. The suggested role of clostridial infection as the cause of this condition has been discounted by many authors. The potential causes and pathogenesis of “porcine intestinal distension syndrome” have been reviewed by Martineau et al. (2008). Other forms of intestinal catastrophe, such as volvulus of a short length of small intestine followed by strangulation of the affected portion, occur more rarely.

**Muscular Hypertrophy of the Ileum.** Muscular hypertrophy of the ileum can be seen as an incidental finding in finishing pigs at slaughter. Approximately 30–50 cm of the terminal ileum is thickened, pale, and firm. Internally, the lumen is narrowed and the mucosa and submucosa are normal, but both internal and external muscular layers are hypertrophied, suggesting that the
process is secondary to a functional obstruction. Some cases show an annular stricture in the terminal ileum or at the ileocecal orifice. Many samples of ileum submitted for examination do not extend to the junction with normal tissue so the cause cannot be confirmed. Although the basis of this condition is unknown, it is possible that a previous focus of inflammation and ulceration has led to stricture formation. Externally, and on palpation, the lesion can be confused with porcine proliferative enteropathy (PPE)/necrotic ileitis associated with *Lawsonia intracellularis* infection. Histopathology readily differentiates the two conditions.

**Intestinal Emphysema.** Intestinal emphysema, pneumatosis intestinalis, or “bubbly gut” is another apparently incidental finding at slaughter (Lazier et al. 2008). The serosal surface shows numerous gas-filled, thin-walled cystic structures along the intestinal wall, in the mesentery, and occasionally, in the mesenteric lymph nodes (Figure 15.3). Lazier et al. (2008) described a 20% incidence of cases from one herd, and although there were no clinical signs of illness in affected pigs, condemnation of the intestines caused significant financial loss for the packer. The pathogenesis of the condition involves gas accumulation in the lymphatics, but the cause is unknown. Possible theories include gas extrusion from the lumen in cases that have suffered from transient bloat associated with liquid/whey feeding, or opportunistic colonization of lymphatics by gas-producing bacteria. The latter theory seems less likely as there is no associated serosal inflammation or edema in affected pigs.

**Infectious Conditions Affecting the Intestines**

Many forms of inflammatory and degenerative changes can affect small and large intestine. Mechanisms of diarrhea include hypersecretion, malabsorption, inflammation, and increased intestinal permeability (Table 15.1). Hypersecretory diarrhea is watery, without gross lesions, and is associated with enteropathogenic *E. coli* and sometimes rotavirus. Entercyte necrosis and loss of surface enterocytes occurs with *Isospora* and some of the *E. coli*, as well as viral infections, including rotavirus and coronaviruses. Fluid exudation is a consequence of epithelial loss and results in watery diarrhea and dehydration. Villous atrophy is particularly associated with coronavirus and rotavirus infections. In these conditions, the intestines are fluid filled and flaccid, with no gross evidence of inflammation. Intestinal erosion and necrotizing, hemorrhagic enteritis in young pigs is often associated with *C. perfringens* type C infection. *Cryptosporidium* (*Cryptosporidium parvum*) infection in neonatal piglets is an infrequent cause of villous atrophy, stunting, and fusion of villi, with diarrhea due to malabsorption. Intestinal ulceration is associated with salmonellosis and sometimes *Lawsonia*. Large intestinal ulcerative typhlocolitis is present with swine dysentery, with *Salmonella* and *Lawsonia* as possible causes as well. So-called button ulcers alert concerns of CSF, but they are also associated with *Salmonella choleraesuis* infection and, infrequently, PDNS. Similar lesions have also been recorded in pigs infected with bovine viral diarrhea virus (Terpstra and Wensvoort 1988). Degeneration of intestinal crypt epithelium is associated with coccidial infection and bovine viral diarrhea virus infection. In coccidiosis, due to *Isospora suis*, damage caused by coccidial development in the villous epithelium will result in villous atrophy, intestinal erosions, and fibrinonecrotic enteritis, mainly affecting the distal jejunum and ileum. The enteric lesions of pseudorabies are characterized by foci of crypt necrosis that extend to necrosis of the mucosa, submucosa, and the muscular layers of the intestinal wall (Narita et al. 1998). Hyperplasia of crypt epithelium is the major feature of *L. intracellularis* infection.

### Table 15.1. Mechanisms of diarrhea

<table>
<thead>
<tr>
<th>Infectious Insult</th>
<th>Primary Pathophysiological Mechanism of Diarrhea</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hypersecretion</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>+++</td>
</tr>
<tr>
<td><em>C. perfringens</em> type A</td>
<td>+</td>
</tr>
<tr>
<td><em>C. perfringens</em> type C</td>
<td>+</td>
</tr>
<tr>
<td><em>C. difficile</em></td>
<td>+</td>
</tr>
<tr>
<td>TGE</td>
<td>+++</td>
</tr>
<tr>
<td>Rotaviruses</td>
<td>+</td>
</tr>
<tr>
<td><em>Salmonella</em></td>
<td>+</td>
</tr>
<tr>
<td><em>Lawsonia</em></td>
<td>++</td>
</tr>
<tr>
<td><em>Brachyspira</em></td>
<td>+</td>
</tr>
</tbody>
</table>

**15.3. Intestinal emphysema (pneumatosis, “bubbly gut”) in a 5-month-old slaughtered pig.** The lesion prevalence was approximately 20% in pigs from this herd (courtesy of M. Hazlett).
and leads to thickening of the mucosa in the ileum and/or colon. While this is generally a widespread and fairly uniform change in the affected areas, occasionally it can cause polypoid-like growths or larger protruding lesions resembling intestinal tumors and, in some cases, fibrinonecrotic enteritis is also present.

Inflammatory cell infiltration occurs in response to any cause of disruption to the epithelial barrier as well as enteropathogenic infections. Mucosal microabscesses are a feature of yersiniosis; both *Yersinia enterocolitica* and *Yersinia pseudotuberculosis* affect pigs. Granulomatous inflammation is associated with viral enteropathogens such as PCV2 and certain coronavirus infections. Hemorrhage into the intestinal tract can occur with acute clostridial enteritis in unweaned piglets. Porcine proliferative hemorrhagic enteropathy (*L. intracellularis*) results in major blood loss into the ileum and is uniquely associated with large clots of fresh blood that can be found in the absence of any obvious intestinal ulceration. Watery, port-wine colonic contents and diarrhea may be seen. Gastric ulcers can give rise to mild subclinical blood loss or major hemorrhage resulting in obvious melena. In ulcerative typhlocolitis due to swine dysentery, there is hemorrhagic colonic content and increased mucus production resulting in mucohemorrhagic diarrhea production containing fresh-looking blood. *Trichurus* ( whipworm) may also cause mucohemorrhagic diarrhea. Fresh blood in feces can also occur in cases that have suffered anorectal trauma, such as rectal prolapse cases. All these hemorrhagic conditions are eminently life-threatening and require prompt diagnosis and treatment where possible. Severe intestinal blood loss occurs in intestinal torsion and intestinal hemorrhage syndrome, but death occurs before fecal hemorrhage is seen.

Diseases affecting the intestinal tract are among the most important economic problems affecting pig production. The prevalence of diseases in pigs varies between countries, farming systems, and units with different health status. Within farms, the disease situation is dynamic, and the prevalence can change quite dramatically between batches for no apparent reason. Multiple enteric infections can occur concurrently, giving rise to complex clinical disease patterns and difficulties in arriving at successful control measures. The major diseases are mentioned only briefly here; detailed descriptions of all are given in respective chapters (Tables 15.2 and 15.3).

### Table 15.2. Differential diagnosis of some common gastrointestinal conditions of swine

<table>
<thead>
<tr>
<th>Cause</th>
<th>Age</th>
<th>Key Clinical Signs</th>
<th>Chapter</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Escherichia coli</em> (ETEC, EPEC)</td>
<td>Neonatal; 1–4 days old</td>
<td>Watery, yellowish diarrhea; dehydration; sudden death</td>
<td>Chapter 53</td>
</tr>
<tr>
<td>Postweaning; 2–3 weeks after weaning</td>
<td>Diarrhea, ill-thrift, deaths; neurological signs, edema, sudden deaths (edema disease)</td>
<td>Chapter 53</td>
<td></td>
</tr>
<tr>
<td>Rotaviruses</td>
<td>1 day to 7 weeks; most frequent at 2–4 weeks of age</td>
<td>Watery to pasty diarrhea; may be subclinical; varying degrees of dehydration</td>
<td>Chapter 43</td>
</tr>
<tr>
<td><em>Clostridium perfringens</em> type C</td>
<td>1–14 days (rarely older)</td>
<td>Hemorrhagic/watery diarrhea; sudden death</td>
<td>Chapter 52</td>
</tr>
<tr>
<td><em>Clostridium perfringens</em> type A</td>
<td>2–10 days (rarely older)</td>
<td>Creamy, watery diarrhea (mild) decreased growth</td>
<td>Chapter 52</td>
</tr>
<tr>
<td><em>Clostridium difficile</em></td>
<td>1–5 days (rarely older)</td>
<td>Creamy diarrhea and dehydration</td>
<td>Chapter 52</td>
</tr>
<tr>
<td><em>Cryptosporidium</em> spp.</td>
<td>3 days to 6 weeks</td>
<td>Mild yellowish diarrhea; varying degrees of dehydration</td>
<td>Chapter 66</td>
</tr>
<tr>
<td><em>Isospora suis</em></td>
<td>5–21 days (sometimes older)</td>
<td>Watery/yellowish diarrhea; dehydration</td>
<td>Chapter 66</td>
</tr>
<tr>
<td>Coronaviruses: TGEV and PEDV</td>
<td>All ages</td>
<td>Profuse watery diarrhea; rapid dehydration; deaths; vomiting often seen</td>
<td>Chapter 35</td>
</tr>
<tr>
<td>Porcine circovirus type 2</td>
<td>6–16 weeks, occasionally older</td>
<td>Ill-thrift, depression, diarrhea; often respiratory or systemic signs</td>
<td>Chapter 26</td>
</tr>
<tr>
<td>Gastric ulceration</td>
<td>Any age after weaning, mainly grow–finish pigs</td>
<td>Asymptomatic or if severe, melena, anemia, pallor, deaths</td>
<td>Chapter 15</td>
</tr>
<tr>
<td><em>Lawsonia intracellularis</em></td>
<td>From approximately 5 weeks old to young adults</td>
<td>Usually sloppy diarrhea; PHE has watery hemorrhagic (port wine color) diarrhea, pale carcass, weakness, ataxia</td>
<td>Chapter 59</td>
</tr>
<tr>
<td><em>Brachyspira hyodysenteriae</em></td>
<td>6 weeks old to adult</td>
<td>Pasty, sloppy diarrhea</td>
<td>Chapter 50</td>
</tr>
<tr>
<td><em>Brachyspira pilosicoli</em></td>
<td>4 weeks to 4 months old</td>
<td>Pasty, sloppy diarrhea</td>
<td>Chapter 67</td>
</tr>
<tr>
<td><em>Salmonella</em> spp.</td>
<td>All ages after weaning (rarely preweaning)</td>
<td>Variable, watery; feces with fibrin, necrotic or blood Flecks; most infections are subclinical</td>
<td>Chapter 60</td>
</tr>
<tr>
<td><em>Oesophagostomum dentatum</em></td>
<td>From weaning to adult</td>
<td>Mild, sloppy diarrhea</td>
<td>Chapter 67</td>
</tr>
<tr>
<td><em>Trichurus suis</em></td>
<td>From weaning to adult</td>
<td>Pasty, sloppy occasionally mucohemorrhagic</td>
<td>Chapter 67</td>
</tr>
<tr>
<td><em>Yersinia</em> spp.</td>
<td>From approximately 6 weeks to 4 months old</td>
<td>Pasty, sloppy reproductive</td>
<td>Chapter 64</td>
</tr>
</tbody>
</table>

ETEC, enterotoxigenic *E. coli*; EPEC, enteropathogenic *E. coli*; PHE, proliferative hemorrhagic enteropathy; TGEV, transmissible gastroenteritis virus; PEDV, porcine epidemic diarrhea virus.
<table>
<thead>
<tr>
<th>Cause</th>
<th>Gross Lesions</th>
<th>Histological Lesions</th>
<th>Common Methods for Laboratory Confirmation</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Escherichia coli</em> (ETEC, EPEC)</td>
<td>Fluid, watery gut content; stomach with milk in neonates; gut and eyelid edema</td>
<td>Mucosal congestion with bacterial attachment to intestinal epithelium; angiotopathy; malacia in brain</td>
<td>Culture, serotype and/or genotype of isolates; PCR detection; tissue IHC</td>
</tr>
<tr>
<td>Rotavirus</td>
<td>Fluid ingesta, pale intestines; sparse stomach contents</td>
<td>Moderate villus atrophy</td>
<td>Virus detection: PCR, antigen-capture ELISA (agELISA), EM, PAGE; antigen-tissue IHC, FAT</td>
</tr>
<tr>
<td><em>Clostridium perfringens</em> type C</td>
<td>Hemorrhagic enteritis; mucosal necrosis; suppurative ulcerative enteritis</td>
<td>Mucosal necrosis, hemorrhage; gram-positive rods associated with lesions</td>
<td>Histopathology; culture with PCR confirmation of beta-toxin gene; beta-toxin ELISA</td>
</tr>
<tr>
<td><em>Clostridium perfringens</em> type A</td>
<td>Watery-creamy intestinal content</td>
<td>Mild, loss of epithelium from villus tips, mild suppurative inflammation</td>
<td>Histopathology; culture with PCR confirmation of beta2-toxin gene; beta2-toxin ELISA</td>
</tr>
<tr>
<td><em>Clostridium difficile</em></td>
<td>Mesocolonic edema; creamy colon contents</td>
<td>Multifocal superficial ulcers and suppuration in colon</td>
<td>Histopathology; toxin detection (ELISA); culture</td>
</tr>
<tr>
<td>Cryptosporidium</td>
<td>Fluid ingesta</td>
<td>None or mild villous atrophy; observe oocysts adjacent to surface epithelium</td>
<td>Histopathology–mucosal smear for oocysts; PCR</td>
</tr>
<tr>
<td><em>Isospora suis</em></td>
<td>Fluid ingesta, mild to moderate fibrinous exudates or necrosis of distal small intestine mucosa</td>
<td>Villous atrophy, fibrinonecrotic enteritis, intracellular coccidial forms</td>
<td>Histopathology; observe immature forms in lesions or mucosal impression smear</td>
</tr>
<tr>
<td>Coronavirus</td>
<td>Thin-walled pale intestine, sparse contents</td>
<td>Severe villous atrophy</td>
<td>Histopathology; detect in feces by PCR, ISH, or by IHC, FAT of tissue; serology</td>
</tr>
<tr>
<td>Porcine circovirus type 2 (PCV2)</td>
<td>Generalized lymph node enlargement, edema of mesocolon, pneumonia</td>
<td>Lymphoid depletion, histiocytic infiltration of lymphoid tissues; granulomatous enteritis</td>
<td>Histopathology; detection by IHC or ISH</td>
</tr>
<tr>
<td>Gastric ulceration</td>
<td>Ulceration and fibrosis of pars esophagea, possible hemorrhage into stomach and blood in intestines; melena</td>
<td>Mild: hyperkeratosis, epithelial loss; severe: full depth necrosis, exposed lamina propria, hemorrhage, granulation, fibrosis</td>
<td>Gross pathology; close examination of the pars esophagus</td>
</tr>
<tr>
<td><em>Lawsonia intracellularis</em></td>
<td>Small or large intestine (ileum most common) has thickened mucosa, sometimes necrotic or ulcerated; in PHE, blood clots in ileum, melena, carcass pale</td>
<td>Enterocyte hyperplasia with cells containing small curved rods (silver stain); in PHE, blood exudation into crypts through intact epithelium</td>
<td>Histopathology; detection by IFA, IHC, ISH on tissue; bacterial detection by PCR (feces or tissue); antibody detection by serology</td>
</tr>
<tr>
<td><em>Brachyspira hyodysenteriae</em></td>
<td>Typhlocolitis with fibrinous exudates, diffuse erosions, and hemorrhage; mucus and blood in colon</td>
<td>Epithelial erosions, crypt mucus–goblet cell hyperplasia, fibrin exudation; large spirochetes present</td>
<td>Bacterial detection by culture or PCR of feces or tissue; histopathology with silver stains, IHC, ISH</td>
</tr>
<tr>
<td><em>Brachyspira pilosicoli</em></td>
<td>Mild to moderate colitis; lesions milder than <em>B. hyodysenteriae</em></td>
<td>Similar to but milder than <em>B. hyodysenteriae</em>; end-on attachment of organisms to surface epithelium seen in some cases</td>
<td>Bacterial detection by culture, PCR of feces or tissue; histopathology with silver stains, IHC, ISH</td>
</tr>
<tr>
<td><em>Salmonella</em> spp.</td>
<td>Fibrino-hemorrhagic, local, or diffuse ulcers, lesions present in small and/or large intestine</td>
<td>Diffuse or focal ulcers; neutrophil infiltration, fibrinonecrotic thrombi; foci of inflammation in liver</td>
<td>Histopathology; bacteria by culture; serotype, phage type; antibody by Mix-ELISA</td>
</tr>
<tr>
<td><em>Oesophagostromum dentatum</em></td>
<td>Erosions, edema, granulomas in cecum and proximal colon</td>
<td>Granulomatous typhlocolitis with nematode parasites</td>
<td>Fecal parasitology; histopathology</td>
</tr>
<tr>
<td><em>Trichuris suis</em></td>
<td>Typhlocolitis, erosions, sometimes mucus or blood</td>
<td>Erosion/ulceration and inflammation associated with migrating or adult phases of infestation</td>
<td>Gross (not visible for 3–4 weeks); histopathology; ova detected in feces 7 weeks prepatent</td>
</tr>
<tr>
<td><em>Yersinia</em> spp.</td>
<td>Mild enteritis and/or colitis</td>
<td>Mild chronic, active enteritis and/or colitis, granulomas, microabscesses</td>
<td>Bacterial detection by culture</td>
</tr>
</tbody>
</table>

ETEC, enterotoxigenic *E. coli*; EPEC, enteropathogenic *E. coli*; PHE, proliferative hemorrhagic enteropathy; TGE, transmissible gastroenteritis; PED, porcine epidemic diarrhea; EM, electron microscopy; PCR, polymerase chain reaction; IHC, immunohistochemistry; FAT, immunofluorescence test; ISH, in situ hybridization; PAGE, polyacrylamide gel electrophoresis; IFA, indirect fluorescent antibody.
Enteric Diseases in Suckling Piglets

In neonatal piglets, ETEC remains a very important disease, with C. perfringens type C causing problems in some geographical areas, most notably in outdoor farming systems (Figure 15.4). Both diseases can be controlled by vaccination of breeding stock provided piglets receive adequate colostrum intake within hours of birth, prior to intestinal “closure.” The availability of breeding stock that is genetically resistant to enteric disease through genetic selection.

Other infections that are commonly reported in young unweaned piglets include rotaviruses and coccidiosis (I. suis), with cryptosporidia also being implicated in some production units. It is relatively rare for Salmonella infection to cause significant enteric disease in unweaned pigs though subclinical infection and shedding can occur as a result of contact with sow feces. The viral diseases TGE and porcine epidemic diarrhea (PED) cause severe morbidity and mortality in susceptible piglets. Vomiting and wasting disease caused by hemagglutinating encephalomyelitis virus is associated with vomiting and wasting in suckling piglets, but it appears to have reduced in prevalence globally over the last 10-15 years. Porcine adenovirus infection is usually asymptomatic but is occasionally associated with diarrhea and vomiting, with reduced growth rates in piglets from 2 to 7 weeks of age. Bovine viral diarrhea virus, though a known pathogen of pigs, is rarely reported. Clostridium difficile is a recognized cause of necrotizing colitis in suckling piglets and may be of potential zoonotic importance. Strongyloides sp. can affect pigs ranging from 10 days of age up to 3 months old. Parasites colonize the anterior half of the small intestine and cause villous atrophy and granulomatous enteritis, resulting in diarrhea and ill-thrift. Enterococcus durans may also be a cause of enteropathy in suckling piglets (Cheon and Chae 1996). The bacteria adhere to microvilli of the enterocytes and, in the event of heavy colonization, cause loss of enterocytes and mild-to-moderate villous atrophy. This can be a primary cause of diarrhea in piglets but more commonly, its role is secondary to other pathogens. Other organisms that have been cited occasionally as causes of diarrhea in piglets include Bacteroides fragilis (Collins et al. 1989) and Chlamydia suis (Rogers and Andersen 2000). Detection of the latter three organisms in porcine fecal samples should be interpreted with caution as all can be carried by normal healthy piglets. New and emerging diarrhea syndromes in suckling pigs can result in major economic losses until the cause and specific control measures can be devised. Investigation of serious diarrhea syndromes merits considerable effort to characterize the condition and determine the cause (Astrup et al. 2010; Gin et al. 2010).

Diseases from Weaning Onward

A number of the aforementioned preweaning infectious agents are also capable of causing disease in young weaners. Postweaning E. coli continues to be a major cause of postweaning diarrhea and mortality, and diseases such as rotavirus, coccidiosis, and cryptosporidiosis can affect piglets up to 6 weeks of age. Edema disease caused by Shiga-toxin (Stx2e)-producing strains of E. coli results in sudden deaths or neurological signs in weaner and grower pigs. Intestinal mucosal lesions are not apparent, although edema of the gastric submucosa, mesocolon, eyelids, and forehead can be prominent. Edema of the mesocolon has also been reported in neonatal piglets infected with C. difficile, and in weaner pigs affected with PMWS.

The disease patterns in swine production units change dramatically when immunosuppression is a feature. The global emergence of PMWS in the first decade of the 21st century dramatically altered patterns of enteric disease. The generalized lymphoid depletion that occurs in weaners with PMWS includes depletion of intestinal Peyer’s patches and mesenteric lymph nodes. In units with PMWS, the age range of susceptibility to diseases such as rotavirus, cryptosporidiosis, and coccidiosis increases, and these enteric diseases play a significant role in the general ill-thrift problems suffered by pigs.

Diarrhea is a feature of PMWS outbreaks and can be a direct consequence of the viral-type enteritis associated with PCV2 infection (Kim et al. 2004; Ségalès et al. 2004). The distal small intestinal mucosa is thickened and may have evidence of necrosis in the ileum and colon, with edema of the mesocolon. Histologically, there is villus atrophy, granulomatous inflammation, and histiocytosis with multinucleated giant cells. The gut-associated lymphoid tissue shows lymphocytolysis and atrophy of the follicles, as also occurs in other lymphoid organs. PCV2 viral inclusions can be visualized microscopically and other antigen
Deposits can be demonstrated readily by immunohistochemistry. Coinfection with other agents such as *Salmonella* species and *Brachyspira* species can be common (Zlotowski et al. 2008). Macroscopically, PCV2-associated enteritis can resemble PPE, and differentiation by histopathology should be carried out (Jensen et al. 2006). Another equally important feature associated with PMWS is mild nonspecific colitis. Often, bacterial cultures fail to show any recognized bacterial pathogen but yield a mixed growth of commensal-type organisms. Histology of the colon shows mixed bacterial infection of crypts and surface epithelium, accompanied by crypt inflammation and goblet cell hyperplasia in chronic cases. This suggests that there is an opportunistic bacterial overgrowth or dysbacteriosis in the colon of affected pigs, possibly associated with altered enteric immune processes.

Through growing and finishing periods, the most important economic-limiting diseases continue to be swine dysentery, PPE, salmonellosis, and porcine colonic spirochetosis. With PPE, caused by *L. intracellularis*, significant progress has been made with the development of serological tests that can be used for herd profiling, and an orally administered vaccine has proved successful in controlling the disease in commercial units. In contrast, there are no commercially available serological tests for swine dysentery or porcine colonic spirochetosis, and to date, studies indicate that vaccines are unsuccessful. The prevalence of *Brachyspira pilosicoli* varies between countries. *Brachyspira* infections are becoming increasingly recognized as a cause of diarrhea and reduced growth rates. The infection may also be mild or subclinical. Mixed infections involving *B. hyodysenteriae*, *B. pilosicoli*, *L. intracellularis*, *Salmonella* sp., and *Yersinia* sp. are common in grow–finish herds with diarrhea and colitis. Achieving the correct diagnosis and finding effective control measures pose significant challenges for the swine veterinarian.

The prevalence of parasitic infections varies considerably between units. Many units have achieved total freedom of enteric parasites, such as *A. suum*, *Oesophagostomum* sp., *Trichuris* sp., and *Hyostrongylus* sp. However, “white spot liver” is commonly reported on abattoir surveys indicating that *A. suum* infection is still a notable health problem in some units (Figure 15.5). Heavy intestinal infestations can cause intestinal obstructions in piglets, with migration of parasites up the pancreatic and bile ducts affecting their outflows. Such piglets are invariably in poor bodily condition and are reported to have poor feed intakes. *Ascaris suum* has been found to severely limit the growth rate and productivity of pigs in units with organic production status where routine anthelmintic use is not permissible.

*Trichuris suis* and *Oesophagostomum* sp. can be causes of colitis that are often overlooked until no response to antibiotic agents results in further investigation. Heavy infestations with *T. suis* can cause mucohemorrhagic typhlocolitis that grossly resembles swine dysentery. Differentiation by gross lesions is difficult until the parasites become visible, starting about 3 weeks after infestation. Diarrhea, dehydration, and chronic ill-thrift can all ensue.

Some *Eimeria* spp. are considered to be potentially pathogenic, causing villous atrophy and enteritis and can affect older pigs when exposed to yards and pasture contaminated with oocysts. Infrequently, coccidiosis (*I. suis* or *Eimeria* sp.) can cause acute enteritis and colitis, including hemorrhage, in naive young adult pigs that are introduced into a heavily infected environment. Similarly, the hemorrhagic enteropathy form of PPE can cause acute hemorrhagic diarrhea and deaths in naive young adult pigs introduced into an infected environment.

An acanthocephalan parasite that can be found in the small intestine of grazing pigs is *Macracanthorhynchus hirudinaceus*, the so-called thorny-headed worm. These parasites can be up to 40 cm in length and attached to the intestinal mucosa by six rows of hooks in the proboscis. It causes granulomatous nodules that can be seen from the serosal surface. Affected pigs suffer ill-thrift and anemia, and occasionally, intestinal perforation occurs, with fatal consequences. *Balantidium coli* is commonly present in the large intestine of pigs. It is a commensal organism but commonly invades the mucosa if other degenerative or necrotizing lesions are present. Other incidental parasites that can occur in the colon are the paramphistomes *Gastrodiscoides* and *Gastrodiscus*. They are not considered to be of any pathogenic significance.

In addition to the common endemic diseases already mentioned, the serious epidemic diseases such as CSF and African swine fever (ASF) have an important enteric component. Suspected outbreaks should be reported and investigated appropriately in order to control the outbreak and limit the spread of infection. TGE and
PED can cause severe diarrhea in all age groups of pigs in addition to the serious morbidity and mortality that occur in suckling piglets in particular. In pseudo-rabies, necrotizing enteritis can affect the distal small intestine in addition to the more commonly recognized lesions of necrotizing rhinitis and tonsillitis. Prevention and control strategies for TGE, CSF, ASF, and PRV vary considerably. Vaccination is used in some countries where disease eradication is not possible due to geographical, social, or political reasons or where there is a constant and uncontrollable threat from wildlife vectors.

The Peritoneal Cavity
The peritoneal cavity is intrinsically involved with the health and normal function of the digestive system. The area of the peritoneal lining is considerable, exceeding the area of skin. When normal, it is smooth and its moisture is maintained by hydrostatic peritoneal fluid that is readily moved in and out of the peritoneal cavity.

The most common congenital abnormalities that affect the peritoneal cavity of pigs are hernias, as discussed earlier in the chapter in relation to intestinal entrapments. Occasionally, the remnants of vestigial structures can be encountered as incidental findings at postmortem examination. Rarely, these can be responsible for intestinal entrapment, with fatal consequences.

Abnormal contents of the peritoneal cavity include blood, excess serous fluid, inflammatory products, intestinal contents, or urine. Severe trauma and rupture of organs can lead to abnormal intra-abdominal contents. Hemoperitoneum associated with a ruptured liver is a common finding in piglets overlain by sows. Ascites can arise for a number of reasons including hypoproteinemia, anemia, uremia, hepatic diseases, heart failure, systemic illnesses, intoxications, and endotoxemia. Discussions of all these circumstances are beyond the scope of this chapter, but the basis of ascites in relation to specific conditions is discussed in the relevant chapters.

Peritonitis associated with systemic bacterial infections is common in growing pigs. These include *Haemophilus parasuis* (Glässer’s disease), streptococcal infections, *Actinobacillus suis*, and mycoplasmal infections. The most important of these is Glässer’s disease in which diffuse fibrinous peritonitis occurs as part of a generalized polyserositis. Pigs that recover spontaneously or following treatment often develop fibrous intra-abdominal adhesions. These impair normal gut function and are thought to cause considerable discomfort. Such cases show reduced appetite and slower growth rates that affect the overall productivity of the unit if significant numbers are involved.

Other circumstances that can give rise to peritonitis are umbilical infection in neonatal piglets and infection associated with castration wounds. A variety of environmental-type organisms such as *E. coli* and *Arcanobacterium pyogenes* are usually involved. Peritonitis can occur secondary to colonic serositis in pigs with overdistension of the organ secondary to rectal stricture, and in pigs with enteritis or typhlocolitis associated with invasive bacterial infections. Rare forms of peritonitis in pigs include intestinal anthrax where the mesentery is gelatinous and hemorrhagic associated with lymphatic spread of infection, and tuberculosis, where lesions are more localized and often adhered to the spleen. In pigs with *Stephanurus dentatus* infection, localized peritonitis with inflammation and edema of perirenal tissue can be caused by larval migration from the liver to the kidneys.

Ossification of the mesentery or the peritoneal lining is seen occasionally in adult pigs. The lesion is usually confined to a focal area and is thought to arise as a long-term consequence of tissue injury or scarring. At the root of the mesentery, there can be radiating spicules of calcification and bone formation. The cause is uncertain, but we postulate that it could result from “stretch and shear” damage, in the event of transient partial torsions at a younger age. Generally, these lesions are limited in extent and of no consequence. However, intestinal obstruction, perforation, and mesenteric torsion involving heterotopic mesenteric boney elements have been described (Forsythe 1987; Sanford and Rehmtulla 1994). In the latter reference, a y-shaped bony lesion measuring approximately 28 cm served as a fulcrum for the mesenteric torsion.

Tumors involving the peritoneal cavity are relatively rare in pigs. Cases of multicentric lymphoma invariably involve the mesenteric lymph nodes in addition to the liver and spleen. Marked edema of the mesentery can result from obstructions to the efferent lymphatic drainage caused by tumor metastasis.

Splenic torsion involving the lengthy gastroplenic ligament in pigs is a common finding. Complete torsions are rapidly fatal as the spleen becomes engorged with blood and ultimately ruptures. Pigs with partial torsions can survive successfully, depending on the amount of spleen affected. The resulting lesions can be seen as incidental findings in slaughtered animals or those that have died from other causes. Typically, one-third to half of the spleen has been entrapped by the ligament and mesentery resulting in chronic adhesions and atrophy of the affected portion of the spleen.

The Liver
Congenital anomalies affecting the pig liver are rare. One recognized condition is a cystic anomaly affecting bile ducts. The liver is enlarged and shows numerous fluctuating cystic lesions containing bile. The enlarged liver causes abdominal distension, and piglets have poor viability.
Traumatic conditions affecting the liver are important. In neonatal piglets, rupture of the liver and fatal hemorrhage is a consequence of trauma, usually inflicted accidentally by the sow. Torsion of one or more liver lobes can affect pigs of any age. It usually affects the left lateral lobe and results in infarction of the lobe. Death occurs due to shock or hepatic rupture and hemorrhage. Hepatosis dietetica is a diet-associated condition in which there is massive hepatic necrosis. Experimentally, concurrent deficiencies of sulfur-containing amino acids, tocopherols, and selenium are required for the development of hepatosis dietetica. The pathogenesis is not fully understood, but it is thought to be associated with the formation of free radicals and their subsequent adverse effects. It affects rapidly growing pigs and causes sudden death. The related condition "mulberry heart disease" in which there is acute cardiomyopathy and myocardial hemorrhage results in congestive heart failure and marked congestion of the liver. Such livers are enlarged, heavy, and turgid on account of congestion. Similar changes can occur with vegetative endocarditis resulting in right-sided circulatory failure, usually associated with chronic streptococcal or Erysipelothrix infection.

Many systemic diseases cause nonspecific changes in the liver, including congestion and inflammatory cell infiltration. Hemorrhages are a feature of septicemia. Salmonellosis, especially S. choleraesuis infection, can cause multifocal white nodules, so-called paratyphoid nodules.

Parasitism is undoubtedly the most common condition affecting the liver. Migrating A. suum larvae cause mechanical damage in the form of hemorrhagic tracts that initiate intense inflammation. Reactive changes, both repair of tissue and hypersensitivity reaction to excretory and secretory products of larvae, cause eosinophil infiltration and fibrosis. “White spot” livers are an economic loss to the farmer (Figure 15.5). In heavy infestations, adult ascarids can migrate up the bile duct or pancreatic duct causing obstruction, jaundice, and cholangitis. Other parasitic infections affecting the liver include Cysticercus tениcollis, the metacestode of the tapeworm Taenia hydatigena (of dogs). Cysts can sometimes be found in the pig peritoneal cavity, often attached to the liver. Immature cysticerci migrate through the liver causing tortuous hemorrhagic tracts before emerging to encyst. Stephanurus dentatus infection results in migratory tracts and hepatitis. Portal phlebitis with thrombus formation in the portal vein is an additional feature following oral infection.

Toxicities affecting the liver can be acute or chronic in nature. Cresol toxicity is caused by exposure to tar compounds that might have been used in the construction of piggery buildings, accidental environmental spillage, or “clay pigeons” used as shooting targets. Lesions include severe hepatic necrosis leading to sudden death. Chronic cresol toxicity results in jaun-

dice, ascites, and anemia on account of chronic, progressive destruction of hepatic tissue. Iron toxicity occurs occasionally in neonatal piglets, with deaths occurring within 24 hours of iron-dextran administration. Toxicity is associated with marginal or deficient vitamin E and selenium status. Iron-catalyzed lipoperoxidation occurs in the liver and muscle resulting in hepatic necrosis and hepatic hemorrhages.

Aflatoxicosis is caused by the use of cereals contaminated with Aspergillus species or Penicillium puberulum. Lesion development is a chronic process in which there is liver hypertrophy and progressive fibrosis. The condition results in reduced growth rates and liver condemnations.

Tumors of the liver are uncommon apart from metastatic lymphoma (Figure 15.6).

The Pancreas

There has been little focus on pancreatic conditions in pigs. Pancreatic hypoplasia is rare and associated with poor growth in individual weaner pigs. The pancreatic duct can be invaded by A. suum in piglets with heavy infestations. This can lead to obstruction of the pancreatic duct resulting in pancreatic necrosis and acute pancreatitis.
ACKNOWLEDGMENTS

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REFERENCES


SECTION II

BODY SYSTEMS

ACKNOWLEDGMENTS

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REFERENCES


INTRODUCTION
Probably no area of veterinary medicine has seen a greater explosion in knowledge than the immune system and its implications in disease and vaccination. In this chapter, we expand on the information provided in past editions by including figures for key concepts important for swine immunity and vaccine responses.

Response starts where microorganisms breach the various barriers and engage the immune system (Figure 16.1). The first line of defense is the innate immune response with phagocytic cells and production of various cytokines, chemokines, and proteins that not only provide antimicrobial protection but also recruit cells through the inflammatory process and activate the acquired immune response.

The acquired immune response with its myriad of B cells, T cells, cytokines, and antibodies provides the pathogen-specific memory with continued duration protective for subsequent infections with the same pathogen. Pattern-recognition receptors (PRRs), including the toll-like receptors (TLRs), monitor pathogen-associated molecular patterns and induce different signaling pathways to activate the immune system against infection.

The innate immune system is typically activated shortly after infection (Figure 16.2). Natural killer (NK) cells, like many cellular components of the innate immune system, have a dual function: an innate response to attack virus-infected cells and cytokine production for assisting in the activation of acquired immunity (Gerner et al. 2009). The acquired immune response in the naïve animal results in T-cell activation, cytokine production, B-cell activation, and antibody production (Figure 16.2).

UNIQUE FEATURES OF THE SWINE LYMPHOID SYSTEM
The swine lymphoid system consists of five major organized tissues: lymph nodes, lymphoid follicles, tonsils, thymus, and spleen (Rothkotter 2009). Porcine lymph nodes are structurally inverted compared with other domestic species. The B-cell germinal centers are located in the interior of the node rather than the cortical regions, and there is a much larger cortical and para-cortical region that normally contains the T cells (Binns et al. 1986; Pabst and Binns 1986). The lymphoid follicle organization of the gut is also unique. Rather than the discrete lymphoid follicles (Peyer's patches [PPs]) seen in the ileum in many species, the pig has discrete follicles in the jejunum and a continuous PP in the ileum (Liebler-Tenorio and Pabst 2006). The continuous ileal PPs are only present for the first few weeks of life and its exact immune role is not clear, although B-cell development may be important.

The tonsils are the entry point for many pathogens to the immune system. The largest tonsils are the tonsils of soft palate, sometimes erroneously referred to as palatine tonsils. The pig also has pharyngeal and lingual tonsils. The tonsils contain many T and B cells in their crypts and on the surface of the tonsil; because of these high numbers of lymphocytes, the tonsil is described as lymphoepithelium. The tonsil has been found to be a very important specimen for the detection of infectious disease agents (Horter et al. 2003).
CONTROLS infection until the adaptive immune system can be activated; it is important in directing the immune system to be activated to produce both antibody and cell-mediated immune responses.

**Physical, Chemical, and Microbial Barriers**

Physical, chemical, and microbial barriers to infection at body surfaces are a very important part of resistance to disease (Figure 16.1). These factors include the epithelial cells, bactericidal fatty acids, normal flora, and the mucous layer, and are modulated by the flow of mucus, low pH, bile, and numerous enzymes.

**Cells**

One of the more important functions of the cells of the innate immune system is phagocytosis to remove pathogens. Phagocytic cells engulf, kill, and digest invading bacteria, and play important roles in controlling viral and fungal infections and in killing cancer cells. The two main types of phagocytic cells are the granulocytes, or polymorphonuclear leukocytes, which include neutrophils, basophils, mast cells, and eosinophils, and the mononuclear phagocytes, which include the circulating blood monocytes and tissue macrophages (Figure 16.3).

Phagocytic cells are capable of all the reactions that are described below for neutrophils. Macrophages also play an important role in processing antigens; they are critical antigen-presenting cells (APCs) that interact with lymphocytes to stimulate cell-mediated and humoral immune responses.

**Granulocytes.** Neutrophils (PMN) are produced in the bone marrow and are released into the blood. The half-life of neutrophils in the bloodstream is approximately 8 hours. In healthy pigs, the neutrophils are eliminated
with bactericidal activity and antibacterial peptides (Sang and Blecha 2009). Neutrophils die after a short time at sites of inflammation. The hydrolytic enzymes are released and contribute to the inflammatory response and tissue destruction. Neutrophil granule proteins induce adhesion and emigration of inflammatory monocytes to the site of inflammation.

The eosinophil is capable of the same phagocytic and metabolic functions as the neutrophils but focuses the host's defense against the tissue phase of certain parasitic infections. Eosinophils are more capable of exocytosis than phagocytosis; that is, rather than ingesting and killing small particles, they efficiently

via the intestinal tract and lungs. Neutrophils tend to marginate in the capillaries by loosely associating with endothelial cells and are activated very early in the inflammatory response (Figure 16.2).

The principal function of the neutrophil is the phagocytosis and destruction of invading microorganisms. The neutrophil must first come into the vicinity of the invading microorganism by the chemotactic attraction of the neutrophil to the site. Chemotactic factors may be produced directly by microorganisms, by the cleavage of complement (C') components, by endothelial activation with inflammatory mediators and chemokines, or by factors released by lymphocytes at the site of infection or inflammation. These factors diffuse from the inflammatory site to form a gradient, cause adhesion molecules in capillary endothelial cell membrane, and result in an increase in neutrophil numbers in the capillaries. Neutrophils enter the tissues and migrate along the gradient toward the site of infection and ingest microorganisms susceptible to phagocytic activity. Most pathogenic microorganisms must be opsonized by the attachment of specific antibody and/or C' to their surface before they can be ingested by neutrophils.

The neutrophil cytoplasm contains membrane-bound lysosomes with numerous hydrolytic enzymes
attach to and kill migrating parasites that are too large to be ingested. Eosinophils are also important in helping to control certain types of allergic responses.

Basophils and mast cells have been associated primarily with allergic reactions because of their binding of immunoglobulin E (IgE). These cells have an important regulatory role. They release inflammatory mediators necessary for the activation of the acquired immune response (Abraham and St. John 2010; Galli and Tsai 2010). In pigs, mast cells have also been shown to have a major effect on intestinal barrier integrity particularly in early-weaned pigs (<21 days) (Moese et al. 2007; Smith et al. 2010). Early-weaned pigs have higher numbers of mast cells in the intestinal lamina propria (LP), and treatment of early-weaned pigs with a mast cell degranulation blocker inhibits intestinal mucosal dysfunction.

Mononuclear Phagocytes: Macrophages and Monocytes. The mononuclear phagocytic system is made up of circulating monocytes, tissue macrophages, migrating macrophages (histiocytes), and dendritic cells (DCs) (Figure 16.3). Monocytes are produced in the bone marrow and released into the bloodstream where they will circulate before migrating into the tissues to become macrophages and DCs (Esquerra et al. 2009). The fixed macrophages are found lining the endothelium of capillaries, particularly in the lungs, as well as in the sinuses of the spleen, bone marrow, and lymph nodes. Tissue macrophages are important for trapping and removing foreign antigens from the bloodstream and lymph as well as serving as APC for T-cell stimulation. Migrating macrophages are derived from blood monocytes and are found throughout the tissues of the body. In certain locations, they differentiate into DCs that present antigens to T lymphocytes. Specialized APCs include the glial cells in the nervous system, Langerhans cells in the skin, and Kupffer cells in the liver. Immature DCs are located in various tissues; upon activation, they migrate to the peripheral lymphoid organs where they mature and become important cells in the activation and differentiation of T lymphocytes (Bautista et al. 2002; Summerfield and McCullough 2009).

Macrophages are capable of all the bactericidal activities described for neutrophils. Macrophages are the second line of innate cell defense. They are slower to arrive at sites of inflammation and are not as aggressive as neutrophils following infection. However, unlike neutrophils, macrophages sustain activity against pathogens, allowing them to kill bacteria that are resistant to killing by neutrophils. This is especially true if the macrophages have been activated by cytokines secreted by T lymphocytes. Macrophages and DCs process antigen and present it to T lymphocytes. This is an essential step in the initiation of a cell-mediated acquired immune response and for facilitating an efficient antibody response by B lymphocytes.

There are specialized tissue macrophages. Alveolar macrophages phagocytize inhaled particles or pathogens, including low numbers of bacteria that they may encounter (Chitko-McKown et al. 1991). After ingesting particles, they leave the alveolus by one of two pathways: through the airways and then moving up the mucociliary escalator, or out of the alveolus between alveolar epithelial cells entering the lymphatic drainage. Alveolar macrophages then enter the local lymph nodes where they present captured antigens to lymphocytes to initiate an immune response. Alveolar macrophages are a major target of porcine respiratory and reproductive disease virus (PRRSV), thus preventing effective antiviral responses and leading to secondary bacterial infections (Gómez-Laguna et al. 2010; Jung et al. 2010).

Pulmonary intravascular macrophages are found adhered to endothelial cells in the vasculature of the lungs (Chitko-McKown and Blecha 1992). They are prominent in pigs and are primarily involved in defense against septicemia rather than protection from respiratory disease. Pulmonary intravascular macrophages that are actively clearing bacteria (especially gram-negative bacteria or free endotoxin) from the bloodstream may release cytokines and arachidonic acid metabolites that contribute significantly to pulmonary inflammation.

Natural Killer Cells. Natural killer (NK) cells are lymphoid cells of the innate immune system and can kill a variety of nucleated cells without previous antigenic stimulation. They are activated quickly (within 1–2 days) following infection (Figure 16.2). Like macrophages, NK cells are an important component of the innate defense mechanisms and participate in activating the adaptive immune response (Figure 16.3).

NK cells in the pig differ markedly from NK cells found in other species in that their activity is mediated by small granular lymphocytes that have the cluster of differentiation 2 (CD2) T-cell marker (Gerner et al. 2009; Kim and Ichimura 1986). Three international workshops have defined the CD antigens for the pig (Haverson et al. 2001; Lunney et al. 1994; Piriou-Guzylack and Salmon 2008; Saalmüller et al. 2005).

The killing activity of swine NK cells is enhanced in the presence of a variety of cytokines: interferon-γ (IFN-γ), interleukin (IL)-2, IFN inducer (poly I:C), human IFN-α, and human IL-1α (Gerner et al. 2009). Stimulated NK cells produce IFN-γ that activate components of the cell-mediated immune system, such as cytotoxic T lymphocytes (CTLs), macrophages, and NK cells, and induce T-helper (Th) cell differentiation to the Th-1 pathway that is important for cell-mediated immunity and memory (Pintaric et al. 2008). Overall, NK cells are an important part of the innate defense mechanisms.
and participate in a cell-mediated immune response by enhanced activity through cytokine activation.

**INNATE IMMUNE FACTORS**

**Defensins**
An important family of molecules that helps to form a chemical barrier to limit infection at epithelial surfaces and attack invading bacteria is the antimicrobial or host defense peptides (HDPs). In pigs, >30 HDPs have been identified and characterized (Brogden et al. 2003; Sang and Blecha 2009). The HDPs are relatively small cationic peptides and are found predominantly at mucosal surfaces and in phagocytic cells. The HDPs vary in structure and antimicrobial activity and can be functionally differentiated during cellular processing. The concentration of some HDPs increases in response to inflammation or microbial infection. It is likely that HDP antimicrobial and immunoregulatory functions are separate, thus opening up major opportunities for new designs for antimicrobial drug alternatives.

**Complement**
The complement (C') system is an enzyme cascade system composed of at least 20 serum proteins. Since the cascade sequence involves enzymes, the C' system is greatly amplified as it proceeds. The components of the mammalian C' system can be divided into the classical pathway, the alternative pathway, and the mannan-binding pathway, and involve the membrane attack complex and regulatory proteins. The C' system mediates the inflammatory response and controls bacterial infections; it plays a prominent role in allergic and hypersensitivity reactions. The classical C' pathway is triggered primarily by antigen–antibody complexes consisting of IgG and IgM. The alternative C' pathway may also be activated by antigen–antibody complexes (IgA and IgE) and by certain bacterial products such as endotoxin or proteases released by tissue damage. The mannan-binding pathway recognizes molecules on the surface of bacteria that differ from those present on the host cells. All three pathways end in the splitting of the third component of C' and start the formation of the membrane attack complex.

Activation of any of these C' pathways causes vaso-dilation and increased vascular permeability resulting in serum components (including antibody and C') entering the tissues to help control infection. C' components produced during activation are chemotactic and attract phagocytic cells to the site of infection. They also coat or opsonize infectious agents to increase their uptake by phagocytic cells. C' components also destroy pig cell membranes and some bacterial cell membranes.

The C' system is important for mediating inflammation and controlling bacterial infections; however, it is also capable of causing serious and even life-threatening damage, if it is activated in an unregulated fashion. Therefore, there are numerous C' regulators that help to control and stop the C' reaction once it has started.

**Toll-Like Receptors**
TLRs are a key component of innate immunity (Uenishi and Shinkai 2009). TLRs are a family of cell surface molecules that bind to various molecules derived from microbes, such as lipopolysaccharide, peptidoglycans, cytosine guanine dinucleotide (CpG)-rich unmethylated oligonucleotides, and double-stranded RNA (Table 16.1). They are the primary method for early detection of, and response to, microbial invasion (Figure 16.2). Binding of microbial components to TLRs initiates an inflammatory response that helps to activate other aspects of innate immunity and to initiate the acquired immune response.

Bacteria-derived vaccine adjuvants can enhance immune response to vaccines through binding to TLRs. As in humans, IL TLRs have been described in swine (Table 16.1). Major efforts are under way to identify functional single-nucleotide polymorphisms (SNPs) that may identify pigs with improved innate responses against pathogens associated with SNPs in TLR genes. Toka et al. (2009) demonstrated that TLR7 and TLR8 agonists indirectly or directly activate porcine NK cells but that optimum levels of activation require cytokine secretion by accessory cells activated by these compounds. Such activated NK cells were cytotoxic against foot-and-mouth disease virus (FMDV)-infected cells in vitro.

**Cytokines: Type I Interferons, Tumor Necrosis Factor-α, IL-6, and IL-8**
Cytokines are small protein or glycoprotein molecules that are secreted by cells and serve as intercellular signaling molecules. All cells of the immune system are capable of secreting and being influenced by cytokines (Figure 16.4). Cytokine secretion is usually transient, occurs in response to specific stimuli, and typically acts locally in low concentrations. A cytokine will only act on a cell that has specific receptors for it; regulation of cytokine receptor expression is an important mechanism for controlling the response to cytokines. Because of the economic and biomedical research importance of pigs, considerable information has been published regarding porcine cytokines (Bailey 2009; Charley et al. 2006; Dawson et al. 2005; Murtaugh et al. 2009). Most porcine cytokines that have been studied are similar to their orthologs in humans or mice.

One group of cytokines is important in mediating innate immunity. This includes the type I IFNs (IFN-α/β) and the proinflammatory cytokines, IL-1β, IL-6, and tumor necrosis factor-α (TNF-α). Sang et al. (2010) showed that porcine type I IFNs comprise at least 39 functional genes with diverse expression profiles and
Toll-like receptors, their ligands, and the effect on the immune response

<table>
<thead>
<tr>
<th>Gene</th>
<th>Ligand</th>
<th>Effect on Immune Response</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>TLR1</td>
<td>Lipopeptides</td>
<td>Response to gram-positive bacteria</td>
<td>Uenishi and Shinkai (2009)</td>
</tr>
<tr>
<td>TLR2</td>
<td>Peptidoglycan, lipoteichoic acid, heat shock protein</td>
<td>Increased expression of TLR2 in the intestinal tissues of gnotobiotic pigs after treatment with <em>Escherichia coli</em>, <em>Lactobacillus fermentum</em></td>
<td>Willing and Van Kessel (2007)</td>
</tr>
<tr>
<td>TLR2, TLR6</td>
<td></td>
<td>Recognition of <em>Mycoplasma hyopneumoniae</em> cell bodies in porcine alveolar macrophages</td>
<td>Muneta et al. (2003)</td>
</tr>
<tr>
<td>TLR3</td>
<td>Double-stranded RNA</td>
<td>Activation TLR3—increased IFN-α expression, decreased PRRSV infection of macrophages; response to viruses</td>
<td>Miller et al. (2009)</td>
</tr>
<tr>
<td>TLR4</td>
<td>Lipopolysaccharide (LPS) (endotoxin), heat shock protein</td>
<td>Response to gram-negative bacteria—LPS and <em>Salmonella enteric</em> serovar Typhimurium or <em>S. choleraesuis</em>; response to damaged host tissue</td>
<td>Burkey et al. (2009b)</td>
</tr>
<tr>
<td>TLR5, TLR9</td>
<td>Flagellin, DNA containing high unmethylated CpG</td>
<td>Upregulation after feeding of <em>Salmonella enteric</em> serovar Typhimurium or <em>S. choleraesuis</em></td>
<td>Burkey et al. (2007)</td>
</tr>
<tr>
<td>TLR6</td>
<td>Lipopeptides</td>
<td>Response to gram-positive bacteria</td>
<td>Uenishi and Shinkai (2009)</td>
</tr>
<tr>
<td>TLR7</td>
<td>Single-stranded RNA</td>
<td>Enhanced transcriptional activation of TLR7-induced genes in swine macrophages; response to viruses</td>
<td>Fernandez-Sainz et al. (2010)</td>
</tr>
<tr>
<td>TLR7, TLR8</td>
<td>Single-stranded RNA</td>
<td>NK cells activated by TLR agonists cytotoxic against foot-and-mouth disease virus infected cells; response to viruses</td>
<td>Toka et al. (2009)</td>
</tr>
<tr>
<td>TLR9</td>
<td>DNA containing high unmethylated CpG</td>
<td>Porcine circovirus 2 infection CpG-TLR9 signaling cytokine inducers and inhibitors; response to bacteria and viruses</td>
<td>Vincent et al. (2007), Wikstrom et al. (2007), Shimosato et al. (2003)</td>
</tr>
<tr>
<td>NOD1</td>
<td>Immunobiotic lactic acid bacteria, peptidoglycan</td>
<td>Induction of proinflammatory cytokines</td>
<td>Tohno et al. (2008a,b)</td>
</tr>
<tr>
<td>NOD2</td>
<td>Muramyldipeptide (MDP) response; immunobiotic lactic acid bacteria</td>
<td>Induction of proinflammatory cytokines</td>
<td>Jozaki et al. (2009), Tohno et al. (2008b)</td>
</tr>
</tbody>
</table>

Adapted from Table 6.2 (Lunney et al. 2010a).

**16.4.** *T*-cell and *B*-cell activation of the adaptive immune response of the pig.
The proinflammatory cytokines (IL-1, IL-6, and TNF-α) are produced primarily by macrophages in response to bacterial infection and require no previous exposure. They may also be produced in response to viral, protozoal, or fungal infections, or tissue damage. The proinflammatory cytokines stimulate the liver to produce acute-phase proteins, stimulate the release of amino acids from muscle tissue, and may induce cachexia or wasting in chronic infections. In addition, they induce fever, loss of appetite, and fatigue if present in high enough concentrations. In low levels, these cytokines promote leukocyte adhesion to endothelial cells and diapedesis of leukocytes into the tissues as well as migration of macrophages and DCs to the secondary lymph nodes, resulting in the activation of the adaptive immune response. Their presence in small amounts is required for effective stimulation of adaptive immune responses. However, in large quantities, they can induce hypovolemic shock and death.

**ADAPTIVE IMMUNITY**

**Lymphocyte Populations**

B and T lymphocytes and their products are the components of the adaptive immune response system. This antigen-driven system requires 2–3 weeks to reach optimal functional capacity after the first exposure to antigen. Upon second exposure to antigen, the specific immune response system reaches optimal activity much more rapidly due to the anamnestic, or memory, response. A major mechanism by which B and T lymphocytes enhance resistance to disease is by activating the innate defense mechanisms (phagocytic cells, NK cells, and C) and increasing their efficiency.

Lymphocyte subpopulations in the blood of pigs differ markedly from other species. Young pigs have high blood lymphocyte counts compared with most other mammals (approximately 107/mL). Porcine T cells can be divided into a number of subpopulations, including the most abundant T cells expressing T-cell receptors (TCRs) with αβ-TCR chains (αβ T cells) and a prominent fraction of T cells with γδ-TCR chains (γδ T cells) (Duncan et al. 1989; Hirt et al. 1990; Saalmüller and Bryant 1994). Pigs and ruminants have a much higher population of γδ T cells in the blood than other mammals that have been studied. γδ T cells do not recirculate between the blood and lymphatic tissues and do not have NK cell activity. Like αβ T cells, these γδ T cells can express CD8α and SLA class II, potential activation markers of T cells, suggesting that they express cytolytic activity or are involved in antigen presentation (Gerner et al. 2009). As in other species, αβ T cells are either SLA class-I-restricted CD8+ CTL, CD4+ Th, or regulatory T (Treg) cells. In pigs, Th cells express other activation-related markers, including CD8-α, SLA class II, and CD45RC. Certain pig Treg cells have a phenotype similar to humans and mice and can suppress proliferation of other T cells and produce IL-10 (Gerner et al. 2009).

Swine αβ T lymphocytes have at least three unusual properties compared with other species (Lunney and Pescovitz 1987). First, approximately 25% of swine peripheral blood T cells express both the CD4 and CD8 antigens on their surface rather than just a single antigen like most other species. It has been suggested that many of these dual-expressing T cells are memory cells; however, the functional significance of having both CD4 and CD8 on the same cells is not known (Pescovitz et al. 1994; Zuckermann and Husmann 1996). Second, the ratio of CD4+ to CD8+ T cells is normally approximately 0.6 in pigs, which is reversed compared with other species. A normal ratio of CD4+/CD8+ in humans is 1.5–2.0. Third, resting CD8+ cells in swine preferentially express class II MHC antigens. The significance of these differences between swine T lymphocytes and those of other species is not completely understood.

γδ T cells are located predominantly along mucosal surfaces, especially as intraepithelial lymphocytes in the intestine, and are thought to be important in protecting mucosal surfaces from infection and perhaps in oral tolerance (Thielke et al. 2003). γδ T cells proliferate in the intestine and actively recirculate through the intestinal lymphatics to the bloodstream and back to the gut. The role of the thymus and intestinal epithelium in the development of γδ T cells is not well understood. A subset of circulating porcine γδ T cells can act as APCs and are capable of producing IFN-γ, proliferating in response to recall antigens in vitro and being cytotoxic (Lee et al. 2004; Takamatsu et al. 2002).

**Lymphocyte Circulation**

The inverted lymph node structure provides for recirculation of lymphocytes from blood to lymphoid tissues and is very important for bringing antigen into contact with lymphocytes for recognition and for facilitating cellular interactions needed for the induction of the immune response. Lymphocytes are produced in the bone marrow, but mature in the thymus (αβ T cells) and the secondary lymphoid tissues (B cells) in the pig. T and B lymphocytes circulate in the blood for approximately 30 minutes before entering the tissue. Lymphocytes in swine and other species enter the lymph nodes through two routes. Lymphocytes, which leave the bloodstream and enter the subcutaneous tissue, are carried to the lymph node in the afferent lymphatics. Lymphocytes may also directly enter the lymph node by adhering to high endothelial cells in the venules of the lymph node, then migrating into the node. In other species, the lymphocytes exit the lymph node in the efferent lymphatics and are carried through the thoracic duct back to the circulatory system (Roth and Thacker 2006). In swine, the efferent lymph contains very few lymphocytes; lymphocytes in the lymph node
class I molecules. T cells do not respond to free soluble antigen or to whole bacteria or viruses; thus, SLA class I and class II molecules play a key role in antigen presentation and have significant influence on the nature of the immune response. The SLA genes are highly polymorphic, that is, differ genetically between individuals (Chardon et al. 1999; Lunney et al. 2009). The type of SLA molecules that a pig inherits has some influence on their immune response to pathogens and their ability to resist some infectious diseases.

In addition to antigen and class II MHC molecule contact, the Th cell requires the presence of cytokines released by the APCs and other T cells, and contact with costimulatory molecules on the surface of the APC for complete activation (Figure 16.4). IL-1, released by macrophages, is a key mediator of the host response to infection through its ability to induce fever and neutrophilia, among other things. IL-1 acts on Th cells to cause secretion of IL-2 that induces T cells to undergo mitosis and clonal expansion. B cells are also capable of processing antigen and presenting it to Th cells on MHC II molecules. During secondary immune responses, B cells are thought to act as APC.

Th cells are critical in initiating the B-cell response resulting in antibody production (Figure 16.4). B cells contact antigen through IgS bound to their surface, which act as the B-cell receptors (BCRs). B-cell antigens do not have to be presented on MHC class II molecules directly reenter the blood in swine rather than go to the next draining lymph node through the efferent lymphatics (Binns et al. 1986) (Figure 16.5).

Lymphocyte subpopulations in swine show a distinct preference for circulation to either gut-associated lymphoid tissues (GALTs) or surface nodes. For instance, mesenteric lymph node cells (both T and B lymphocytes) preferentially home to the gut (Salmon 1986) (Figure 16.6). In swine, approximately equal numbers of lymphocytes in the mammary gland come from GALT or from peripheral lymph nodes. The dual origin of mammary lymphocytes suggests that the local mammary immune response may not depend solely on oral immunization (Salmon 1987).

Cell-mediated Immunity

Antigen Presentation. The induction of clonal expansion and the immune response requires complex interactions of macrophages, T, and B lymphocytes. Macrophages phagocytize and destroy infectious agents, then present antigenic fragments bound to SLA molecules as they contact T lymphocytes. CD4 Th cells can only efficiently recognize foreign antigens that are on a cell surface bound to SLA class II molecules. CD8+ cytotoxic T cells are important for killing cells infected with intracellular pathogens and cancer cells. They recognize foreign antigens that have been processed intracellularly and transported to a cell surface bound to SLA class I molecules. T cells do not respond to free soluble antigen or to whole bacteria or viruses; thus, SLA class I and class II molecules play a key role in antigen presentation and have significant influence on the nature of the immune response. The SLA genes are highly polymorphic, that is, differ genetically between individuals (Chardon et al. 1999; Lunney et al. 2009). The type of SLA molecules that a pig inherits has some influence on their immune response to pathogens and their ability to resist some infectious diseases.

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come in contact with antigen on APC (DCs or macrophages) following their activation in the periphery. B lymphocytes can directly bind antigen that enters the lymph node. Each lymphocyte's TCR or BCR responds to only one specific antigen that it recognizes through its antigen receptor. Swine T-cell specificity is defined by the TCR variable regions as they interact with foreign antigen peptides presented by SLA molecules on the APC (Gerner et al. 2009; Piriou-Guzylack and Salmon 2008).

The vast majority of lymphocytes that contact an antigen in the lymph node cannot respond to it. Thus, in an animal that has never been exposed to a particular infectious agent before, there are relatively few lymphocytes in each clone that can recognize a particular antigen. The first step in producing an effective primary immune response is expansion of the clone of lymphocytes that recognize the antigen. Within a few days, there will be enough lymphocytes in the clone to mount an effective humoral and/or cell-mediated response.

by macrophages; although optimal B-cell response to antigen requires Th-cell release of cytokines and contact with costimulatory molecules on the Th-cell surface. This Th-cell help is needed for B-cell mitosis and clonal expansion and for switching the class of antibody produced from IgM to IgG, IgA, or IgE.

**Clonal Selection and Expansion.** An important concept that is basic to understanding the immune response is the clonal selection process. Each mature T or B lymphocyte in the body has receptors on its surface that it uses to recognize antigen. All of the antigen receptors (TCR and BCR) on one lymphocyte recognize exactly the same antigen. All of the lymphocytes that recognize exactly the same antigen make up a “clone,” and they have all arisen from the same ancestor cell. Lymphocytes are in a resting stage as they circulate through blood and migrate through the lymph nodes and reenter the bloodstream. In the lymph nodes (or other secondary lymphatic tissues) T lymphocytes come in contact with antigen on APC (DCs or macrophages) following their activation in the periphery. B lymphocytes can directly bind antigen that enters the lymph node. Each lymphocyte's TCR or BCR responds to only one specific antigen that it recognizes through its antigen receptor. Swine T-cell specificity is defined by the TCR variable regions as they interact with foreign antigen peptides presented by SLA molecules on the APC (Gerner et al. 2009; Piriou-Guzylack and Salmon 2008).

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immune response. If the animal has been exposed to the antigen previously, the clone of lymphocytes that has already been expanded, so fewer cycles of cell division are required to produce enough lymphocytes to mount an immune response, as occurs by vaccination or previous pathogen exposure, leading to faster protection against infection. The cells present in the expanded clone are called memory cells. If the previous exposure has been relatively recent, there will still be circulating antibody and effector T lymphocytes that can act immediately to control the infection.

**Th-1, Th-2, and Treg Cells.** Cytokines secreted by macrophages, DCs, and other T cells play crucial roles in the initiation and maintenance of immune responses against both viral and bacterial pathogens in pigs (Dawson et al. 2005; Thanawongnuwech and Thacker 2003; Thanawongnuwech et al. 2000, 2001; Zuckermann et al. 1998) (Figure 16.4). Similar to other species, the CD4+ T cells differentiate into Th cells with characteristic cytokine secretion profiles includes the production of IFN-γ by Th-1 cells, activating macrophages and stimulating T- and B-cell proliferation. The role of IL-4 in Th-2 cells in swine is controversial (Murtaugh et al. 2009). Release of IL-10 is associated with the induction of one subset of Treg cells and is important in suppressing macrophage function and maintaining homeostasis of the respiratory tract. Other Treg cells (CD25+FoxP3+) have been identified in pigs (Silva-Campa et al. 2010), but assessments and details of specific interactions remain to be elucidated. The timing and balance between expression of cytokines following PRRSV infection clearly affect the efficiency of the antiviral response and likely viral persistence (Charerntananakul et al. 2006; Lunney et al. 2010b; Suradhat and Thanawongnuwech 2003).

A group of cytokines regulates lymphocyte activation, growth, and differentiation. IL-2 stimulates T and B cells that have recognized antigen to proliferate and activates NK lymphocytes to have increased cytotoxic activity. IL-4 is important for effective IgE-mast cell-eosinophil inflammatory reactions required to control some parasites and may result in allergic signs to non-parasite antigens. In other species, IL-12 activates NK lymphocytes and induces CD4+ cells to differentiate; in swine, the lack of upregulation of the IL-12 receptor means that such activation is less prominent (Solano-Aguilar et al. 2002). IFN-γ causes cells to be resistant to virus infection (similar to IFN-α/β) and is a potent activator of macrophages, neutrophils, and NK cells. TNF-α often acts synergistically with IFN-γ to activate phagocytic cells. TNF-α can also activate endothelial cells resulting in diapedesis of leukocytes into sites of inflammation.

Another group of cytokines, the colony-stimulating factors (CSFs), stimulate hematopoiesis through the expansion and differentiation of bone marrow progenitor cells. They include IL-3, which stimulates the production of all of the types of leukocytes; granulocyte-macrophage CSF(GM-CSF) that stimulates the production of granulocytes and macrophages; and granulocyte CSF (G-CSF) that stimulates the production of granulocytes only. The CSFs also enhance the antimicrobial activities of mature neutrophils and macrophages.

**Acquired Immune Defense Mechanisms.** Soluble products released by stimulated lymphocytes mediate important components of lymphocyte activities in host defense. T lymphocytes are the predominant population of cells that secrete cytokines as well as being cytolytic to abnormal cells through secretion of perforins and granzymes. Antibodies produced by B cells are specific for the antigens to which they are induced whereas cytokines are not. The cytokines produced during an immune response play an important role in orchestrating host defense against pathogens partially through their direct activities and partially by enhancing the activity of both the innate immune system (i.e., C′, phagocytic cells, and NK cells) and the adaptive immune response by Th cells as described earlier.

CTLS are an important part of the cell-mediated immune response to virus infection and tumors. CTLs are CD8+ and only recognize antigen associated with MHC class I molecules on a cell surface. MHC class I molecules present peptide antigens derived from proteins synthesized within the cell, such as viral proteins. CTLs directly attack host cells that have foreign antigen (e.g., viral antigen) presented on MHC class I molecules on their surface; they do not attack free bacteria or viruses. CTL activity specific for hog cholera virus, African swine fever virus, and pseudorabies virus (PRV) has been demonstrated in pigs that have recovered from infection (Martins et al. 1993; Pauly et al. 1995; Zuckermann et al. 1990) but no CTL responses to PRRSV (Costers et al. 2009). CTL cells kill target cells by making direct contact, releasing granzymes onto the cell surface, and inducing apoptosis (programmed cell death) in the target cells. Production of cytokines, including IL-12 and IFN-γ, by Th-1 cells are required for the activation of CTL and elimination of cells infected with intracellular pathogens, especially viruses.

**Humoral Immunity Immunoglobulins.** Swine B-cell development is a function of immunoglobulin (Ig) gene rearrangement and modification (Butler et al. 2009a,c; Schroeder and Cavancini 2010) (Figure 16.7). After birth, B-cell development begins in the bone marrow and is independent of antigen stimulation. With development, the fate of the B-cell becomes increasingly dependent on its response to antigen. Immature B cells express IgM but leave the bone marrow and become mature B cells that begin to express both IgM and IgD (Figure 16.7).
The B cells recirculate through the blood, the secondary lymphoid organs, and the bone marrow. Encounter with cognate antigen can cause the cell to become a memory B cell or a plasma cell. B lymphocytes from clones that have never been stimulated by antigen have monomeric IgM on their surface, their BCR; all of the IgM molecules on one B cell are specific for the same antigen. When stimulated by antigen and cytokines produced by CD4+ Th cells, a B cell begins to undergo mitosis resulting in many more B cells with IgM recognizing the same antigen; some differentiate into plasma cells that secrete IgM. As the antigen-specific IgM level begins to increase in the blood, activated Th cells produce the cytokines that signal B cells to undergo class switch from IgM to IgD, IgG, IgA, or IgE production (Crawley et al. 2003). These B cells then rearrange their genetic material to produce antibody molecules with the same antigenic specificity (i.e., the same light-chain structure and variable portion of the heavy chain) but of a different antibody class (i.e., the constant heavy portion of the antibody molecule is changed). Changing the antibody class gives the antibody molecules different properties. The class of Ig that the Th cells cause the B cells to switch to depends to a large extent on the nature of the antigen and the location in the body where the antigen was trapped; Th cells located in lymph nodes and the spleen tend to induce B cells to switch to IgG production. Those located in PPs or under other mucosal surfaces tend to induce B cells to switch to IgA and/or IgE production, depending on the nature of the antigen and the genetic predisposition of the individual.

The molecular cascade involved in swine Ig expression has been explored in depth by Butler and his colleagues (Butler 2009a–c; Lunney et al. 2010a). Regions of each Ig heavy-chain gene are encoded by genes (e.g., IGHV, IGHD, IGHJ, IGHG1) that must be joined as illustrated in Figure 16.7 to form the mature IgG antibody molecule. There are six IgG subclass genes in swine. As in other mammals, major differences among swine subclasses are located in the hinge region. Evolutionary divergence of subclasses appears to have been through a combination of gene duplication and genomic gene conversion. B-cell lymphogenesis is initiated when lymphocyte stem cells begin to rearrange their Ig genes. For IgG, this starts with IGHD to IGHJ (D-J) rearrangements followed by rearrangement to an IGHV gene. Recombination–activating gene (RAG)-mediated somatic hypermutation of Ig gene segments involves the excision of the intervening sequences.
between exons. Because swine have only two functional IGDH segments and one functional IGJH segment, the process of B-cell lymphogenesis is much easier to follow than in mice or humans.

Antibody molecules have a variety of activities in host defense, although they alone cannot kill infectious agents. Antibody molecules can coat infectious agents to prevent them from attaching to or penetrating host cells, agglutinate infectious agents to reduce their infectivity, and directly bind to and neutralize toxins. A very important function of antibody is that it marks infectious agents for destruction by C′, phagocytic cells, and/or cytotoxic cells.

**Classes of Immunoglobulins.** Characteristics of the various classes of porcine Iggs were thoroughly reviewed in a previous edition of this book (Porter 1986) and in more recent reviews (Butler et al. 2009b,c; Crawley et al. 2003). IgG is the predominant Ig class in the serum of the pig and other species, accounting for more than 80% of the Ig in serum and colostrum. As in other mammals, IgA is the major mucosal Ig for swine, including in mature milk (Klobasa and Butler 1987; Porter 1969). The IgA and IgM repertoires were more diverse in ileal than in jejunal piglet PPs, reflecting a more diversified microflora in the ileal PP (Levast et al. 2010). IgM accounts for approximately 5–10% of the total Ig in serum and colostrum; it is a large pentameric protein held together by disulfide bonds. Zhao et al. (2002) affirmed the presence of IgD genes in artiodactyls, suggesting that IgD may have some as yet unknown biological properties, distinct from those of IgM.

The porcine immune system produces far more IgA than any other class of antibody; however, most of the IgA is found on mucosal surfaces rather than in the serum (Figure 16.6). IgA is present in swine serum as monomers and dimers (two monomers bound together with a J chain) (Porter and Allen 1972). IgA at mucosal surfaces is mostly dimeric IgA with a J chain and associated secretory component (see the section on “Mucosal Immunity”).

Porcine IgE has been shown to have the same physicochemical properties as other species, including the characteristic of losing biological activity when serum is heated to 56°C (Roe et al. 1993). A polyclonal antiserum for porcine IgE inhibited a passive cutaneous anaphylaxis reaction, identified a sparse population of plasma cells in the LP of the gut and mesenteric lymph nodes of parasitized pigs, and reacted with human IgE in Western blotting.

**Polyclonal and Monoclonal Antibodies.** Antibody produced by an animal in response to an infection or vaccination is polyclonal and recognizes multiple antigens. Infectious agents are complex antigens with many different antigenic specificities (epitopes) on their surface; therefore, they stimulate many clones of B and T lymphocytes to respond. This results in a heterogeneous mixture of antibodies that recognizes a wide variety of surface molecules on the microorganism. The broad spectrum of antibodies that are produced and are present in the serum is most helpful to the animal in overcoming infection. It is sometimes a disadvantage, however, if one wishes to use the serum for developing diagnostic reagents. The polyclonal antibodies produced in response to one infectious agent may cross-react with another infectious agent and thus interfere with the specificity of the assay.

Monoclonal antibodies (mAbs) are commonly produced in research laboratories and often overcome many of the disadvantages of polyclonal antisera for diagnostic and (less commonly) for therapeutic purposes. Mouse mAbs are the result of expansion of one clone from a single B lymphocyte and therefore are all identical and specific for the same antigenic determinant. Since mAbs can be selected for their specificity, this reduces the problem of cross-reactivity between microorganisms in diagnostic tests. In the future, mAb produced against a protective antigen on a microorganism could be used in therapy or for prevention of disease. Since mAb can be produced in very high concentrations and purity, a much lower volume of mAb compared with a polyclonal antibody solution can be used to passively immunize animals. This reduces the risk of serious reaction to the passively administered antibody and its extraneous protein. Just as mouse mAb can be humanized, it is expected that in the future, mAb will be modified for use as pig therapeutics (Presta 2008).

**MUCOSAL IMMUNITY**

**Mucosal Immunity: Role of the Mucosal Epithelium**

The mucosal immune system provides the first immune defense barrier for over 90% of potential pathogens. The gut mucosal immune system alone contains more than 10^{12} lymphocytes and has a greater concentration of antibodies than other tissue in the body (Burkey et al. 2009a). It must not only protect against harmful pathogens but also tolerize the immune system to dietary antigens and normal microbial flora. The mucosal immune system is very rudimentary in the newborn pig and gradually develops in four stages over the first 6 weeks of life (Table 16.2).

The mucosal immune system includes not only immune cells but the epithelial cells of the mucosa that help with antigen recognition and immune modulation. The epithelial cells are coated with mucus–glycocalyx layer that helps protect cells but at the same time the epithelial cells of the mucosa are continually in contact with commensal and pathogenic organisms. The epithelial cells have TLR but not on their surface; rather, they are found on the inner cell membranes and
Table 16.2. Stages in the development of the mucosal immune response in the neonatal pig

<table>
<thead>
<tr>
<th>Stage</th>
<th>Pig Age</th>
<th>Immune Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>The newborn pig</td>
<td>Rudimentary Peyer’s patches</td>
</tr>
<tr>
<td></td>
<td>1 day to 2 weeks</td>
<td>Small numbers of mucosal APCs and T cells</td>
</tr>
<tr>
<td>2</td>
<td>2–4 weeks</td>
<td>Appearance of mature helper T cells in lamina propria</td>
</tr>
<tr>
<td>3</td>
<td>4–6 weeks</td>
<td>Expansion of B-cell repertoire to IgA+ B cells in intestinal crypt areas</td>
</tr>
</tbody>
</table>

Adapted from Bailey et al. (2005b) with permission from Elsevier and Bailey and Haverson (2006).

will only be activated when the cell is infected (Philpott et al. 2001). Epithelial cells express chemokines like CCL25 that is chemotactic and binds the chemokine receptor CCR9 on mucosal system T cells (Cheroutre and Madakamutil 2004) (Figure 16.6). The production of CCL25 by epithelial cells recruits these lymphocytes to the LP and into mucosal epithelium.

**Intraepithelial Lymphocytes and Lamina Propria Immune Cells**

Intraepithelial T lymphocytes are important mediators of immunity at mucosal surfaces (Figure 16.6) (Burkey et al. 2009a; Dunkley et al. 1995). This is especially true for respiratory and enteric infections. T lymphocytes also play a role in immunity in the intestinal tract. Pigs have high numbers of intraepithelial lymphocytes that are predominantly γδ T cells and CTLs (Salmon 1987; Thielke et al. 2003). These intraepithelial cells appear at 4–6 weeks of age (Table 16.2). CTLs in contact with intestinal epithelial cells are likely to be important in destroying virus-infected epithelial cells. The γδ T cells proliferate in the intestine and recirculate through the lymphatic and blood vessels back to the intestine. They can produce IFN-γ, be cytotoxic, and act as APCs through MHC II molecules (Lee et al. 2004; Takamatsu et al. 2002).

The LP is the thin layer of connective tissue that lies beneath the mucosa epithelium of the respiratory, gastrointestinal, and urogenital tracts. Besides the smooth muscle cells, blood vessels (including high endothelial venules [HEVs]), and lymphatics, the LP contains many immune cells including macrophages, DCs, neutrophils, mast cells, and lymphocytes. DCs are the first cells to appear in the LP, increasing in the first few weeks. Mast cells also appear in the LP within the first few weeks, and their numbers are much higher in early-weaned pigs (<21 days) than in pigs weaned at 28 days (Moeser et al. 2007; Smith et al. 2010). LP lymphocytes appear within the first week of life while mature Th cells appear at 2–3 weeks of age (Table 16.2). LP T lymphocytes require more signals for activation, produce higher levels of cytokines, and are memory cells; CTLs appear later, at 4–6 weeks of age, and are also memory cells. B cells first appear near the crypt areas in the LP at 2–4 weeks of age (Table 16.2) and IgA+ B cells do not appear until 4–6 weeks of age (Figure 16.6; Table 16.2).

Homing is an important function of LP lymphocytes (Bailey 2009; Cheroutre and Madakamutil 2004). After T and B cells recognize antigen and mature in mucosal lymphoid follicles like PPs, they express adhesive molecules like α4β7 integrin. The lymphocytes then travel to mesenteric lymph node, enter the blood circulation, and home back to the LP where the lymphocyte adhesion molecule binds to homing receptors like mucosal addressin cell-adhesion molecule 1 (MADCAM1) on the HEV and the lymphocytes emigrate out of the venules into the LP (Figure 16.6).

**Secretory IgA**

The predominant Ig secreted by the mucosal immune system is IgA. Dimeric IgA is secreted by plasma cells in the LP, binds to the polyimmunoglobulin receptor on the basal membrane of mucosal epithelial cells, and is transported to the mucosal surface of the epithelial cell. The cleavage product is called the secretory component and remains bound to the dimeric IgA. The secretory component is important for protecting the IgA molecule from proteolytic enzymes and also serves to anchor the IgA into the mucous layer so that it forms a protective coating on the mucosal surface (Figure 16.6).

Secretory IgA plays an important role in immunity at mucosal surfaces by agglutinating infectious agents, preventing attachment of infectious agents to epithelial cells, and neutralizing toxins. Other components of the immune response may also be important in protection against various types of infection at mucosal surfaces. For example, neutrophils in the pig can migrate into the intestinal lumen in large numbers in response to antigen–antibody complexes. The recruitment of neutrophils into the intestinal lumen is dependent on the presence of circulating IgA antibody, collostral antibody, or locally induced IgA class antibody. The migration of neutrophils into the lumen of the gut and their subsequent destruction has been shown to result in an increased concentration of lactoferrin, lysozyme, and cationic proteins. These substances may also contribute to immunity to bacterial infections in the gut.

**Organized and Diffuse Mucosal Lymphocytes**

Organized mucosal-associated lymphoid tissues (MALTs) are widely distributed in mucosal surfaces throughout the body (Liebler-Tenorio and Pabst 2006).
MALT is the initial induction site for mucosal immunity for antigens that are sampled from mucosal surfaces. These mucosal aggregates or follicles of B cells, T cells, and APCs are covered by epithelium that contains specialized epithelial cells called dome cells or M cells that are found in the gut and bronchus-associated lymphoid tissues (BALTs) (Figure 16.6). These dome cells pinocytose antigen and transport it across the epithelial layer. The antigen may then be processed by APCs and presented to T and B lymphocytes. These follicles are organized like lymph nodes with T-cell areas and B-cell germinal centers. The lymphocytes that emigrate from these organized areas into the surrounding LP are referred to as diffuse lymphocytes (Bailey and Haverson 2006). The hallmark of this system is that local stimulation will result in memory T and B cells in the nearby mucosal tissue but also in other mucosal tissues.

Common Mucosal System

Lymphocytes can be divided into two populations: (1) those that circulate between the bloodstream and the systemic lymphoid tissues, and (2) those that circulate between the bloodstream and lymphoid tissues associated with mucosal surfaces. In the mucosal lymphoid tissues, mature T cells and B cells that have been stimulated by antigen and induced to switch to produce IgA will leave the submucosal lymphoid tissue and reenter the bloodstream. These lymphocytes will exit the bloodstream through HEV as described above and locate in the LP (Figure 16.6). The B cells will differentiate into plasma cells that will secrete dimeric IgA. Many of these cells will return to the same mucosal surface from which they originated (Bailey 2009, but others will be found at different mucosal surfaces throughout the body. This homing of lymphocytes to other MALT sites throughout the body is referred to as the “common immune system” (Figure 16.5). Therefore, oral immunization can result in the migration of IgA precursor cells to the bronchi and subsequent secretion of IgA onto the bronchial mucosa. There is a special affinity for lymphocytes, which have been sensitized in the gut of the sow to migrate to the mammary gland to become plasma cells and secrete IgA into the milk.

Environmental Influences and Development of Mucosal Immunity

Mucosal immunity, particularly the GALT, is greatly influenced by environmental factors on the surface of the mucosa (Bailey and Haverson 2006; Bailey et al. 2005a,b; Inman et al. 2010). The GALT of the pig is poorly developed and undergoes a rapid period of development and expansion (Table 16.2) that is not completed when pigs are weaned commercially at 14–24 days (Lalles et al. 2007). The developing GALT has to make one of two different responses to antigens: an active protective response against pathogens or an active tolerance to commensal organisms and dietary antigens. The two most important critical control points for environmental influences are immediately after birth and at weaning (Bailey et al. 2005a). Colostrum is important for gut development and growth and non-antigen-specific immune development (Bailey and Haverson 2006). Colostrum is also important for providing pathogen-specific Ig. Colostrum contains high levels of transforming growth factor-β (TGF-β) that has anti-inflammatory effects and accelerates the switch of IgA antibody to common food proteins along with preventing expression of active immune responses and promoting the development of tolerance against nutrient antigens (Bailey et al. 2005a). The presence of commensal microbial flora is absolutely essential for the development of GALT in the neonatal pig (Bailey and Haverson 2006). Studies in rearing conditions using either high hygiene (pigs reared in an isolator) or low hygiene (pigs nursed on the sow) demonstrated that a substantial number of the low-hygiene pigs had a more complex microbial flora similar to older pigs raised in low hygiene, and they had more DCs (Inman et al. 2010). These pigs also had higher levels of IL-4, a cytokine associated with an anti-inflammatory effect and the production of IgA.

The second major environmental control point for GALT is weaning. At weaning, the pig can make active immune responses (Bailey et al. 2005b). The weaning period is characterized by diet change and sometimes low feed intake, poor growth and development, diarrhea, and increased risk for disease from enteric pathogens. Unfortunately, the maternal milk factors that modulate the immune response (TGF-β) and provide specific immunity in the newborn (Ig) are no longer available at weaning, and the balance between tolerance and active immunity at weaning is disturbed. The magnitude and severity of this “weaning” GALT crisis is dependent on how much the immune system was expanded during the preweaning period (Bailey et al. 2005a). Unfortunately, the point where the production system determines the weaning age and the point where the immune system is ready for “weaning” are not the same, and managing the immune system for optimal disease prevention at early-weaning ages will continue to be problematic.

IMMUNITY IN THE FETUS AND NEONATE

All components of the native and acquired immune systems of the pig develop in utero and are functional at birth. However, they are generally less efficient than in the adult (Hammerberg et al. 1989). Since the normal newborn piglet has not yet been exposed to antigen, humoral and cell-mediated immune responses to infectious agents have not yet been developed (Table 16.2; Figure 16.8). After exposure to infectious agents, it will take 7–10 days for a primary antibody or cell-mediated immune response to develop (Figure 16.2).
During this time, resistance to infection depends on the actions of the innate defense mechanisms and antibody that is passively transferred from the sow to the piglet. In the pig, there is virtually no transfer of antibody across the placenta due to the epitheliocorial placentation of the sow; there are several tissue layers between maternal and fetal circulation that effectively prevents all antibody transfer. In the sow, as in other large domestic species, passive transfer of antibody from mother to offspring occurs through the colostrum. The sow concentrates antibody in the colostrum during the last days of gestation. This antibody is largely transferred intact across the gut epithelial cells into the circulation of the newborn piglet. The passive transfer of antibody from sow to piglet in the colostrum and milk is very important for neonatal survival and is discussed in more detail below.

**Innate Defense Mechanisms**

The newborn piglet has low levels of hemolytic C’ activity that is related to birth weight; heavier pigs have significantly higher C’ concentrations in the serum (Rice and L’Ecuyer 1963). Piglets allowed to suckle colostrum have higher titers of hemolytic C’ than colostrum-deprived piglets during the first 3 weeks of life. The level of natural IFN-α production by porcine blood mononuclear cells (PBMCs) is low at birth and gradually increases until adult age, with a significant increase around puberty (Nowacki et al. 1993).

Phagocytic cells are present in newborn animals but generally have reduced phagocytic activity as compared with adult animals (Osburn et al. 1982). Alveolar macrophages from 1-day-old pigs had reduced oxidative killing mechanisms compared with adult pigs; by 7 days of age, these aspects of alveolar macrophage function had reached adult levels (Zeidler and Kim 1985). Neonatal pigs have low numbers of pulmonary intravascular macrophages that can increase up to 14-fold by 30 days of age (Winkler and Cheville 1987). Since phagocytes depend on C’ and/or antibodies to opsonize many infectious agents, the overall efficiency of phagocytosis may be reduced due to inadequate levels of C’ and antibodies. Neutrophils from fetal pigs have been shown to have ADCC activity that is comparable with that of adult pigs. Neutrophils from neonatal pigs have also been shown to rapidly emigrate into the lumen of the gut in response to the presence of E. coli and colostral antibody (Sellwood et al. 1986; Yang and Schultz 1986).

**Acquired Immune Mechanisms**

The percentage of CD2’, CD4’, and CD8’ blood T lymphocytes increases with age over the first several weeks of life in specific pathogen-free pigs (Bianchi et al. 1992; Joling et al. 1994). The PBMC blastogenic responsiveness to mitogens was low at birth and increased by 4 weeks of age (Becker and Misfeldt 1993). The mucosal lymphoid system is also less developed at birth and matures over the next few weeks of life (Jericho 1970; Ramos et al. 1992). NK cell activity is absent in peripheral blood of fetal pigs and low in pigs of less than 2 weeks of age (Yang and Schultz 1986).

**Passive Transfer in the Neonate**

Due to the epitheliocorial placentation of the sow, pigs are born with almost no serum antibody and absorb colostrum that is three- to fourfold higher in IgG, IgG2, and IgA than serum (Roth and Thacker 1963). During this time, resistance to infection depends on the actions of the innate defense mechanisms and antibody that is passively transferred from the sow to the piglet. In the pig, there is virtually no transfer of antibody across the placenta due to the epitheliocorial placentation of the sow; there are several tissue layers between maternal and fetal circulation that effectively prevents all antibody transfer. In the sow, as in other large domestic species, passive transfer of antibody from mother to offspring occurs through the colostrum. The sow concentrates antibody in the colostrum during the last days of gestation. This antibody is largely transferred intact across the gut epithelial cells into the circulation of the newborn piglet. The passive transfer of antibody from sow to piglet in the colostrum and milk is very important for neonatal survival and is discussed in more detail below.

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Colostrum has approximately the same concentration of IgM as serum. As the pig suckles, the colostrum is replaced with milk, which has fivefold lower IgG content. From 3 days of age until the end of lactation, IgA is the predominant Ig found in sow milk. The majority of milk Ig is synthesized in the mammary gland whereas colostral Ig is mostly derived from serum, although this varies by Ig class.

All three major classes of Ig (IgG, IgA, and IgM) are absorbed from the colostrum into the circulation of newborn pigs (Curtis and Bourne 1971; Porter 1969). IgA, however, is absorbed less efficiently than the other classes of antibody (Hill and Porter 1974; Porter 1973), apparently because porcine colostrum is dimeric IgA lacking secretory component. Neonatal colostrum-deprived piglets have been shown to express secretory component in the gut that tends to localize in the mucus of the crypt areas. Because of the affinity of the dimeric IgA and IgM for secretory component, it has been suggested that IgA and IgM are bound in association with secretory component and held in the mucus of the crypt areas and are, therefore, less efficiently absorbed from the colostrums (Butler et al. 1981). The IgA present in sow’s milk throughout the suckling period may also bind to the secretory component in the crypt areas and provide relatively continuous protection against intestinal pathogens.

Intestinal absorption of Ig from the colostrum normally ceases by 24–36 hours after birth. If pigs suckle normally, the efficiency of absorption decreases with a half-life of about 3 hours (Speer et al. 1959). Lecce and Morgan (1962) found that the period of time that the intestine could absorb antibodies was extended up to 5 days in starved pigs that were maintained by parental administration of nutrients. Therefore, piglets that have not had an opportunity to eat during the first 24–36 hours may still benefit from colostrum ingestion. Neonatal pigs have been shown to absorb colostal lymphocytes from their intestinal tract into the bloodstream (Tuboly et al. 1988; Williams 1993). By 24 hours, cells derived from colostrum were found in the liver, lungs, lymph nodes, spleen, and gastrointestinal tissue. Pigs that had absorbed the colostral lymphocytes had higher lymphocyte blastogenic responses to mitogens than control pigs. It is not clear if the passively transferred lymphocytes also transfer clinically significant cell-mediated or antigen-specific immunity from the sow to the piglet.

**STRESS, IMMUNOSUPPRESSION, NUTRITION, AND IMMUNITY**

**Physical and Psychological Stress: Interactions of the Central Nervous, Endocrine, and Immune Systems**

There is ample evidence that both physical and psychological distress can suppress immune function in animals, which may lead to an increased incidence of infectious disease. Excess heat or cold, crowding, mixing, weaning, limit-feeding, shipping, noise, and restraint are stressors that are often associated with intensive animal production and have been shown to influence immune function in various species (Blecha et al. 1985; Kelley 1985; Westly and Kelley 1984; Yen and Pond 1987). Also, social status, genetics, age, and the duration of stress (chronic vs. acute) have been shown to be important in the pig’s response to stress (Salak-Johnson and McGlone 2007).

The immune system and the central nervous system (CNS) are a bidirectionally linked “two-way street,” each influencing the other (Borghetti et al. 2009) (Figure 16.9). In particular, there is a critical balance that exists between hormones (growth hormone (GH), glucocorticoids (GCs), prolactin (PRL), catecholamines, and insulin), and the proinflammatory mediators (IL-1, IL-6, and TNF-α) of the immune system.

The immune system and CNS interaction is influenced through two pathways of the nervous system: the neuroendocrine (hypothalamic–pituitary axis) and the autonomic nervous systems (hypothalamic–sympathetic) (Borghetti et al. 2009; Salak-Johnson and McGlone 2007). Stress on the CNS affects both inflammatory/innate and adaptive responses through the neuroendocrine activation of the hypothalamic–pituitary–endocrine responses. Activation of the hypothalamus results from either stress (i.e., transportation,
dehydration, handling) or the acute-phase response following infection when proinflammatory cytokines, IL-1, TNF-α, and IL-6, are released (Figure 16.9). This hypothalamic activation then stimulates hormones from the pituitary gland. The hormones released by the pituitary include thyroid-stimulating hormone (TSH), PRL, and GH, which are linked to insulin-like growth factor 1 (IGF-1) activity, and adrenocorticotropin (ACTH), which activates a number of different endocrine systems including the adrenal gland (GCs, i.e., cortisol).

The study of these multisystem endocrine interactions initially focused on the secretion and influence of GC, which suppress several aspects of immune function directly and indirectly by the production of anti-inflammatory cytokines like IL-10. However, pigs are more resistant to the immunosuppressive effects of GCs compared with some other species (Flaming et al. 1994). It is also recognized that there are many mechanisms by which the neuroendocrine system can alter immune function. Neurotransmitters (catecholamines, acetylcholine, neuropeptides, vasoactive intestinal peptide, and substance P) affect the immune system. Catecholamines inhibit IL-12 and enhance IL-10 production (Salak-Johnson and McGlone 2007). Somatotrophic hormones like GH and IGF-1 have a positive effect on the immune response including increased proliferation of immune cells in the bone marrow, production of the proinflammatory cytokines, increased cytotoxic activity of CTL and NK cells, and T-cell development in the thymus to name a few (Borghetti et al. 2009). TSH, GH, ACTH, PRL, and neuropeptides influence the thymus and the development of T cells.

Recently, the importance of adipokines, the cytokines produced by adipose tissue, has been recognized (Borghetti et al. 2009). Leptin, the most well-studied adipokine, has many positive effects on the immune system including survival of T cells in the thymus, increased killing activity in monocytes and neutrophils, and maturation of DCs. Adipose tissue also produces proinflammatory cytokines. Interestingly, runt pigs have higher adipokine gene expression prior to weaning including higher proinflammatory cytokines (Ramsay et al. 2010).

The immune system is capable of altering the activity of the neuroendocrine system (Kelley 1988). Immune activation at very early age (10–16 days of age) resulted in much lighter pigs at 28 days of age (Fangman et al. 1998; Schinckel et al. 1995). Pigs receiving high levels of antigens had decreased weight gain, feed efficiency through 107 days of age, and 5.6 more days to reach 230lb and 3.6 days to reach market weight of 264lb (Schinckel et al. 1995). This likely involves an effect of immune cytokines on IGF-1 production. Activated Th, CTL, and thymic cells, like thymic epithelial cells, can produce hormones like GH, PRL, ACTH, and TSH. The thymic hormone, thymulin, can have a direct effect on the pituitary to release endocrine hormones (Borghetti et al. 2009).

One of the most important areas in immune development affected by stress is Th-1 and Th-2 balance. In most cases, a balanced Th-1/Th-2 response means that both a cell-mediated response (Th-1) and a humoral response (Th-2) will be produced. Skewing it in one direction or the other may be inappropriate for certain pathogens—typically, extracellular organisms require a Th-2 response, while intracellular organisms require a Th-1 response, for eliminating the organism. Mixing, cold stress, heat stress, crowding, restraint, and weaning age all affect the Th-1/Th-2 balance (Salak-Johnson and McGlone 2007).

Weaning is a stressful event for the young pig. Research indicates that weaning before 3 weeks results in long-term negative effects on the immune system and mucosal development (Davis et al. 2006; Hameister et al. 2010; Moeser et al. 2007; Niekamp et al. 2007; Smith et al. 2010). Weaning at 2, 3, or 4, but not 5 weeks, of age has been shown to decrease the in vivo and in vitro response of porcine lymphocyte proliferation to undergo clonal expansion to initiate an immune response (Blecha et al. 1983). These same parameters were suppressed in artificially reared neonatal piglets compared with their sow-reared littermates (Blecha et al. 1986; Hennessy et al. 1987). Early weaning of pigs at 3 weeks of age suppressed the ability of mesenteric lymph node cells to produce IL-2 (Bailey et al. 1992). Immunizations 24 hours after weaning (at 5 weeks of age) decreased the antibody response while vaccinating 2 weeks prior to weaning did not decrease the antibody response (Blecha and Kelley 1981). Early weaning results in prolonged increase in corticotropin-releasing factor (CRF) as well as increases in mast cells, which results in intestinal mucosa dysfunction (Moeser et al. 2007; Smith et al. 2010). However, successful vaccination strategies at the time of weaning are frequently reported in the field.

An area of increased interest is the effect of stress on the pregnant sow and its subsequent effects on the immune system of the developing piglet (Bate and Hacker 1985; Bellinger et al. 2008; Tuchscherer et al. 2002). Cold stress 2 days prior to parturition resulted in increased serum IgG in the piglet but decreased the ability of the piglet to produce IgG. Heat stress in the last 2 weeks of gestation resulted in decreased serum IgG and globulin through day 21 of age (Machado-Neto et al. 1987). Restraint stress (5 minutes a day for 35 days prior to farrowing) resulted in decreased IgG serum levels in the 1- and 3-day-old pigs and the thymus was smaller in 1- and 35-day-old pigs. Further study demonstrated that a variety of T- and B-cell proliferation responses, along with NK killing activity, were lower at 35 days of age in pigs from the stressed sows (Tuchscherer et al. 2002). Another report indicated that tethering (continuous restraint) of sows suppressed...
antibody synthesis, which reduced the amount of antibodies that were transmitted through the colostrum into the blood of the piglets (Kelley 1985).

Social stress (mixing gilts together twice a week for 4 weeks) resulted in significant decrease in the total numbers of white blood cells, lymphocytes, and granulocytes from 26 days of age to study termination at 60 days of age (Couret et al. 2009). It also decreased the CD4+/CD8+ T-cell ratio on day 4. Endotoxin induced TNF-α production on day 60 and increased the mitogen-induced lymphocyte proliferation on D4 and D60. Another social stress study in sows with a similar design demonstrated that there was an increase in pituitary and hypothalamus neurotransmitters of the piglets both at 28 days of age (weaning) and at 60 days of age following another stressor (relocation) (Otten et al. 2010). Although this study did not directly show an effect on the immune system, changes to neurotransmitters can influence the immune system both directly and indirectly (Figure 16.9).

Immunosuppressive Infectious Agents

Many infectious agents are capable of suppressing immune function, particularly the innate response, making the animal more susceptible to secondary infections. For example, infection with Mycoplasma hyopneumoniae, Actinobacillus pleuropneumoniae, virulent or vaccine strains of hog cholera virus, PRRSV, or PRV increases the severity of Pasteurella multocida-associated pneumonia (Fuentes and Pijoan 1986, 1987; Pijoan and Ochoa 1978; Smith et al. 1973). A number of swine pathogens impair or kill tissue macrophages (often alveolar macrophages) and monocytes including A. pleuropneumoniae (Dom et al. 1992; Tarigan et al. 1994), PRV (Chinsakchai and Molitor 1992; Iglesias et al. 1989a,b, 1992), swine influenza virus (SIV) (Kim et al. 2009), PRRSV (Bautista et al. 1993; Charley 1983), and PCV2 (Chang et al. 2006). PRV also inhibits IFN-α (Brukmann and Enquist 2006). Porcine parvovirus replicates in alveolar macrophages, as well as lymphocytes, and has been shown to impair macrophage phagocytosis and lymphocyte proliferation (Harding and Molitor 1988).

PRRSV and PCV2 both modulate the immune response at many levels. Specific details about the pathogenesis and immunosuppression of these two viruses are covered elsewhere in this book. However, it is important to recognize that much of the impact of PRRSV and PCV2 on the swine industry is due to their ability to modulate or alter the ability of the immune system to control other pathogens.

The functions of neutrophils, another innate cell, are compromised following bacterial infection; Mycoplasma hyopneumoniae, Salmonella typhimurium, or Salmonella choleraesuis alters porcine neutrophil function (Roof et al. 1992a,b). In addition, M. hyopneumoniae also appears to induce a preferential Th-2 response that may further decrease the ability to control respiratory pathogens.

Nutritional Influences on Immunity

Immune system functions require energy, protein, vitamins, and trace minerals. Both malnutrition and over-feeding may result in impairment of immune function and increased susceptibility to disease. Swine in modern production typically have a completely controlled diet. Key vitamins and minerals for optimal immune function include vitamins A, C, E, and B complex vitamins, and copper (Cu), zinc (Zn), magnesium (Mg), manganese (Mn), iron (Fe), and selenium (Se). The balance of these constituents is especially important since an excess or deficiency in one component may influence the availability or requirement for another (Wintergerst et al. 2007).

Zn metabolism is greatly influenced by acute-phase reaction (APR) following infection (Borghetti et al. 2009). Zn is an essential cofactor for the thymic hormone thymulin and T-cell development. High levels of GCs and IL-1 and IL-6 result in hypozincemia due to redistribution of Zn among various tissues, particularly the liver, and the consistent loss of Zn in urine and feces. Zn deficiency associated with stress and high levels of GC causes a decrease in the resistance to infection and a continuous imbalance of Th-1/Th-2 favoring Th-2 bias. Zn deficiency also decreased recruitment of naive T cells, NK cell activity, and the precursors of CTL and hypoplasia of lymphoid organs (lymph nodes, thymus, spleen, PPs) (Borghetti et al. 2009).

There are very little research data to predict the optimal diet for immune function for swine. The dietary requirements for optimal immune function may differ from the requirements to avoid deficiencies as judged by traditional methods. In addition, stress or the demands of rapid growth may change dietary requirements for optimal immune function.

VACCINES AND IMMUNITY

General principles regarding vaccine efficacy and vaccine failure will be discussed here. Information regarding protective immunity and vaccination for specific diseases may be found in other chapters of this book.

Developing a Vaccination Program

A swine vaccination program should start with assessment of the disease risks in a particular herd. The common “blanket vaccination” programs suggested for many pathogens are not appropriate for all herds. A careful review of endemic disease agents and risk of external introduction of agents is warranted before recommending a vaccination program. Second, the effect of maternal immunity and the age of the pig must be considered. The relationship is generally linear: The
immune development over older generation adjuvants like alum (Wilson-Welder et al. 2009) (Table 16.3). Therefore, in most instances, if primary vaccination occurs after 3 weeks of age, booster vaccination beyond 3 weeks and even longer will be efficacious. The dogma that revaccination must occur within 2 weeks of the primary vaccination is not true; the anamnestic response will be better if the interval between vaccines is longer.

**Route of Vaccination**
Protecting the animal from infection at mucosal surfaces such as the intestinal tract, respiratory tract, mammary glands, and reproductive tract is especially difficult for the immune system. The antibodies responsible for humoral immunity and the lymphocytes responsible for cell-mediated immunity are predominantly in the bloodstream and tissues, and they are typically not found on the mucosal surfaces. Therefore, while lymphocytes assist in preventing systemic invasion through the mucosal surface, they are often not very effective at controlling infection on the mucosal surface. Even in the lungs and the mammary gland, where IgG and lymphocytes are found in relative abundance, they are not able to function as effectively as in the tissues. Protection on mucosal surfaces is due in large part to secretory IgA, CTL, and $\gamma\delta$ T cells as discussed earlier.

**Adjuvants**
Adjuvants provide several opportunities to improve vaccine performance (Wilson-Welder et al. 2009). They are used with inactivated vaccines to enhance innate immune response and antigen presentation. They can

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**Interval between Vaccinations**
In all animals following vaccination, there is expansion in the populations of responding T- and B-cell clones (Figure 16.10). However, to have a complete and mature immune response, this clonal expansion must not only stop but an active process of cell death (apoptosis) must also occur. This “waning process” allows “culling” by apoptosis of T or B cells that may be poor responders or even cause autoimmunity (Wagner 2007). This whole process from vaccination to achieving mature immune response homeostasis takes at least 3 weeks. This fully developed mature primary response can then be boosted to get a true anamnestic secondary response; typically, swine vaccine primary and booster doses are administered at 2-week intervals. In young pigs, this is done to provide an opportunity to make sure that the pigs develop a primary response in the face of maternal immunity. The adjuvants that are used with most commercial vaccines provide superior

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**Figure 16.10.** Importance of vaccine timing and the booster response (courtesy of D. Topham, PhD, Rochester, NY).
be immunostimulants that can direct the immune response to either Th-1 or Th-2 responses. Adjuvanted parenteral vaccines are often used to overcome preexisting immunity (maternal or active immunity) (Morein et al. 2002). There are more adjuvants available for veterinary use than human use, with the most commonly used adjuvants in swine being alums, oil in water, and carbopol (Table 16.3).

Adjuvants have also been used to enhance the immature immune system and to stimulate Th-1 response to balance the response in young pigs, where the response is skewed to Th-2, or included in vaccination formulation of immunosuppressive pathogens like PRRSV to aid the immune system to develop an efficacious response (Charerntantanakul 2009). Two experimental vaccine systems have demonstrated the ability to break the Th-2 bias in very young pigs. Small DNA sequences called oligodeoxynucleotides (ODNs) containing one or more unmethylated CpG motif (CpG ODN) have been shown to be potent stimulators of Th-1 immune responses when used as vaccine adjuvants. One-day-old piglets vaccinated with attenuated PRV with an adjuvant system containing CpG ODN induced significant cellular proliferation and IFN-γ production in response to vaccine antigen within the first week after vaccination (Linghua et al. 2007). This vaccine also induced significant antibody titers. An even better Th-1 immune response was obtained by adding a plasmid expressing the proinflammatory Th-1 inducing porcine IL-6 to CpG ODN adjuvanted PRV vaccine (Linghua et al. 2006).

### Use of Autogenous Vaccines and “Planned Exposure”

Autogenous vaccines are comprised of agents or antigens that are derived from the individual herd in which they are to be used. The use of autogenous vaccines for a variety of bacterial and viral agents is a common practice in swine medicine (Chase 2004). The greatest value of autogenous vaccine is when diagnostic and molecular techniques are used to differentiate the field strains from those organisms in commercial vaccines. The science-based application of autogenous vaccines is important for prevention of certain diseases; any cost savings from using autogenous vaccines should not be the major factor in selecting an autogenous vaccine over a commercial vaccine.

Infectious agents do not uniformly infect all animals simultaneously. The intention of a planned or controlled exposure procedure is to expose all animals in a population to a live infectious agent derived from the herd while minimizing the impact of any associated disease. From the immunology perspective, this practice is simply letting an infection (rather than vaccine) generate an immune response. An example is the management practice of using serum containing live PRRSV to infect certain populations of pigs. This is done to achieve uniform exposure and immunity against PRRSV. This practice is not without risks. Careful consideration of benefits and risks is warranted before executing controlled exposure programs.

### United States Department of Agriculture Vaccine Licensing

Commercial vaccines that are licensed by the United States Department of Agriculture (USDA) have been tested to determine that they are safe and effective by standardized tests. It does not mean that the vaccine must be able to induce complete immunity under all conditions that may be found in the field. This would not be realistic since the immune system is not capable of such potent protection under adverse conditions.

To be federally licensed by the USDA, the vaccine must have been tested under controlled experimental conditions. The vaccinated group must be able to induce complete immunity under all conditions that may be found in the field. This would not be realistic since the immune system is not capable of such potent protection under adverse conditions.
tious diseases, or exposed to a high dose of infectious agents due to overcrowding or poor sanitation.

**Vaccination Failure**

There are many reasons why animals may develop disease even though they have been vaccinated (Roth 1999). The major reasons for these failures include (1) vaccine administered in the face of maternal immunity, (2) vaccine administered after infection, (3) improper handling of vaccines and/or administration equipment, (4) poor timing, and (5) immunosuppression at the time of vaccination.

One of the major challenges in developing an active immune response in young pigs has been interference from maternal immunity (Hodgins et al. 2004; Ma and Richt 2010; Opriessnig et al. 2008). The timing of many vaccines administrated by the parenteral route involves estimating when the level of maternal antibody is low enough for an active immune response to progress sufficiently to provide vaccine immunity (Hodgins et al. 2004; Opriessnig et al. 2008). Maternal antibody half-life in pigs is 11.3–20 days compared with cattle with a half-life of 16–28 days for maternal antibody (Fulton et al. 2004). The half-life for swine maternal antibodies is 16.2 days for PRRSV (Yoon et al. 1995), 14 days for SIV (Fleck and Behrens 2002), 19 days for PCV2 (Opriessnig et al. 2004), 11.3 days for PRV (Mueller et al. 2005), 11 days for classic swine fever virus (Mueller et al. 2005), 20 days for parvovirus (Paul et al. 1982), and 15.8 days for *M. hyopneumoniae* (Morris et al. 1994).

The prime window for vaccination can be anywhere from a few weeks to 3 months. As illustrated in Figure 16.8, this can vary by animal and depends on the level of maternal antibody and the vaccine antigen. This presents a major challenge to getting an adequate vaccine response. Antibody levels often decay to a level still high enough to block responses to vaccine, but not high enough to resist a field infection; this creates a window of opportunity for infecting organisms.

The host requires several days after vaccination before an effective immune response will develop. If the animal encounters an infectious agent prior to or near the time of vaccination, the vaccine may not have time to induce immunity. The animal may come down with clinical disease resulting in an apparent vaccination failure. In this situation, disease signs will appear shortly after vaccination and may be mistakenly attributed to vaccine virus causing the disease. Modified live vaccines consisting of attenuated organisms may be capable of producing disease in immunosuppressed animals.

Improperly handled and administered vaccines may fail to induce the expected immune response in normal, healthy animals. Modified live bacterial and viral vaccines are only effective if the agent in the vaccine is viable and able to replicate in the vaccinated animal. Observing proper storage conditions and proper methods of administration are very important for maintaining vaccine viability. Failure to store the vaccine at refrigerator temperatures or exposure to light may inactivate the vaccine. Even when stored under appropriate conditions, many vaccines lose viability over time. Therefore, vaccines that are past their expiration date should not be used. The use of chemical disinfectants on syringes and needles can inactivate modified live vaccines if there is any residual disinfectant. The use of an improper diluent or the mixing of vaccines in a single syringe may also inactivate modified live vaccines. Diluent for lyophilized vaccines are formulated specifically for each vaccine. A diluent that is appropriate for one vaccine may inactivate a different vaccine. Some vaccines and diluents contain preservatives that may inactivate other modified live vaccines. For these reasons, and many others, different vaccines should not be mixed and given as a single “vaccine” for injection.

The timing of the vaccination is important. Vaccination of young animals may be ineffective due to age of the animal and/or because of the presence of maternal antibody. If the vaccine is administered after all maternal antibodies are gone from animals in the herd, there may be a prolonged period of vulnerability before they develop their own immune response. Most veterinarians and producers decide that because of time and expense considerations, it is impractical to vaccinate young pigs frequently. However, frequent vaccination may be justified in cases of unusually high disease incidence.

Immunosuppression due to a variety of factors including stress, malnutrition, concurrent infection, or immaturity or senescence of the immune system may also lead to vaccination failure. If the immunosuppression occurs at the time of vaccination, the vaccine may fail to induce an adequate immune response. If the immunosuppression occurs sometime after vaccination, then disease may occur due to reduced immunity in spite of an adequate response to the original vaccine. Therapy with immunosuppressive drugs, for example, GCs, may also cause this to occur.

**ACKNOWLEDGMENTS**

The authors have adapted their chapter from that written for the 9th edition (Roth and Thacker 2006).

**REFERENCES**


STRUCTURE AND FUNCTION

The skin acts as both a barrier and a communication between the external environment and the internal organs. Its functions include maintenance of body fluids, electrolytes, and macromolecules; protection against chemical, physical, and microbiological damage or invasion; and sensory perception of touch, pressure, pain, itch, and temperature change. Skin also regulates body temperature through support of hair coat, regulation of cutaneous blood supply, and sweat gland function (Scott 1988). Skin has an important function of immunoregulation.

The skin is the largest body organ and can, in some animals, constitute 12–24% of body weight. In the pig, the skin represents between 10% and 12% of body weight at birth and around 7% in adult animals, although in some breeds, such as the Meishan, skin can be 10–12% of adult body weight.

The skin of swine is basically similar in histological structure to that of other domestic animals and, compared with other species, has many more similarities to human skin. The skin of the pig is divided into two layers: the epidermis and dermis. In most areas, the epidermis consists of four layers, since the stratum lucidum is absent except in the snout. The epidermis is relatively thick, the major cells being the keratinocytes in the stratum basale, the polyhedral cells in the stratum spinosum, flattened cells in the stratum granulosum, and cornified cells in the stratum corneum. The thickness of the epidermis varies considerably in different areas of the body (Meyer et al. 1978). Generally, the skin of the dorsum is thicker and hairier than the skin of the ventrum. Maximum thickness is between the toes and on the lips, the snout, and the shield, which is unique to the scapular and costal region in older boars. The thinnest layers are found in the axilla, eyelids, and ventral areas of the thorax and abdomen (Marcarian and Calhoun 1966).

The dermis consists of two ill-defined layers; beneath these layers is a prominent layer of adipose tissue (hypodermis). The two layers of the dermis, the stratum papillaris and stratum reticularis, are composed of connective tissue in which blood vessels, nerves, lymphatics, and associated epidermal appendages are situated. Cells found in the dermis include fibroblasts, melanocytes, and mast cells. Origins of hair follicles and sweat glands are found in the hypodermis.

The holocrine sebaceous glands of the pig are branched alveolar and open into the neck of the main hair follicle. The sweat glands are coiled, tubular, and apocrine, and are found in all areas, although there are relatively few (about 25/cm²) compared with other species, except on the snout.

In swine, the short, stout hair follicles possess arrectores pilorum muscles attached to the outer root sheath; the bristles occur either singly or in groups of two or three. The hair coat consists of 60–70% bristles and 30–40% fine downy hair. Specialized forms of hair are also found—in particular, tactile hairs in the region of the snout (Marcarian and Calhoun 1966; Mowafy and Cassens 1975). Specialized seromucoid glands are found in the carpal glands, located behind the carpus, and in the mental (mandibular) organ, located in the intermandibular space.

CLINICAL EXAMINATION AND DIAGNOSIS

Skin diseases may involve the skin only or be cutaneous manifestations of internal disease (Tables 17.1 and
252  SECTION II  BODY SYSTEMS

Table 17.1.  Causes of diseases of the skin in swine

<table>
<thead>
<tr>
<th>A. Infectious</th>
<th>Bacterial</th>
<th>Viral</th>
<th>Fungal</th>
<th>Parasitic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exudative epidermitis</td>
<td>Swine pox</td>
<td></td>
<td></td>
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<tr>
<td>Streptococcus</td>
<td>Swine vesicular disease</td>
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<tr>
<td>Ear necrosis</td>
<td>Vesicular stomatitis</td>
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<td>Spirochosis</td>
<td>Vesicular exanthema</td>
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<td>Facial necrosis</td>
<td>Porcine parovirus</td>
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<tr>
<td>Abscesses</td>
<td>Idiopathic vesicular disease</td>
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<tr>
<td>Erysipelas</td>
<td>Classical swine fever</td>
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<tr>
<td>Salmonellosis</td>
<td></td>
<td>African swine fever</td>
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<tr>
<td>Pasteurellosis</td>
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<td>Mastitis</td>
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<td>Edema disease</td>
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<td>Anthrax</td>
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<tr>
<td>Malignant edema</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>B. Noninfectious</th>
<th>Environmental</th>
<th>Nutritional</th>
<th>Hereditary</th>
<th>Neoplastic</th>
<th>Miscellaneous</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sunburn</td>
<td>Parakeratosis</td>
<td>Pityriasis rosea</td>
<td>Melanoma</td>
<td></td>
<td>Porcine dermatitis/ nephropathy syndrome</td>
</tr>
<tr>
<td>Photosensitization</td>
<td>Fatty acid deficiency</td>
<td>Dermatosis vegetans</td>
<td>Rhabdomyoma</td>
<td></td>
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</tr>
<tr>
<td>Skin necrosis</td>
<td>Iodine deficiency</td>
<td>Epitheliogenesis imperfecta</td>
<td>Lymphangioma</td>
<td></td>
<td></td>
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<tr>
<td>Bursitis</td>
<td>Riboflavin deficiency</td>
<td></td>
<td>Papilloma</td>
<td></td>
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<tr>
<td>Callosities</td>
<td>Pantothenic acid deficiency</td>
<td></td>
<td>Fibroma</td>
<td></td>
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<tr>
<td>Limb and foot lesions</td>
<td>Biotin deficiency</td>
<td></td>
<td>Hemangiomia</td>
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<tr>
<td></td>
<td>Vitamin A, C, and E deficiencies</td>
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<td></td>
<td>Sweat gland adenoma</td>
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<td></td>
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<td>Polyp</td>
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</tbody>
</table>

17.2). Examples of diseases restricted to the skin are ear necrosis, pityriasis rosea, and swine pox. Examples of skin lesions symptomatic of a more general pathophysiological condition are erysipelas, classical swine fever, and dermatitis/nephropathy syndrome. It is therefore essential that an accurate history be taken, followed by a thorough clinical examination involving the entire animal first and then the skin itself. Examination of the skin should aim to define the nature of lesions (primary or secondary) or abnormality (vesicles or pustules, edema or erythema). This should be followed by the formulation of a differential diagnosis. Tests should then be carried out to confirm a diagnosis (skin scraping, culture, or biopsy) in order to determine the approach to treatment and subsequent prevention.

History

Information obtained should be by on-farm examination and be complete. The type of husbandry and housing system provides clues for risk factors. Extensive or free-range systems may predispose to sunburn or photosensitization. Intensive indoor housing often leads to conditions such as pressure sores in sows or teat necrosis in piglets. Infectious conditions such as exudative epidermitis are more frequently seen in continuous flow-through systems than in all-in/all-out systems.

Specifically examine the environmental conditions; poor hygiene and high ambient temperatures, relative humidity, and stocking density may predispose to outbreaks of staphylococcal and streptococcal pyoderma. Pityriasis rosea is also more frequently seen with high stocking densities and high humidity.

A seasonal pattern may be evident for some skin diseases. Also, determine if there has been recent movement of pigs or introductions of new stock. The mixing of pigs at weaning, for example, may result in fighting and biting and increase the incidence of exudative epidermitis in the nursery.

Examine animals for signs of trauma. Self-inflicted trauma may be due to pruritus associated with sarcoptic mange or lice infestation. Evaluation of nutrition and diet may be helpful, as deficiencies of the B group vitamins, zinc, or essential fatty acids can result in dry, scaly, dandruff-like dermatitis or parakeratosis.

Breed predilection may indicate a congenital or hereditary condition. Pityriasis rosea has been commonly seen in Landrace pigs and in the progeny of pigs that have had pityriasis rosea as weaners. Other inherited conditions include dermatosis vegetans and epitheliogenesis imperfecta.
# Table 17.2: Differential diagnosis of skin diseases

<table>
<thead>
<tr>
<th>Location</th>
<th>Lesions and Signs</th>
<th>Diseases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Head and neck</td>
<td>Macules, vesicles, pustules, greasy exudate (seborrhea), crusts in sucking piglets and weaners especially around the eyes</td>
<td>Exudative epidermitis</td>
</tr>
<tr>
<td></td>
<td>Pustules, erosions, crusts, and abscesses</td>
<td>Streptococcosis</td>
</tr>
<tr>
<td></td>
<td>Plaques, pustules, crusts, alopecia with pruritus</td>
<td>Sarcoptic mange</td>
</tr>
<tr>
<td></td>
<td>Pustules, erosions, necrosis, crusts below the eye, cheek, and lips in sucking piglets</td>
<td>Facial necrosis</td>
</tr>
<tr>
<td></td>
<td>Edema around the eyes, conjunctiva, and frontal area mainly in weaners and young growers</td>
<td>Edema disease (<em>E. coli</em>)</td>
</tr>
<tr>
<td></td>
<td>Edema of the head and throat</td>
<td>Malignant edema (<em>Clostridium</em> sp.)</td>
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<tr>
<td></td>
<td>Red to purple discoloration of the snout, face, and neck (jowls)</td>
<td>Septicemia</td>
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<tr>
<td></td>
<td>Discrete ulcer and crust over mandible in sows</td>
<td>Pressure necrosis</td>
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<td></td>
<td>Vesicles, pustules, erosions on the snout, lips, mouth, and tongue</td>
<td>Foot-and-mouth disease</td>
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<td></td>
<td>Swine vesicular disease</td>
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<tr>
<td></td>
<td></td>
<td>Vesicular exanthema</td>
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<tr>
<td></td>
<td></td>
<td>Vesicular stomatitis</td>
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<td></td>
<td></td>
<td>Porcine parvovirus</td>
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<td></td>
<td></td>
<td>Idiopathic vesicular disease</td>
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<tr>
<td></td>
<td></td>
<td>Swine pox</td>
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<tr>
<td></td>
<td></td>
<td>Ear necrosis</td>
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<tr>
<td>Ears</td>
<td>Vesicles, erosions, black crusts</td>
<td>Salmonellosis</td>
</tr>
<tr>
<td></td>
<td>Black necrosis, ulcers on the tips and posterior edge of the pinna in piglets</td>
<td>Erysipelas</td>
</tr>
<tr>
<td></td>
<td>Deep ulcers at the base of the pinna in growers, often bilateral</td>
<td>Ulcerative spirochetosis</td>
</tr>
<tr>
<td></td>
<td>Erythema, red to purple blotchy discoloration</td>
<td>Septicemia</td>
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<td></td>
<td></td>
<td>Classical swine fever</td>
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<td></td>
<td>African swine fever</td>
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<td></td>
<td></td>
<td>Sunburn</td>
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<td></td>
<td></td>
<td>Sarcoptic mange</td>
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<td></td>
<td></td>
<td>Exudative epidermitis</td>
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<td></td>
<td></td>
<td>Streptococcosis</td>
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<td></td>
<td></td>
<td>Ringworm (microsporosis)</td>
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<tr>
<td>Dorsum</td>
<td>Plaques, brown or gray crusts on the inner ear, ear sharking, pruritus, gray thick crusts on adult animals</td>
<td>Essential fatty acid; vitamin A, C, or E; or zinc deficiency</td>
</tr>
<tr>
<td></td>
<td>Macules, pustules, black crusts</td>
<td>Sarcoptic mange</td>
</tr>
<tr>
<td></td>
<td>Circular macules, patches, small scales, pink to red discoloration behind ears and neck</td>
<td>Epitheliogenesis imperfecta</td>
</tr>
<tr>
<td>Shoulder</td>
<td>Hyperkeratosis, dry scales along spine, some alopecia</td>
<td>Pressure sores, due to confinement in farrowing crates, pressure from crate bars or prongs</td>
</tr>
<tr>
<td></td>
<td>Complete absence of epithelium (large red shiny areas) in newborn piglets</td>
<td>Pressure sore due to confinement on solid or mesh floors, low energy intake</td>
</tr>
<tr>
<td></td>
<td>Abscesses and pressure necrosis over spine between last ribs and lumbar area in sows</td>
<td></td>
</tr>
<tr>
<td>Ventral abdomen</td>
<td>Large deep discrete ulcer, necrosis and crust over spine of scapula in sows often in poor body condition</td>
<td>Exudative epidermitis</td>
</tr>
<tr>
<td></td>
<td>Erythema, pustules, dark brown crusts, exudate</td>
<td>Streptococcosis</td>
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<td></td>
<td></td>
<td>Sarcoptic mange</td>
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<td></td>
<td></td>
<td>Candidiasis</td>
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<td>Biotin deficiency</td>
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<td></td>
<td></td>
<td>Erysipelas</td>
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<td></td>
<td></td>
<td>Pityriasis rosea</td>
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<td></td>
<td></td>
<td>Ringworm (microsporosis, trichophytosis)</td>
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<td></td>
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<td>Zinc deficiency (parakeratosis)</td>
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<td>Dermatosis vegetans</td>
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<td></td>
<td></td>
<td>Exudative epidermitis</td>
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<td></td>
<td>Swine pox</td>
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<td></td>
<td>Acute mastitis</td>
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<td></td>
<td></td>
<td>Trauma and infection due to rough floors and poor hygiene in farrowing crates</td>
</tr>
</tbody>
</table>

(Continued)
Determine ages affected and age distribution. A number of diseases are more frequently seen in certain age groups. Exudative epidermitis rarely affects pigs older than 6 weeks of age, and pityriasis rosea is seen only in pigs between 2 and 6 weeks of age. Teat necrosis usually occurs within 24 hours after birth. Nutritional deficiencies are unlikely to occur before weaning.

Determine the chronology of lesions. Information relating to initial lesions observed and their location may indicate a typical clinical evolution. In swine pox, for example, macules are observed initially, and then vesicles and pustules that rupture to form a dark circumscribed crust before healing. Exudative epidermitis often commences as macules and pustules around the eyes before spreading over the entire body of piglets.

Skin lesions may be obvious but also examine animals for other clinical signs. A history of other signs such as anorexia, depression, loss of body condition, reluctance to move, or diarrhea may indicate the skin lesions are the result of an internal disease.

Incidence, the number of animals affected over time, may suggest a contagious nature. The history of mortalities may suggest an infectious disease such as exudative epidermitis or erysipelas, which tends to spread rapidly, whereas congenital and hereditary conditions such as pityriasis rosea are seen at a constant rate within a herd.

Critical evaluation of response to therapy can aid in differentiating diseases. Variation in response to therapy may help to differentiate between viral, bacterial, and
fungal infections—for example, swine pox, streptococcal dermatitis, and ringworm. Pityriasis rosea does not respond to any treatment, whereas parakeratosis responds dramatically to the addition of zinc and essential fatty acids to the diet.

Clinical Examination
Before carrying out a detailed examination of the skin, a general clinical examination is necessary to determine whether the skin disease is symptomatic of an internal disease.

Internal diseases causing skin lesions or skin abnormalities such as abnormal color changes include erysipelas, salmonellosis, pasteurellosis, mastitis, classical swine and African swine fever, and dermatitis/nephropathy syndrome. Almost any septicemia or toxemia can cause erythema or cyanosis characterized by red to purple discoloration, especially on the extremities and easily seen in the white breeds. Urticaria, seen as multiple pink to purple raised areas of skin, commonly occurs in cases of erysipelas, beta-hemolytic streptococcal infections, food allergy, or insect bites. Blue to black skin discoloration with necrosis can indicate gangrene. Necrosis of the ears and tail of piglets is also black. Pallor is often an indication of anemia, perhaps due to blood loss.

Pruritus causes scratching and should be looked for as a possible sign of sarcoptic mange or lice infestation. Scratching is frequently accompanied by shaking of the head due to the presence of mites in the ears and can result in aural hematomas. Aural hematoma is the acute distention of the ear with blood, usually the result of trauma from fighting or head shaking. Ear hematomas are more commonly observed in breeds with longer, dependent ears. Intervention beyond segregation is usually not warranted since draining or lancing the hematoma usually results in continued hemorrhage or infection. Alopecia and excoriation may also be an indicator of intense scratching seen in chronic sarcoptic mange.

Edema of the skin can indicate a systemic disease, for example, hypoproteinemia, vasculitis, increased vascular permeability such as in malignant edema caused by Clostridium septicum and in edema disease associated with Escherichia coli, the last two conditions producing edema around the head.

Skin lesions are classified as primary, the direct result of the insult or disease, or as secondary, a result of evolutionary changes to the skin dictated by factors such as the cause of the disease, secondary infection, and self-trauma.

It is essential for the clinician to differentiate between primary lesions and secondary lesions; however, when the animal is presented, only secondary lesions may be seen. Examination of the entire body of several animals may be necessary to locate primary lesions on recently affected cases.

Primary Lesions.

Macules, defined as circumscribed flat discolorations less than 1 cm in diameter, and papules, more solid, raised areas of skin of varying color, are seen in the early stages of exudative epidermitis, erysipelas, and swine pox.

Plaques, elevated superficial lesions more than 0.5 cm in diameter, scattered over the whole body surface of young growing pigs have been associated with erysipelas, pityriasis rosea, and Aujeszky's disease.

Vesicles are well-demarcated, dome-shaped lesions (<1 cm) usually containing serum or inflammatory exudates. They are pale or translucent and are characteristic of a number of the viral skin diseases in swine, such as swine pox, foot-and-mouth disease, swine vesicular disease, and vesicular stomatitis. Similar lesions have been reported in swine with porcine parvovirus infection (Kresse et al. 1985).

Pustules are elevated lesions filled with inflammatory cells (leukocytes) and can be follicular or epidermal. They are white, yellow, or red (hemorrhagic) and, in some cases, are surrounded by erythema. Pustules in swine are commonly associated with streptococcal infections, exudative epidermitis, and swine pox.

Wheals are circumscribed, raised, round or oval areas of skin due to edema. They may be blanched or slightly erythematous. The edema is usually associated with the dermis. Fly and mosquito bites frequently cause wheals.

Secondary Lesions.

Scales or flakes can indicate abnormal keratinization and shedding caused by ectoparasites, such as Sarcoptes scabiei, or by bacterial skin diseases. Scales are seen on the thinner skin of piglets with exudative epidermitis, on the inner side of the margin of the ringlike lesions of pityriasis rosea, and on the outer periphery of ringworm lesions. Scales may be mixed with sebum and sweat, giving a greasy or oily appearance—seborrhea.

Crusts are a very common secondary skin lesion of swine and are due to a combination of serum, sebum, blood, and cutaneous debris adhering above the normal skin surface. Crusts are seen following bacterial infections and viral vesicular diseases, especially swine pox, and as a result of pruritus associated with sarcoptic mange or lice infestation.

Hyperkeratosis, an increased thickening of the stratum corneum, develops with nutritionally related metabolic disorders, such as vitamin A, zinc, and fatty acid deficiencies, or with local callus formation due to trauma associated with pressure and friction. Erythema and intense pruritus with hyperkeratosis and acanthosis have been associated with in-feed tiamulin therapy (Laperle et al. 1988).

Erosions involving the epidermis only and ulcers involving also the dermis are caused by deep bacterial infections (Staphylococcus spp., Streptococcus spp., Fusobacterium necrophorum, and a spirochete referred to as “Borrelia suis”) or may be due to trauma or pressure.
Severe scratching will result in alopecia, commonly seen over the shoulders and hindquarters in pigs with sarcoptic mange or lice and in pigs irritated by insects such as flies and mosquitoes. The characteristic lesions of pityriasis rosea are typical epidermal collarettes, described as ruptured pustules spreading peripherally with a ring of scales on the inner margins of the ring.

**Diagnostic Tests**

Diagnosis of skin diseases can be confirmed by a number of relatively simple tests. In swine, the most frequently used tests include skin biopsy for histopathological examination, direct smears for identification of bacteria and fungi, and culture for isolation and identification of bacteria and viruses.

**Skin Biopsy.** Skin biopsy should be used on all neoplastic lesions, any persistent ulceration, and skin lesions not responding to treatment. Fully developed primary lesions or early vesicles and pustules are best for biopsy, whereas secondary lesions may be of little value. The technique described by Scott (1988) involves the removal of 6–9 mm of skin using a biopsy punch or surgical excision with a scalpel, which may be more suitable for larger lesions, vesicles, and pustules and where the skin is very thick. Local anesthetic may be indicated. The biopsy should be gently blotted to remove blood and surface material, and immersed within 1–2 minutes in a fixative such as 10% neutral phosphate buffered formalin. Skin biopsies are usually stained with hematoxylin and eosin (H&E).

Skin biopsy, unfixed, can also be used for isolation of bacteria and viruses. For virus isolation, the skin should be cleaned with water or saline only and not with alcohol. Samples should be stored and transported at 4°C in a virus transport medium.

Examination of direct smears is commonly used for the identification of bacteria or fungi. For bacteria, samples of pus or exudate from pustules, macules, or ulcers can be smeared on glass slides, air-dried, and stained with methylene blue, Gram’s stain, or Diff-Quik for light microscope examination to identify the type of bacteria (e.g., cocci or rods and gram-positive or gram-negative) (Scott 1988). Skin scrapings or direct touch impression can be used for suspected fungal diseases. Skin scrapings should be made after removing fat from the skin with alcohol. Scrapings are warmed in a 20% solution of sodium hydroxide, and spores appear as round highly refractile bodies in chains or mosaics in hair follicles, in epithelial scales, and on the surface of hair fibers. Skin scraping is useful for identification of mange (see section on “Parasitic Diseases”).

**Culture.** Best results are obtained by aspirating samples from intact pustules, vesicles, or abscesses with a needle and syringe. Cultures of open sores (erosions, ulcers, and sinuses) generate confusion (Scott 1988). Bacterial culture is usually done on blood agar or in thioglycolate broth. Virus identification can be done from tissue culture or by electron microscopy. Hair and skin scrapings (surface keratin) can be inoculated onto Sabouraud’s dextrose agar or dermatophyte test medium (DTM) for fungal culture (Scott 1988).

**BACTERIAL DISEASES**

**Exudative Epidermitis (Greasy Pig Disease, Impetigo Contagiosa, and Seborrhea Contagiosa)**

Exudative epidermitis is caused by *Staphylococcus hyicus*, certain strains of which produce a heat-labile exfoliative toxin and is discussed fully in Chapter 61. Suckling piglets are the most commonly and severely affected age group. In weaned piglets, the morbidity can be up to 80% in some groups but mortality is usually low. The disease can occur sporadically in older swine as well.

Disease may be acute, subacute, or chronic. Lesions are frequently seen around the eyes, nose, lips, and gums, and behind the ears as macules increasing in size to form damp and oily greasy exudates of sebum, sweat, and serum (Figure 17.1A). Erythema is marked, often over the entire body. Lesions may develop around coronary bands and heels of hooves. Occasionally, the conjunctiva is affected, causing the eyelids to become swollen and matted together. In severe cases, lesions are generalized, affecting nearly the entire body. The disease has also been associated with lesions of the kidney involving the renal pelvis and tubules (Blood and Jubb 1957). Ulcerative glossitis and stomatitis have been reported by Andrews (1979). Nervous signs were recorded in one outbreak of exudative epidermitis by Blood and Jubb (1957). The differential diagnosis includes sarcoptic mange, parakeratosis associated with zinc and other nutritional deficiencies, swine pox, pityriasis rosea, pustular dermatitis, and ringworm.

**Pustular Dermatitis (Contagious Pyodermata)**

Pustular dermatitis is caused by streptococcal infections (Chapter 62) that result in skin necrosis and pustular dermatitis. Transmission can be directly from sows to newborn piglets and through skin abrasions and tissue damage associated with tail docking, ear notching, needle teeth clipping, bite wounds, or trauma. Wounds on any region of the body can become infected, resulting in cellulitis, necrosis, abscess formation, and ulceration. Differential diagnosis includes ulcerative dermatitis, exudative epidermitis, sarcoptic mange, swine pox, and erysipelas.

**Ear Necrosis (Necrotic Ear Syndrome and Ulcerative Spirochetosis of the Ear)**

Ear necrosis is a syndrome seen in pigs aged from 1 to 10 weeks. It is characterized by bilateral or unilateral necrosis of any part of the ear, but especially in young
pigs on the tip and around the posterior edge of the pinna (Figure 17.1B). In growers, necrosis at the base of the ear can occur, with many pigs being affected at any one time. Outbreaks often occur in one pen of pigs, with up to 80% affected.

It is likely that the lesions are the result of a mixed infection following damage to the skin. It has been suggested that infection with *S. hyicus* may take place first, followed by the more invasive streptococci and spirochetes (see section on “Ulcerative Dermatitis”), resulting in necrosis and ulceration. Biting following mixing of pigs is a common predisposing factor. In older pigs, ear biting can be a vice similar to flank and tail biting, and can lead to infection. Self-inflicted trauma resulting from the irritation of tail biting, and can lead to infection. Some pigs may show signs of inappetence, unthriftiness, and eventual loss of some of the ear or the entire ear. Some pigs may show signs of inappetence, unthriftiness, and fever; death may occur in a few cases.

**Ulcerative Dermatitis (Granulomatous Dermatitis)**

Ulcerative dermatitis can occur as ulcerative, necrotic, or tumorlike lesions found on most areas of the body surface and around the buccal cavity of pigs. More specific syndromes such as ear necrosis, facial necrosis, infected bursae, and calluses over joints and bony prominences can be forms of spirochetosis. The etiology involves initial trauma of the skin, followed by infection, often involving several organisms. Microscopy of lesions from ear, feet, and leg granulomas, or shoulder ulcers may demonstrate spiral-shaped organisms. Original reports implicated *B. suis*, *S. hyicus*, and beta-hemolytic streptococci as the most likely contributors. *Arcanobacterium pyogenes* is a common secondary invader (Cameron 1984).

Skin trauma or damage that results in infections can be associated with bite wounds, especially around the face and head and on the flank and tail. Infection following castration, pressure sores, and ulceration of swollen bursae and calluses often leads to spirochetosis. Gum damage following teeth clipping can result in lesions in the buccal cavity.

Lesions are most commonly seen in young pigs or, in the case of pressure sores and bursitis, in older growers and adults. The initial lesions are characterized by erythema and edema followed by necrosis, ulceration, or tumefaction, with fistulae discharging a grayish-brown glutinous pus. Lesions may continue to enlarge for several months and involve deeper structures of the body. The central area will often slough.

A differential diagnosis would include foreign-body abscesses, neoplasms, other infectious granulomas, and pressure necrosis.

**Facial Necrosis (Facial Pyemia)**

Facial skin necrosis is a common condition in suckling pigs less than 1 week of age and is characterized by bilateral necrotic ulcers that are often covered by hard brown crusts and that extend from the side of the face to the lower jaw area.

The condition is the result of infection of wounds inflicted by piglets on each other during feeding, often because inexperienced stock persons have failed to carry out teeth clipping correctly. Lacerations to the side of the face become infected with organisms such as *F. necrophorum*, *Streptococcus* spp., and *B. suis*.

Facial necrosis is commonly seen in large litters and especially in the disadvantaged weaker piglets and when milk letdown is slow, that is, when sows suffer from agalactia or hypogalactia. Facial necrosis occurs during the first few days of life and any number of piglets in a litter can be affected. Initially, lesions can be seen as striated lacerations caused by bites from other piglets. The lesions become infected, resulting in shallow ulcers covered with hard brown crusts. The encrustation may extend over a large area involving the lips and eyelids, making it difficult for the piglet to open its mouth or eyes. These animals have difficulty in feeding and may starve. Facial necrosis can predispose to outbreaks of exudative epidermitis.

The condition is easily diagnosed by the nature and distribution of the lesions on the face of young piglets. Bacteriological examination of the lesions will help identify the organisms involved.

Careful removal of the crusts and application of a mild disinfectant solution of chlorhexidine or iodophores or of an antibiotic cream will help remove the infection as well as soften the lesions. Prevention is by clipping the canine and lateral incisor teeth just above the level of the gum surface during the first 24 hours of life in all piglets in the litter. Instruments used should be thoroughly disinfected. Fostering piglets to eliminate large litters will help reduce competition for teats at feeding. Hygiene in the farrowing accommodation and prevention of milk letdown problems are also important.

**Specific Bacterial Diseases**

Erysipelas is an infectious disease of pigs that manifests in several forms, including septicemia, nonsuppurative arthritis, vegetative endocarditis, and skin lesions caused by *Erysipelothrix rhusiopathiae* (Chapter 54). In acute erysipelas, the skin of the extremities—including the snout, ears, lower limbs, tail, and scrotal area, as
well as the jowls and ventral surface of the abdomen—is erythematous. The color varies from pink to purple, typical of many systemic infections and not necessarily diagnostic of erysipelas. The more specific skin lesions associated with erysipelas first appear as small pink or red raised areas (papules) or larger plaques ranging from 3 to 6 cm in diameter (Figure 17.1C). Many of these lesions will develop the characteristic diamond or rhomboidal shape and are raised, firm, and easily palpated. The outer area is pink in color and the center becomes blue to purple (necrosis) as the disease progresses. These discrete lesions are associated with arteritis; the small arterioles show acute cellular infiltration and cellular thrombi with the presence of mainly neutrophils (Jubb et al. 1985). In the chronic stage, skin lesions become more necrotic; appear dark, dry, and firm; and easily peel away from the underlying tissues. Occasionally, sloughing of the ears, tail, or a foot can occur (Scott 1988). Alopecia may be seen in more long-standing cases.

Salmonellosis (Chapter 60) in pigs can cause septicemia and thus skin lesions and changes in skin color generally caused by Salmonella choleraesuis. All age groups of pigs can be affected, although pigs from weaning to 4 months of age are most frequently affected.

Cyanosis of the extremities and abdomen may be seen. The discoloration of the skin is due to intense capillary dilation and congestion in the dermal papillae, followed by thrombosis in the capillaries and venules and, to a lesser extent, arterioles, leading to necrosis and sloughing of the skin. Skin necrosis commonly involves the ears, tail, and feet in young pigs. Discoloration of the skin is similar to that in other septicemic diseases—that is, swine fever, erysipelas, and pasteurellosis—which therefore have to be considered in a differential diagnosis. Ear necrosis also has to be differentiated from necrosis associated with other infectious agents.

Viral Diseases
Swine pox (contagious impetigo, louse-borne dermatitis) is a typical poxvirus infection mainly affecting young pigs. There is little or no systemic illness, and lesions are usually confined to the ventrolateral abdomen and thorax (Chapter 30). The lesions follow the typical pox evolution of erythematous macules becoming papules, and then vesicles progressing to pustules, which rupture and form crusts. Lesions are seen mainly on the side of the body, ventral abdominal wall, and inner thighs. Occasionally, lesions are seen on the back, face, and udder (Figure 17.1D). Uncommonly, piglets may be infected in utero and be born with generalized pox lesions.

Classical swine fever (hog cholera) is caused by a pestivirus of the family Togaviridae. Diffuse erythema followed by purplish discoloration of the skin over the abdomen, snout, ears, and thighs is common in acute cases. Necrosis of the edges of the ears, tail, and vulva may develop. Purple blotching of the ears is characteristic, with generalized hypotrichosis in the chronic form of the disease. Congenital alopecia has been reported in piglets infected in utero (Carbrey et al. 1966).

The vesicular diseases include foot-and-mouth disease (Chapter 42), swine vesicular disease (Chapter 42), vesicular stomatitis (Chapter 45), and vesicular exanthema (calicivirus), all of which cause vesicular skin lesions in swine. The diseases all produce very similar lesions with similar distribution.

African swine fever (Chapter 25) is caused by a DNA virus at present unclassified. Besides general signs of fever, depression, anorexia, and incoordination similar to hog cholera, skin changes include cyanotic blotching and purple discoloration of the limbs, snout, abdomen, and ears. Hemorrhages may also occur on the skin of the ears and flanks.

Fungal Diseases (Ringworm)
Fungal diseases of swine tend to be superficial mycoses involving the keratinized epithelial cells and hair only. Fungi reported in swine include Microsporum nanum, Microsporum canis, Microsporum gypseum, Trichophyton mentagrophytes, Trichophyton rubrum, Trichophyton tonsurans, Trichophyton verrucosum, and Candida albicans.

Ringworm is found in both extensively and intensively reared swine. All age groups can be affected, and the incidence appears to be higher where sanitation is poor and stocking densities are high with moderate temperatures and high humidity. Bedding may be an important source of infection. Fungal spores can remain viable for many years in a dry and cool environment. Mycelial growth is promoted when the environmental conditions are warm and humid with a slightly alkaline skin pH. Ringworm fungi are strictly aerobic. Microsporum nanum is the most common fungal infection in swine; however, M. canis produces ringworm in piglets and M. gypseum is also found on pigs. Ringworm lesions can be found on almost any part of the body (Figure 17.1E). Lesions begin as circumscribed spots, which tend to enlarge in a circle, some to enormous size covering the complete side of the pig. The skin is reddish to light brown in color, roughened but not raised. Dry crusts form around the periphery, the hair is usually not lost, and no pruritus develops. Dirt and dust may obscure the lesions, which often are not noticed until the pigs are washed. Experimental infection with M. nanum (Connole and Baynes 1966) produced lesions that first appeared as pustules or moist brown areas of desquamated tissue 2 cm in diameter. As the lesions extended, fresh pustules were often seen near the periphery. Scales, crusts, and deposits of black material similar to the natural infection appeared. Lesions developed in 2–3 weeks and resolved by 9
weeks. Chronic infections are often seen behind the ears of adult swine and appear as thick, brown crusts that spread over the ear and neck.

Trichophytosis is most commonly associated with *T. mentagrophytes* in swine, but *T. rubrum*, *T. tonsurans*, and *T. verrucosum* can also cause ringworm in swine. Lesions are found on the thorax, flank, and neck, behind the ears, and on the legs. The size and shape of lesions vary; some measure up to 12.5 cm across and are roughly circular. Typical lesions are red or covered by a thin brownish dry crust. The disease tends to be self-limiting and lasts about 10 weeks (McPherson 1956). Similar lesions have been described by Pepin and Austwick (1968) in a herd of Wessex pigs. The same authors reported an outbreak of ringworm in a litter of Large White piglets caused by *T. verrucosum*. Arora et al. (1979) described lesions caused by *T. rubrum* as rough and reddish and appearing on several sites; 10% of piglets and 4% of sows in a herd were affected.

Cutaneous candidiasis in swine is caused by the yeast *C. albicans* and appears to cause disease when the host’s resistance is lowered. The disease has been reported in grower pigs fed garbage and kept in unsanitary conditions. The morbidity was 40%. The lesions on the most severely affected animals consisted of circular areas approximately 2 cm in diameter coated with moist gray exudate. Lesions were found on all limbs and the lateral and ventral surfaces of the abdomen. The skin was thickened, wrinkled, devoid of hair, and hung loose in folds (Reynolds et al. 1968).

Treatment, if indicated, consists of removal of the crusts and local application of products such as a weak solution of iodine, Whitfield’s ointment, copper sulfate or copper naphthenate, or thibendazole ointment (2–4%) as a suspension in glycerine. Agriculture Bordeaux mixture (an aqueous solution of copper sulfate and unslaked lime) has been used with good results (Blood and Radostits 1989). Systemic treatment employs oral administration of griseofulvin at a dose rate of 1 g/100 kg body weight daily for up to 40 days.

Control is by maintaining good sanitation. Housing can be disinfected with phenolic disinfectant (2.5–5%), sodium hypochlorite (0.25% solution), or a 2.0% formaldehyde and 1.0% caustic soda solution used as a spray.

**Parasitic Diseases**

Sarcoptic mange (scabies) is the most commonly encountered parasitic skin disease of swine. It is one of the most important skin diseases and of major economic importance (Chapter 65). The disease is caused by the mange mite *S. scabiei* var. *suis*.

The first skin lesions appear about 3 weeks after contact with mites as small encrustations around the ears, eyes, and snout that develop into plaques about 5 mm in diameter. The lesions in the ear may regress and disappear in 12–18 weeks. Early pruritus is due to the local irritation from the mites establishing themselves in the skin. As the ear lesions regress, focal erythematous papules associated with hypersensitivity occur on the rump, flank, and abdomen. Mites are not usually found in these lesions. The hypersensitivity causes further pruritus, which results in excessive rubbing and the liberation of tissue fluids, giving the animal a greasy or shiny appearance. This is followed by coagulation and drying of the serum, sebum, and sweat to form crusts. In more chronic cases, excessive keratinization and proliferation of the connective tissue occur, with the result that the skin becomes thickened and wrinkled (Figure 17.1F). A common sign seen in grower pigs is shaking of the ears and, in some, the development of large hematomas on the inner surface of the ear. Chronic cases, usually in adults, develop thick gray-colored, loosely attached scales lining the inner surface of the ears, around the neck, and down the lower limbs, especially over the hock joints. Considerable loss of hair is associated with chronic mange.

Demodectic mange (follicular mange) is of little economic importance in swine (Chapter 65). Clinical signs are seen when pigs are in a poor or debilitated condition. The disease is caused by *Demodex phylloides*, which lives in the hair follicles or sebaceous glands of the skin. The mites usually invade the soft skin of the snout and around the eyes but can spread over the entire body. Infection is not uncommon on the abdomen between the legs. Lesions start as small red spots that become scaly with a nodular appearance. The nodules contain white caseous material and many mites. Mites can be found on skin scrapings from around the eyes in pigs showing no clinical signs. Treatment is usually not successful and severely affected animals should be culled.

The pig louse, *Hematopinus suis*, which affects pigs only, causes severe irritation resulting in continual scratching and rubbing against objects (Chapter 65). Lice are easily found around the neck, base of the ears, inner ears, and inside the legs and flank, and the white eggs can also be seen on the bristles, especially in colored pigs. The blood feeding causes considerable irritation; the resultant scratching and rubbing against objects damages the skin, which becomes lacerated and bleeds. Lice tend to congregate around the areas where skin damage has taken place. The continual irritation results in loss of body weight and reduces weight gains. Lice may spread the swine pox virus and erysipelas.

Fleas, mosquitoes, and flies commonly affect swine (Chapter 65), most commonly the fleas (*Ctenocephalides canis*, *Ctenocephalides felis*, *Pulex irritans*, and *Echidnophaga gallinacea*), mosquitoes (*Aedes* spp.), flies (*Musca domestica* and *Stomoxys calcitrans*), and screwworm flies (*Callitroga* spp.). Clinical signs can include varying degrees of rubbing, scratching resulting in alopecia, excoriations, and bleeding from the skin and
Skin becomes dry, hard, and fissured and peels. In young pigs, the tail and ears become necrotic and slough.

A simple and effective treatment is to cover the skin with bland oil, for example, vegetable oil or light mineral oil. Animals should be removed from direct sunlight, and adequate shade should be provided for prevention.

**Photosensitization**

Photosensitization is a condition seen in extensive, free-range-managed swine exposed to photodynamic agents and sunlight. Photosensitivity (hypersensitivity to light) results from the ingestion of photodynamic agents such as hypericin found in St. John's wort (*Hypericum perforatum*) and fagopyrin found in buckwheat (*Fagopyrum esculentum* and *Polygonum fagopyrum*). Other plants, including rape (*Brassica* sp.), lucerne (*Medicago sativa*), and *Trifolium* sp., cause photosensitization of unknown etiology. Other substances causing photosensitization in swine are phenothiazine, tetracyclines, sulfonamides (Amstutz 1975), and possibly aphids (McClymont and Wynne 1955).

Lesions are seen in white-skinned breeds and on areas most directly exposed to the sunlight. The severity of the condition depends on the concentration of the photodynamic agent and the length of exposure to light (Jubb and Kennedy 1970).

Erythema and edema develop and serum may exude from the skin and become dry and matted in the hair. Pain causes swine to walk carefully, and severely affected animals may suddenly drop into sternal recumbency and immediately rise again or stagger sideways (Hungerford 1990), similar to acute sunburn. The ears become thickened; congestion of the conjunctiva may occur with matting together of the eyelids (Amstutz 1975). Skin becomes dry, hard, and fissured and extremely pruritic. Areas of skin may become necrotic and peel off in strips. Ears and tail may slough.

Sunburn, erysipelas, and sarcoptic mange have to be included in a differential diagnosis. The typical lesions confined to unpigmented or white areas of skin exposed to sunlight and a history of ingestion of a photodynamic agent or plants known to cause photosensitization will suggest a diagnosis.

Affected animals should be placed in darkened housing. Parenteral use of corticosteroids or antihistamines may be of value. Local application of antibiotic creams may also be useful. Control is by preventing access to photodynamic agents, grazing only at night, or keeping pigs indoors.

**SKIN NECROSIS AND TRAUMA**

Skin necrosis in piglets most frequently affects the knees, fetlocks, hocks, elbows, teats, coronets, and soles of the feet (Figure 17.2A). Necrosis of the hocks, vulva, and tail is common in piglets with splayleg. In sows, skin necrosis is common on the shoulder, over the hip region, and on the side of the jaw. In piglets, necrosis starts as small abraded areas often developing 12–24 hours after birth and reaching maximum severity in 7 days (Penny et al. 1971). Lesions are due to trauma from hard abrasive floors, especially rough concrete in farrowing crates. The alkaline pH of new concrete floors and slats may also affect older pigs. Necrosis of the soles of the feet can be caused by the abrasive surface of rusting wire mesh or metal floors. Teat necrosis is best looked for at 3 days of age (Stevens 1984). The lesions develop as blackish-brown scales or crusts that easily peel off, leaving a new, fresh wound. Teat necrosis usually affects the pectoral (first four) teats, resulting in blind, nonfunctional teats. The highest incidence was found in pigs on heated concrete floors, with decreasing incidence on expanded metal floors, rubber mats, and plastic-coated wire mesh. A genetic basis for teat necrosis associated with sire lines was demonstrated, but nongenetic causes were more common than genetic causes (Stevens 1984). Females are most commonly affected. Necrosis of the knees (carpus) is very common in the weaker smaller piglets in large litters and where sows have problems of milk letdown or mastitis. Necrosis of the tail starts at the base, usually encircling the whole tail, which becomes black and may slough. Ear necrosis may be due to fighting or infections.

Skin necrosis in sow's teats is due to a combination of pressure from lying for long periods on hard floors (both concrete and mesh) and poor body condition associated with rapid weight loss during lactation or old age. The condition can occur in young sows after their first litter.

Control in piglets should be aimed at avoiding rough, wet contact surfaces, and providing bedding or rubber mats in the creep area of farrowing crates. Skin necrosis in sows is due to a combination of pressure from lying for long periods on hard floors (both concrete and mesh) and poor body condition associated with rapid weight loss during lactation or old age. The condition can occur in young sows after their first litter.

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necrosis of sows is best prevented by maintaining good body condition through appropriate feeding before and during lactation, using plastic-coated floors in farrowing crates, and encouraging sows to stand and exercise frequently.

Calluses are epidermal hypertrophy with fibrosis of the skin over joints and bony prominences. Calluses are seen mainly over the fetlocks, elbows, hocks, and tuber ischiil. They become very large and hard and contain fluid, which may become infected and result in subcutaneous abscesses. Pigs with leg weakness, foot lesions, or muscular weakness, or that spend a lot of time lying down due to illness frequently develop callosities or bursitis.

NUTRITIONAL DISEASES

Swine parakeratosis (Scott 1988) is a nutrition-related metabolic disorder of growing pigs characterized by a generalized nonpruritic, crusting dermatosis (Figure 17.2B). The cause of this condition is now considered to be complex, involving deficiencies of zinc and essential fatty acids or high levels of calcium, phytates, and other chelating agents that affect zinc absorption. Gastrointestinal diseases may also predispose to a more severe zinc deficiency and parakeratosis.

Early lesions (macules and papules) develop on the ventral surface of the abdomen, medial thighs, and distal parts of the legs. The lesions rapidly become covered with scales and then hard dry crusts. The typical keratinous lesions are characterized by crusts and deep fissures. The surface of the skin may be dry and rough, but moist brownish sebum, dirt, and debris accumulate in the fissures. In severe cases, animals will have reduced growth rates and reduced appetite, diarrhea, and in some cases vomiting. Testicular development may be affected. Mortalities are rare.

The condition has to be differentiated from chronic sarcopptic mange, exudative epidermitis, and deficiencies of the B group vitamins and iodine. A history of feeding a diet likely to be deficient in zinc or essential fatty acids or including factors that may interfere with zinc absorption, together with the characteristic lesions and their distribution, will suggest parakeratosis.

Skin biopsy for histopathology will be of value. Serum alkaline phosphatase and zinc levels may be decreased. Response to supplementary zinc and essential fatty acids will support a diagnosis.

CONGENITAL AND HEREDITARY DISEASES

Pityriasis Rosea (Pustular Psoriaform Dermatitis)

Pityriasis rosea in swine is the name used to describe a pustular dermatitis that takes on the appearance of epidermal collarettes, or rings, seen only in young swine, mainly on the ventral abdomen and inner thighs (Figure 17.2C). The disease is self-limiting. The condition, however, does not resemble pityriasis rosea in humans clinically or pathologically; therefore, pustular psoriaform dermatitis may be a more suitable name (Scott 1988). Although the actual cause is unknown, the condition appears to be inherited. Swine that have had the condition are more likely to produce affected progeny. The incidence may be higher in Landrace swine. Attempts to transmit the disease or demonstrate an infectious agent have failed. The condition is seen in piglets derived by hysterectomy and reared in isolation.

The disease is seen in young pigs 3–14 weeks of age. Entire litters or only a few piglets in a litter may be affected. The disease begins as small erythematous papules on the skin of the abdomen and inner thighs. The papules are raised with a central crater and rapidly expand to produce a ring, or collarette, with a raised bright red periphery behind which are scales. As the ring expands, the central area returns to normal. The rings coalesce as they expand to produce mosaic patterns. The hair is usually not lost and little pruritus is seen. The condition usually lasts about 4 weeks, regressing slowly and leaving normal skin as lesions heal. The extent and severity of the lesions appear to increase when pigs are reared in weaner cages where stocking densities are high with high temperatures and high humidity. Lesions may become infected with bacteria and resemble an exudative dermatitis. The condition has to be differentiated from ringworm, dermatosis vegetans, and swine pox. Failure to isolate fungi or microorganisms will help confirm a diagnosis.

Skin biopsy will show psoriaform epidermal hyperplasia and superficial perivascular dermatitis. There is mild to moderate mucinous degeneration of the superficial dermis, and the predominant inflammatory cells are eosinophils and neutrophils. Parakeratotic hyperkeratosis is usually prominent (Scott 1988).

Treatment does not appear to affect the course of the disease. Good hygiene will reduce the chance of secondary infections, whereas overstocking with high humidity and high temperatures appears to increase the incidence. It may be best to cull breeding stock known to produce progeny that develop the condition from the breeding herd.

Dermatosis Vegetans

Dermatosis vegetans is a hereditary and often congenital disease of swine due to a semilethal autosomal recessive factor believed to have originated in the Danish Landrace breed (Done et al. 1967). The condition is seen as an erythematous maculopapular dermatitis with lesions on the coronary band and hoof together with pneumonia.

The main features of the condition are skin lesions, abnormalities of the feet, poor growth, and respiratory dysfunction. The skin lesions may be present at birth.
or, more commonly, develop within 2–3 weeks of birth. They first develop on the abdomen and inside the thighs as small (0.5–2.0 cm in diameter) raised pink swellings that enlarge rapidly. The lesions spread over the flanks and back of the pigs and become covered with yellowish-brown, brittle, papillomatous crusts that are easily removed, leaving a pink granular surface. The lesions become very thick with a hard, horny surface that develops cracks and fissures, giving a characteristic papillomatous appearance. Swine may die after 5–8 weeks, but some will survive and the skin lesions regress.

Lesions of the hoof are usually present at birth and consist of marked swelling and erythema over the coronary band of both the main and the accessory digits and are covered with a yellowish-brown greasy exudate. The walls of the hooves are thickened with ridges and furrows parallel to the coronary band. The horn becomes discolored and blackened.

Respiratory dysfunction is due to giant-cell pneumonia. Respiratory signs of interstitial pneumonia or bronchopneumonia precede death, usually following a course of 4–6 weeks. However, some pigs may survive for 2–3 months but become weak and emaciated. On rare occasions, affected pigs have reached maturity and bred.

The condition has to be differentiated from pityriasis rosea, chronic exudative epidermitis, and vitamin deficiencies. The clinical appearance of the skin and hoof lesions seen in young pigs 2–3 weeks of age is, however, characteristic. The very thick papillomatous crusts of the skin lesions are also typical and together with respiratory distress will suggest a diagnosis of dermatosis vegetans.

Skin biopsy reveals intraepidermal pustular dermatitis and microabscesses containing eosinophils and neutrophils, with parakeratotic hyperkeratosis. Older lesions show hyperplastic superficial perivascular dermatitis with multinucleated giant cells in the dermis (Percy and Hulland 1967, 1968). Histopathology of the lungs will help a diagnosis. Done et al. (1967) recommended that lungs should be fixed by filling with, as well as immersing in, 10% neutral formalin, and several samples from each lung should be examined.

There is no treatment for the condition, and prevention is aimed at detection and removal of breeding stock known to have produced affected progeny.

**Epitheliogenesis Imperfecta (Aplasia Cutis)**

Epitheliogenesis imperfecta is an inherited congenital condition of both white and colored swine, and is caused by a simple autosomal recessive trait thought to result in a primary failure of embryonic ectodermal differentiation.

The lesions appear as clearly demarcated discontinuities of the squamous epithelium of varying sizes and shapes but usually on the back, loins, or limbs (Figure 17.2D). The condition may be seen in individual piglets or with a familial incidence in litters. The defect may also affect the dorsal and anterior ventral surface of the tongue with concurrent hydroureret and hydronephrosis (Jubb and Kennedy 1970). Lesions develop as large ulcers and frequently become infected; they may fail to heal or cause septicemia, which may lead to death.

**NEOPLASTIC DISEASES**

Tumors are reported relatively rarely in swine, probably because the majority are slaughtered at 6–8 months of age or around 4–5 years of age for breeding stock. However, a variety of neoplastic conditions have been reported, including lymphangiomata, rhabdomyomata, papilloma, sweat gland adenoma, fibroma, and hemangioma.

Melanomas, the result of proliferation of melanoblasts, have been reported most commonly in the Duroc breed and are often found at birth. The tumors are frequently seen on the flank region and have been described as being 1–4 cm in diameter, raised with an irregular black shiny surface. Metastases have been seen in lymph nodes, kidney, liver, lungs, heart, brain, and skeletal muscle.

Rhabdomyosarcomas have been reported as solitary or multiple tumors on piglets less than 1 week old in at least 25 piglets in a short period of time in one herd in The Netherlands, indicating a common, possibly genetic cause (Vos et al. 1993).

Small fibrous polyps or wart-like lesions have been seen on sows, especially around the neck, along the back, and on the ears. These lesions frequently bleed and can easily be removed under local anesthetic.

**PORCINE DERMATITIS AND NEPHROPATHY SYNDROME**

Porcine dermatitis and nephropathy syndrome (PDNS) has relatively recently been reported in pigs (Cameron 1995; Hélie et al. 1995; Smith et al. 1993; White and Higgins 1993) and is characterized by multifocal skin lesions, weight loss, edema of the limbs, vasculitis, and glomerulonephritis. Additional discussion of the renal component is found in Chapter 22. The cause is unknown, but histopathological and immunological findings suggest the pathogenesis involves an immune-complex disorder (antibody–antigen complex deposition) possibly due to an infectious agent. Thibault et al. (1998) suggested that porcine reproductive and respiratory syndrome virus (PRRSV) infection may play a role in the pathogenesis of the disease, because PRRSV antigens were detected by immunochemistry in macrophages located around vessels of skin and kidney tissue examined in acute and chronic cases.
Wellenberg et al. (2004) considered that porcine circovirus type 2 (PCV2) was likely to be the primary agent in the development of PDNS. They reported excessively high PCV2 antibody levels in a case-control field study of PDNS and hypothesized that PCV2 plays an important clinical and immunopathological role in the development of PDNS. They suggested that the excessive high levels of PCV2 antibodies trigger the development of fibrinous deposits (immune complexes) in, for example, kidneys that can initiate an inflammatory process when deposited within the vascular or glomerular capillary wall. They also found an epidemiological association in herds that had experienced postweaning multisystemic wasting syndrome (PMWS) also caused by PCV2. However, their study did not indicate that PRRSV infection was a primary cause of PDNS, nor did they consider Pasteurella multocida a primary agent as has previously been suggested (Sierra et al. 1997; Thomson et al. 2001).

Seen mainly in growing swine, 20–65 kg live weight, the most obvious clinical signs are skin lesions and a rapid loss in body weight with concurrent depression. The skin lesions range from large areas of erythema, macules, and hemorrhagic papules to dark brown to black thick crusts of necrosis on the ears, face, lower limbs, hindquarters, scrotum in boars, and vulva of sows (Figure 17.2E,F).

Other clinical signs include subcutaneous edema along the ventral abdominal wall and limbs. The lower parts of the legs are obviously swollen, and swelling of the joints is not uncommon. An outbreak in a large specific pathogen-free (SPF) herd soon after it had been established by medicated early weaning was investigated. Typical cases were seen in at least 20 growing pigs over a 3-month period. Most pigs had to be destroyed or died within a few weeks (R. Cameron, unpublished data).

The condition could be confused with erysipelas, with skin necrosis, or, in its early stages, with sarcotic mange. Of serious concern is that the clinical signs and lesions closely resemble those of classical swine fever and African swine fever. The autopsy findings will reveal enlarged, pale, spotted (petechiation) kidneys, fluid in the body cavities, subcutaneous fluid, and excessive synovial fluid in the joints. Gastric ulceration and hemorrhage are commonly seen. The histopathology of the kidney lesions is consistent with a diffuse necrotizing and proliferating glomerulonephritis. Glomerular spaces contain precipitated protein, necrotic cells (particularly polymorphs), and red blood cells. Secondary renal changes include formation of hyaline/granular casts and distended tubules. Necrotizing vasculitis of arterioles in the dermis and subcutis is associated with skin lesions. Small-vascular vasculitis can be detected in other organs, including lymph nodes, spleen, stomach, liver, bladder, brain, and joints (Higgins 1993). A significant increase in plasma urea and creatinine with a decrease in sodium and chloride and very high levels of protein and red and white blood cells in urine are characteristic. Because the actual causative agent is unknown, control is difficult.

REFERENCES

THE FEET AND CLAWS

INTRODUCTION

Reports suggest that the prevalence and severity of lesions of the feet and claws have increased as a result of pigs being raised on solid, particularly concrete, floors. Lesions of feet and claws are frequently associated with lameness, which can adversely affect growth rates and reproductive performance (caused by early culling in the breeding herd), as well as having a significant impact on welfare (Allerson et al. 2008; Anil et al. 2005; Bradley et al. 2008; Penny et al. 1963, 1965). Foot lesions have been reported in suckling piglets (Zoric et al. 2009), postweaned pigs (Gillman et al. 2009), and finisher pigs (Mouttotou et al. 1997, 1999) as well as sows and boars (Anil et al. 2007). Factors influencing the prevalence or severity of lesions include foot/claw conformation, housing and floor surface, nutrition, and infectious agents.

STRUCTURE AND FUNCTION OF THE FOOT AND CLAW

The pig is tetradactylous having two large weight-bearing digits (third and fourth) and two non-weight-bearing accessory digits or dewclaws (DCs) (second and fifth), the first digit is absent in the pig. The third digit is medial, and the fourth is lateral on each foot. The term “foot” usually refers to the entire region from the toe to the top of the DCs. Each digit includes the claw or hoof, which usually describes the keratinized horn tissue made up of the hard wall, hard sole, and soft heel. Each claw covers the third phalanx and the distal part of the second phalanx. The accessory digits are caudal to the larger digits and have a full complement of bones (phalanges). The volar surface of the claws consists of the large and prominent heel (which houses the digital cushion), the sole, and the wall. The sole in the pig covers a relatively small area, and a nonpigmented soft wall (white line [WL]) forms the junction between the wall of the claw and the sole. The lateral digit is larger than the medial digit on both the front and hind feet. The disparity in size is usually more prominent on the hind feet.

A cross section of the claw consists of bone of the phalanx, subcutus, corium or dermis, vascular basement membrane, and epidermis. The corium located just below the epidermis forms the supportive connective tissue layer for the epidermis, containing blood vessels and nerves. From this layer, nutrients and hormones are provided to the stratum basale or germinal layer for the production of the epidermal cells. All distal layers of the epidermis are derived from these cells by a process of proliferation and differentiation. The corium and its basement membrane are critical structures for keratin or soft/hard horn formation. Keratins are produced by a complex process of differentiation (keratinization) of the epidermal cells. Formation and biochemical binding of keratin proteins, synthesis and exocytosis of intracellular cementing substance are the hallmark of keratinization (Tomlinson et al. 2004).

The corium is covered by the densely arranged cells of the stratum basale, which are pushed into the next layer and enter the process of differentiation to make up the stratum spinosum. Toward the end of differentiation, basophilic dense keratohyalin granules accumulate in the cells, which are described as stratum granulosum. It is this layer that forms the border of cornification in which the cells die, that is, cornify and turn into horn cells, which establishes the stratum corneum. The process is dependent on an appropriate supply of nutrients, including vitamins, minerals, and trace elements. These nutrients are essential for the integrity of the hoof horn. Decreasing nutrient supply to keratinizing epidermal cells can lead to inferior horn quality and increase its susceptibility to chemical, physical, or microbial damage from the environment, which may result in clinical lesions of the claws and lameness.

CLASSIFICATION AND DESCRIPTIVE PATHOLOGY OF LESIONS

Foot and claw lesions can involve the heel, sole, WL, wall, and accessory digits. Anil et al. (2007) characterized claw lesions as erosion, cracks, and overgrowths.

Heel lesions commence as bruising seen as dark discolored areas under the volar surface. This is followed by erosion, which appears as a rough “ragged” lesion with underlying hemorrhage. Ulceration of the heel may follow. Continuous trauma can result in hyperkeratinization, excessive granulation, and necrosis (Penny et al. 1963) often extending to the interdigital cleft. Bradley et al. (2008) described heel erosion occurring in a circular pattern resembling tissue degeneration or irritation with cracks resembling a cut or tissue separation crossing the heel diagonally from the WL to the rear of the heel.

Sole lesions are also first seen as bruising with dark brown to red areas of hemorrhage under the surface, most frequently seen along the junction of the heel and sole. Hyperkeratinization of the sole can be a sequel to erosion and ulceration.

WL lesions of the claw, axial or abaxial, start as hard tissue wear (erosion) and separation between the hard and soft tissue of the hoof wall and sole or heel or both (Bradley et al. 2008; Penny et al. 1965). The lesions can result in extensive separation, which becomes impacted.
with dirt, feces, and foreign material often resulting in deformity of the claw and separation of the wall. WL lesions provide a site for infection to enter the hoof and corium.

Cracks of the wall can be vertical starting at the WL (false sand cracks) or starting at the coronary band (true sand cracks). Horizontal cracks can also occur. False sand cracks can vary in severity from a fine crack to a deep fissure with necrotic edges (Penny et al. 1963).

Coronary band abscesses (bush foot) are most likely an infection of the laminae with an abscess-like lesion along the coronary band. The area above the coronary band becomes very swollen, and a granulating sinus develops producing a purulent discharge. Infection may also involve deeper areas of the hoof and joints of the foot causing severe lameness.

Accessory digit lesions, common in sows and boars, can involve loss of the horn with hemorrhage and infection of the underlying tissue or lengthening of the digit (overgrowth).

A number of claw lesions were found by Da Silva et al. (2010) to be highly correlated with inflammation of the corium in lame sows. The authors considered the pathology and etiology of inflammation of the corium may not be the same for each lesion type and that there was the potential for claw lesions to contribute to additional inflammation of the corium. Due to the large number of nerves associated with the corium, inflammation is likely to result in pain and lameness.

Deen and Winders (2008) suggested using a consistent scoring system of 0–3, where 0 = no lesion, 1 = mild, 2 = moderate, and 3 = severe for the following types of pathology: heel overgrowth and erosion (HOE), a common lesion ranging from slight overgrowth and/or erosion of the soft heel tissue to severe overgrowth and cracks; heel–sole crack (HSC) lesions at the junction between the heel and the sole with severe lesions involving most or all of the junction; WL lesions involving separation along the WL in the juncture of the claw wall; cracked wall horizontal (CWH), cracks horizontally along the claw wall with severe lesions involving multiple or deep cracks; cracked wall vertical (CWV), vertical cracks in the claw wall and severe lesions involving multiple or deep cracks; toe (T) lesions involving excess lengthening of one or more claws where severe lesions significantly affect gait; and DC, moderate or severe lengthening of the claw (score 1 or 2) or torn or missing claw (3).

PREVALENCE OF FOOT AND CLAW LESIONS

Foot lesions are common and have been reported in all age groups of swine. The prevalence in suckling piglets of sole bruising ranged from 49.4% to 100% and sole erosion from 15.5% to 49.1% in the studies of Mouttotou and Green (1997) and KilBride et al. (2009), respectively. Zoric et al. (2004) found 87% of suckling piglets with sole bruising at 3–10 days of age and 39% still affected at 17 days.

Prevalence in weaner pigs has been reported as 50.2% with at least one digit affected (Mouttotou et al. 1998) and an overall prevalence of 39.6% reported by Gillman et al. (2009).

In two studies, the prevalence of lesions in finisher pigs was reported as 65.1% and 93.8% of pigs with at least one lesion by Penny et al. (1963) and Mouttotou et al. (1997), respectively. Lesions in weaners and finishers were similar and included heel, sole, and toe bruising and erosions; heel flaps; WL lesions; wall separation; and false sand cracks.

The early reports of Osborne (1950), Hogg (1952), and Penny et al. (1963) noted a high prevalence of foot lesions in sows. A more recent study found more than 96% of culled sows from loose housing and 80% from confined housing had at least one lesion (Gjein and Larssen 1995a). Anil et al. (2007) found that out of 184 gestating sows, 88.6% had at least one wall lesion and 86.4% had one heel lesion. Lesions between the junction of the heel and sole were seen in 66.3% of sows and 60.9% had WL lesions. Lesions were more severe on the forelimbs and a higher proportion of sows had lesions on the lateral claws than the medial claws.

ASSOCIATION BETWEEN FOOT LESIONS AND LAMENESS

The early studies of Osborne (1950) and Penny et al. (1963, 1965) reported an association between the more severe lesions of the claw and lameness especially in sows and boars. Lameness was also more likely to be seen where the lesions had become infected and in the more severe cases of heel erosion, severe WL cracks, infected vertical cracks of the wall, and abscesses of the coronary band.

Anil et al. (2007) found a significant association between lameness and WL lesions in sows while other claw lesions were not associated with lameness. However, in a later report by Anil et al. (2008), lesions of the heel as well as the WL were found to be associated with lameness.

In a study of the prevalence of lameness and claw lesions in two commercial herds where the prevalence was relatively low (less than 4% of sows had serious lameness), the most common lesions related to lameness were heel erosion and overgrowth of DCs. WL and sidewall lesions were not a feature of lameness in these herds (Sonderman et al. 2009).

Early recognition and assessment of lameness in sows is important for early intervention and treatment. However, Anil et al. (2008) showed that clinical assessment in correctly grading lameness scores was difficult for mild cases and nonlame sows. Accurate assessment of lameness requires experience and specific training.
FACTORS ASSOCIATED WITH THE PREVALENCE OF FOOT AND CLAW LESIONS

Research into the prevalence and severity of foot and claw lesions has identified a number of associated factors including claw conformation, environment in particular housing and floor type, nutrition, and infectious agents.

Claw Conformation

Numerous studies have shown that with the presence of disparity in claw size, where the lateral claw is generally larger than the medial claw, lesions tend to be more prevalent and/or more severe on the lateral claw. The disparity between claw size also tends to be more frequent on the hind feet.

Penny et al. (1963) measured claw size and found that the average ratio of length of lateral claw to medial claw was 1.11:1 and width 1.13:1. In 3195 pigs examined, significantly more lesions were found on the lateral claw especially on the hind feet. They concluded that the difference in claw size was most likely hereditary and that the smaller medial claw was protected to some extent by the larger lateral claw. Therefore, the medial claws take less weight and less trauma, while the lateral claw is subject to more trauma due to its size and position.

Subsequent reports are in general agreement with these earlier findings (Anil et al. 2007, 2009; Bradley et al. 2008; Gjein and Larssen 1995b). Anil et al. (2007) found lesions more severe on forelimbs than on hind limbs, and the proportion of sows with lesions on the lateral claws was greater than the proportion of sows with lesions on the medial claw. In discussion and with reference to other studies, Anil et al. (2007) considered weight distribution of sows may be important in determining the development of lesions on different claws and different limbs (Kroneman et al. 1993). Hoof lesions may be more common on lateral claws because of a greater weight-bearing surface than medial claws (Tubs 1988). More than 75% of the weight of the pig is born by the lateral digit and 80% of the injuries affect these digits (Webb 1984). Citing Kroneman et al. (1993), the same authors suggested that the strength of different parts of the foot may vary. Junctions between hard and soft areas may be more susceptible to injury. Maximum weight is born by the heel bulb, followed by the junction between the heel bulb and abaxial hoof wall of the lateral digit. The tip of the toe is the greatest weight-bearing region on the medial digit.

Amstel et al. (2009) found that the front feet have better weight-bearing stability than the rear feet, with the rear medial claw as the least stable. It was suggested that this may result in more weight being transferred to the more stable rear lateral claw, which may explain the higher reported incidence of lesions observed in that claw.

The disparity in claw size in piglets would suggest it is hereditary (Penny et al. 1963). R. Walters (personal communication) reviewed the literature on uneven claw size and found a large range in heritability due to genotype differences and different scoring (measurement) methods. The average however was 0.27 similar to the heritability of growth rate. Walters considered that in an “average” population, we could expect progress through selection. Jørgensen and Andersen (2000) found values of 0.13 and 0.19 in Landrace and Large White pigs, respectively. Tarres et al. (2006) examined sow longevity and feet and leg scores in Large White sows in Swiss herds and concluded that culling on extreme feet and leg scores from 1999 to 2003 have contributed to the improvement of sow longevity in these herds.

Type of Flooring

The association between housing and type of flooring and the prevalence of foot/claw lesions has been the subject of reports in suckling piglets, weaners, finishers, and sows.

Foot lesions are found in suckling piglets where farrowing crate floors and creep areas are solid, especially solid concrete (Mouttotou et al. 1999; Zoric et al. 2004, 2009). By reducing the roughness and abrasiveness of farrowing crate floors or by covering with sufficient straw, the prevalence and severity of lesions in piglets can be reduced (Zoric et al. 2009). Sole bruising increases in prevalence in piglets on slatted floors compared with solid concrete floors even with bedding. Slatted floors increase the risk of sole erosions (KilBride et al. 2009).

The prevalence of lesions in weaners up to 14 weeks old held on solid, totally or partially slatted floors, or outside on a soil base all with varying amounts of bedding was studied by Gillman et al. (2009). Toe erosion was positively associated with deep bedding, whereas deep bedding on a soil base was negatively associated with the prevalence of heel and sole erosion. Heel flaps and heel/sole bruising were also associated with slatted floors.

Claw lesions were more common in finisher pigs kept on solid concrete floors especially wall cracks and WL cracks (Osborne 1950). Finishers kept on straw-bedded floors were found to have less sole/heel erosions and heel flaps but more WL lesions, false sand cracks, wall separation, and toe erosion compared with pigs on bare concrete. These lesions can increase where the floors are partially or fully slatted (Mouttotou et al. 1999).

Smith and Morgan (1998) found finisher pigs kept on totally or partially slatted concrete had claw lesions on both floor types. The prevalence of lesions rose as floors became more abrasive. The prevalence on good quality totally slatted floors (low to moderate values of abrasion, slipperiness, slat edge, slat gap, and nature of...
slat edge) was lower than on floors with unsuitable slat quality. The authors recommended the edge of slats should be rounded and smooth, and have a slat width of 13–14 cm, a void width of 1.8–2.4 cm, and a total linear edge of 10.0–17.7 m/pig.

The prevalence of claw lesions in pregnant sows has been found to be higher in loose housing systems compared with sows confined in stalls especially where the floors are partially or fully slatted. However, deep litter based on straw reduces the incidence and severity of lesions, while poor floor hygiene and high stocking density increase the severity of claw lesions (Gjein and Larssen 1995b; Holmgren et al. 2000).

Roughness of floors contributes to a higher incidence of claw lesions and increased lameness. The pH of concrete from 7.4 to 8.3 did not appear to influence prevalence; however, dirty wet conditions may soften the volar aspect of the feet, making them more susceptible to trauma (Penny et al. 1965). All types of claw lesions tend to be higher in sows kept in loose housing with fully slatted floors compared with sows confined to fully slatted stalls (Anil et al. 2007). The use of electronic feeders, which may contribute to aggression on entry to the feeder, may further increase prevalence of claw and foot lesions. Increase body weight has been associated with increased wall lesions, and higher back fat was associated with more heel lesions but less overgrowth of heels (Anil et al. 2007).

**Nutrition and Hoof Horn Integrity.** In a review of keratins and hoof horn development in the bovine, Tomlinson et al. (2004) stated that keratinization of hoof epidermis is controlled and modulated by a variety of bioactive molecules and hormones dependent on an appropriate supply of nutrients including vitamins, minerals, and trace elements. Nutrient flow to the epidermal cells plays a central role in determining the quality and consequently, the functional integrity of the hoof horn.

Hormones are also now considered important in horn growth. Insulin, epidermal growth factor, prolactin, and glucocorticoids are likely to be involved in horn growth by their impact on nutrient flow.

Nutrients required for horn growth include amino acids (cysteine, histidine, and methionine), minerals (calcium, zinc, copper, selenium, and manganese), and vitamins (A, D, E, and biotin). Except for biotin, very little peer-reviewed literature related to nutritional requirements for hoof growth in the pig is available. Present knowledge is based on hoof development and integrity researched in cattle and horses.

Amino acids play key roles in establishing the structural integrity of the keratinocyte. Calcium, zinc, and copper are all important in the keratinization and cornification process. Zinc is important for tissue healing and as with copper, strength and elasticity of the hoof tissue.

Zinc supplementation in dairy cows has shown a reduction in cases of foot rot, heel cracks, interdigital dermatitis, and laminitis (Moore et al. 1989). Also in cattle, copper deficiency can result in heel cracks, foot rot, and sole abscesses. Selenium is an antioxidant and may contribute to the protection and maintenance of physiological function of the lipid-rich intracellular cementing substance (Tomlinson et al. 2004). Excessive selenium, however, may result in inferior hoof horn, resulting in lameness and deformed claws as in cattle (Larson et al. 1980).

Manganese is essential for skeletal and tendon development, and joint and cartilage strength. Vitamins A, D, and E all play an integral role in developing the structure and quality of keratinized horn tissue. Vitamin D is also important in calcium metabolism.

The role of biotin for hoof and claw integrity has been extensively researched in the pig (Hill 1992). Tomlinson et al. (2004) considered biotin was possibly the vitamin of greatest importance to the keratinization process and integrity of keratinized tissues (skin, hair, claws, and foot pads). Biotin is essential for the formation of the complex lipid molecules in the intracellular cementing substance (Mülling et al. 1999). Hill (1992) cited a number of early experiments involving individual biotin deficiencies in pigs resulting in alopecia, dermatosis, cracked hooves, and lameness.

In 1977, Brooks et al. supplemented the diet of breeding sows with biotin and demonstrated a 28% decrease in total foot lesions. Penny et al. (1980) reported using biotin supplementation in a herd of 116 sows and gilts with severe lameness problems. The herd was divided into two groups, half given a supplement of 1160 µg/day of biotin during pregnancy and 2320 µg/day in lactation over a 12-month period, the other half were used as controls (no supplementation). Feet, including the volar aspect, were examined in all animals at the beginning of the trial and subsequently at three monthly intervals. Sows with existing foot lesions and lameness showed no improvement over the 12 months from biotin supplementation. However, gilts entering the herd as replacements and receiving biotin supplement showed significantly fewer lesions of heel and WL. The number and severity of lesions on the lateral hind claw were also significantly improved. The authors suggested that biotin was an essential vitamin for young stock entering the breeding herd as the hoof horn would be better able to withstand the trauma of the environment.

Subsequent studies (Misir and Blair 1986; Simmins and Brooks 1988) also reported the benefit of biotin-supplemented rations in relation to hoof lesions, hoof wall hardening, and foot pad resilience. The results showed the response to biotin was dose and time dependent. In the report by Misir and Blair, the supplement of biotin resulted in a significant reduction in the
incidence of lesions on foot pads, above the DC but no effect on hoof cracks.

In a study of reproductive performance and the incidence and severity of foot lesions in sows fed a corn–soybean-meal–based, gestation–lactation diet supplemented with 440 µg/kg d-biotin, Watkins et al. (1991) found the biotin supplement did not significantly affect the incidence or severity of foot lesions. However, foot lesion scores tended to be lower in the biotin-supplemented sows.

However, in a similar study by Lewis et al. (1991) involving three research herds, biotin supplementation in gestating and lactating sows did not decrease the incidence of foot lesions. In fact, there was a higher incidence in feet cracks in sows receiving biotin supplement than in nonsupplemented sows. This difference was significant for sidewall cracks. Also, the incidence and severity of bruises was higher in sows receiving biotin supplement, and there was a significant herd × treatment interaction.

Kornegay (1986) reviewed evidence that biotin increases the hardness of the hoof wall but decreases the hardness of heel bulb tissue, suggesting biotin supplementation could decrease incidence of foot cracks and increase incidence of foot bruises. Kornegay was of the opinion that numerous environmental and nutritional factors may influence the occurrence of biotin deficiency in swine and alter biotin required in the diet.

A number of studies looking at microscopic changes in relation to the hoof/horn have been reported. Results of these studies were to some extent conflicting. Brooks and Simmins (1980) found no difference in horn strength as a result of biotin supplement in induced biotin-deficient pigs. However, Webb et al. (1984) found strength and hardness improved in the midabaxial sidewall in pigs fed biotin supplement. They considered that this would reduce the susceptibility of the foot to injury. Microscopic changes in the hoof horn of pigs supplemented with biotin indicated improved strength and hardness in experiments reported by Webb et al. (1984) and Kempson et al. (1989).

Watkins et al. (1991) considered that the discrepancy in the efficiency of biotin supplementation seen in the literature most likely reflects the wide range of environmental, nutritional, and management practices used in the swine industry. Availability of biotin for the pig may vary significantly depending on the type of grain and storage conditions. Presence of mold can reduce the availability of biotin in corn (Hamilton and Veum 1984). Biotin in grains has been reported to be poorly absorbed in the pig and diets based on wheat or barley may not contain adequate levels of bioavailable biotin (Misir and Blair 1986). Other factors such as age, lactation, antibiotic medication, enteric disease, and access to feces (coprophagic pigs) may also influence biotin levels in the pig.

REFERENCES

MAMMARY GLAND ANATOMY

The microscopic and macroscopic anatomy of the porcine mammary gland has been described by Barone (1978), Schummer et al. (1981), and Calhoun and Stinson (1987). The mammary glands of swine are located in two parallel rows along the ventral body wall from the thoracic region to the inguinal area. The actual number of glands varies between 12 and 18 (Labroue et al. 2001) in commercial swine, whereas pigs of the Meishan breed can have up to 22 mammary glands. Data from France indicates that 65% of purebred sows had 16 or more functional teats compared with approximately 18% in 2002. As suggested by Muirhead (1991), boars and gilts retained for breeding should have 14 well-placed normal nipples with the rows of teats parallel for proper teat presentation and accessibility to the piglets. Poor teat placement could be a major reason for failure of sows to rear 11 or 12 piglets.

The glands (two thoracic, four abdominal, and one inguinal) are attached to the ventral body wall by adipose and connective tissue. Each mammary gland normally has one teat (nipple) with two separate teat canals. When the teat orifice is not visible (inverted teat), it has a 50% chance of remaining blind. Functional supernumerary smaller teats can also be found. Paired vestigial nonfunctional accessory teats, not connected to glandular tissue, may also occur (Labroue et al. 2001; Molenat and Thibeault 1977).

In the nulliparous sow, the mammary gland consists of cell buds distributed among fat and connective tissue, whereas in the lactating gland, the connective tissue is largely displaced by glandular parenchyma. The mammary glands of the lactating sow are composed of a compound tubuloalveolar tissue with the secretory units arranged in lobules. The lobules are lined by epithelial cells (lactocytes) that synthesize milk. These secreting units are connected by a nonsecreting duct system to an ostium found on the teat. There are usually two complete gland systems within each mammary gland of the pig. The glandular tissue of one system usually interdigitates with the other, but the components of the two systems are independent, emerging as two orifices for each nipple (Figure 18.1). There is no muscular sphincter around the teat orifice; therefore, intramammary treatment by way of the teat opening is not possible.

The arterial, venous, and lymphatic circulation is provided on each side of the ventral midline by a network that extends longitudinally from the axillary to the inguinal regions (Barone 1978; Lignereux et al. 1996; Schummer et al. 1981). Moreover, in swine, there is a venous anastomosis between the right and left mammary gland of each pair of glands.

Nerve supply to the cranial mammary glands differs from that to the inguinal glands. Cranial mammary glands receive their innervation from the last eight or nine thoracic nerves, while inguinal mammary glands receive their innervation mainly from the pudendal nerve (Gandhi and Getty 1969a,b; Ghoshal 1975). A more complete anatomical and histological description of the porcine mammary gland can be found in the 7th edition of Diseases of Swine (Smith et al. 1992).

MAMMOGENESIS

The extent of mammary growth and milking capacity is related to the number of lactocytes present. Mammary growth starts at the fetal stage but occurs mainly...
et al. 1999a). The period of maximal mammary mass coincides with the estimated peak of lactation (Hurley 2001). Parity can have an effect; between day 113 of gestation and day 26 of lactation, sows of parities 1, 2, and 3 had increases of mammary gland wet weight of 70%, 20%, and 30%, respectively (Beyer et al. 1994).

Factors Affecting Mammary Gland Development

Estrogens are important for mammogenesis (Kensinger et al. 1986). The increase in rate of mammary development at 90 days of age in gilts is linked to the onset of ovarian activity (Farmer et al. 2004b; Sorensen et al. 2002). Prolactin is shown to have an impact on mammary development taking place before puberty by stimulating an increase in the number of milk secretory cells (Farmer and Palin 2005).

During pregnancy, the formation of lobuloalveolar cells is related to increases in estrogen and progesterone concentrations in the maternal circulation (Ash and Heap 1975) while the concentrations of prolactin remain low (Dusza and Krzymowska 1981). After day 105 of pregnancy, decreasing progesterone and increasing estrogen concentrations are related to a significant increase in metabolic activity of the mammary gland (Kensinger et al. 1986; Knight et al. 1977; Robertson and King 1974), perhaps by induction of prolactin receptors in the mammary gland. Inhibition of prolactin in the last third of pregnancy drastically decreases mammary development in gilts (Farmer et al. 2000), specifically from days 90 to 109 of gestation (Farmer and Petitclerc 2003).

Buttle (1988) reported that ovariectomy (but not the removal of only the corpora lutea) of gilts at day 60 of gestation delays the onset of lobuloalveolar development in the mammary glands. Hurley et al. (1991) later demonstrated that relaxin plays a major role in promoting mammogenesis in gilts during the last third of pregnancy. Relaxin increases growth of parenchyma and decreases the mammary fat pad while having no effect on the cellular composition of mammary parenchyma. The mechanism is unknown, yet it seems likely that it requires estrogen priming as well as interaction with other mammotropic hormones, such as prolactin (Hurley et al. 1991).

Nutrition in the two periods of rapid mammary development (i.e., from 90 days of age until puberty and during the last third of gestation) impacts the extent of development. Sorensen et al. (2006) demonstrated that a period of ad libitum feeding from 3 months of age to puberty is needed to maximize mammary growth in gilts. Lyvers-Peffer and Rozeboom (2001) showed that decreasing energy intake of gilts between 9 and 25 weeks of age reduces weight of parenchymal tissue and lowers mammary DNA at the end of gestation, also demonstrated by Farmer et al. (2004b). While effects of energy are clearly demonstrated, the
feeding of a lower-protein diet (14.4% vs. 18.7% crude protein) during that same period of time does not hinder mammary development of gilts as long as a feed protein) during that same period of time does not feeding of a lower-protein diet (14.4% vs. 18.7% crude levels (Kim et al. 2001). Individual mammary mammogenesis in swine.

neonatal life may have long-lasting repercussions on changes that occur in utero and/or in early changes during the initial 7 days after weaning, with significant changes occurring as early as 2 days after weaning (Ford et al. 2003). Mammary gland involuition involves losses of over two-thirds of the parenchymal mass and cells present on the day of weaning (Ford et al. 2003). This regression occurs rapidly during the first 7–10 days postpartum and is affected by dietary nutrient levels (Kim et al. 2001). Individual mammary glands that are not regularly suckled during lactation undergo involuition during lactation with no further reduction in parenchymal tissue during the first 7 days after weaning (Ford et al. 2003). Mammary gland involuition is reversible within 24 hours, but milk yield will remain lower for the remainder of lactation (Kim et al. 2001). Involution is irreversible after 3 days (Theil et al. 2005).

Mammary glands that are suckled during lactation are larger than the nonsuckled glands at the end of involuition, which suggests more mammary tissue available for redevelopment and greater productivity in the next lactation (Ford et al. 2003). Fraser and Rushen (1992) found that teat use during lactation has a positive effect on milk yield of that gland in the next lactation; in contrast, a Norwegian study showed that inactive mammary glands for one or two lactations may have full milk yield in the subsequent lactation (Gut-à Porta et al. 2004).

The abrupt cessation of lactation in sows when milk production is at a maximum (3–4 weeks) brings about drastic changes in metabolic activity and endocrine status of the mammary glands and sow. There is a decrease in milk lactose and a transient decrease in milk glucose followed by an increase in plasma lactose and glucose (Atwood and Hartmann 1995). This is probably due to alterations in the tight junctions between mammary epithelial cells. In the week following weaning, the mammary alveoli regress (Hacker 1970) and the secretory glandular mass is replaced by adipose tissue in which a new alveolar system can develop in the following pregnancy (Delouis 1986). The absence of stimulation of the mammary glands stops the regular secretion of prolactin (Benjaminsen 1981), while the concentrations of gonadotropic hormones start to increase, allowing ovarian cycles to resume (Stevenson et al. 1981). Ford et al. (2003) administered estrogens during the postweaning period, which did not enhance overall rate of mammary involution in sows weaned at 21 days of lactation.

Weaning age, on the other hand, affects the process of mammary involution since tight junctions between mammary epithelial cells become leaky as lactation advances from 22 to 44 days (Farmer et al. 2007). It was suggested that completion of the functional changes occurring in mammary glands during involution may be required for the gland to redevelop fully in the subsequent lactation (Hurley 1989). The best management practices to maximize milk yield the following lactation still need to be established.

MAMMARY GLAND INVOLUTION

Suckled mammary glands of sows undergo dramatic changes during the initial 7 days after weaning, with significant changes occurring as early as 2 days after weaning (Ford et al. 2003). Mammary gland involuition involves losses of over two-thirds of the parenchymal mass and cells present on the day of weaning (Ford et al. 2003). This regression occurs rapidly during the first 7–10 days postpartum and is affected by dietary nutrient levels (Kim et al. 2001). Individual mammary glands that are not regularly suckled during lactation undergo involuition during lactation with no further reduction in parenchymal tissue during the first 7 days after weaning (Ford et al. 2003). Mammary gland involuition is reversible within 24 hours, but milk yield will remain lower for the remainder of lactation (Kim et al. 2001). Involution is irreversible after 3 days (Theil et al. 2005).

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PHYSIOLOGY OF MILK PRODUCTION

Lactogenesis

Lactogenesis is the ability of the mammary gland to synthesize milk components such as lactose, casein, and lipids, and is often described as a two-phase process.
Lactogenesis phase I refers to the preparation of the mammary tissue for the synthesis of milk components, and lactogenesis phase II describes the start of milk synthesis and secretion around parturition (Hartmann et al. 1995).

Milk components appear in the alveoli between days 90 and 105 of gestation indicating the beginning of phase I (Kensinger et al. 1982); however, little secretion can be obtained until parturition. At the end of gestation and during the Colostral phase, the junctions between epithelial cells surrounding the alveoli are not tight. This allows serum transudate to leak from the bloodstream into the mammary secretions and milk components from the mammary gland alveoli to leak back to the bloodstream (Figure 18.2). Although serum transudation may contribute to the volume of Colostrum obtained by the piglets, its contribution is most likely very short in duration.

During the Colostral phase, lactose is present in high concentrations (>200 mmol/L) in the plasma of sows (Hartmann et al. 1984), and all of the immunoglobulins found in mammary secretion originate from the plasma (Bourne and Curtis 1973). During lactation, plasma lactose concentrations are low (<100 mmol/L) (Hartmann et al. 1984), and most of the immunoglobulins present in the mammary secretions are synthesized locally (Bourne and Curtis 1973). Similar patterns were found for the plasma concentrations of milk whey proteins (α-lactalbumin and β-lactoglobulin) during the Colostral and lactation phases in swine (Dodd et al. 1994).

The onset of milk component synthesis is closely related to the decline in serum progesterone concentrations at farrowing (Hartmann et al. 1984; Robertson and King 1974). However, exogenous progesterone administration during late pregnancy delays the onset of parturition without inhibiting lactogenesis (Foisenet et al. 2010c; Whitely et al. 1990). Nevertheless, progesterone withdrawal (i.e., lysis of the corpora lutea via prostaglandins) is considered to be a major hormonal signal for the initiation of copious milk synthesis in swine by priming the glands, whereas withdrawal of Colostrum from the glands would trigger the initiation of copious milk secretion (Hartmann et al. 1995).

Relaxin is involved in the onset of parturition since its concentration always increases before those of progesterone begin to decline at the end of pregnancy. The preparturient rise in prolactin followed by the decline in both progesterone and relaxin appear to form important components of the lactogenic hormonal complex (Whitely et al. 1990). Prolactin is known to be a key hormone for the onset of lactation (Tucker 1985). In the pregnant sow, suppression of the prepartum peak of prolactin inhibits subsequent milk production (Farmer et al. 1998; Taverne et al. 1982; Whitacre and Threlfall 1981).

**Milk Ejection**

During the Colostral phase, particularly during parturition and the first hours afterward, Colostrum ejection is elicited with ease. At parturition, distension of the cervix for the passage of the piglets and movements of the sow are enough to lead to Colostrum ejection (Castren et al. 1989; Fraser 1984). Colostrum ejections are as frequent as every 10–20 minutes, and the period of high intramammary pressure permitting Colostrum withdrawal may be sustained for a minute or more. Once 50–100 mL of Colostrum is removed, the intramammary pressure is reduced to the point that further withdrawal is more difficult (Fraser 1984).

After the Colostral phase, and for the rest of lactation, milk ejection is cyclical and actively synchronized by sow gruntings (Castren et al. 1993; Chaloupková et al. 2007; Lewis and Hurnik 1985; Whittemore and
Fraser 1974). The removal of milk from the alveoli and ductal system of the porcine mammary glands requires a neuroendocrine milk ejection reflex. This is elicited by piglets massaging the udder (Fraser 1980), stimulating the release of oxytocin and the ejection of milk (Hartmann and Holmes 1989). Oxytocin stimulates the contraction of myoepithelial cells surrounding the alveolar lumen to force milk through the ductal system to the teats (Ellendorf et al. 1982). Udder stimulation is needed to trigger oxytocin release (Algers et al. 1990), but growth rate of piglets is not affected by the peak amplitude in intramammary pressure (Kent et al. 2003), which is linked to oxytocin levels. The increase in circulating oxytocin concentrations can occur up to 30 seconds before milk ejection (Ellendorf et al. 1982), which is very short in duration and lasts only for 10–20 seconds (Fraser 1980). Whitely et al. (1985) observed acute episodic releases of relaxin in the blood of sows both when piglets sucked and after the administration of oxytocin, which suggested that relaxin could oppose the action of oxytocin and/or provide a negative feedback on the hypothalamus for the suppression of oxytocin secretion.

Not all nursing attempts are successful during lactation. Failed nursing attempts may affect some or all piglets in the litter. Among the latter, one must distinguish between sucklings terminated by the piglets and those terminated by the sow (Illmann and Madlafousek 1995). Unsuccessful sucklings affecting the whole litter become more frequent after the colostral phase (Castren et al. 1993; Fraser 1977). These are characterized by an absence in the rise of intramammary pressure associated with a lack of increase in plasma oxytocin (Ellendorf et al. 1982). During the first 10 days of lactation, the proportion of unsuccessful sucklings is reported to be between 20% and 40% (Fraser 1977; Jensen et al. 1991). Although frequent and requiring expenditure of energy from the piglets, ejection failures may play a role in maintaining lactation (Algers 1993), and plasma concentrations of lactogenic hormones tend to rise after an unsuccessful nursing (Rushen et al. 1993). Rushen et al. (1995) demonstrated that external stress, such as placing sows in a novel environment, increases the chance that the subsequent nursing attempt will fail. This was not due to increased concentrations of cortisol or adrenocorticotropic hormone (ACTH), but more likely caused by an opioid-mediated inhibition of oxytocin. The stress of a novel environment did not lead to a general increase in the threshold of stimulation of the mammary gland that is required for oxytocin release.

**Measuring Milk Production**

Measuring milk production in the sow is difficult because the small and numerous teats make hand or mechanical milking difficult. Moreover, milk is not available continually after the colostral phase; therefore, milk ejection must be stimulated with exogenous oxytocin. Sows would have to be milked more than 24 times a day to mimic the piglets (Hernandez et al. 1987). Consequently, sow milk production is usually estimated by taking a number of measurements during the day and extrapolating to the whole day. Seven to eight measurements over a 24-hour period are reported to provide a good estimate of daily milk production (Mahan et al. 1971; Salmon-Legagneur 1965).

Piglet weights before and after sucking used to estimate sow milk output underestimate milk production capacity in the first part of lactation because milk production is greater than the ingestion capacity of the piglets. This can be compensated by using more than one litter to suckle the milk production of a single sow. In mid- and late lactation, milk supply is a limiting factor for piglet growth, and these methods give a good estimate of the milk production capacity of the sow. Milking machines are reported to give more repeatable estimates of milk production and have been used to compare milk production between teats (Fraser et al. 1985) and breeds (Grun et al. 1993a).

Early studies reported that piglet growth was a poor predictor of sow milk production (Lewis et al. 1978; Salmon-Legagneur 1956), but recent work demonstrated that milk production over different parts of lactation can be adequately predicted by using piglet growth (Noblet and Etienne 1989). To do so, it is essential to ensure that litter size is standardized and that piglets do not have access to solid feed.

**Pattern of Milk Production**

Sow milk production is usually described in four phases: the colostral, ascending, plateau, and descending phases. Sows in modern production units usually do not reach the descending phase because they are weaned during the plateau phase at less than 28 days of lactation. Typical sow milk production curves are presented in Figure 18.3 (Toner et al. 1996) and are similar to those presented by others (Grun et al. 1993b; Noblet and Etienne 1986; Shoenherr et al. 1989). Reports of the end of the ascending phase vary from 14 to 28 days postpartum. This is most likely related to differences in breeds, nutrition, and parities of the sows, or in the methods used to estimate milk production (Elsley 1971; Harkins et al. 1989).

**Colostral Phase.** Colostrum contains more proteins (mainly immunoglobulins), less fat, and less sugar than milk (Dorland 1985). The transition between colostrum and milk occurs around 24 hours after the onset of parturition and is characterized by a drop in immunoglobulin concentrations (Klobasa et al. 1987) with concomitant rises in fat and lactose. In the early colostral phase, mammary secretions are continuously available.
to the piglets, whereas later in the colostral phase, milk availability becomes cyclical (de Passillé and Rushen 1989a). Within 48–72 hours postpartum, piglets establish a teat order in which each piglet consistently sucks from one or two specific teats (de Passillé et al. 1988; Fraser 1976; Roychoudhury et al. 1995). Within 48 hours after parturition, any unsuckled mammary gland goes through involution and becomes nonfunctional (Atwood and Hartmann 1993). Milk production during lactation is proportional to the number of suckled mammary glands (Auldist et al. 1998).

A recent review focusing on the nutritional, hormonal, and environmental controls of colostrogenesis in swine was published (Farmer and Quesnel 2009). Sow colostrum production ranges between 2.5 and 5 kg over the first 24 hours postpartum for a litter of 8–12 piglets, with an average of 3.5 kg. In comparison, milk production on day 4 of lactation ranges between 5 and 10 kg/day with an average of about 8 kg/day. The total yield of colostrum is roughly half that of milk in early lactation while being just as variable (Devillers 2004; Devillers et al. 2004a,b, 2005, 2006, 2007; Farmer et al. 2006, 2007; Foisnet 2010; Foisnet et al. 2010a–c; Le Dividich 2006; Le Dividich et al. 1994a, 2004, 2007).

**Ascending Phase.** During the ascending phase of lactation (until approximately day 10), there is an increase in nursing frequency as well as an increase in the volume of milk obtained at each nursing. Nursing frequency doubled (17 vs. 35 nursings/day) between day 2 and day 10 of lactation (Jensen et al. 1991) and the quantity of milk at each nursing increased from 29 to 53 g between the first and the third week of lactation (Campbell and Dunkin 1982). During the ascending phase, sow milk production is adjusted to the needs of the piglets with litters of heavier piglets consuming more milk than litters of lighter piglets (King et al. 1997). During the first 2 weeks of lactation, the heavier piglets at birth ingest more milk than their lighter littermates; however, the milk consumption relative to body weight (gram per kilogram of live weight) is similar for all piglets (Campbell and Dunkin 1982). The larger consumption observed among the heavier piglets was explained by a larger volume ingested at each nursing.

**Plateau Phase.** After approximately day 10 of lactation, milk production is at a maximal level (King et al. 1997). Therefore, piglet growth is limited by the insufficient milk supply during the later part of lactation, which is magnified by longer lactation and larger litters (Table 18.1). The weight of suckling piglets supplemented with cow’s milk between days 7 and 28 of lactation was greater by 140 g on day 14, 756 g on day 21, and 1761 g on day 28 (Reale 1987). Supplementation with milk replacer from day 3 to weaning also increased average piglet weight by 120 g on day 7, 340 g on day 14, and 910 g on day 21 (Wolter et al. 2002). In a 21-day lactation, the growth limitation of suckling piglets can be more than 2 kg per pig (Harrell et al. 1993). This problem of inadequate milk supply to sustain optimal piglet growth has been exacerbated in recent years with increasing litter size of hyperprolific sows.

### CONTROL OF MILK PRODUCTION

#### Milking Frequency

Cyclical nursings start around 10 hours after the onset of farrowing (Lewis and Hurnik 1985) and develop gradually (Algers and Uvnäs-Moberg 2007). Nursing frequency has a major role in determining milk output and reaches a maximum around days 8–10 of lactation and decreases thereafter (Puppe and Tuchscherer 2000). Nursing intervals of 36–40 minutes were reported in early lactation (day 5 or 6) and of 39–48 minutes in late lactation (day 18 or 20) (Farmer et al. 2001; Fisette et al. 2004). Nursing frequency seems to be similar

### Table 18.1. Least-square means of piglet weight (kilogram) at different ages from randomly chosen 59 litters of different sizes after correction for birth weight (these litters had no mortality for the 28-day lactation period)

<table>
<thead>
<tr>
<th>Litter Size</th>
<th>Age of the Piglets (Days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>3 7 14 21 28</td>
</tr>
<tr>
<td>9</td>
<td>15 1.87 2.82 4.62 6.42 8.21</td>
</tr>
<tr>
<td>10</td>
<td>15 1.85 2.78 4.38 6.18 7.91</td>
</tr>
<tr>
<td>11</td>
<td>14 1.88 2.72 4.40 6.18 7.89</td>
</tr>
</tbody>
</table>

Adapted from Klopfenstein et al. (2006).
Hormonal Control

The activation of neural receptors within the mammary glands by piglets stimulates oxytocin from the posterior pituitary as well as the release of prolactin, growth hormone (GH), ACTH, and thyroid-stimulating hormone from the anterior pituitary. Hormones from the anterior pituitary have the function of maintaining the synthesis of milk from the mammary epithelial cells (Delouis 1986).

The role of GH for milk production is both direct (as a regulator of nutrient partitioning for milk component synthesis) or indirect (Flint and Gardner 1994). The indirect role of GH is via an increase in the concentrations of insulin-like growth factor 1 (IGF-1), which acts on the mammary epithelial cell via IGF-1 receptors. When circulating levels of GH and IGF-1 were reduced in lactation by immunizing sows against growth hormone-releasing factor (GHRF), milk yield was significantly decreased, yet growth rate of the piglets was unaltered (Armstrong et al. 1994). It was concluded that GH may have a facilitative rather than an essential role in support of lactation in sows. Ruan et al. (2005) showed that mammary transgenic overexpression of IGF-1 did not impact lactation sow milk yield.

When the secretion of prolactin was systematically inhibited at various stages of lactation, the weight gain of piglets was also suppressed (Farmer et al. 1997). These results demonstrate that prolactin is essential, not only for the initiation of lactation, but also for the maintenance of milk production in sows. Plaut et al. (1989) showed that the binding of prolactin to its receptor is a major effector of milk production in sows.

Thyroid hormones are required for various metabolic functions and protein synthesis by the mammary gland (Tucker 1985). Thyrotropin-releasing factor (TRF) also stimulates the release of thyroid hormones and of prolactin into the circulation of sows (Dubreuil et al. 1990), which could lead to a possible involvement in the control of milk production.

Recent studies used an injection of azaperone at the time of expulsion of the placenta was shown to facilitate nursing behavior, to increase colostrum intake by piglets (Biermann et al. 2010) and, consequently, to improve piglet conditions (Miquet and Viana 2010; Tseng et al. 2010).

Water Availability

Hourly water intake increases from approximately 1 L/h at the end of gestation to attain 2.6 L/h 12 hours before the end of parturition (Klopfenstein 2003). Water intake can be very low (less than 10 L/day) in some sows during the first 24 hours following parturition. After this period of transition, water intake increases gradually to reach 20–35 L/day during lactation. The increased water intake just prior to farrowing is partly due to greater water needs. During the hours preceding parturition, there is a rapid increase in the water
In the processes of parturition (Dobson 1988), the content of the reproductive system is modified to allow the process of parturition (Dobson 1988).

### COMPOSITION OF SOW MILK

Sow milk contains more than 100 different chemical components (reviewed by Xu 2003). The major components are lactose, proteins (casein, α-lactalbumin, β-globulins, serum albumins, immunoglobulins), lipids, lactocytes, leukocytes, bivalent ions (calcium, phosphorus, and magnesium), and electrolytes (sodium, potassium, and chloride). The relative concentrations of these components vary according to the stage of lactation (Table 18.2). Wu et al. (2010) reported that concentrations of immunoglobulins and lactoferrin are greater in milk from anterior glands than from posterior glands. This suggests that anterior mammary glands are more active in protein synthesis than posterior glands.

### Somatic Cells of Normal and Mastitic Milk

The somatic cell count (SCC) of mammary secretions from healthy sows is 1–4 million cells/mL (Drendel and Wendt 1993; Evans et al. 1982; Hurley and Grieve 1988; Klopfenstein 2003; Magnusson et al. 1991; Schollenberger et al. 1986). Somatic cells found in the milk of infected glands are mainly leukocytes (>75%). During sow lactation, a cellular content over 12 million cells/mL with an increased proportion of leukocytes is suggestive of mammary gland alteration. Persson et al. (1983) used SCCs in mammary secretions to differentiate subclinical from normal cases; however, SCC used as a diagnostic tool has not found practical clinical application due to lack of sensitivity, specificity, and low predictive value.

### MANIPULATION OF MILK PRODUCTION

#### Feeding Strategies

It is generally accepted that sows should maintain body condition throughout the production cycle (Einarsson and Rojkittikhun 1993). This is an increasingly difficult challenge due to increasing litter sizes and milk production (Foxcroft et al. 2007), and because low parity sows are expected to gain weight and grow over the first two parities.

Milk production requires substrates derived from the diet and the sow’s body reserves; the relative importance of which changes as lactation progresses. Body reserves might be sufficient in early lactation but not in late lactation to compensate for inadequate nutrient intake (Pettigrew 1995). Accordingly, severe restriction of feed intake during lactation has no impact on litter.
growth in the first week postpartum, whereas it mark-
edly decreases litter growth during the fourth week of
lactation (Mullan and Williams 1989). The magnitude
of reduction depends on the dam’s body reserves at
farrowing, with gilts with lower body reserves being
affected the most (Mullan and Williams 1989). A survey
of 25,000 lactating sows (Koketsu 1994, as reported by
Pettigrew 1995) showed that the impact of lactating
sows’ feed intake on litter weight becomes larger as
lactation progresses.

The intake of protein/amino acids by lactating sows
is critical for lactation performance. Lysine is typically
the first limiting amino acid for lactating sows, with
26 g of dietary lysine needed per kilogram of litter
growth per day (Sohn and Maxwell 1999). To achieve
a zero protein (nitrogen) balance, Dourmad et al. (1998)
demonstrated that 45–55 g/day of crude lysine are
required for normal and high-yielding sows, respec-
tively. Valine and isoleucine, but not leucine, appear
to increase milk production as indicated by increased
litter weight gain (Kerr 1997, as cited by Sohn and
Maxwell 1999). These amino acids can be metabolized
to succinyl-CoA and can therefore potentially serve as
a source of energy for the mammary gland (Sohn and
showed that sows can mobilize amino acids from
muscle to support mammary growth and milk produc-
tion, yet they also demonstrated that an increased
energy intake can partially relieve the effects of
decomposed protein intake on milk production.

From various studies designed to determine the rela-
tion between milk production and dietary energy, Wil-
liams (1995) noted that each suckling piglet grows an
extra 1 g/day for each megajoule of metabolizable
energy consumed by the sow. Matzat et al. (1990)
showed a linear relationship between milk output and
energy intake of sows, whereas Pluske et al. (1995b)
demonstrated that piglet growth did not respond
beyond 75 MJ of metabolizable energy in gilts. Such a
ceiling for lactational performance was also observed
in first-parity sows offered increasing amounts of
protein (King et al. 1993).

Various feeding management systems have been
used to ensure that lactational feed intake of sows is
maximized and that lean-tissue gain during pregnancy
is sufficient so that the sows’ milk production capacity
is not compromised. Increasing feed consumption of
sows by 8% through wet feeding had no impact on
average daily gains (ADGs) of piglets over an 18-day
lactation period (Genest and D’Allaire 1995). Similarly,
the use of a bulky diet in gestation increased average
lactational feed intake of sows by 8% without improv-
ing mean litter weight (Farmer et al. 1996). Increasing
daily feeding frequency from 2 to 3 (Genest and
D’Allaire 1995) or 2 to 4 (Farmer et al. 1996) during
lactation also had no effect on feed intake. The addi-
tion of fat to the sows’ diet to increase energy density
and energy intake of sows during lactation did not lead
to a less negative energy balance in the sows but
resulted in fatter piglets when sows were fed at a high
feeding level (van den Brand et al. 2000).

Exogenous Hormones
Early studies with GH and GHRF reported an important
increase in milk yield of 15–22% (Harkins et al. 1989),
but those results could not be reproduced in further
noticed that the effect of GH on milk yield is not greater
in sows having large litters (13 vs. 8 piglets). However,
reduced voluntary feed intake during lactation and
greater body weight and back-fat losses of sows receiv-
earth during lactation are consistent findings in all
studies.

Sows with spontaneous lactation failure have abnor-
ma 
aply low concentrations of prolactin (Whitacre and
Threlfall 1981). This may explain the 8% increase in
litter weight gain observed in gilts (but not sows)
receiving a single injection of porcine prolactin on
day 1 of lactation (Dusza et al. 1991). Two studies
injected prolactin from day 107 of gestation through
lactation (Crenshaw et al. 1989) but did not show any
effect on milk yield. Since injections were started before
there were any piglets to remove milk from the
mammary glands, premature involution of the secre-
tory units of the mammary glands might have taken
place (Boyd et al. 1995). Daily injections of prolactin
to sows from days 2 to 23 of lactation did not alter
sow and piglet performance (Farmer et al. 1999).
Together, studies suggest that virtually all prolactin
receptors are saturated in lactating sows, thereby pre-
venting any beneficial effects of further increasing pro-
 lactin concentrations.

SOW BEHAVIOR
Eating, drinking, and sow behavior can be used to
monitor the status of sows (Oliviero et al. 2008a),
which may be useful to determine those sows and
newborn piglets that are at risk for postpartum dysga-
lactia syndrome (PPDS). Although eating activity of the
sow goes down within 24 hours before parturition,
healthy animals seldom go completely off feed when
measured on a daily basis (Peltoniemi et al. 2009;
Quesnel et al. 2009). In contrast, sows contracting
PPDS often go completely off feed. Loss of appetite is
part of a general response to systemic inflammatory
mediators (cytokines and interleukins), which may be
stimulated by endotoxin (lipopolysaccharide). Prod-
ucts of inflammation induce fever as well as promote
the function of the immune system and thereby the
recovery of the body (Johnson 2002; Weary et al. 2009).
Water intake substantially increases prior to parturi-
tion, and a decrease in water intake may be taken as a
sign indicating increased risk leading to PPDS. Piglets
When milk production exceeds piglets’ needs during the first days after parturition, most (>85%) of the sucklings are initiated by the sow and are terminated by the piglets. When the milk supply becomes less than the needs of the piglets (weeks 3–4), most sucklings are initiated by the piglets and terminated by the sow (Jensen 1988; Jensen et al. 1991). Sows terminate suckling by leaving the piglets or by limiting teat access by rolling to her belly (de Passillé and Rushen 1989b). Both the duration and intensity of teat stimulation of each piglet on each udder segment influence milk production during the first 3 days of lactation (Algers and Jensen 1991). Nursing frequency is shown to influence piglet weight gain (Auldist et al. 2000; Spinka et al. 1997; Valros et al. 2002). Valros et al. (2002) found that the duration (200–250 min/day) and frequency (20–30 times/day) of nursing were rather stable for each sow throughout lactation, indi-

![Figure 18.4](image-url)

18.4. (A) Average duration and number of times standing up per hour in the different intervals of time before and after farrowing (black arrow). Adapted from Oliviero et al. (2008b). (B) Individual average daily water intake of sows fed a lactation diet low (3.8%) in crude fiber (LACT) and a high (7%) crude fiber (FIBER) diet before farrowing. Adapted from Oliviero et al. (2009). The asterisk symbols indicate the power of statistical results (*0.05 and **0.01).
cating the existence of a stable individual nursing behavior pattern.

PIGLET GROWTH

The efficacy of conversion of milk into live weight gain ranges from 3.5 to 4.2 (Beyer et al. 1994; Le Dividich et al. 2007; Noblet and Etienne 1986; Pluske et al. 1998). This efficiency is lower in restricted-fed piglets due to a greater proportion of intake devoted to maintenance (Le Dividich et al. 2007). Energy and nitrogen in sow milk have an apparent digestibility of approximately 98% (Le Dividich et al. 2007), and digestibilities of colostral lactose and fat likely approximate 100% (Le Dividich et al. 1994b).

Bottle-fed colostrum to piglets during the first 24 hours after birth and kept in a similar environment as sow-reared piglets have a voluntary intake exceeding 450g/kg of birth weight, twice the average consumption of sow-reared piglets (210–370g/kg of birth weight). This suggests that the sow limits the colostrum intake of her piglets, a potential problem for hyperprolific sows. However, 60–88% of sows produce enough colostrum to ensure survival of their litters since an intake of 160g of colostrum per kilogram of birth weight is necessary for piglet survival.

From birth to weaning, piglets from larger litters tend to be lighter than piglets from smaller litters (Dyck and Swierstra 1987; Le Dividich et al. 2004; Van der Lende and de Jager 1991). This difference is mainly due to differences in piglet birth weight and/or lower milk supply per piglet in large litters later in lactation. Hyperprolificacy also affects growth rate because the number of live piglets exceeds the number of mammary glands, and low-birth-weight piglets are often less vigorous for nursing. Data on hyperprolific sows showed that more than one-third of the litters had over 15 total born piglets (Dourmad et al. 2010). These sows were generally “lean sows,” which can have negative consequences (Table 18.3).

Growth rate, usually measured as ADG, is directly related to individual piglet weight at birth (Castren et al. 1991; Le Dividich et al. 2004; Tyler et al. 1990). When piglet weights are mathematically corrected for a standard birth weight, the effect of litter size is absent on day 3, is small on day 7, and becomes greater as lactation proceeds (Klopfenstein 2003). Klopfenstein also showed that milk production becomes a limiting factor for piglet growth when the sow has attained her maximal milk production capacity (days 10–15).

The coefficient of variation (standard deviation [SD]/mean) is probably a better tool to describe heterogeneity of birth weight than within-litter SD. When litter size increases from less than 10 to more than 15, mean litter birth weight decreases by 0.5 kg and the coefficient of variation increases by 10% to reach 24%. In large litters, there is an increase in small piglets from 6% to 16% of total born. For a given sow, there is no repetition of the coefficient of variation with subsequent parity (Quesnel et al. 2008). Homogeneity is greater in young sows (parities 1 and 2) than in older sows (Table 18.4) in both normoprolific sows (Bolet and Etienne 1982) and hyperprolific sows (Quesnel et al. 2008). It therefore seems to be a parity effect per se. Indeed, the coefficient of variation is similar for P1 and P2 sows although the number of total born piglets is less in P2 than in P1 (Table 18.4). This parity effect is important because the majority of published scientific data were obtained in young sows. Sow body condition in early gestation also has an impact on heterogeneity of piglet birth weight. Heterogeneity increases as sows are heavier at mating or farrowing.

The correlation between mammary gland wet weight and ADG of pigs is high, from 0.59 to 0.68 (Kim et al. 2000; Nielsen and Sorensen 1998), suggesting a strong relationship between pig growth and mammary size. The significant correlation between mammary wet weight and cross-sectional area supports observations that cross-sectional area is an effective indicator of mammary gland mass (Kim et al. 1999b).

Milk production differences between mammary glands are believed to be a major source of piglet weight variation (Fraser and Jones 1975). Mammary glands

<table>
<thead>
<tr>
<th>Litter Size</th>
<th>≤9</th>
<th>10 –11</th>
<th>12 –13</th>
<th>14 –15</th>
<th>≥16</th>
</tr>
</thead>
<tbody>
<tr>
<td>% of litters (# of litters)</td>
<td>12.2 (195)</td>
<td>9.6 (154)</td>
<td>17.3 (276)</td>
<td>24.7 (394)</td>
<td>36.3 (579)</td>
</tr>
<tr>
<td>Parity</td>
<td>2.6</td>
<td>2.3</td>
<td>2.5</td>
<td>2.6</td>
<td>3.5</td>
</tr>
<tr>
<td>Litter size: total born</td>
<td>7.1</td>
<td>10.6</td>
<td>12.6</td>
<td>14.5</td>
<td>17.7</td>
</tr>
<tr>
<td>Litter size: live born</td>
<td>6.9</td>
<td>10.2</td>
<td>12.0</td>
<td>13.7</td>
<td>16.1</td>
</tr>
<tr>
<td>Litter size: stillborn</td>
<td>0.3</td>
<td>0.4</td>
<td>0.6</td>
<td>0.8</td>
<td>1.5</td>
</tr>
<tr>
<td>Mean birth weight (kg)</td>
<td>1.88</td>
<td>1.67</td>
<td>1.57</td>
<td>1.48</td>
<td>1.38</td>
</tr>
<tr>
<td>Within-litter SD (kg)</td>
<td>0.28</td>
<td>0.29</td>
<td>0.32</td>
<td>0.32</td>
<td>0.33</td>
</tr>
<tr>
<td>Coefficient of variation (%)</td>
<td>15</td>
<td>18</td>
<td>21</td>
<td>22</td>
<td>24</td>
</tr>
<tr>
<td>% small piglets (&lt;0.75% mean birth weight)</td>
<td>6</td>
<td>9</td>
<td>12</td>
<td>13</td>
<td>16</td>
</tr>
</tbody>
</table>

Adapted from Quesnel et al. (2008).
have different shapes depending on their anatomical location. The space available for growth of the anterior and middle glands during lactation may be limited, resulting in greater ratios of wet weight to cross-sectional area compared with posterior glands. Posterior glands may be better able to expand elliptically in a longitudinal manner compared with anterior and middle glands, which expand laterally and medially. Scheel et al. (1977) showed that pigs with greater birth weight had a preference for anterior glands, but Hemsworth and Winfield (1976) and Kim et al. (2000) showed no significant relationship between birth weight of pigs and the preference for mammary glands. To summarize, pigs nursing the well-developed anterior and middle mammary glands have a greater ADG than pigs nursing the remaining glands. Weight variation among pigs nursing the anterior and middle glands is not statistically significant.

**PIGLET MORTALITY**

Despite efficiencies gained in modern swine production, piglet losses at birth and during lactation appear to be increasing as litter sizes increase (Boulot et al. 2008). Preweaning mortalities often exceed 10% of live-born piglets, and most of these occur during the first week after parturition (de Passillé and Rushen 1989b; Dyck and Swierstra 1987; English and Morrison 1984; Le Cozler et al. 2004; Oliviero et al. 2008b). The effect of litter size on mortality is quadratic; piglet losses increase in the larger litters (Boulot et al. 2008; Dyck and Swierstra 1987; Fahmy and Bernard 1971; Guthrie et al. 1987) and are probably related to an insufficient number of functional mammary glands to supply milk for all the piglets (Bilkei et al. 1994; Chertkov 1986).

## IMMUNE PROTECTION OF THE PIGLET

Newborn piglets rely on ingestion of colostrum for passive transfer of immunity (Bourne 1976). The absorption of immunoglobulins from the sow’s colostrum causes closure of the intestine for the passage of these large proteins (Klobasa et al. 1991), suggesting that absorption is possible only during the first feedings after birth. Le Dividich et al. (2004) showed that colostrum immunoglobulin G (IgG) content decreased by 31% by 3 hours following the birth of the first piglet. Passive immunity in piglets 48 hours old was 50% greater in the first- than in the last-born piglet, the difference remaining significant at weaning. Piglets artificially reared and receiving six hourly feedings of 25 mL of sow colostrum followed by hourly feedings of cow’s milk had plasmatic immunoglobulin concentrations similar to those of naturally fed piglets at 24 hours of age (Klobasa et al. 1991). Increasing the number of hourly feedings of colostrum from 6 to 12, 18, or 24 did not increase the concentrations of plasma immunoglobulins in piglets. Fasting newborn piglets for periods of up to 24 hours after birth before giving them access to their first colostrum intake did not decrease serum immunoglobulins concentrations 12 and 18 hours after feeding (Klobasa et al. 1990). Therefore, closure of the gut system for the passage of immunoglobulins is dependent on the quantity of colostrum ingested rather than on time since birth. Six feedings of colostrum should be sufficient to give adequate immune protection to the piglets.

Studies based on the radioactive labeling of immunoglobulins in colostrum have shown that almost 100% of IgG, 40% of IgA, and 85% of IgM are derived from sow serum (Bourne and Curtis 1973). IgG is preferentially transferred from the blood into mammary secretions during colostrogenesis, resulting in a marked decrease in serum IgG levels at this time. The uptake of colostral immunoglobulins in neonatal piglets may not be mediated by a receptor, although neonatal Fc receptors are present in gut epithelial cells. Indeed, IgG, IgM, and IgA (not secretory IgA) undergo selective transcytosis into enterocytes (Devillers et al. 2006).

The quantity of IgG ingested by each piglet is affected by birth order due to the rapid changes in colostrum composition taking place between onset of farrowing and birth of the last piglet (Klobasa et al. 2004; Le Dividich et al. 2004). Piglets dying before weaning have lower plasma immunoglobulin concentrations after parturition (Hendrix et al. 1978; Tyler et al. 1990); yet this association disappears when birth weight is used as a covariate (Tyler et al. 1990). Moreover, the probability of dying is not increased among last-born piglets, even though they obtain less immunoglobulin than first-born piglets (Le Dividich et al. 2004). These results, although surprising, can suggest that most mortalities

### Table 18.4. Effect of parity (P) on piglets’ characteristics at birth (French observations on 1596 litters from a single herd)

<table>
<thead>
<tr>
<th>P</th>
<th>L1</th>
<th>L2</th>
<th>L3</th>
<th>L4</th>
<th>L5</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1</td>
<td>432</td>
<td>349</td>
<td>470</td>
<td>261</td>
<td>86</td>
</tr>
<tr>
<td>Litter size: total born</td>
<td>14.0</td>
<td>12.3</td>
<td>14.5</td>
<td>15.3</td>
<td>15.1</td>
</tr>
<tr>
<td>Litter size: live born</td>
<td>13.2</td>
<td>11.7</td>
<td>13.5</td>
<td>14.4</td>
<td>13.3</td>
</tr>
<tr>
<td>Litter size: stillborn</td>
<td>0.8</td>
<td>0.6</td>
<td>0.9</td>
<td>1.3</td>
<td>1.8</td>
</tr>
<tr>
<td>Mean birth weight (kg)</td>
<td>1.45</td>
<td>1.64</td>
<td>1.57</td>
<td>1.47</td>
<td>1.44</td>
</tr>
<tr>
<td>Within-litter SD (kg)</td>
<td>0.28</td>
<td>0.31</td>
<td>0.33</td>
<td>0.34</td>
<td>0.35</td>
</tr>
<tr>
<td>Coefficient of variation (%)</td>
<td>20</td>
<td>20</td>
<td>22</td>
<td>24</td>
<td>25</td>
</tr>
<tr>
<td>Losses in small piglets</td>
<td>11</td>
<td>11</td>
<td>13</td>
<td>15</td>
<td>16</td>
</tr>
</tbody>
</table>

Adapted from Quesnel et al. (2008).
are the consequence of inadequate nutrition rather than infectious diseases.

**MASTITIS AND POSTPARTUM DYSGALACTIA SYNDROME**

Mastitis may be limited to one or a few mammary glands (uniglandular mastitis) or may involve all mammary glands (i.e., multiglandular effects, hard udder syndrome). Acute mastitis is usually accompanied by systemic and local signs, whereas the hard udder syndrome does not have systemic signs in the sow. Both conditions occur mainly within the first 3 days of parturition and rapidly lead to piglet starvation. It can be difficult to differentiate between acute multiglandular mastitis and the hard udder syndrome. Consequently, these two conditions are often reported by producers as “acute mastitis” or “mastitis, metritis, agalactia (MMA) syndrome,” although metritis is uncommon.

Postweaning or dry-sow mastitis usually affects one or a few glands. Drying off mastitis is uncommon in sows (compared with cows) because of inherent differences in anatomy and process of mammary gland involution.

Chronic mastitis is characterized by the formation of abscesses and granulomas in the mammary tissue, usually seen at the time of weaning or shortly after (Hultén et al. 2003). Common environmental bacteria can enter the mammary glands through teat wounds inflicted by piglets during suckling, by entry from traumatic wounds of sows that are group-housed postweaning, or by trauma associated with the particular anatomy of inguinal mammary glands of old sows.

The term MMA complex is commonly used in European countries and literature but is misleading, as metritis is only occasionally found in affected sows. Furthermore, instead of showing total agalactia, sows usually continue to produce milk but at a reduced level. MMA should be considered to be a severe form of PPDS.

PPDS is when piglets have signs of growth retardation or starvation but mammary glands themselves are seemingly unaffected. The limit between PPDS and MMA is unclear.

**Mastitis**

Acute or chronic uniglandular mastitis is common in lactating or weaned sows, most often in older sows and the inguinal glands. The microorganisms involved are the same as in acute multiglandular mastitis. Important risk factors are traumatic lesions to teats and glands or inaccessibility of teats to piglets. Piglets suckling inguinal mammary glands of old sows are often unable to reach the teat during milk ejection, and a nonsuckled mammary gland is a candidate for infection. Usually, piglets have selected a specific gland by 24 hours after birth; hence, a piglet suckling an affected gland will show growth retardation while littermates retain a normal growth. Milk secretion may be restricted by acquired problems of mammary conformation (as in old sows), traumatic lesions, or other teat abnormalities. Since teat lesions may develop anytime, the integrity of the mammary gland should be checked before each farrowing. Risk factors involved in the development of blind teats should be identified.

Traumatic teat lesions can be the consequence of injuries induced by piglets or other sows, or by trauma from the environment. The cranial teats appear to be more at risk of trauma compared with other teats (Hultén et al. 2003). Unfortunately, primary lesions often go unnoticed; hence, the gland is lost for the ongoing lactation(s). The number of nursing piglets should be limited to functional glands or the sow should be culled.

Mastitis is a pathological entity. Infected glands have inflammation, edema, and skin congestion with fever (>40.3–40.5°C) and anorexia in the sow (Van Gelder and Bilkei 2005). Mastitis can affect individual, multiple, or all mammary glands. Gram-negative coliform (Escherichia, Enterobacter, Citrobacter, and Klebsiella) bacteria are most frequently isolated from mastitis-affected sows (Klopfenstein et al. 2006) with gram-positive bacteria (streptococci, staphylococci, and Aerococcus spp.; Menrath et al. 2010) being less frequent. Pyogenic organisms (Arcanobacterium, streptococci, staphylococci) are not uncommonly isolated from chronic infections. Because coliforms predominate, the word “coliform mastitis” (CM) was commonly reported (Gerjets and Kemper 2009); however, most studies on CM were conducted between 1970 and 1990. Recent data from Kemper and Gerjets (2009) has shown that the prevalence of different types of common bacteria in anterior and posterior glands in PPDS-positive and -negative sows does not differ.

**Postpartum Dysgalactia Syndrome**

PPDS in sows is characterized by insufficient colostrum and milk production during the first days after farrowing. The consequences of PPDS depend on the severity. Acute cases with clinical signs in sows lead to high mortality in piglets, whereas PPDS without signs in sows leads to “only” poor growth in piglets during the neonatal period (Foisnet et al. 2010b), the latter sometimes referred to as “problem litters.” The variation of clinical signs in sows from subclinical to severe systemic disease confounds clinical diagnosis as well as estimates of prevalence of PPDS. Estimates of within- or across-herd prevalence depends on the criteria used for the assessment of occurrence and severity. Variations in criteria, assessments, and reporting explain why it is difficult to give a precise definition of PPDS. The multifactorial nature of PPDS makes implementation of preventive and therapeutic measures very challenging for pig veterinarians.
Pathophysiology of Postpartum Dysgalactia Syndrome. The pathophysiology of PPDS has not yet been fully elucidated, and a single pathway is unlikely to exist (Figure 18.5). There are at least three different pathways in connection with lactogenesis and galactopoiesis. One is mediated by endotoxins and acts via the innate immune system. Another one is mediated by stress, and the last one is linked with the “body building syndrome.” The latter is further subdivided into two entities, namely the “fat sow syndrome” (FSS) and the “over-muscled sow syndrome” (OMSS). As illustrated in Figure 18.5, it is necessary to consider the impact on lactogenesis and galactopoiesis (step 2) in order to understand the links between the suggested mechanisms of action of PPDS (step 3) and homeorhesis and behavior (step 1).

Clinical Signs and Symptoms. Martineau et al. (1992) summarized a list of early and late signs of PPDS that may be present in the sow, in piglets, and in herd productivity. Scalable measures in sows include local signs (structurally absent nipples or glands, udder edema, mastitis with agalactia, vaginal discharge) and/or general signs (absence of any secretion, fever, prostration, anorexia). In piglets, early signs include mortality, diarrhea, or poor growth among the litter, whereas late signs consist mainly of variation in growth within the litter and lower weaning weights. At the herd level, measures include the consequences of PPDS on productivity (number of piglets weaned/sow/year) as well as all other consequences associated with low or variable weaning weights.

Not all sows exhibit the same range or intensity of clinical signs, and the number of affected sows may vary. The most visible sign of insufficient milk and colostrum is growth retardation of piglets. Unfortunately, growth retardation can be identified with certainty only after piglets show retarded growth or high mortality. Close observation of the behavior of piglets is one of the best methods for early detection of problem litters (Whittemore and Fraser 1974). These piglets fight more and for longer periods, lose weight, and remain close to the sow between sucklings (Algers and de Pas-sille 1991). Detecting early signs in sows is difficult because most do not show differentiable signs (Klopfenstein 2003) even though milk production of sows nursing such litters is less. As reported by Foisnet et al. (2010b), the clinical presentation of sows with poor colostrum production is apparently normal.

Criteria for assessing mastitis in the literature can be confusing. For example, the normal rectal temperature of healthy gestating sows is reported to be between 38.3 and 38.5°C (Elmore et al. 1979; King et al. 1972; Klopfenstein 2003; Klopfenstein et al. 1997; Messias de
2004; Madec and Leon 1992; Persson et al. 1989). A recent study demonstrated that rectal temperatures of sows nursing “problem litters” and characterized as PPDS were similar to those of sows nursing “normal litters” both at the end of gestation and at the beginning of lactation (Klopfenstein 2003). The greater rectal temperature observed in lactating sows must consider physiological hyperthermia and should not be confused with fever.

Body temperature should not be misinterpreted or used as a single tool in clinical evaluation of PPDS (Marnell et al. 2005; Meisner 2005; Pepys and Hirschfield 2001). The belief that higher rectal temperature identifies sows with mastitis or PPDS is so generally accepted that many researchers use this criterion without estimating piglet growth and preweaning mortalities; hence, many such published results on the MMA syndrome are not included in this chapter.

**Postpartum Dysgalactia Syndrome Prevalence.** The prevalence of PPDS either at animal or herd level depends on the criteria used to assess the occurrence and the severity of the syndrome. Symptoms and criteria used to define PPDS vary largely, depending on the study (reviewed by Papadopoulos 2008). Reports from “classical” studies can be used to further illustrate the diversity of proposed diagnostic criteria. In one study, the classification of MMA was used if one or more of the following signs were present: agalactia, anorexia, constipation, vaginal discharge, inflammation of mammary gland, or a rectal temperature higher than 39.8°C (Jorsal 1986). In contrast, postpartum agalactia was assigned when the cell count in the milk was greater than 10 million/mL in mammary secretions, even if rectal temperature was normal (<39.5°C) and no clinical mastitis was present (Persson et al. 1996). Bäckström et al. (1984) defined sows as being hypogalactic or agalactic by the presence of starving piglets and one or more of the clinical signs (anorexia, fever, depression, signs of mastitis, vaginal discharge) within 3 days after parturition. Sows were classified as hypogalactic, agalactic without clinical mastitis, and agalactic with clinical mastitis using an individual sow score based on severity of fever, state of milk production, degree of disturbed appetite, and assessments of the condition of the piglets (van Gelder and Bilkei 2005).

Bäckström et al. (1984) defined sows as being hypogalactic or agalactic by the presence of starving piglets and one or more of the clinical signs (anorexia, fever, depression, signs of mastitis, vaginal discharge) within 3 days after parturition. Sows were classified as hypogalactic, agalactic without clinical mastitis, and agalactic with clinical mastitis using an individual sow score based on severity of fever, state of milk production, degree of disturbed appetite, and assessments of the condition of the piglets (van Gelder and Bilkei 2005). Hirsch et al. (2003) first reported a detailed scoring system by classifying sows as affected by PPDS when they showed at least slight disturbance of the general demeanor, a reduction of feed intake by up to one-third of normal, drops of pathological vaginal discharge, barely perceptible inflammation of mammary glands, and a body temperature equal or higher than 39.5°C.

As a result of variation in criteria for classification, reported prevalence data is difficult to compare and likely overestimates actual prevalence. Bäckström et al. (1984) reported PPDS in 6.9% of 16,405 farrowings.
recorded over 1 year in 31 swine herds in Illinois with
in-herd prevalence range from 1.1% to 37.2% and no
relationship to herd size. The mean prevalence of PPDS
was 13% in multiparous sows, and 4.2% in primiparous
sows was not significant. Threlfall and Martin (1973)
studied 27,656 farrowings in the state of Missouri and
found that 13% of the sows were affected by PPDS. The
range of incidence of PPDS in Swedish herds has been
reported from 5.5% in small herds to 10.3% in large
herds (Bäckström et al. 1982). More recently, in
Denmark, Larsen and Thorup (2006) used a definition
of PPDS as one or more of the following: inappetence,
abnormal mammary glands (reddening, swelling), or
temperature >39.4°C, and found a prevalence of 32.5%
on the first day postfarrowing, 31.5% on the second
day, and 10.1% on the third day postfarrowing.
Although PPDS is observed in many herds, prevalence
varies in large part due to the variation in criteria used
to assess affected sows.

Postpartum Dysgalactia Syndrome Risk Factors and
Prevention. Risk factors for PPDS are typical of mul-
tifactorial diseases. There are many factors that are suf-
ficient to increase the probability of occurrence of PDS
but by themselves are not necessary for causation. One
of the keys in reducing the incidence of problem litters
in a herd is the identification and correction of specific
risk factors. Recent studies (Maes et al. 2010; Papado-
poulos et al. 2010) suggest that modern pig herds need
to utilize control measures, which include optimizing
management and feeding practices.

Because different pathophysiological pathways may
lead to PPDS (Figure 18.5), there are many potential
risk factors identified for each of these pathways. We
propose to report risk factors using the six major risk
categories globally present in herds: animals, housing,
nutrition and water, management, microorganisms,
and caretaker (Martineau and Morvan 2010).

Animal Factors and Body Type. Information on the
role of genetics in the occurrence of PPDS is limited.
Awad et al. (1990) reported a genetic predisposition
for CM in Austria. A French investigation of a large herd
with many different genetics found the main factors
influencing the initial colostral immune quality were
the breeding lineage, the genotype, and parity with sows
belonging to a female line having IgG concentra-
tions in colostrum greater than sows from a male line.
Similarly, sows of the Large White genotype had colos-
trum with more IgG than that from Pietrain sows, but
this is to be interpreted with care due to the low number
of Pietrain sows in the study (Voisin et al. 2006).

Sow constipation and endotoxemia are considered
to be linked risk factors. As sows approach farrowing,
water absorption in the intestine increases due to the
fluid needed for the onset of milk production (Mroz
et al. 1995) which, in turn, alters feces consistency. The
threshold between “normal dry feces” and constipation
is not clear. Daily fecal scores (Oliviero et al. 2009)
allowed diagnosis of constipation at the sow as well as
the herd level. Postpartum constipation was observed
in some sows nursing problem litters (Hermansson
et al. 1978b; Ringarp 1960). Feeding high-fiber diets in
late gestation has been widely used in order to decrease
the incidence of early-lactation constipation related to
PPDS (Ringarp 1960; Wallace et al. 1974). Added fiber
decreases concentrations of the other components but
nutritional needs can still be met (Goransson 1989a;
Jensen 1981; Sandstedt and Sjögren 1982; Sandstedt
et al. 1979). Offering feed low in volume and fiber can
worsen constipation and increase the risk for absorbing
bacterial endotoxins (Smith 1985a,b). Studies of con-
stitipated sows showed higher rates and direct effects on
occurrence of PPDS (Hermansson et al. 1978a; Persson
et al. 1996).

A common feeding strategy that aims to reduce the
amount of feed offered and to increase the energy of
the ration often limits the amount of fiber. Although
this strategy aims to ensure that sows receive enough
energy for late pregnancy and milk production (Ein-
arsson and Rojiktikhun 1993), the combination of a con-
centrated and low-fiber diet during a period of
physiologically low intestinal activity can cause severe
constipation. A solid mass of feces may create a physi-
ological obstacle for birthing (Cowart 2007) and perhaps be
a source of intestinal pain for the sow, thus jeopardiz-
ing animal welfare.

Insufficient water intake just before farrowing can
enhance postpartum constipation. Low postpartum
water intake and low activity level of sows were pro-
posed as risk factors for early lactation problems (Fraser

Two “body building syndromes," namely FSS and
OMSS, are recognized as very important risk factors for
PDS. Moreover, for both FSS and OMSS, control begins
at the gilt level. Evaluation of visual “body score” is
an approximate tool; a medium body score (body score
of 3 or 4 on a scale of 1–5) has a very large variation
of the back fat (Charette et al. 1996) and back lean
(H. Solignac, personal communication). Body scores
only approximate true body condition. Fat sows at far-
rowing are long recognized as a risk factor for PPDS
(Goransson 1989a). Control of the FSS is not easy
because small errors in the amount of feed distributed
over the whole gestation period can lead to overweight
or underweight sows at the time of parturition (Mar-
tineau and Klopfenstein 1996). Sows maintained in pens
have more variable body weights, the most aggressive
sows often being overweight while submissive animals
are underweight (Marchant 1997; Martineau 1990).

Control of the OMSS is also not easy, as it is a new
emerging syndrome (Solignac et al. 2010) and as there
is no consensus on the best feeding strategy for
the modern hyperprolific sow. This OMSS is the direct
consequence of genetic improvement and hyperprolificacy (Solignac 2008; Solignac et al. 2010). Modern and hyperprolific sows are leaner, which increases the risk of an early catabolism phase before farrowing. An important contributor is that the accretion weight rate of individual fetuses is 4–5 g/day during early gestation and around 50 g/day during the last 10 days of gestation (Ji et al. 2005), with protein deposition occurring faster during late gestation (Whittemore 1998). Mammary gland growth also increases during gestation. Therefore, around and often before farrowing, the sow has already switched to a catabolic state that continues during lactation, using her body reserves to produce large quantities of milk (van den Brand and Kemp 2006).

Rising levels of blood nonesterified fatty acids (NEFAs) is a clear indicator of a catabolic state associated with severe loss of body weight and low feed intake (Messias de Braganca and Prunier 1999). Le Cozler et al. (1998) and Oliviero et al. (2009) found that circulating concentrations of NEFAs increased rapidly a few days before farrowing, reaching a peak on the day of parturition. A catabolic state can also be quantified by circulating urea and creatinine concentrations; creatinine is a more efficient indicator of muscle catabolism than urea since it is a direct product of creatine metabolism in the muscles (Mitchell and Scholz 2001). As sows approach farrowing, intestines are less active (Kamphues et al. 2000). It was found that increasing the dietary energy in late pregnancy can negatively affect feed intake during early lactation due to a reduction in glucose tolerance and to insulin resistance caused by an excess of energy intake during late pregnancy (Boren and Carlson 2006).

Housing and Environment. Oliviero et al. (2010) showed that duration of the farrowing process in crates is longer than in pens, implying that the housing effect is indirect via an increase of farrowing duration. This effect may be a factor where there were more litters with starving piglets in herds where the sows farrowed in confinement than on the pasture (Bäckström et al. 1982). The incidence of PPDS was higher in sows housed in crates of 60 cm width than in crates of 67 cm width (Cariiolet 1991). The incidence of PPDS was greater in sows gestated on pasture and transferred to crates several days before farrowing (Bäckström et al. 1984) than cohorts. Although sows are exposed to change when moved from the gestation to the farrowing area, research results do not suggest that these changes are harmful to sows. Indeed, allowing sows to adapt to the new environment for more than a week, rather than only a few days, before parturition was not associated with a lower prevalence of problem litters (Klopfenstein et al. 1995). On the other hand, Papadopoulos et al. (2010) found that moving pregnant sows to the farrowing unit 4 days before the expected farrowing date compared with moving sows 7 days or earlier before farrowing increased the risk for PPDS. A period of adaptation of the sow to the new farrowing-lactation environment therefore appears to be necessary. The use of slatted floors in the farrowing pens was associated with a decreased risk of chronic mastitis in sows (Hultén et al. 2004). Fully slatted floors may be hygienic, but they do not appear to support social behavior (Munsterhjelm et al. 2008) and may sometimes reduce air quality.

The effects of heat stress in lactating sows include decreased feed intake and milk production (Quiniou and Noblet 1999). Other studies have suggested that high ambient temperatures have a direct effect on sow milk production, independent of the reduction in feed intake (Messias de Bragança et al. 1997). Temperature control in the farrowing room is certainly a crucial factor affecting sow lactation performances. Newborn piglets require a localized warm environment, but the temperature requirement of the sow is less since her zone of thermoneutrality is much lower. It is generally recommended that room temperature be maintained warm (20–22°C) for 2–3 days after farrowing to favor piglet survival. However, after this crucial period, room temperature can be gradually decreased to attain 18°C or even 15°C on day 10 of lactation (Farmer et al. 1998b). Indeed, when an adequate dry, draft-free heat zone is provided for the piglets, the ambient temperature can be decreased to 15°C on day 8 of lactation with no detrimental effects on sow and litter performances. Overheating of the mammary glands by inappropriate placement of heating lamps decreases milk production (Muirhead and Alexander 1997). Supplementary heat should be directed away from the sow and her udder and be removed when not needed to favor the sow’s well-being and maximize her milk production. The position of the heat lamps has an impact on the spatial behavior of neonatal pigs (Titterington and Fraser 1975).

Providing plenty of space and adequate enrichment material for nest building may reduce the incidence of PPDS as compared with housing sows in farrowing crates (Bäckström et al. 1984; Oliviero et al. 2009). Improving the level of hygiene of the farrowing unit was reported to be associated with lower preweaning mortality (Ravel et al. 1996). Stray voltage is sometimes suspected as a source of lactation problems (Gillessie 1984), but suspicion is not easy to verify (Robert et al. 1996).

Nutrition, Feed, and Feeding. Reduced feed consumption in the first days of lactation decreases the incidence of lactation failure, and gradually increasing feed intake of sows in the first week postpartum (rather than ad libitum) decreased the risk of PPDS substantially (Moser et al. 1987; Neil et al. 1996; Papadopoulos et al. 2010). The inclusion of fiber in the feed is probably the
most-cited nutritional factor to reduce PPDS. According to Papadopoulos (2008), a diet with a low n-6:n-3 ratio (fish diet) provided 8 days before expected farrowing, improved feed intake during the first days postpartum and was associated with a better metabolic adaptation and inflammatory profile in periparturient sows.

A relationship between diets deficient in selenium and vitamin E and sow lactational problems was proposed (Trapp et al. 1970; Whitehair and Miller 1986) because these antioxidant micronutrients can decrease effects of endotoxins (Elmore and Martin 1986). A low level of vitamin E (16 or 33 IU/kg vs. 66 IU/kg) was reported as a risk factor for PPDS (Mahan 1991), but Mahan et al. (2000) later reported that increasing dietary levels of vitamin E from 30 to 60 IU did not decrease the prevalence of MMA diagnosed at parturition. In another study, injections of vitamin E (4001 IU) and selenium (3 mg) to sows three times during gestation, at days 30, 60, and 100 of gestation, while fed normal levels of these nutrients, increased the survival rate of piglets but did not affect litter weights at weaning (Chavez and Patton 1986).

Mycotoxins are often reported by swine veterinarians as a risk factor for PPDS, yet the only well-recognized mycotoxin is ergot intoxication (Kopinski et al. 2007). Grains contaminated with ergot derivatives of Claviceps purpurea may hinder milk production in sows. Indeed, sows fed 1.5% ergot for 6–10 days preceding farrowing produced no milk, while ergot inclusions of 0.6–1.2% caused lesser problems in milk release and neonatal piglet mortality (Kopinski et al. 2007). The effects are likely due to suppressed prolactin secretion by ergot toxins.

Both the sow and piglets should have easy access to fresh, good quality water. Slippery and dirty floors are one of the main causes of low activity of lactating sows and may lead to many health problems including PPDS or to reduced feed and water intakes.

**Management.** In a survey done by Papadopoulos (2008), two of the four significant risk factors for PPDS were related to management: (1) moving pregnant sows to the farrowing unit 4 days compared with 7 days or earlier before expected farrowing, and (2) farrowing induction.

Farrowing induction increased the risk of occurrence of PPDS, but there are conflicting studies. In some herds with a significant percentage of gilts and sows showing PPDS, induction of parturition with prostaglandins has proven effective in reducing the incidence of PPDS (Cerne and Jochle 1981; Holtz et al. 1983), whereas it had no effect in other herds (Ehnvall et al. 1977; Hansen 1979). Prostaglandins could be effective in treating PPDS caused by retarded lactogenesis since incomplete luteolysis of corpora lutea can lead to high progesterone concentrations, which could potentially inhibit lactogenesis. However, farrowing induction at 113 days of pregnancy did not modify the chronology of endocrine changes taking place during the peripartum period, nor did it affect colostrum yield and IgG concentrations (Foisnet et al. 2010a,c), albeit some hematological variables of piglets were modified by the farrowing induction.

Postpartum administration of prostaglandins can have a beneficial effect on uterine involution and prevention of severe clinical endometritis (Waldmann and Heide 1996). Prostaglandins are luteolytic agents causing a prepartum decline in progesterone and the release of relaxin from the corpora lutea. They are widely used for the induction of farrowing (Ehnvall et al. 1977), resulting in an immediate and sharp increase in prolactin concentrations lasting approximately 6 hours (Hansen 1979).

**Producer.** Recently, Papadopoulos et al. (2010) reported that frequent farrowing supervision and neonatal care given by the stock person compared with no farrowing supervision decreased the risk of PPDS. Obstetric intervention is, however, reported among the noninfectious factors increasing the occurrence of CM in sows (Bostedt et al. 1998). Obstetric aid by either herd managers or veterinarians results in a fourfold increase in the sow's risk of acquiring early lactation problems (Jorsal 1986) and increases the incidence of postpartum vulvar discharges and endometritis (Bara and Cameron 1996). Depending on the criteria used to define the problem of PPDS (mastitis or dysgalactia), interpretation of observations may differ. Assisting a sow showing signs of dystocia may reduce the occurrence of PPDS. Similarly, sow productivity was positively correlated with practical procedures associated with enhanced supervision, including frequent manual assistance, frequent use of pharmaceuticals, and alternative options for cross-fostering piglets, such as sows from other farrowing batches and artificial suckling machines (Martel et al. 2008). Induction of farrowing by prostaglandins was reported to increase the risk of PPDS (Papadopoulos et al. 2010). With new technology such as photosensors, prediction of the onset of parturition becomes feasible (Oliviero et al. 2008b).

**TREATMENT OF THE DISEASED SOW**

Treatment of affected sows needs to be early and given a high priority, while simultaneously considering risk factors and preventative measures for PPDS. The herd health plan should include specific instructions for caretaking personnel to guide them on procedures to achieve accurate diagnosis and proper treatment. Since the disease is a multifactorial clinical condition with changing environmental and management contributors, treatment protocols need critical and frequent updating. In any case of PPDS, the use of nonsteroidal anti-inflammatory drugs (NSAIDs) is indicated, as is the
use of oxytocin for milk letdown. Antimicrobials may be warranted, depending on individual situation.

NSAIDs are targeted to alleviate effects of inflammation and endotoxemia, and are shown to have benefit for affected sows. Drugs used include flunixin (2 mg/kg) (Cerne et al. 1984), tolfenamic acid (2–4 mg/kg) (Rose et al. 1996), and meloxicam (0.4 mg/kg) (Hirsch et al. 2003). Treatment strategies usually consist of one treatment on the day of parturition and sometimes a second treatment the next day. NSAID appear to make up one-third of the costs related to PPDS treatment, while most of the costs are due to antibiotics. With a 1% improvement in piglet mortality assumed from the treatment, the benefits outweigh the treatment costs (Hirsch et al. 2003).

The selection of an antibiotic should be based on its spectrum of activity against those bacterial organisms identified or thought to be related to PPDS. Actually identifying the most important bacteria to the diseased sows is difficult. Initiation of antimicrobial treatment should be based on the clinical picture as a whole rather than relying on increased body temperature, which may simply be physiological or normal variation. Use of antimicrobials is indicated if generalized signs are present.

Another important objective in treatment is to stimulate milk flow as rapidly as possible to minimize the consequence of PPDS. Repeated use of oxytocin in clinical cases of PPDS is likely to be the most frequent treatment administered to sows to stimulate milk production. Oxytocin may be given at 5–10 IU/sow (IV) for four to five times at two to three hourly intervals until milk letdown is restored (Martineau 2005). There should be at least 30 minutes between two subsequent injections. In fact, in most cases, 5 IU appears to be enough to induce milk letdown, and by using this concentration, repeated doses may be given without side effects. Parenteral administration of synthetic oxytocin is a very efficient way to trigger milk ejection. The intramuscular route of administration is most common, but using the intravenous route may further improve the effect. Although efficient and considered as safe, repeated use of oxytocin might have some detrimental effects on sows. Indeed, overuse may be related to poor piglet growth (Bilkei Papp 1994; Ravel et al. 1996) and increased SCCs in milk (Garst et al. 1999).

Vaccination for PPDS is not widely practiced. The Escherichia coli strains causing neonatal diarrhea are not those usually detected in sows with PPDS but at least one report found them useful (Fairbrother 2006). The variation in bacteria found in farrowing units as well as the lack of randomized, blinded controlled study data would bring into question the use of vaccination for PPDS. Reduction in CM in dairy cows is reported following vaccinations with antigenic endotoxin (lipopolysaccharide) products. Vaccination against E. coli-related urinary tract infections at 4 and 2 weeks before parturition increased the overall lactation performance of sows (Pejsak et al. 1988).

Supportive treatment for the sow and piglets, especially fluid therapy, is an important part of treatment of serious cases of PPDS. The main target of supportive therapy is to alleviate dehydration. Sows with endotoxemia suffer from dehydration (Reiner et al. 2009). If monitoring determines that the sow is not drinking, then fluids can be administered intravenously or per rectum (Peltoniemi and Halli 2004). Daily fluid requirement is considered to be approximately 7% of the body weight. If given per rectum, a backflow of about 50% is expected, and therefore, volumes need to be larger than for intravenous administration.

In the case of piglets, the first step is to provide an alternative source of energy and/or to transfer affected low-birth-weight piglets to another healthy sow. Piglets of heavier birth weights appear to transfer with greater success as they do have some energy reserves. Piglets may drink appreciable amounts of tap water on the first day following birth, particularly if milk supply is limited (Fraser et al. 1988). Water intake is further increased when using a specially designed water dispenser with air bubbles (Phillips and Fraser 1991). Water with electrolyte or glucose administration may help to prevent dehydration and promote survival of the piglets. Intra-peritoneal infusion of approximately 15 mL of 5% glucose will temporarily alleviate dehydration and starvation (Fairbrother 2006). The piglets may thereafter be alert enough to find the teat and start suckling. Piglets may also be raised with a combination of milk replacers and highly digestible adapted feed. However, immunoglobulins included in some milk replacers are unlikely to contain antibodies specific for a farm and cannot be considered as replacers of colostrum. Applying additional heat for piglets suffering from hypothermia is always of utmost importance.

REFERENCES


INTRODUCTION
Outbreaks of disease with higher morbidity and mortality are most often associated with the alimentary and respiratory systems by clinicians. However, disorders and diseases involving the nerves, bones, muscles, and joints are very common (Anil et al. 2009), occur across all management systems, and are seen as individual animal afflictions with occasional herd outbreaks.

Many of the nervous disorders are sudden onset or acute outbreaks that may be infectious, toxic, or possibly of foreign animal origin. On the other hand, most of the disorders of the bones are chronic disorders involving faulty formation of skeletal structures or the effects of chronic infection or trauma. Most of the locomotor disorders are difficult to differentiate from each other by clinical examination.

In the aggregate, locomotor disorders contribute to losses in all stages of production. There are hundreds of reports on the economic loss associated with lameness. Importantly, the causes of these losses are different on individual farms, with countless risk factors contributing to occurrences (D’Allaire 1987; D’Allaire et al. 1987; Jones 1967; Smith and Robertson 1971; Svendsen et al. 1979).

NORMAL STRUCTURE AND FUNCTION
The fundamental resources for the anatomy, physiology, and pathology necessary for the effective study of the four body systems under discussion in this chapter are shown in Table 19.1. The combined systems (nervous and locomotor) are responsible for the perception, locomotion, reflex, and voluntary movement; the key fundamentals of which follow.

Nerves
The brain, normally white to yellowish in color, weighs about 35 g in the newborn piglet and 110–120 g in the adult (Widdowson and Crabb 1976). The spinal cord weighs about 4 g in the newborn and 30–40 g in the adult. The wild pig may have a brain that is 20% heavier (Rohrs and Kruska 1969). The brain grows rapidly from 5 weeks before birth to 8 weeks after birth, and thereafter, the ratio of brain weight to total body weight decreases.

Myelination begins about 55–60 days of gestation and peaks around birth, which explains why myelination is affected by virus infection in gestating dams. There is often a major surge in the rate of myelination at around 3 weeks of age (Dickerson and Dobbing 1967; Patterson and Done 1977; Sweasey et al. 1976), and it is usually complete by around 6 weeks. The neuronal complement of the central nervous system (CNS) is complete by about 6 weeks of age—Neurons lost after this are not replaced, but once mobilized, Schwann cells can provide limited repair of myelin.

Joints and Muscles
The joints are composed of articular cartilage supported by bone, synovial membrane that secretes synovial fluids, joint capsule, and insertions of tendons and ligaments, and may communicate with tendon sheaths. The joints are the most stressed areas in the locomotor system and are the most prone to be infected following local trauma or septicemia. Suspected joint problems should be examined first when performing postmortem examination.

The muscles of the pig are described in Sisson (1975). There are no special features of porcine mycology.
Growth plates have many shapes and sizes; some bones have a single physis, others more than one. The physes or growth plates should not be called epiphyseal plates. The epiphyseal growth component of the articulo-epiphyseal cartilage complex is responsible for the increase in size of the secondary centers of ossification such as the head and greater trochanter of the humerus. As the epiphysis matures, the epiphyseal growth cartilage becomes redundant and thinner. Not all long bones have epiphyses.

In the fetus and the newborn, the epiphyseal regions are continuous and are replaced by cancellous bone leaving just a thin rim of articular cartilage. The compared with the other species that can be considered important in pathology and medicine.

**Bones**

Bones are not inert. They are highly metabolically active in mineral metabolism and are constantly undergoing change as a result of the mechanical forces of muscles and tendons as well as the homeostatic process of remodeling (Goff 2010). In the pig, skeletal growth is still visible at 3–3.5 years of age because the growth plates (or physes) continue to be functional (Getty 1975). The physeal closure times are shown in Table 19.2.

### Table 19.1. References for anatomy, physiology, and pathology of the neurolocomotory system

<table>
<thead>
<tr>
<th>Subject</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anatomy</td>
<td>Sack (1982), Ashdown et al. (2009)</td>
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<tr>
<td>Neuroanatomy and nerves</td>
<td>Dellmann and McClure (1975), De Lahunta (1977), Yoshikawa (1968)</td>
</tr>
<tr>
<td>Autonomic nervous system</td>
<td>Swenson (1977), De Lahunta (1983)</td>
</tr>
<tr>
<td>Development of the brain</td>
<td>Widdowson and Crabb (1976)</td>
</tr>
<tr>
<td>Brain of the wild pig</td>
<td>Rohrs and Kruksa (1969)</td>
</tr>
<tr>
<td>Neuroanatomy and behavior</td>
<td>Signoret et al. (1975)</td>
</tr>
<tr>
<td>Brain development</td>
<td>Marrable (1971), Larsell (1954), Done and Hebert (1968), Dickerson and Dobbing (1967)</td>
</tr>
<tr>
<td>Eye</td>
<td>Prince and Diesem (1960), Diesen et al. (1975), King (1978)</td>
</tr>
<tr>
<td>Inner ear</td>
<td>Dellmann (1971)</td>
</tr>
<tr>
<td>Bones</td>
<td>Ham and Cormack (1979), Sisson (1975)</td>
</tr>
<tr>
<td>Ossification</td>
<td>Ham and Cormack (1979)</td>
</tr>
<tr>
<td>Muscles</td>
<td>Handel and Stickland (1986), Ham (1979), Sack (1982), Sisson and Gandhi (1975)</td>
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<td>Muscle tone</td>
<td>Simpson (1972), Palmer (1976)</td>
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<td>Neumuscular junction</td>
<td>McComas (1977)</td>
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<td>Joint</td>
<td>Ghadially (1983), Ham and Cormack (1979), Doige and Horowitz (1975)</td>
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<td>Necropsy of the pig</td>
<td>Van Kruiningen (1971), Wells (1978), King et al. (1979)</td>
</tr>
<tr>
<td>Neuropathology taxonomy</td>
<td>Done (1957, 1968), O’Hara and Shortridge (1966)</td>
</tr>
<tr>
<td>Patterns of neurobiology</td>
<td>Done (1976), Nietfield (2010)</td>
</tr>
<tr>
<td>CNS disorders</td>
<td>Done (1995), Done and Wells (2005)</td>
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### Table 19.2. Physeal closure times in bones of the thoracic and pelvic limbs of the pig

<table>
<thead>
<tr>
<th>Bone</th>
<th>Physis</th>
<th>Closure Time (Years)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Humerus</td>
<td>Proximal</td>
<td>3.5</td>
</tr>
<tr>
<td></td>
<td>Distal</td>
<td>1</td>
</tr>
<tr>
<td>Radius</td>
<td>Proximal</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Distal</td>
<td>3.5</td>
</tr>
<tr>
<td>Ulna</td>
<td>Proximal</td>
<td>3.5</td>
</tr>
<tr>
<td></td>
<td>Distal</td>
<td>3</td>
</tr>
<tr>
<td>Metacarpal III</td>
<td>Proximal</td>
<td>Before birth</td>
</tr>
<tr>
<td></td>
<td>Distal</td>
<td>2</td>
</tr>
<tr>
<td>Phalanx I</td>
<td>Proximal</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Distal</td>
<td>Before birth</td>
</tr>
<tr>
<td>Phalanx II</td>
<td>Proximal</td>
<td>Before birth</td>
</tr>
<tr>
<td></td>
<td>Distal</td>
<td>Before birth</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Bone</th>
<th>Physis</th>
<th>Closure Time (Years)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Femur</td>
<td>Proximal</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Distal</td>
<td>3.5</td>
</tr>
<tr>
<td>Tibia</td>
<td>Proximal</td>
<td>3.5</td>
</tr>
<tr>
<td></td>
<td>Distal</td>
<td>2</td>
</tr>
<tr>
<td>Fibula</td>
<td>Proximal</td>
<td>3.5</td>
</tr>
<tr>
<td></td>
<td>Distal</td>
<td>2–2.5</td>
</tr>
<tr>
<td>Metatarsal</td>
<td>Proximal</td>
<td>Before birth</td>
</tr>
<tr>
<td></td>
<td>Distal</td>
<td>2</td>
</tr>
<tr>
<td>Phalanx I</td>
<td>Proximal</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Distal</td>
<td>Before birth</td>
</tr>
<tr>
<td>Phalanx II</td>
<td>Proximal</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Distal</td>
<td>Before birth</td>
</tr>
</tbody>
</table>
metaphysis is mainly cancellous bone. A thin shell of cortical bone surrounds the cancellous bone. The metaphysis merges with the diaphysis or shaft of the bone so the cortical bones become thicker. In the diaphysis, cancellous bone is virtually absent, creating a medullary or marrow cavity filled with bone marrow.

The surface of bone is covered by the periosteum except on the articular surfaces and sites where tendons are inserted. In older animals, the bone marrow is replaced by fat except in the ribs, vertebrae, and pelvis. There is a proliferating zone that contains tightly packed horizontal columns of flattened chondrocytes. The hypertrophic zone has flattened chondrocytes that enlarge to form columns of spherical chondrocytes. The chondrocyte columns of the proliferating zone extend into the metaphysis as long bony spicules. They form a lattice on which bone is deposited, called the primary spongiosa.

The secondary spongiosa forms nearer to the diaphysis. In the diaphysis itself, the trabeculae are eroded to produce the medullary cavity.

INVESTIGATION OF NERVOUS AND LOCOMOTOR PROBLEMS

Central to a proper diagnostic investigation is the collection of the past history, present history, clinical inspection (examination from a distance without disturbance), environmental assessment, and individual animal pathological examination. Floors, equipment, pigs, and human interactions all contribute to locomotor disturbances.

A systematic examination approach starts with the foot and leg. At most locations, there are no subcutaneous tissues to protect joints and muscles. Here, adventitious bursae may develop in response to pressure and may subsequently become infected with bacteria.

Abnormalities that occur in fetuses or at parturition may indicate infectious, toxic, genetic, or developmental abnormalities. Clinical signs may include failure to farrow, abortion, mummification, stillbirth, or anomaly. The occurrence of disease in the neonate is particularly influenced by both prenatal and postnatal sow factors, as well as maternal immunity and the environment into which the piglet is born. The weaner-grower phase is particularly susceptible to infectious insults because of withdrawal of maternal immunity, the acquisition of new infections, the mixing and moving of pigs, and, especially, weaning. Locomotor disturbance in the grower finishing stage has all the sequela of the neonatal and grower stages and the conditions brought about by incorrect feeding. These also extend into the adult phase of life.

In larger animals, examination may be difficult because of handling constrains, but palpation is extremely important. If possible, the animal should be moved to stand on a clean, level surface. Localizing the injury can help to suggest whether the origin is in the nerves, bones, muscles, or joints. Changes in limb angulation and joint congruency may indicate where the problem lies. Specific observations on gait can also be made on a level, clean surface. Pigs will walk on their carpus if their foot hurts. If there is pain in the hind feet, they shift weight from one leg to another. Myalgia (pain in muscles) associated with myodegeneration may cause pigs to tremble and crouch.

Swine veterinarians should adopt a systematic approach to examination whereby all aspects of all components of locomotion are examined. For example, start at the foot and work upward to the pelvis and shoulder, then the trunk and head. The pelvis is often neglected, but it should be examined for symmetry. Pain around the hip may suggest epiphysiodesis, fracture, or dislocation of the hip. Movement of the hip may produce crepitus. Abnormalities of body or leg posture may indicate leg abnormalities, back muscle necrosis, spondylitis, or spondylolisthesis. Restraint may allow a more thorough examination, using the minimum amount that is safe for both the patient and handling staff. Detailed, skillful, complete examination of the individual, pen, group, and herd is essential and described elsewhere in this book.

Often, accurate etiological diagnosis will require laboratory testing via hematology, chemistry, serology, microbiology, feed analysis, and pathological assessment. When warranted, a veterinarian should be capable of performing a thorough postmortem examination of several typically affected pigs, including efficiently accessing the joints, brain, and spinal cord. Foreign animal disease (FAD) introductions should always be considered in outbreaks of locomotor disease.

CLINICAL ASSESSMENTS

It is always important to use the correct descriptive terms to effectively communicate clinical signs of locomotor disease (Table 19.3). Sometimes, clinical signs are not observed if animals are found dead. Careful examination of other animals and thorough investigation of the premises for risk factors or recent changes are warranted. The most important part of the assessment is a thorough systematic necropsy. In the absence of diagnostically significant gross lesions, the collection of a full range of samples for laboratory investigation should follow. The sites of the systems (brain, spinal cord, peripheral nerves, bones, muscles, and joints) are discussed, with possible causes listed in various tables in this chapter.

Nervous System

The clinical signs, possible sites of origin, and potential cause(s) of nervous disturbance can be difficult to differentiate. Signs include visual difficulties, proprioceptive losses and loss of purposeful movement, altered
postnatal life with Teschen, pseudorabies, bacterial meningitis, and middle ear disease. The cerebellum can herniate through the foramen magnum due to increased intracranial pressure from internal hydrocephalus or cranial construction.

The cerebrum is responsible for voluntary movement, consciousness, and behavior. Seizures are common in salt poisoning and also in visceral larval migrans and arsanilic acid poisoning. It is important to remember that this is only a general guide and many conditions can be seen in other ages than the ones listed. Spinal cord injuries are most likely to occur when there is excess load on disks or osteochondrosis. In growing animals, spinal abscesses are often related to the presence of tail biting.

### Joints

While clinical examination, palpation, and manipulation can implicate joint disease, diagnosis usually requires a postmortem examination and laboratory analysis (cultures, joint fluid cytology, or histology). The nature of the capillary bed in joints means that they are particularly likely to trap infective circulating septic emboli, particularly in young animals not actively protected or immunocompromised by viral infections. If joint disease is suspected, specimens should be obtained aseptically as the first step in pathological examination.

<table>
<thead>
<tr>
<th>Descriptive Terms</th>
<th>Clinical Signs</th>
<th>Possible Location of Lesion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blind, loss of smell, loss of control of eye movement, pupil reflex</td>
<td>Impaired smell, vision, eye reflexes, eye control</td>
<td>Cranial nerve deficits (CN1–4, 6)</td>
</tr>
<tr>
<td>Loss of sensation of the face</td>
<td>Facial sensory deficit, mastication muscle deficits</td>
<td>Cranial nerve deficit (CN5)</td>
</tr>
<tr>
<td>Facial paralysis</td>
<td>Facial muscle deficits, expression</td>
<td>Cranial nerve deficit (CN7)</td>
</tr>
<tr>
<td>Seizure</td>
<td>Episodic uncontrolled brain activity, convulsion</td>
<td>Cerebrum</td>
</tr>
<tr>
<td>Tremors</td>
<td>Involuntary rhythmic muscle contraction, shaking</td>
<td>Cerebrum, spinal cord, peripheral nerve, cold/pain</td>
</tr>
<tr>
<td>Fasciculation</td>
<td>Muscle twitch, involuntary muscle contractions</td>
<td>CNS, muscle, other</td>
</tr>
<tr>
<td>Nystagmus</td>
<td>Involuntary rhythmic eyeball movements</td>
<td>CN6–8, brain stem, meningitis</td>
</tr>
<tr>
<td>Vestibular syndrome</td>
<td>Circling, head tilt, lateralizing signs, recumbent and paddling</td>
<td>Brain stem, CN8, otitis</td>
</tr>
<tr>
<td>Pain</td>
<td>Reluctant to move, vocalization</td>
<td>Report location</td>
</tr>
<tr>
<td>Incoordination</td>
<td>Abnormal gait, hypermetria, postural defects</td>
<td>Cerebellum, spinal cord</td>
</tr>
<tr>
<td>Proprioceptive deficits</td>
<td>Improper positioning of legs and feet, knuckling</td>
<td>Cerebellum, spinal cord, peripheral nerves</td>
</tr>
<tr>
<td>Flaccidity</td>
<td>Decreased muscle tone</td>
<td>Spinal cord</td>
</tr>
<tr>
<td>Spasticity</td>
<td>Increased muscle tone</td>
<td>Cerebrum, spinal cord</td>
</tr>
<tr>
<td>Plegia/paralysis</td>
<td>Complete loss of sensory and motor function, failure of withdrawal reflexes</td>
<td>Spinal cord</td>
</tr>
<tr>
<td>Paresis</td>
<td>Complete or partial loss of motor function</td>
<td>Cerebrum, vestibular system, spinal cord</td>
</tr>
<tr>
<td>Tetraplegia</td>
<td>All four limbs paralyzed</td>
<td>Spinal cord</td>
</tr>
<tr>
<td>Paraplegia</td>
<td>Both hind limbs paralyzed</td>
<td>Spinal cord</td>
</tr>
<tr>
<td>Hemiplegia</td>
<td>Front and hind limbs on one side</td>
<td>Spinal cord, brain stem</td>
</tr>
<tr>
<td>Monoparesis</td>
<td>A single limb paralyzed</td>
<td>Peripheral nerve, spinal cord</td>
</tr>
<tr>
<td>Anal tone</td>
<td>No sphincter response, unable to pass feces or urine</td>
<td>Spinal cord</td>
</tr>
<tr>
<td>Incoordination</td>
<td>Lack of coordination of muscle movement</td>
<td>Cerebellum, spinal cord, muscle</td>
</tr>
<tr>
<td>Proprioceptive deficit</td>
<td>Compromise of placement of limbs, knuckling</td>
<td>Cerebellum, spinal cord, lower brain stem</td>
</tr>
</tbody>
</table>

mental status, altered posture or gait, and evidence of trauma. In many cases, assessment of the nervous system will be most difficult and nearly as difficult as the differential diagnosis of sudden death. A routine for investigation has been described in Kornegay and Seim (1996).

Lesions in ganglia produce increased irritability and increased sensitivity of spinal reflexes and this effect is most pronounced in Teschen, less so in Talfan and other enterovirus conditions. Lesions in lower motor neurons usually produce changes in the muscles with lack of reflex activity and flaccid paralysis. Loss of either motor or sensory components will result in the loss of reflex activity. Upper motor neurons (UMNs) are affected by vertebral fracture, dislocation of disks, tumors, or spondylitis. Irritation of the UMN may produce signs of motor excitement, for example, salt poisoning, pseudorabies, or lead poisoning. The location of a lesion in the spinal cord will determine whether front, hind, or all limbs are affected as well as impact the ability to urinate and defecate.

Cerebellar lesions produce abnormalities of posture, balance, coordination, and regulation of fine muscle movements. Cerebellar cortical damage is characterized by ataxia, nystagmus, and tremor. The cerebellum is very prone to damage in the pig, particularly in fetal development, but can also be specifically attacked in postnatal life with Teschen, pseudorabies, bacterial meningitis, and middle ear disease. The cerebellum can herniate through the foramen magnum due to increased intracranial pressure from internal hydrocephalus or cranial construction.

The cerebrum is responsible for voluntary movement, consciousness, and behavior. Seizures are common in salt poisoning and also in visceral larval migrans and arsanilic acid poisoning. It is important to remember that this is only a general guide and many conditions can be seen in other ages than the ones listed. Spinal cord injuries are most likely to occur when there is excess load on disks or osteochondrosis. In growing animals, spinal abscesses are often related to the presence of tail biting.

### Joints

While clinical examination, palpation, and manipulation can implicate joint disease, diagnosis usually requires a postmortem examination and laboratory analysis (cultures, joint fluid cytology, or histology). The nature of the capillary bed in joints means that they are particularly likely to trap infective circulating septic emboli, particularly in young animals not actively protected or immunocompromised by viral infections. If joint disease is suspected, specimens should be obtained aseptically as the first step in pathological examination.
Muscles
Examination of muscle is by visual inspection and palpation to give an overall impression of size, tone, strength, normal contours, symmetry, normal motion, function, and gait. Generally, clinic abnormalities of the muscle involve extra musculature, paucity of the muscle, trauma, inflammation, or infection. Muscle atrophy as a result of nerve damage can take a long time to develop as does repair, which usually occurs by fibrosis. In slow progressive muscle disorders, such as the “Pietrain creeper” disease, muscle changes are progressive and irreversible.

Bones
Clinical examination of the bones is not easy as they are covered by muscle. Obviously, fractures and separation of epiphyseal heads should be detectable, but much else depends on history, progression, and postmortem examination with detailed dissection. Many bone abnormalities are a result of fetal or neonatal nutritional inadequacies or development faults, which are magnified by trauma and specific deficiencies in later life, particularly in the sow.

IMPORTANT FOREIGN OR REPORTABLE DISEASES
It is essential to consider and, if indicated, rule out FADs when considering the combined nervous and locomotor diseases and disorders. Specific diseases of concern vary from country to country, but generally will involve reporting to the federal or state authorities. Vigilance and due diligence for a suspected FAD or a disease requiring notification to state or federal authorities are a veterinarian’s responsibility. When in doubt, seek assistance for the necessary investigations. Field cases rarely mimic the classical disease presentations described in the textbooks.

Vesicular diseases can cause lameness and/or pyrexia, but it is not possible to clinically differentiate the vesicular virus diseases. The most important of the four is foot-and-mouth disease (FMD), followed by vesicular stomatitis (VS). Swine vesicular disease is important only as it confuses diagnosis of FMD, and vesicular exanthema has not been seen for 50 years.

Diseases that manifest as hemorrhagic diatheses include classical swine fever (CSF) and African swine fever (ASF) and cannot be differentiated in the field. A wide variety of CNS signs are seen with these diseases including conjunctivitis (a very important early sign that is often neglected), drooping head, head resting, hanging tail, huddling due to pyrexia, prostration, staggering, ataxia, convulsions, and death.

Rabies is uncommon in pigs but usually manifests as the “dumb” form with posterior prostration. Pigs are relatively resistant to anthrax and have to consume a large number of spores to be affected. Affected pigs have an extreme state of depression, and perhaps cervical lymphadenitis with dyspnea.

Blue eye disease caused by paramyxovirus is an important cause of CNS signs where it occurs, primarily in Mexico. In older animals, there may be just reproductive failure. It also affects the peripheral nerves.

Pseudorabies virus (PRV) is the important differential for “blue eye.” In some countries, PRV is eradicated; in others, it is controlled but notifiable as an FAD; and in yet other countries, PRV is endemic. Like “blue eye,” it is more clinically significant in young animals, often with 100% morbidity and sometimes 100% mortality. In some cases, the lesions of bilateral encephalomalacia (Nunoya et al. 1985) can be confused with other malacias.

Teschen disease is severe and caused by enterovirus type 1. The severe disease has only been seen in the area around the town of the same name in Czechoslovakia (Trefny 1930), but less virulent strains were isolated from a Welsh town called Talfan (Harding et al. 1972). Many of the mild strains were called Talfan. Since then, many other serotypes are known and are called teschoviruses that are within the family Picorna-viridae. These enteroviruses are sporadic causes of paresis and paralysis in growing pigs.

Japanese B encephalitis is another possible FAD that occasionally causes encephalitis in pigs. Eastern equine encephalomyelitis virus (EEV) occasionally affects pigs and will cause incoordination, depression, seizures, and death in pigs less than 2 weeks of age (Elvinger et al. 1994). The microscopic brain lesions are described.

Menangle virus can cause mummified fetuses, stillborn pigs with arthrogryposis, and craniofacial defects. Pathologically, it is possible to find degeneration of the brain and spinal cord, eosinophilic inclusions in the cerebrum and cord, and nonsupplicative meningitis.

POSTMORTEM EXAMINATION
Systematic postmortem examination adds confidence and accuracy to diagnosis when performed on acutely affected, representative, nonmedicated pigs; sometimes, a necropsy of a series of three to five pigs is necessary to establish herd diagnosis. Samples for laboratory testing, depending on ambient conditions and etiology, should not be collected from animals that have been dead for more than 1 hour prior to collection. Depending on presentation, it is sometimes necessary to change your usual necropsy procedure to collect needed samples immediately after death and without contamination. For laboratory testing, carefully examine and collect samples in this order—joints, brain, peripheral nerves, internal organs, spinal cord, and bone—to confirm the diagnoses and avoid contamination. The gross postmortem procedures are described in detail, most recently by Andrews et al.
(1986). Seize the opportunity to collect appropriate samples irrespective of a planned laboratory submission, since once the carcass has been rendered, the opportunity is lost. Specimens can be retained in formalin and refrigerated or frozen should additional testing be required. Fluids from the eyes, urinary bladder, and joints can also be stored.

The brain is easily removed in young animals by midline longitudinal section with a knife. Brain removal in older animals requires special tools such as a saw or a cleaver. Spinal cord sections can be invaluable for diagnosis; patience and tools (e.g., Barnes dehorner, saw, cleaver, and/or spinal cord elevator) are useful to prevent damage to the nerve roots and ganglia.

The first saw cut to remove the brain is through the frontal bones close to the orbits in the neonate and slightly more rostral in the older animal. The second and third cuts are from the medial angle of occipital condyles to the lateral margins of the first cut at the medial canthus of each eye (45° to the long axis of the skull). Incise the dura mater, remove the tentorium cerebelli, cut through the olfactory lobes, and turn the skull upside down so that the brain hangs suspended by the cranial nerve attachments, which are carefully cut while supporting the brain. The leptomeninges can be carefully examined for cloudiness in the sulci indicating meningitis.

Necrosis in the brain can be detected with an ultraviolet lamp; positivity decays rapidly after death. The brain tissues routinely examined by histology include the olfactory tubercle, the midbrain (at the level of the oculomotor nerve), the medulla at the level of the obex, and a sagittal section of the cerebellum with the rest of the brain preserved in formalin in case extra areas need to be examined.

When lesions are suspected in the spinal cord, the whole cord should be removed. When complete histopathology is critical, the cord can be suspended in formalin in a perspex tower with a weight attached to prevent shrinkage artifacts. A reasonable alternative is to collect representative cross sections of the cord from cervical, thoracic, and lumbar vertebrae. Care should be taken to preserve the dorsal root ganglia on the cord as in some diseases (e.g., Teschen), these are a valuable diagnostic aid. Representative segments of the cord should be preserved in formalin as well as chilled or frozen to confirm etiology.

Sections of cranial or peripheral nerves are useful but should be attached to a card with stitches or pins through the connective tissues to prevent shrinkage artifacts. If myelin degeneration neurochemistry, muscle histochemistry for fiber types, or infectious agent detection is desired, then freeze the cord and muscle.

The middle ear may be exposed by a vertical saw cut at right angles to the long axis of the skull once the external ear has been removed. The eyeball can be removed similar to surgical enucleation, trimmed and fixed, preferably in Bouin’s solution.

Examination of the bones is more difficult, but it is always wise to collect representative samples. It may be necessary to take the ends of the long bones, intact vertebrae, intervertebral areas, and ribs including costochondral junctions. Costochondral junctions are particularly useful as they are manageable and give information on bones, cartilage, and joints and the state of the bone marrow. Longitudinal slices (0.5 cm) of the bone and cartilage can be fixed in formalin, decalcified with acids, and examined histologically. Radiography can confirm the state of mineralization and is a good way to assess the quantity of cortical bone.

Muscle samples should be collected after death as soon as possible, and the name of the muscle should be noted. It is important to clamp the sample before excision to reduce artifacts and to assure there are both a longitudinal section and a cross section of the muscle fibers since diagonal sections are impossible to interpret (Bradley 1978). When investigating a specific disorder (e.g., nutritional myopathy), it may be necessary to target specific muscles as these show the fiber-type variations that need to be assessed. For example, the diaphragm, the gastrocnemius, and the supraspinatus muscles are useful to assess type I fibers and the psosas and longissimus muscles to assess type II fibers (Ruth and van Fleet 1974). The psosas is an excellent muscle to use as a control as it is deep in the abdomen protected by the spinal column and is therefore unlikely to be affected by trauma and surface infection. Tissue from muscle biopsies can be collected in the same way (Bradley 1978; Dubowitz and Brooke 1973).

**Examination of the Joints**

Examination of the joints and collection of laboratory specimens requires aseptic technique to prevent contamination. The best technique is similar to that used in horses by Van Pelt (1974). If a single joint is affected, then sample that joint aseptically before proceeding. Joint samples and tissues can be processed routinely, remembering that at least five joints are commonly sampled—right stifle, right hip, atlanto-occipital, right elbow, and right radiocarpal joints. Collect synovial fluid aseptically before opening the joint or after aseptic exposure. Useful specimens for laboratory examination include smears, swabs, joint fluids, and tissues for culture, polymerase chain reaction (PCR), and microscopy.

The synovial membrane is easily accessed by bacteria through surface abrasions or septicemia. Bacteria release enzymes that damage collagen and proteoglycans and cause excess damage to the joints. Synovial fluid is normally present as clear or lemon-colored viscous fluid in all joints. It is a transudate formed by the synoviocytes and contains hyaluronic acid to act as a lubricant. With inflammation, synovial fluid becomes
turbid and cloudy due to leukocytes or reddish if there is hemorrhage. Nerve endings in the synovial membrane, capsule, and ligaments allow for proprioception and pain perception.

Alteration in size and shape of the joints is inevitable with arthritis as nearly all insults produce excess synovial fluid, therefore swelling. Excess movement also produces fibroplasia. Shape changes may occur in congenital disorders of the joints. Articular cartilage is usually only damaged in developmental problems and not in inflammatory conditions; cartilages have very little ability to repair.

Degenerative joint disease (DJD) follows osteochondrosis dissecans (OCD), suppurative arthritis, and collapse of subchondral bone. Softening of articular cartilage is a result of enzyme action on proteoglycans. Rough fibrillated cartilage can occur with eburnation (polishing of the bone). Pannus formation, which is fibroplasia, can also be seen. It often follows chronic suppurative arthritis such as erysipelas or occasionally in Mycoplasma hyosynoviae infections. Alterations in capsule and synovial membrane usually result from inflammation as there is a very good blood supply.

Synovitis follows as a result of incongruity of the joint, so it is common after OCD, bacterial invasion, and polyarthritis. Severe suppurative arthritis and osteomyelitis may produce local reddening and fibrinopurulent exudates with acute infections with streptococci, staphylococci, Erysipelothrix, Actinobacillus, Mycoplasma, Arcanobacterium, or Haemophilus parasuis. If the process continues, there will be villous hypertrophy of the synovial membranes (e.g., Erysipelothrix rhusiopathiae, Mycoplasma hyorhinis, and M. hyosynoviae). Changes in intervertebral disks are rare, but discospondylitis does occur and it is usually in the upper thoracic and upper lumbar spines. It is associated with either the multibacterial agents of tail biting or by septicemic E. rhusiopathiae. In very rare instances, there is damage to both disks and surrounding bone.

**Examination of the Nervous System**

In fetuses and neonates, there may be a wide range of congenital abnormalities including a reduction in brain size, evidence of abnormal development, anencephaly (absence of the cerebral hemispheres), or complete absence. Microencephaly may follow heat stress during pregnancy as well as hog cholera virus (HCV) infection. A reduction in the gray matter can be from vitamin A deficiency. Cerebellar hypoplasia is a common defect described with Japanese B encephalitis virus or pestivirus infections. The cerebral gyri may also be flattened due to pressure from excess meningeal fluid; the cerebrospinal fluid may also be increased. Dilation of the ventricles is due to poor fluid drainage via the arachnoid villi or obstruction of cerebral aqueduct by bacterial inflammation. Frank hydrocephalus occurs sporadically and can occur with Japanese B encephalitis, often with failure of cranial bones to fuse with an associated meningocele.

Enlargement of the brain from cerebral edema is common. Abscesses, cysts, or neoplasms are more rare possibilities. One of the more useful diagnostic observations is when extra fluid with brain swelling forces the cerebellum to be herniated through the foramen magnum. When present, it is an indication to directly swab through the foramen magnum for bacterial isolation before removing the brain for complete examination.

Examination of the brain may also reveal changes in consistency. Softness of the brain and/or spinal cord (malacia) is associated with reduced blood perfusion. Necrosis and liquefaction may follow. Cerebrocortical necrosis has been seen in young piglets. The malacia in older pigs may be an indication of water deprivation/salt poisoning, edema disease, or possibly selenium toxicity, the latter more likely in the cervical or lumbar cord. Occasionally, foreign bodies in the form of cysts (Cysticercus cellulosae) in the brain parenchyma or the submeningeal areas are found.

Spinal cord lesions include necrosis, which may follow fibrocartilaginous embolism, selenium toxicity, or nicotinamide deficiency. Malacia has also been found in the spinal cord when fractures, abscesses, or hemivertebrae have compressed the cord. Abscesses are usually associated with tail biting and follow septicaemia or lymphatic spread of pyogenic bacteria.

In malacic or tumor sites, there may be metastatic calcification and mineralization. Color changes are rare but may include black (melanin), yellow (jaundice), or paleness, which may indicate malacia and a potential area for UV fluorescence, a test that can indicate necrosis for up to 48 hours after death.

**Examination of the Muscle**

Myositis is the likely result of localized bacterial infections. Myopathies can be developmental (congenital, hereditary) or acquired. Acquired myopathies are by a variety of mechanisms including toxins, autoimmune or endocrine dysfunction, parasite insult, neurogenic dysfunction, cachexia, or metabolic dysfunction. Alteration in color of the muscle is not uncommon. The color of any tissue is a reflection of the blood content on one hand and the cellular content on the other. Paleness of the muscle is seen in splayleg and nutritional muscular dystrophy; redness is seen in septicaemia, gross necrosis, back muscle necrosis, or myositis associated with clostridial infections. Wet muscles are a feature of porcine stress syndrome (PSS), and focal lesions are characteristic of abscesses, foreign body reactions, injection sites, or bacterial infections. Muscle hemorrhage is seen in the hemorrhagic diatheses of CSF or ASF. Rarely is muscle atrophy discernible by gross examination of the actual muscle tissue.
Alteration in the tension or texture of the muscles on palpation is important to note. Rigor mortis may last 24 hours, and during this period, muscle may be hard and the joints are fixed. Rapid onset of rigor after death is a reflection of rapid intense muscular activity (low pH and low glycogen reserves).

Mineralization is a feature of past insults, nutritional myopathy, and chronic cyst formation of parasites. Soft muscle is a feature of PSS and autolysis. Dry and crepitant muscle is a feature of clostridial myositis, whereas dry and sticky muscle often denotes dehydration. Dry, firm, and dark (DFD) muscle is a feature of PSS. Steatosis or excess fat deposition may be a genetic change (Handel and Stickland 1986). Cysts are usually felt as hard gritty nodules.

**Examination of the Bones**

Alterations in shape and size of the bones are relatively common in swine. Congenital hyperostosis is a fatal and heritable thickening in limb bones in fetuses and neonates; piglets affected with congenital hyperostosis cases are born dead or die quickly. There may be thickening of skull bones as in fibrous osteodystrophy. Reduced length of the bones is seen in an inherited disorder of pigs known as chondrodystrophic dwarfism. Similar effects have been noticed with oversupplementation of vitamin A. There may be enlarged ends to the long bones, a feature of rickets, where the metaphysis is enlarged and the epiphysis may be flattened. Enlarged costochondral junctions may indicate fluorine toxicity. Localized enlargements may suggest osteomyelitis, focal lesions, or partially healed fracture sites.

Fractures of long bones and vertebrae can be caused in stunning or electrocutions, including sublethal electrocution, as well as trauma. The shape of bones can be altered by malalignment at the ends of the bones on either side of the fracture. Limb deformity and reduced bone length in pigs can also result from manganese deficiency. In older animals, the most likely cause of abnormalities of the physeal plates are related to osteochondroses.

Alterations in color are seen in congenital porphyria. There may be chocolate brown discoloration of bones and dentine. In ascorbic acid deficiency, there may be hemorrhages both subperiosteal and metaphyseal. Hemorrhages may also follow change in blood flow associated with necrosis and inflammations as in osteomyelitis. CSF may cause lines of discoloration in the metaphysis parallel to the physis.

Bone strength is reduced in rickets, osteomalacia, fibrous osteodystrophy, and osteoporosis. Fragile bone is found in osteoporosis, maternal copper deficiency, and osteogenesis imperfecta. Physeal separation is seen in epiphysiolysis and apophasyysis, usually with hemorrhage around the joints. It is possible to collect bones at postmortem examination and test strength by determining the bone-breaking force.

**DIFFERENTIAL DIAGNOSIS**

The following discussion is organized by stage of growth and system, and will follow the fetus, suckling, and postweaned age groups in a discussion of the most likely disease/disorder diagnoses following the sequence—nerves, bones, muscles, and joints—as the most important tool for locomotor disorder differentiation. The eye, ear, and nose will be discussed first as they are examined in the general view of the external surfaces. Be reminded that postmortem examination is essential for the examination of structures, but is in a different order—joints, then nerves, muscles, and finally bones.

**Special Senses: Eyes**

Examination of the eyes of live pigs using an ophthalmoscope is difficult; hence, critical descriptions of the eye fundus, cornea, and conjunctiva are scant. Assessment of visual function is, in practice, confined to observations of behavioral signs of blindness, testing of simple reflexes. The blink and fixating reflexes are both dependent on the integrity of pathways from the retina to the visual cortex. These reflexes are all lost in conditions affecting the cerebrum or in arsanilic acid poisoning, which specifically damages the optic nerve and tract. Some of the clinical signs related to the eye are shown in Table 19.4 and others are mentioned below.

**Table 19.4.** Observations and lesions of the eye (adapted from Saunders and Jubb 1961)

<table>
<thead>
<tr>
<th>Observed Signs</th>
<th>Possible Cause</th>
</tr>
</thead>
<tbody>
<tr>
<td>Discharges —</td>
<td>Ammonia, other gases, rhinitis, atrophic rhinitis, PCMV, S. hyicus, streptococci, pseudorabies</td>
</tr>
<tr>
<td>—</td>
<td>ASF, CSF, PRV, PRRS, edema disease, H. parasuis</td>
</tr>
<tr>
<td>Cloudiness</td>
<td>Expected change by 24 hours after death</td>
</tr>
<tr>
<td>White</td>
<td>Anemia: blood loss, aplastic (iron deficiency)</td>
</tr>
<tr>
<td>Yellow</td>
<td>Jaundice: liver diseases (toxin, bacteria, virus)</td>
</tr>
<tr>
<td>State of hydration</td>
<td>Reflection of systemic illness</td>
</tr>
<tr>
<td>Hemorrhages</td>
<td>ASF, CSF, septicemia, coagulopathy, trauma</td>
</tr>
<tr>
<td>Facial staining (tears)</td>
<td>PRV, CSF, blocked tear ducts, atrophic rhinitis, irritating gases (ammonia)</td>
</tr>
<tr>
<td>Conjunctivitis</td>
<td>HCV, SIV, PRV, PRRS, PCMV, Chlamyphila, Mycoplasma</td>
</tr>
<tr>
<td>Cataracts</td>
<td>Riboflavin deficiency</td>
</tr>
<tr>
<td>Blindness</td>
<td>Organic arsenic, lead, mercury poisoning; PRV; sodium intoxication; botulism; vitamin A deficiency</td>
</tr>
<tr>
<td>—</td>
<td>No eyes (Hale 1933) and small eyes (Harding 1956)</td>
</tr>
</tbody>
</table>
In the fetus and neonate, there may be congenital abnormalities recognized but most of these may be related to vitamin A deficiency with signs that include anophthalmia, microphthalmia, and entropion.

The most important infection causing conjunctivitis is hog cholera (CSF) and possibly ASF, an often disregarded sign. In the day-to-day life of the pig, excessive lacrimation and conjunctivitis may be associated with dust, ammonia, hydrogen sulfide, and other toxic gases. Pseudorabies is an important cause of conjunctivitis, retinitis, and optic neuritis. Other infections recorded include *Chlamydia*, *Mycoplasma*, porcine cytomegalovirus (PCMV), swine influenza virus (SIV), and porcine reproductive and respiratory syndrome (PRRS), which is particularly associated with periocular edema. PCMV (Edington et al. 1988) can cause a severe rhinitis with nasal discharge, conjunctivitis, and sneezing, and can cross the placenta to produce mumified, stillborn, or weak infected piglets. All strains of swine influenza may also cause conjunctivitis. Rarely, occasional fractures of the orbital bones may distort the eyes. *Cysticercus cellulosae* has also been found in the eye.

Gross lesions are rarely seen in the optic nerve and globe; histopathology is needed for conditions affecting these structures. Hyponpyon, rare in pigs, is suppuration in the anterior chamber of the eye. Corneal edema is occasionally seen in blue eye disease (Stephano et al. 1988) or following trauma. There are other odd conditions such as riboflavin deficiency that will cause cataracts. Enteroviral encephalomyelitis will cause retinitis and neuritis, and swine vesicular disease will also cause optic neuritis.

Histologically, organic arsenical poisoning produces degeneration of the optic nerve, optic tracts, and peripheral nerves. Mercury poisoning will produce blindness, and organophosphate poisoning will produce lacrimation. Hygromycin B toxicity will produce cataracts. Lead poisoning is rare but will produce blindness. Excess vitamin A to sows will produce cataracts in piglets.

Keratosis is rare in the pig but has been seen following trauma, noxious gas exposure, pseudorabies, and *Chlamydia* infection. Pigs with GM2 gangliosidosis may have multiple white foci in the retina.

**Special Senses: Middle and Inner Ears**

These can only be examined properly after the skull has been disarticulated and the mandible has been removed. The tympanic bullae can then be removed and the lumen can be examined for exudate and swabs taken. The nasopharyngeal opening of the auditory tube can be examined by cutting the skull longitudinally along the midline.

Rupture of the tympanic membrane usually follows inflammation of the middle ear. Otitis media is usually due to progressive external otitis often involving a head tilt. Diseases of the inner ear cause lateralizing signs—a head tilt toward the affected side, horizontal nystagmus, ataxia that may include circling or falling—and are common sequelae to *Streptococcus suis* infections. *Mycoplasma hyorhinis* has been suggested as a primary pathogen for otitis media.

**Differential Diagnosis of the Fetus**

Many disorders are found early on in the pig’s life because sows are exposed to infectious diseases, toxic insults, and deficiency states that are often manifested in the fetus or neonate (Table 19.5). The fetus has abnormal development and patterns of fetal growth rather than degenerative disease. Developmental diseases are not limited to only those present at birth (Done 1976); some are manifested as pigs grow. Congenital disorders include developmental accidents and teratogen-induced diseases as well as simple Mendelian and additive hereditary defects.

**Fetal Nervous System.** Done (1976) classified the patterns of abnormal nervous development as follows: dysmorphismogenesis, such as the cerebellar hypoplasia of in utero viral infections; metabolic defects, such as lysosomal storage disease or porcine gangliosidosis; dysmaturity, a difference between chronological and developmental age as seen with myobrillar hypoplasia; and dysfunction, where there is no demonstrable pathological changes as in splayleg.

One of the more common genetic disorders in pigs is hydrocephalus in which there is excess cerebrospinal fluid dilating the ventricles. Meningocele is common as a failure of the bony union of frontal and parietal bones.

Cerebellar hypoplasia can occur with viral (CSF, border disease/bovine virus diarrhea) infection of the sow and is also reported as a sequel to trichlorofon poisoning (Pope 1986). Once common, vitamin A deficiency has serious effects on the nervous system. In pregnancy, low vitamin A levels caused herniation and constriction of dorsal and ventral nerve roots. It results in abortion with stillbirths and small or absent eyes. Other abnormalities include cleft palate, edema, high morbidity, or high mortality. Rarely, toxoplasmosis can cause stillborn piglets and prolonged gestation.

**Fetal Joints and Muscles.** Arthrogryposis, which may occur at birth or be found in fetal pigs, is described below. Congenital abnormalities affecting muscles are, indeed, rare in pigs.

**Fetal Bones.** A whole variety of abnormalities may occur and can be grouped in various classes. Infectious disease rarely causes bone changes, but Menangle virus and HCV can produce changes in bones. Abnormalities of endochondral ossification include bullhead, bent...
Table 19.5. Congenital and newborn diseases affecting the locomotor system

<table>
<thead>
<tr>
<th>Viral</th>
<th>Clinical sign/lesion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Congenital tremors a</td>
<td>Tremors at birth; see Tables 19.10 and 19.11</td>
</tr>
<tr>
<td>Bovine viral diarrhea (BVD)/border virus</td>
<td>Cerebellar hypoplasia, microencephaly</td>
</tr>
<tr>
<td>(pestiviruses)</td>
<td>Cerebellar hypoplasia, hydrocephalus</td>
</tr>
<tr>
<td>Classical swine fever</td>
<td>Mummies, stillbirths, arthrogryposis, craniofacial defects</td>
</tr>
<tr>
<td>Japanese B virus</td>
<td></td>
</tr>
<tr>
<td>Menangle virus</td>
<td></td>
</tr>
<tr>
<td>Feed, toxin, other</td>
<td></td>
</tr>
<tr>
<td>Congenital tremors a</td>
<td>Tremors at birth; see Tables 19.10 and 19.11</td>
</tr>
<tr>
<td>Splayleg a</td>
<td>Stiff hind legs, hypomyelinogenesis, muscle hypoplasia</td>
</tr>
<tr>
<td>Hypoglycemia a</td>
<td>Recumbent, paddling, frothing at the mouth</td>
</tr>
<tr>
<td>Hypoxia a</td>
<td>Huddle, fail to suckle, “squeaking” vocalization</td>
</tr>
<tr>
<td>Vitamin A deficiency or toxicity</td>
<td></td>
</tr>
<tr>
<td>Trichlorfon toxicity</td>
<td></td>
</tr>
<tr>
<td>Manganese deficiency</td>
<td></td>
</tr>
<tr>
<td>Conium (hemlock)</td>
<td></td>
</tr>
<tr>
<td>Nicotiana (tobacco)</td>
<td></td>
</tr>
<tr>
<td>Datum (jimsonweed)</td>
<td></td>
</tr>
<tr>
<td>Heat stress</td>
<td></td>
</tr>
<tr>
<td>Genetic</td>
<td></td>
</tr>
<tr>
<td>Congenital tremors a</td>
<td>Tremors at birth; see Tables 19.10 and 19.11</td>
</tr>
<tr>
<td>Kinky tail, fused vertebrae</td>
<td></td>
</tr>
<tr>
<td>Limbless pig</td>
<td></td>
</tr>
<tr>
<td>Club foot, absence of fibula, extra toes</td>
<td></td>
</tr>
<tr>
<td>Polydactyly</td>
<td></td>
</tr>
<tr>
<td>Syndactyly—one toe</td>
<td></td>
</tr>
<tr>
<td>Chondrodysplastic—dwarfism</td>
<td></td>
</tr>
<tr>
<td>Congenital hyperostosis—thick legs</td>
<td></td>
</tr>
<tr>
<td>Hydrocephalus</td>
<td></td>
</tr>
<tr>
<td>Meningocele—bones not fused</td>
<td></td>
</tr>
<tr>
<td>Hemivertebrae—failure of ossification</td>
<td></td>
</tr>
<tr>
<td>Cleft palate—failure to fuse</td>
<td></td>
</tr>
<tr>
<td>Congenital muscle hyperplasia</td>
<td></td>
</tr>
</tbody>
</table>

Reference

Donald (1949), Nordby (1934)
Johnson (1940)
Palludan (1961), Nordby (1939)
Hughes (1935), Malynicz (1982)
Detiefsen and Carmichael (1921), Leopold and Dennis (1972), Ross et al. (1944)
Jensen et al. (1984)
Doige and Martineau (1984), Roels et al. (1996)
Hereditary; multiple causes
Multiple causes
Done et al. (1998)
Painter et al. (1985)
Done et al. (1990)

*Most common.

legs, deformed feet, hyperostosis (Kaye 1962), congenital carpal flexion, and kyphosis associated with a congenital hemivertebrae. A second group causing fetal or neonatal abnormalities is associated with the consumption of toxic plants during pregnancy. Consumption of *Conium maculatum* will produce bone abnormalities, particularly cleft palate, as will tobacco poisoning.

**Differential Diagnosis in Newborn and Suckling Pigs**

**Nervous System.** Pigs born alive with hydrocephalus will show stiff joints, quiver with pain, are uncoordinated if they move (Hughes and Hart 1934), and stand with difficulty.

Vitamin A deficiency can cause a generalized flaccid congenital paralysis of all four limbs, head tilt, incoordination, stiff gait, lordosis, excitability, muscle spasms, night blindness and paralysis, progressive dysfunction with incoordination, swaying gait, restlessness, dog sitting, posterior paralysis, lordosis, blindness, and reduced growth. It causes spasm and paralysis due to degeneration of portions of the spinal cord and sciatic and femoral nerves. At postmortem, there may be hydrocephalus, a small cranium, and herniation of the spinal cord in the lumbar region.

Hypoglycemia is a common cause of death, particularly in the newborn piglet, but it can also occur postweaning. The piglet is born with low glucose levels, poor stores of glycogen, and no brown fat, and to maintain glucose levels, the piglet needs to suckle every hour. Causes of hypoglycemia include sow factors (nutrition, presence of disease, fewer functioning mammary glands than piglets, and poor presentation of both rows of glands). Cold or wet creep areas and poor crate design will exacerbate hypoglycemia by increasing the demand for energy. Piglets that have disease or injury may not suckle. Clinical signs include loss of condition, poor vocalization (“squeaking”), weakness, faltering gait, paddling in lateral recumbency, froth at the mouth, coma, and death.

Hypoxia is probably more common than recognized, vaguely characterized clinically as depression, inability to suck, inappetence, huddling, lethargy, shivering, squealing when moved, coma, convulsions, and death.
hypoxia is often associated with starvation and/or chilling, but it is not possible to definitively differentiate hypoxia or hypoglycemia. Umbilical cord rupture, cord occlusion during birth, dystocia, slow farrowing, or PRRS lesions in the umbilical cord may be associated with the condition. The last pigs of the litter are also prone to be affected. Heated buildings with high levels of carbon monoxide may also cause hypoxia. Congenital tremor (CT), also known as “myoclonia congenita,” “trembling pig syndrome,” or “jumpy pig disease,” particularly affects newly established gilt herds or where boars and gilts are purchased from different sources. Six varieties of the disorder have been described (see Table 19.6) and can only be differentiated by pathology, particularly neurochemistry (Patterson and Done 1977). It generally affects a low proportion of litters. The potential causes and key features are shown in Table 19.7, but the essential lesion is the same in all cases and is a hypomyelination or demyelination of the brain and spinal cord.

The major clinical sign of CT is a very fine tremor that is usually absent during sleep but is particularly obvious on the head and limbs. It can be differentiated from primary head tremor due to cerebellar disease because the tremor becomes less pronounced at rest and more pronounced during excitement. Ataxia has also been seen in these cases. There may be an associated limb weakness along with considerable variation from fine tremor to a coarse twitch. It may also involve different skeletal muscle groups to varying degrees. At times, the tremor may stop and be seen only as a slight twitch. Tremor is aggravated by excitement, cold, and ingestion of cold liquids. Tremor may persist for several weeks or months. There is a good prognosis if the pig can suckle and survives 4–5 days after birth. In splayleg, the demyelination affects just the motor system, whereas in CT, there is an overall reduction of myelin in the spinal cord.

CT type AI is only associated with hog cholera. Type AI is most common in North America and, on the basis of experimental work, was thought to be associated with porcine circovirus type 2 (PCV2) (Hines and Lukert 1994). Others have explored a role for PCV2, including Stevenson et al. (2001) who found PCV2 in neural tissue by in situ hybridization in affected piglets from four farms in the midwest United States; the virus detected in pigs with CT and those with PMWS had 99% sequence identity (Choi et al. 2002). PCV2 can infect nervous tissue in the developing fetus, but unequivocal evidence to implicate PCV2 as sole cause remains lacking. Type AIV is known as “Landrace trembles” and is associated with females, high growth rates,

<table>
<thead>
<tr>
<th>Field observations</th>
<th>AI</th>
<th>All</th>
<th>AI1</th>
<th>AIV</th>
<th>AV</th>
<th>B</th>
</tr>
</thead>
<tbody>
<tr>
<td>% of litters affected</td>
<td>Hog cholera virus</td>
<td>Virus: unknown; PCV2 implicated</td>
<td>Genetic: sex-linked recessive</td>
<td>Genetic: autosomal recessive</td>
<td>Chemical: trichlorfon</td>
<td>Unknown</td>
</tr>
<tr>
<td>% pigs/litter affected</td>
<td>High</td>
<td>High</td>
<td>Low</td>
<td>25%</td>
<td>25%</td>
<td>&gt;90%</td>
</tr>
<tr>
<td>Mortality among affected pigs</td>
<td>Medium-high</td>
<td>Low</td>
<td>High</td>
<td>High</td>
<td>High</td>
<td>Variable</td>
</tr>
<tr>
<td>Gender affected</td>
<td>Both</td>
<td>Male</td>
<td>Both</td>
<td>Both</td>
<td>Both</td>
<td>Any</td>
</tr>
<tr>
<td>Breed</td>
<td>Any</td>
<td>Saddleback</td>
<td>Any</td>
<td>Any</td>
<td>Any</td>
<td>Any</td>
</tr>
<tr>
<td>Recurrence in subsequent mating</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Variable</td>
</tr>
<tr>
<td>Duration of outbreak</td>
<td>&lt;4 months</td>
<td>&lt;4 months</td>
<td>Indefinite</td>
<td>Indefinite</td>
<td>&lt;1 month</td>
<td>Variable</td>
</tr>
</tbody>
</table>

### Table 19.7. Key features and reference for types of congenital tremors described

<table>
<thead>
<tr>
<th>Type</th>
<th>Cause</th>
<th>Key Features</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>Hog cholera</td>
<td>Dysgenesis, cerebellar hypoplasia, small cord, demyelination, swollen oligodendrocytes</td>
<td>Harding et al. (1966), Bradley et al. (1985), Done (1976), Done et al. 1984</td>
</tr>
<tr>
<td>AII</td>
<td>Congenital tremor virus PCV2 implicated</td>
<td>Swollen oligodendrocytes</td>
<td>Done et al. (1986), Vanderkerckhove (1989)</td>
</tr>
<tr>
<td>AI1</td>
<td>Inherited autosomal recessive sex linked in Landrace</td>
<td>Reduced oligodendrocytes, reduced myelination, hypoplasia of the cord</td>
<td>Harding et al. (1973)</td>
</tr>
<tr>
<td>AIV</td>
<td>As above in Saddleback, also Landrace/Saddleback cross syndrome</td>
<td>Demyelination; cerebral, cerebellar, and cord hypoplasia</td>
<td>Berge et al. (1987), Kidd et al. (1986)</td>
</tr>
<tr>
<td>AV</td>
<td>Trichlorfon toxicity</td>
<td>Cerebellar hypoplasia at 45–79 days gestation, particularly 75–79 days</td>
<td>Pope (1986), Wells (1977)</td>
</tr>
<tr>
<td>B</td>
<td>Unknown</td>
<td>No special features</td>
<td>Gedde-Dahl and Standal (1970)</td>
</tr>
</tbody>
</table>
lean carcasses, and pale-colored meat. It has also been described in Landrace crosses by Kidd et al. (1986). Type V is associated with trichlorfon toxicity. It is worth remembering two features of CT—Pigs do not die and pigs can have both CT and splayleg. The incidence of both may sometimes be reduced by selection.

A condition has been described in Iowa as porcine reproductive and neurological syndrome (PRNS) from which an unclassified virus has been isolated; it produced mild encephalomyelitis experimentally (Pogranichniy et al. 2008). Toxoplasmosis rarely may produce tremors, weakness, staggering, and mortality, and survivors may be ataxic and blind.

Porcine reproductive and respiratory syndrome virus (PRRSV) is a possible cause of meningoencephalitis, but the common presence of PRRSV should not override vigilance for simultaneous infections with other primary viral or secondary bacterial infections.

Vomiting-and-wasting disease associated with hemagglutinating encephalitis virus (HEV) can cause neuropathology via spread from the peripheral nervous system (PNS) to the CNS.

Cerebellar abiotrophy is a condition where pigs are normal at birth but develop ataxia and a fine tremor by 3–8 weeks of age. This condition is found in Yorkshire and Large White pigs, probably an autosomal recessive trait. It is progressive over days to weeks until affected pigs are recumbent and unable to rise. It is a condition diagnosed on histopathology as premature degeneration of neurons (Purkinje cells).

Cerebrospinal angiopathy was described by Harding (1966) in weaned pigs 5 weeks of age. The degenerative change in the blood vessels of the cerebrum, midbrain, and brain stem, and the clinical signs of abnormal head carriage and circling for hours at a time were probably a subacute form of edema disease or, less likely, mulberry heart disease.

Vestibular syndrome manifests as turning or tilting of the head to the affected side, with a tendency to circle in that direction. It is probably a consequence of bacterial meningitis affecting the vestibular nerve, brain stem, and cerebellum or pharyngitis with ascending infection of the Eustachian tube.

Cerebral cortical sclerosis results from hypoxia and intermittent ischemia to parts of the cerebellum supplied by the cranial cerebellar artery, which may become kinked or occluded by pressure. It may be seen after streptococcal meningitis, toxoplasmosis, pseudorabies, or vitamin A deficiency.

Trauma in various forms should not be overlooked. Hind limb lameness, often unilateral and perhaps the same leg in multiple pigs, is occasionally observed in young pigs near weaning and through the nursery. Caretaker activities such as catching, transporting, or holding a struggling pig by the hind leg can traumatize the hind limb musculature or the sciatic nerve (Strugnell et al. 2011). Iatrogenic injection of irritating substances into the ham muscle near the sciatic nerve can also lead to sciatic damage and unilateral lameness or paralysis. Injections of adjuvanted vaccines deep in the cervical region of small pigs may occasionally locate or migrate to cause granulomatous myelitis and neuritis leading to posterior paresis or paralysis.

**Joints.** Noninfectious causes of joint disease are uncommon. Arthrogryposis can occasionally affect 40–50% of piglets and litters with the joints being partially mobile or completely fixed. Joints may be fixed in the normal position, but more typically are markedly flexed, entwined around the body, or overextended, especially the hock in the latter. Severely affected pigs are unable to move at all, but moderately affected animals may survive. A variety of causes have been suggested, but etiological diagnosis is rarely confirmed, the common theme being maternal toxicity. Tobacco stalks (*Nicotiana tobacum*), jimsonweed (*Daturum stramonium*), poison hemlock (*C. maculatum*), and wild black cherry (*Prunus scrotina*) have all been suggested as possible causes. Likewise, deficiency of manganese or of vitamin A has also been suggested. Arthrogryposis has also been produced in Menangle virus infections. Lomo (1985) suggested the cause to be a simple recessive trait.

Fluctuating swellings in the region of the joints usually means the presence of excess joint fluid, pus, or blood or the development of adventitious bursae and is fairly common in pigs because of the endless possibilities of trauma, infection, or both. Periarticular edema may be local or extend over much of the limb as many of the joint capsules are also linked to the local tendon sheaths. There are a few conditions that have been associated with joint swellings. Vitamin K deficiency produces swollen joints and connective tissue hematomas. Enlarged, nonpainful joints are seen in vitamin A deficiency due to a premature closing of the epihyseal plates. Enlarged shoulder joints, hemorrhage into joints, and arthritis in the head of the femur have been seen in zinc toxicity.

The most common presentation of joint disease is due to infectious, usually bacterial, insults. Polyarthritis is the most common form of arthritis in preweaned pigs, with prevalence variable. For example, Nielsen et al. (1975) reported that it affects about 18% of litters and 3.5% of pigs after 4 days of age. Most affected pigs eventually die by 3–5 weeks of age. Increased frequency of occurrence is often associated with interventions such as teeth clipping, tail docking, or castration as well as general lack of hygiene.

Arthritis is a common problem in neonatal pigs for several reasons. Their skin is very soft with minimum keratin and is easily eroded particularly over the joints that have no soft tissue protection. Polyarthritis is defined as more than one joint involved, and this is a
very common reason for locomotor dysfunction in baby pigs prior to weaning.

Although the main routes for bacterial infection are the tonsil and small intestine, high prevalence of arthritis is associated with septicemias from navel ill or abrasions of the integument. Clipping of teeth and tails or ear notching in the farrowing house can contribute to the problem. In addition, many of the floors on which piglets are raised are abrasive, erosive, or contaminated with environmental organisms and opportunists. Litters from gilts are often more severely affected, which suggests that immunity plays an important role in the susceptibility to arthritis agents. Males are slightly more prone to infection. Introduction of new stock is also an important feature in an outbreak.

Many of the bacteria that cause arthritis are common, opportunistic, or secondary invaders. By far, the most common are groups C and L streptococci, but this group would also include staphylococci, other streptococci, 

\( \text{Escherichia coli} \), other coliforms, \( \text{Arcanobacterium pyogenes} \), and actinobacilli. The most common causes of polyarthritis are hemolytic streptococci, particularly \( \text{S. suis} \) or occasionally \( \text{Streptococcus equisimilis} \) (63% of cases, Smith and Mitchell 1977. These are usually suppurative infections and may be seen as early as 24 hours postpartum (Sanford 1987a, b) together with meningitis, otitis, or septicemia. The joint lesions are characterized by increased synovial fluid, inflamed synovial membranes, fibrinous periarteritis, and joint swelling due to the exudation and eventual abscission. The carpal, elbow, hock, and hip joints are the ones most often affected. Quite often, there are other associated lesions including turbid cerebrospinal fluid, concurrent pneumonia, endocarditis, and gingivitis. Other causes include \( \text{E. coli} \), \( \text{Staphyloccocus hyicus} \), \( \text{A. pyogenes} \), and other staphylococci. Most of the infectious contributors are common flora (e.g., \( \text{S. equisimilis} \) is a common resident of the vagina of sows) or present in other niches or the environment. Traumatic insults to piglets leading to breeches in the integument are the most common predisposing factors. The immature and developing inflammatory and immune systems play a very important role in the outcomes of infections.

\( \text{Streptococcus suis} \) (and likely other) infections (Gottschalk and Segura 2005) and some strains may develop differently depending on the genetics of the pigs (see Chapter 62). Infections are usually acquired from the sow as septicemia with arthritis following 7–14 days later (Sanford and Tilker 1962). They may be persistent infections (Torremorell and Pijoan 1998; Walsh et al. 1992).

**Muscle.** The muscle mass of an animal is genetically programmed, but nutritional status is the key determinant of muscle size. Well-fed animals without deficiencies or overdoses of essential nutrients have larger muscle masses than those that are not fed correctly. It is important to remember that many myopathies also affect the cardiac muscle as well as the smooth muscle of the other organ systems. Examples include vitamin E/selenium responsive myopathies, gossypol toxicity, and monensin poisoning. Only PSS and back muscle necrosis (possibly part of the same syndrome) affect the skeletal muscle alone.

Confidence of clinical impression of muscle involvement generally requires a full postmortem examination and confirmation by histology, electron microscopy, histochemistry, or biochemistry to determine the extent of the damage and the diagnosis (Patterson and Allen 1972). Swollen muscles, inflammation, abscesses, or cystic structures require careful gross examination. All structures of the lower limb (nerves, tendons, ligaments, bones, and muscles) are liable to ascending infections from the foot because of the permanent exposure to microbial hazards and trauma. Pathological changes in tendons such as calcification, tenonitis, tenosynovitis, and rupture should also be detected at the postmortem examination.

Congenital muscle hypertrophy is a specific muscular disorder (Done et al. 1990) in which a single boar produced rounded bulging ham muscles in over 200 piglets. At birth, affected piglets had no control of their legs and were unable to place their feet on the ground. The forelimbs were stiff if they could walk at all and the hind limbs were adducted. The stifle joints were angled out and the piglets walked on tiptoe. The defects increased with age. No gross lesions were detected, but microscopically, there was hypertrophy of the myocytes and possibly some hyperplasia. The boar used to produce these litters died of progressive cardiac failure but had no gross or microscopic lesions at slaughter.

Muscle pigmentation may be endogenous or exogenous in origin, but is rarely a serious problem. Occasionally, a brownish discoloration of the muscle may indicate hemosiderosis where there has been previous hemorrhage. Blackish deposits in the muscle may indicate melanosis or, in very rare occurrences, the presence of secondary malignant melanomas. The injection of parenteral iron may result in acute toxicity and death (see Chapter 70); increased susceptibility has been linked in some cases to hypovitaminosis E (Lannek et al. 1962). There are a few generalized specific muscle conditions except splayleg.

**Splayleg.** Splayleg is usually found at or within hours of birth. Clinically, it resembles CT, and it is important to realize that both conditions may occur in the same pig. Splayleg is a lateral extension of the hind legs. The piglet cannot adduct the hind legs, but the front legs are not affected. It is widespread, common, and typically only affects one to four pigs in just a few litters; however, some outbreaks may reach 8% (Ward and Bradley 1980). Affected pigs frequently have low birth
weights, more frequently are males, and are more often crushed by the sow because of impaired mobility. At birth, normal pigs have myofibrillar hypoplasia (myofibrils do not fill the muscle cells), but this is more pronounced in the splayleg piglets. Essentially, there are too few type I fibers, particularly in the forelegs, lumbar region, and the hind leg muscles. By about 5–6 days of age, there are no apparent differences between normal and splayleg affected piglets.

The primary pathological change of splayleg is a reduction in axonal diameter and the thickness of myelin sheaths to the hind limb adductors (Szalay et al. 2001) and delayed myelination caused by poor production of myelin by oligodendrocytes, particularly in the ventral and lateral funiculi in the lumbar cord. In the splayleg piglet, prebirth myelination is not sufficient but compensation can occur within the first 2–3 weeks of life. If splayleg piglets survive to 3–5 weeks, they are usually fully myelinated. They will usually recover if they survive being laid on by the sow and can reach a supply of nutrients.

Complicating factors for splayleg include large litters, low-birth-weight pigs, slippery floors, choline deficiency (Cunha 1972), Large White and Landrace genetics, shortened gestation lengths (Ward 1978a,b), and perhaps estrogenic mycotoxins. Splayleg piglets have been described as being born after sows ingested Fusarium (Miller et al. 1973) found in grain stored at cool temperatures. If fed by accident between midpregnancy and 3 months gestation, as many as 85% of the piglets may be splaylegged (Steane 1985). Although recessive genes may be involved, the main reason is myofibrillar hypoplasia, and it is likely polygenically inherited. Treatment of splayleg is by providing supportive care and perhaps leg support via a harness or tape. Hereditary contribution is not documented, but some advocate control by selection of seed stock from the unaffected litters.

**Bone**

Few insults affect the bones of suckling piglets. Significant bone loss is associated with conchal bone atrophy (turbinate atrophy) as it occurs in progressive atrophic rhinitis, which may start in the suckling period. This disease, associated with Bordetella bronchiseptica and Pasteurella multocida type D, is discussed in respective chapters.

**DIFFERENTIAL DIAGNOSIS IN WEANED PIGS**

Many of the neonatal and suckling pig disorders can occur in the postweaning period. Infectious agents are common primary causes of locomotor disturbances postweaning. Often, these agents are endemic in the herd whereby passive or acquired immunity promotes resistance and results in mild signs or variable occurrences. However, strain variations or new introductions of common infectious agents can produce severe outbreaks on occasion.

A variety of acquired viral infections have the ability to produce locomotor signs. PRRSV will occasionally produce CNS signs with underlying encephalitis, myelitis, or meningitis. More severe lesions and clinical signs are described with coinfection with pseudorabies (Narita and Ishii 2004), who reported nonsuppurative encephalitis and polioencephalomalacia in dual-infected pigs and suggested that this was due to the PRRS increasing the replication of the PRV. Similarly, PCV2 does not primarily cause encephalitis, but Youssef et al. (2004) identified PCV2 in an outbreak of sudden death and acute nervous signs. The brain lesions are described in detail by Correa et al. (2007). These signs included paddling, tremors, and lateral recumbency, often linked with secondary bacterial infections.

A malignant catarrhal fever-like condition was described by Pohlenz et al. (1974) in Germany, and subsequently, cases have been described in Norway (Loken et al. 1998), Finland (Syrla et al. 2006), in the United States (Alcaraz et al. 2009; Gauger et al. 2010), and in late 2010 in the United Kingdom (K. Williamson, personal observation). This condition resembles that seen in cattle (lethargy, anorexia, fever, recovery or death) and is associated with ovine herpes virus type 2 (OVH2). Louping ill was described in outdoor pigs as a nonsuppurative meningoencephalitis (Ross et al. 1994). Encephalomyocarditis (EMC) virus infection is fairly common but clinical disease is rare; usually, a nonsuppurative meningoencephalitis accompanies the more usual heart failure associated with nonsuppurative myocarditis as a cause of sudden death in young pigs (Maurice et al. 2005).

An unusual, inherited lower motor neuron disease, known as hereditary porcine neuronal system degeneration (HPNSD), was described by O’Toole et al. (1994). Pigs had muscular tremors, paresis, or ataxia, which developed around 12–59 days of age, with symmetrical or asymmetrical posterior paresis, bilateral knuckling of metatarsophalangeal joints, poor exercise tolerance, and, in one pig, hypermetria. There was axonal degeneration in some spino cerebellar tracts and ventral spinal roots and the muscles had denervation atrophy. Certain virus diseases, in particular, PRRS, PCV2, and SIV, destabilize the “endemic infection versus disease equilibrium.” As a result of compromised immunological defenses, bacterial diseases often appear on the farm as indicators. This is no less true for the nervous system. Erysipelothrix may cause sudden death when it first appears in a unit or when vaccination is discontinued. It produces fever, difficulty in rising, and reluctance or incapacity for walking due to arthritis and pain. Actinobacillus suis can cause septicemia, arthritis, and subsequent nervous infections in the United States. Systemic infection with H. parasuis is particularly common following PRRS and PCV2 infections, with
leptomeningitis and polyserositis typical of Glässer’S disease. *Listeria* can cause encephalitis, but even though exposure is common, disease is not. Many cases are only diagnosed on brain culture or on histopathology when meningitis, nonsuppurative encephalitis with perivascular cuffing and microabscessation can be seen (Lopez and Bildfell 1989). *Streptococcus suis* septicemic infection is very common in young piglets but can occur in growing pigs as well. *Streptococcus suis* type 2 is responsible for most of the meningitis that results (Madsen et al. 2002a,b). A variety of other opportunistic bacteria can cause sporadic disease in the nervous system in individual animals.

There are several major bacterial toxin-mediated diseases, both of which are discussed in respective chapters. Edema disease is an acute, often fatal enterotoxemia of recently weaned pigs. It is widespread and occurs worldwide but has become more sporadic in occurrence as knowledge and awareness have increased. When edema disease does occur, it is usually explosive with a short course and sudden death. It is associated with a few types of *E. coli* (see Chapter 53), which usually produce F18 or F4 (K88) fimbriae and elaborate a Shiga-like verotoxin SLT-IIE (STx2e).

Tetanus (*Clostridium tetani*) is fairly rare but was often associated with castration in poor hygiene conditions. Affected pigs have rapid clinical course to death with signs that include a stiff gait, muscle rigidity, erect ears, straight tail, lateral recumbency, opisthotonus, and legs extended backward as “extensor rigidity syndrome.” Loud noise will often produce tetanic spasms. The actual site of the lesion is difficult to detect; signs are related to elaboration of a potent neurotoxin.

Botulism (*Clostridium botulinum*) rarely affects pigs as they are fairly resistant to botulism by oral ingestion. Type C strains have caused death in pigs after eating dead fish. Doutre (1967) described botulism in pigs fed decomposing brewers’ waste. Affected pigs have a progressive flaccid paralysis of voluntary muscles.

Stachybotryotoxicosis, caused by the toxin of *Stachybotrys atra* acquired from hay or straw, may cause toxemia. Moldy hay or straw may result in depression, vomiting, tremors, sudden death, and abortion. Besides common endemic bacterial flora, it is important to remember that brucellosis can also cause a significant arthritis.

Those chemicals, minerals, or plants discussed as toxins in Chapter 70 will not be discussed, save a few comments of interest. Organophosphate toxicity can be acute, with typical parasympathetic signs. However, there can be delayed effects from certain organophosphate exposures whereby pigs develop ataxia from demyelination of the long myelinated tracts in the spinal cord, with additional signs that include loss of vocalization (recurrent laryngeal nerve), difficulty breathing (phrenic nerves), and long pyramidal tracts in the cord are affected whereby pigs are unable to stand unless helped. The persistent dog sitting leads to retention of urine and ascending cystitis and pylonephritis. Pentachlorophenol, once used as a wood preservative, was associated with posterior paralysis. Phenoxy herbicides are less used now but may be associated with depression, muscular weakness, and posterior paralysis. Excess magnesium may cause generalized anesthesia and complete muscular relaxation. Zinc toxicity was described by Pritchard et al. (1985) in association with copper toxicity and anemia in swill-fed pigs. Thallium toxicity produces weakness, impaired vision, hyperesthesia, convulsions, coma, and death, but recovered animals are usually blind. Other materials to have caused CNS damage include olaquindox, which causes episodic paralysis (Newsholme et al. 1986), poor growth, and adrenocortical injury. Vitamin D overdose will produce tremors and tenesmus (Wimsatt et al. 1988).

Salt poisoning (water deprivation, sodium ion intoxication) is discussed later. Disease can occur with excess sodium chloride ingestion (brines, whey, salted fish, feed mixing error) and is exacerbated by inadequate water (not available, frozen) or water engorgement when water becomes available. Some animals may be found dead, but others often have signs that include epileptiform seizures, snout twitching, contraction of the neck muscles, stepwise movements of the head, pigs moving backward, dog sitting with nose upward, and twitching face/ears. Signs can progress to tremors, lateral recumbency, running movements, prostration, coma, and death. Sequelae include blindness and polioencephalomalacia. Eosinophilic meningoencephalitis is a transient microscopic lesion in the brain. Severity of effects of water deprivation can be mitigated by limiting water intake, gradually increasing to ad libitum over a 4-hour period.

Several mineral deficiencies will produce signs in the CNS, peripheral nerves, and special senses. Calcium and phosphorus deficiency will produce hyperesthesia and posterior paralysis. Magnesium deficiency will produce hyperirritability and tetany. Copper deficiency has been shown to produce a natural disease in pigs (Bennetts and Beck 1942; Fletcher and Banting 1982; McGavin et al. 1962; Wilkie 1959) characterized by swayback, ataxia, posterior paralysis, and paraplegia. Histopathology reveals marked spinal demyelination affecting the dorsal spinocerebellar tracts (Pritchard et al. 1985).

Vitamin B₆ (pyridoxine) deficiency produces a mincing gait, hyperecstibility, ataxia, goose stepping, and epileptiform convulsions with a waddling progression. It used to be associated particularly with swill fed diets composed mainly of bakery waste, which are low in B₆. Naturally occurring pantothenic acid deficiency has been described by Doyle (1937) and Goodwin (1962). It may cause incoordination, poor...
Postweaning: Nervous

The list of agents or insults that can affect neural function is quite long (Table 19.8). Infectious agents, nutritional contributors, and toxicities will be discussed in respective chapters but are highlighted here. A few noninfectious conditions are discussed in detail.

Diagnosis of ataxia and paralysis with central awareness can be challenging, usually requiring careful clinical observation and description coupled with scrupulous postmortem examination and collection of complete and representative samples for testing. Some causes of paralysis are listed in Table 19.9.

Selenium toxicity (see Chapter 70) is a fairly common cause of CNS signs because the margin between the daily requirement for growing pigs (0.3 ppm) and the toxic dose (4.0 ppm) is quite small (Stowe and Herdt 1992; Stowe et al. 1992). Acute toxicity was recently reported by Nathues et al. (2010), where 25 g/kg of selenium was accidently fed and pigs had progressive apathy and paralysis, and all died within 36–72 hours of exposure. Poisoning is seen as a bilateral symmetrical focal encephalomalacia particularly in the cervical and lumbar cords (Casteel et al. 1985; Harrison 1983; Penrith and Robinson 1996; Stave et al. 1992) and also in the nuclei of the facial nerve in the medulla oblongata. Polioencephalomalacia is suggestive of selenium toxicity, but it can also follow nicotinamide deficiency (Wilson and Rake 1972) or other conditions (Table 19.10).

Fibrocartilaginous embolism was first described by Pass (1978) who described a sow in advanced pregnancy with posterior paralysis. Lesions were limited to the lumbar vertebrae and the spinal cord. The sow had degeneration of the intervertebral disk at L5–L6, a longitudinal fracture of the epiphysis of L5 that contained degenerative disk material, a fracture of the epiphyseal growth plate, and cartilaginous emboli in the spinal vessels causing myelomalacia. How the emboli entered the vessels is unclear, but it is likely that they arise from the fibrocartilage of the intervertebral disk or from the hyaline cartilage of the growth plate being pushed into the medullary cavity of the vertebra. A risk factor may be nutritional imbalances leading to metabolic bone disease.

Ataxia is sometimes observed in association with sunburn (Williamson et al. 2009). Vertebral abscesses have been described by Finley (1975). Ortho-cresyl phenols are organophosphates with delayed effect of demyelination and posterior paresis and paralysis also known as delayed organophosphate toxicity. Spinal cord degeneration was investigated in an outbreak of disease in old sows at the time of the bovine spongiform encephalopathy (BSE) outbreak (Davies et al. 1996). It was unusual that the average parity number in the herd was over 10 with most animals in their sixth or seventh year. They had flaccid paralysis of the muscles, were unable to stand unaided, and were euthanized on welfare grounds. They had an axonopathy that was nonsymmetrical, with vacuolation of the superior colliculus. There was also a widespread Wallerian degeneration of the ulnar, tibial, and peroneal nerves.

Most plant poisonings are diagnosed by finding plants in the environment and in the digestive tract. They often occur when adult swine are used to clean derelict land or woodland. Moldy corn poisoning (Fusarium) was suspected when pigs go off feed, have staggering of hindquarters, and may stand with their head in a corner, or press their head against a wall. Cassia spp. seeds cause incoordination, ataxia, staggering, decreased weight gain, and increased mortality in grower pigs (Colvin et al. 1986; Flory et al. 1992). Only a few seedlings of cocklebur (Xanthium strumarium) may cause depression, nausea, weakness, ataxia and spasms of the neck muscles, convulsions, and death. Mortality is sporadic and limited to when plants are emerging numerous. Solanaceae (nightshade) causes stupfaction, depression, ataxia, muscle tremors, loss of appetite, convulsions, and coma with dilation of pupils. They may be very nervous initially, may lie on their side, and kick their feet. Water hemlock ingestion causes nervousness, pawing, spasmodic twitching and contraction of the muscles, paralysis, and death. The pigs squeal before and during the convulsions (Barlow 2006).

Poison hemlock ingestion produces very rapid trembling of the flank muscles followed by paralysis caused by damage to ends of the motor nerves (Hayashi and Muto 1901). Bracken causes muscle weakness and death in 4–5 days. Buttercups will cause muscle twitching in the ears, nervousness, and paralysis in 2–3 days followed by death. Pigweed (Amaranthus or redroot) can cause poisoning with this in the late summer and fall, with clinical signs usually 5–10 days after exposure. Signs include trembling, weakness, incoordination, kicking, and almost complete rear leg paralysis, and often pigs are in sternal recumbency. The morbidity is variable and the mortality can be high. Death is usually within 48 hours of onset of clinical signs. Red squill ingestion causes hyperesthesia, depression, weakness, ataxia, paralysis, and death usually within 3 days. Ergot affecting the nervous system was described by Chennells et al. (2006).

Hepatic encephalopathy perhaps occurs, but naturally occurring cases have not been described in pigs. One would expect multifocal lesions in the CNS as a result of neurotoxic substances due to dysfunction of the liver.
Table 19.8. Some causes and clinical signs of conditions of the nervous system

<table>
<thead>
<tr>
<th>Insults</th>
<th>CNS—Cerebrum</th>
<th>CNS—Cerebellum/Brain Stem</th>
<th>Spinal Cord</th>
<th>Nystagmus</th>
<th>Tremor</th>
<th>Motor Excitement</th>
<th>Seizures</th>
<th>Incoordination/Vestibular</th>
<th>Reflexes</th>
<th>Paresis/Paralysis/Ataxia</th>
<th>Vision/Auditory</th>
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<tbody>
<tr>
<td><strong>Infectious: Viral</strong></td>
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<td>Enterovirus: teschoviruses</td>
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<td>Mycoplasma hyorhinis</td>
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<td>Mycoplasma hyosynoviae</td>
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<td>Staphylococcia</td>
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<td>Streptococcia (groups C, L)</td>
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</table>

*Note: x indicates presence of clinical signs*
Table 19.8

| Pathogen / Condition                  | 1   | 2   | 3   | 4   | 5
|---------------------------------------|-----|-----|-----|-----|-----
| *Streptococcus equisimilis*           | x   | x   | x   | x   |
| *Streptococcus suis*                  | x   | x   | x   | x   |
| *Listeria*                            |     |     |     |     |
| Burkholderia (meliodosis)             |     |     |     |     |
| Botulism (*C. botulinum*)             |     |     |     | x   |
| Tetanus (*Clostridium tetani*)        | x   | x   | x   |     |
| Stachybotryotoxicosis                 |     |     |     |     |
| Visceral larval migrans               |     |     | x   |
| Nutritional Deficiency                |     |     |     |     |
| Hypoglycemia                          | x   |     |     |     |
| Water (Na toxicity)*                  |     |     | x   | x   |
| Copper                                | x   |     |     |     |
| Hypocalcemia                          |     |     |     |
| Magnesium                             | x   |     |     |
| Manganese                             |     |     |     |
| Niacin                                |     |     |     |
| Nicotinamide                          |     |     |
| Pantothentic acid                     |     |
| Pyridoxine (*B_6*)                    |     |
| Rickets: Ca, P, vitamin D             |     |
| Vitamin A deficiency                  |     |
| Toxicity                              |     |
| Hydrogen sulfide*                     | x   |
| Iron toxicity*                        |     |
| Selenium toxicity*                    |     |
| Sodium toxicity*                      |     |
| Congenital tremor—trichlorfon*        | x   | x   |     |
| *Amaranthus* (pigweed)                |     |
| Arsenic, organic                      |     |
| Buttercup                             |     |
| Carbon monoxide/dioxide               |     |
| Cassia                                | x   |
| Chlorinated hydrocarbons              |     |

(Continued)
### Table 19.8. (Continued)

<table>
<thead>
<tr>
<th>Insult(^a)</th>
<th>CNS—Cerebrum</th>
<th>CNS—Cerebellum/Brain Stem</th>
<th>Spinal Cord</th>
<th>Nystagmus</th>
<th>Tremor</th>
<th>Motor Excitement</th>
<th>Seizures</th>
<th>Incoordination/Vestibular</th>
<th>Hyperesthesia/Reflexes</th>
<th>Paresis/Paralysis/Ataxia</th>
<th>Vision/Auditory</th>
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<tbody>
<tr>
<td>Delayed organophosphate</td>
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<tr>
<td>Furazolidone</td>
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<tr>
<td>Hygromycin</td>
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</tbody>
</table>
| Ionophore | | | | | | | | | | | | x
| Lead | | | | | | | | | | | | x
| Mercury | x | | | | | | | | | | |
| Nitrite | x | | | | | | | | | | |
| Olaquindox | | | | | | | | | | | |
| Organophosphate/carbamate | x | | | | | | | | | | |
| Pentachlorophenol | | | | | | | | | | | |
| Phenoxy herbicides | | | | | | | | | | | |
| Red squill | | | | | | | | | | | |
| Solanaceae (nightshade) | x | | | | | | | | | | |
| Thallium | | | | | | | | | | | |
| Vitamin A | | | | | | | | | | | |
| Vitamin D | | | | | | | | | | | |
| Water hemlock | | | | | | | | | | | |
| Xanthium (cocklebur) | | | | | | | | | | | |
| Noninfectious: Other | | | | | | | | | | | |
| Congenital tremors\(^b\) | x | | x | x | | | | | | | |
| Splayleg\(^b\) | | | | | | | | x | | | x
| Sunburn\(^b\) | | | | | | | | | | | |
| Trauma\(^b\) | | | | | | | | | | | |
| Arthrogryposis | | | x | | | | | | | | |
| Fibrocartilagenous emboli | | | | | | | | | | | |
| Heat stress | | | x | | | | | | | | |
| Hepatic encephalopathy | | | | | | | | | | | |
| Hydrocephalus | | | | | | | | | | | |
| Cerebellar abiotrophy\(^b\) | x | | | | | | | | | | x
| Hyperostosis\(^b\) | | | | | | | | | | | |
| Pietrain creeper\(^b\) | | | | | | | | | | | |
| Porcine stress syndrome\(^b\) | | | | | | | | | | | |

\(^a\) Most common in the United States.

\(^b\) Hereditary, multifactorial.
Table 19.9. Some causes of posterior paresis and paralysis

<table>
<thead>
<tr>
<th>Insult Class</th>
<th>Specific Insults</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trauma</td>
<td>Fracture, electrocution, misplaced injections (vertebra/spinal cord), fibrocartilaginous emboli</td>
</tr>
<tr>
<td>Bacterial</td>
<td>Vertebral abscess, myelitis (e.g., \textit{Haemophilus}, streptococci)</td>
</tr>
<tr>
<td>Viral</td>
<td>Enteroviruses (teschovirus, Teschen, Talfan, pseudorabies, PCV2, rabies)</td>
</tr>
<tr>
<td>Toxin</td>
<td>Selenium, aryl phosphate/delayed organophosphate, arsenic, mercury, pigweed</td>
</tr>
<tr>
<td>Genetic</td>
<td>Cerebellar abiotrophy</td>
</tr>
<tr>
<td>Nutritional</td>
<td>Nicotinamide, pantothenic acid, metabolic bone diseases</td>
</tr>
<tr>
<td>Miscellaneous</td>
<td>Apophysiolysis, epophysiolysis, neoplasm</td>
</tr>
</tbody>
</table>

Table 19.10. Sites of malacia in the CNS

<table>
<thead>
<tr>
<th>Sites of Malacia in the CNS</th>
<th>Causes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cord</td>
<td>Selenium toxicity, nicotinamide deficiency, spinal injury, fibrocartilaginous emboli</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>Mulberry heart disease, cerebrospinal angiopathy, meningitis</td>
</tr>
<tr>
<td>Brain stem</td>
<td>Edema disease, mulberry heart disease</td>
</tr>
<tr>
<td>Cerebral cortex</td>
<td>Salt poisoning/water deprivation, congenital hypoxia, carbon monoxide poisoning, mulberry heart disease, pseudorabies</td>
</tr>
</tbody>
</table>


Enteroviruses other than Teschen usually cause a much less severe clinical disease, which is often difficult to diagnose without careful collection of samples for virus isolation, laboratory tests, and particularly histopathology of the spinal cord (Done et al. 2005; Mills and Nielsen 1968; Pogranichnyi et al. 2003).

There is no evidence for the naturally occurring cattle type of transmissible spongiform encephalopathy (TSE) occurring in the pig (Wells et al. 1987). In the past, pigs have not become infected when kept in close association with humans with Kuru or when inoculated with human Kuru material. In experimental administration of bovine TSE material by parenteral routes to pigs, one pig developed (after 69 weeks) mild aggressive behavior to attendants, inappetence and depression, and progressive pelvic limb ataxia (Dawson et al. 1990). Pigs were exposed to large amounts of BSE material and no signs developed (Wells et al. 2003). All relevant evidence suggests pigs are not susceptible to a natural TSE. Only an experimental intracerebral inoculation offers a different proposition (Ryder et al. 2000).

Table 19.11. Diseases and insults that affect the joints

<table>
<thead>
<tr>
<th>Insult/Disease</th>
<th>Clinical Signs/Lesions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Osteochondosis \textit{laetus}, manifesta, dissecans</td>
<td>The result of defective bone growth, shifting joint pain; see text and Tables 19.16 and 19.17</td>
</tr>
<tr>
<td>Bacterial infections*</td>
<td>Usually abrupt onset of joint pain, joint swelling, fever, lethargy, reluctant to move</td>
</tr>
<tr>
<td>\textit{Erysipelothrix}</td>
<td></td>
</tr>
<tr>
<td>\textit{Haemophilus parasuis}</td>
<td></td>
</tr>
<tr>
<td>Streptococci</td>
<td></td>
</tr>
<tr>
<td>\textit{Actinobacillus suis}</td>
<td></td>
</tr>
<tr>
<td>\textit{Mycoplasma hyosynoviae}</td>
<td></td>
</tr>
<tr>
<td>\textit{Mycoplasma hyorhinis}</td>
<td></td>
</tr>
<tr>
<td>\textit{Arcanobacterium pyogenes}</td>
<td></td>
</tr>
<tr>
<td>Other bacteria (sporadic)</td>
<td></td>
</tr>
<tr>
<td>Trauma*</td>
<td></td>
</tr>
<tr>
<td>Anticoagulant/vitamin K deficiency</td>
<td>Joint hemorrhage</td>
</tr>
<tr>
<td>Viruses, toxins</td>
<td>Congenital arthrogryposis</td>
</tr>
</tbody>
</table>

*Most common.

Postweaning: Joints

Arthritis is most common in pigs from 2 to 4 months of age with multiple infectious causes as well as many noninfectious causes or risk factors (See Table 19.11). Many of the infectious causes are resident microflora that can take advantage of opportunity. Commingling of ages or sources of pigs increases the likelihood of introduction of new or different organisms or strains of organisms. Flooring, trauma, handling, management, and nutrition have significant impact on occurrence of joint diseases in modern production systems.

Joint lesions are characterized by increased synovial fluid, inflamed synovial membranes, fibrinous periarteritis, and joint swelling due to the exudation and eventual abscessation. Joint fluid is normally scant, clear, and viscous. Excess synovial fluid with color change, cloudiness, or change in viscosity is an important indicator of pathology. The carpal, elbow, hock, and hip joints are the joints most often affected. Quite often there are other associated lesions including turbid cerebrospinal fluid, concurrent pneumonia, endocarditis, and gingivitis in affected pigs or penmates.

Acute arthritis is concomitant with organisms that can also cause septicemia and polyserositis, is relatively common in pigs between 4 and 12 weeks, and perhaps is more prevalent in autumn and winter (Miniats et al. 1986). Infections may cause pyrexia, inability to rise or move, lameness, and death. The main organisms involved include \textit{M. hyosynoviae}, \textit{H. parasuis}, \textit{S. suis}, \textit{E. rhusiopathiae}, \textit{M. hyorhinis}, and \textit{A. suis}. A potpourri of other organisms, especially streptococci, \textit{Arcanobacterium}, coliforms, or pyogenic organisms, can sometimes be isolated from chronic cases.

Arthritis associated with \textit{Mycoplasma} spp., \textit{Erysipelothrix}, \textit{Haemophilus}, and \textit{Streptococcus} is fully discussed...
in the respective chapters. Mycoplasma hyosynoviae is usually an uncomplicated, nonsuppurative arthritis of stifles with hocks, shoulder, and elbow, possibly affected in pigs greater than 30 kg and after 12–14 weeks of age (Blowey 1993; Hagedoorn-Olsen et al. 1998, 1999). The inability to stand occurs within 24 hours of infection and is difficult to differentiate from other causes of severe lameness, particularly E. rhusio pathiae. Animals are reluctant to rise (Ross and Duncan 1970; Ross and Spear 1973; Ross et al. 1971). Diagnosis is by culture or, more recently, by PCR (Platts et al. 2008).

Mycoplasma hyorhinis is a widespread infection and an uncommon cause of disease, usually with low morbidity and mortality, affecting pigs from 3 to 10 weeks of age (Frisi and Feenstra 1994; Ross and Spear 1973). The organism is carried in the nose, produces a bacteremia with a strong predilection for serosal surfaces, and sometimes localizes in joints (tarsal, carpal, shoulder, stifle, hock, and occasionally the atlanto-occipital) (Roberts et al. 1963a,b; Ross et al. 1973). The subject has recently been reviewed in detail by Rosales and Nicholas (2010). Mycoplasma arthritidis has been isolated from the joints of pigs with conjunctivitis (Binder et al. 1990).

Haemophilus parasuis is widespread and a common infection and a fairly common cause of disease. It causes acute lameness, depression, fever, dyspnea, hot swollen joints, a reluctance to stand or move, tremor, paralysis, meningitis, and sudden death (Hoefling 1994; Nielsen and Danielsen 1975; Smart et al. 1986). Experimental infections produce severe lesions within 60 hours of infection (Vahle et al. 1997). Cases may develop into chronically affected animals. Importantly, etiological diagnosis is virtually impossible in chronic cases with fibrous adhesions; only specimens from acutely affected, nonmedicated pigs should be submitted for culture or PCR.

Erysipelothrix rhusiopathiae can cause severe disease whereby acutely affected animals are pyrexic, prefer to lie down, have stilted gait, and are in pain so they shift their weight from foot to foot and lie down quickly (Grabell et al. 1962). With intervention, they may recover or, more usually, will become chronically affected (Franz et al. 1996; Johnston et al. 1987). Diagnosis is by culture, PCR, or immunohistochemistry. A hypersensitivity reaction with pannus formation, periarticular fibrosis, and exostoses (Vaughan 1969) occurs in chronic erysipelas. Granulation tissue forms in the articular cavity, and there is proliferation of the connective tissues with elongated tags attached to the synovial membrane (Grabell et al. 1962).

Arcanobacterium pyogenes is associated with abscesses, tenosynovitis, and vertebral infections, which are suppurative and which can cause complete collapse of the vertebral body, often following tail biting. Actinobacillus suis and sometimes Salmonella can cause septicemia with tissue localizations that includes the joints. Similarly, other common bacteria can occasionally be isolated from joints of individual animals.

### Postweaning: Muscle

Primary myopathies are uncommon since PSS has been largely mitigated. Myopathies may affect different groups of muscles. The most common signs are muscular weakness, trembling while standing, pain, muscle swelling, or muscle wasting. All are manifested as locomotor or postural disorders. They are accompanied by sensory dysfunction except in the case of acute back muscle necrosis. There is usually a rise in the plasma creatine phosphokinase (CPK) levels, which indicates muscle damage, followed by a transient myoglobinuria. Table 19.12 summarizes some conditions primarily affecting the muscle.

**Porcine Stress Syndrome.** Porcine stress syndrome (PSS) and pale, soft, and exudative (PSE) muscle is a complex of conditions associated with an autosomal recessive gene of variable penetrance (Bradley and Fell 1986).

<table>
<thead>
<tr>
<th>Insult/Disease</th>
<th>Clinical Signs/Lesions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Splayleg*</td>
<td>Hind legs adducted, myofibrillar hypoplasia (see text)</td>
</tr>
<tr>
<td>Nutritional myopathy (vitamin E and/or selenium deficiency)*</td>
<td>Pale muscle, white streaks, gritty (see text)</td>
</tr>
<tr>
<td>Trauma/injection sites granuloma or abscess*</td>
<td>Note: The heart is more likely to be affected in pigs.</td>
</tr>
<tr>
<td>Bacterial infections*</td>
<td>Bruising, hemorrhage, inflammation, fibrosis, abscess</td>
</tr>
<tr>
<td>Actinobacillus suis</td>
<td>Usually initiated by trauma or by sepsis</td>
</tr>
<tr>
<td>Clostridium septicum, C. perfringens, C. chauvoei</td>
<td>Necrosis, hemorrhage, edema; usually, poor hygiene, poor injection techniques, and/or trauma initiate disease</td>
</tr>
<tr>
<td>Porcine stress syndrome (PSS/PSE/DFD)</td>
<td>Muscle rigidity, skin flushed, pyrexia, rapid rigor, lactic acidosis, malignant hyperthermia, heritable (see text)</td>
</tr>
<tr>
<td>Hemorrhages: improper stunning</td>
<td>Muscle hemorrhages in carcasses at slaughter</td>
</tr>
<tr>
<td>Hemorrhages: ASF, CSF</td>
<td>Focal hemorrhages in muscle, other clinical signs and lesions</td>
</tr>
<tr>
<td>Acute hypocalcemia</td>
<td>Weak, tremors, muscle fasciculation</td>
</tr>
<tr>
<td>Steatosis</td>
<td>Excess fat in the muscle, heritable</td>
</tr>
<tr>
<td>Gossypol toxicity</td>
<td>Cardiomyopathy: toxin found in cottonseed meal</td>
</tr>
<tr>
<td>Monensin toxicity</td>
<td>Myopathy: synergism with tiamulin or salinomycin</td>
</tr>
<tr>
<td>Congenital muscle hypertrophy</td>
<td>Ataxia at birth with very large hams, heritable</td>
</tr>
<tr>
<td>Parasites (Trichina, Taenia)</td>
<td>Myalgia, inflammatory nodules (see Chapter 67)</td>
</tr>
</tbody>
</table>

*aMost common.
Positive steps by breeders to reduce the prevalence of the gene have much reduced its incidence and prevalence, which was once at 95% in Pietrain and 35% of Landrace. Well-muscled pigs die suddenly from a variety of stressors under natural conditions. The Pietrain, which has been genetically selected for high lean meat/low fat content, is particularly susceptible. Susceptible animals must have a hereditary predisposition that is then triggered by an exertional stress in the form of transportation, mating, excessive pre-slaughter tension, high environmental tension, or excitement. Exposure to halothane is a particularly effective stimulant and was used to test for the predisposition (then called malignant hyperthermia; Jones et al. 1972).

Clinically, pigs have abrupt onset of respiratory distress, staggering, rigid firm muscles, and discolored skin. Pyrexia, muscle rigidity, and increased lactate in muscles cause fairly rapid death. The pig enters a premature rigor with pyrexia that persists for an hour or so after death. If pigs have high glycogen levels at death, the meat, particularly from the loin and ham (the high value parts of the carcass), takes on the appearance of the so-called PSE muscle (Briskey 1964). There is an excess of watery leakage from the muscle (drip), and it is unattractive for sale. If there is little glycogen, the pH rises and the muscle does not store well and ends up as a DFD meat, which is equally unattractive. Essentially, the stress has induced an accelerated glycolysis.

This complex is a genetically transmitted myopathy triggered by exertional stress, which produces the inherited defects in the uptake storage and release of calcium in the muscle fibers. Bonca (2009) recently described the small changes in the normal “ryrl” gene, which is located on the sixth chromosome that encodes for the protein ryanidine, which is responsible for the calcium channel control in the skeletal muscle fibers. The consequence of the change at the molecular level is that thymidine is substituted for the normal cytosine in the 1843rd “ryrl” gene position. Animals with the normal gene can properly adjust the ion transfer, but those with the mutation cannot; hence, a double elimination rate of Ca²⁺ ions results in comparison with the normal animal.

Back muscle necrosis seems to be a special manifestation of the PSS (Bradley et al. 1979). The condition is sporadic and again affects heavy-weight pigs over 50 kg. There is pain, difficulty in moving, and swelling of the back muscles (Mm multifidii and longissimus) with heat and pain on palpation. There is loss of sensation over the affected area and pigs may be reluctant to stand and eventually dog sit. The body curves toward the affected side in unilaterally affected animals, but bilaterally affected animals cannot stand and may die. There may be knuckling of the lower limb joints. Muscle damage is pronounced with high enzyme levels, and diagnosis is easily performed by sectioning the spine across the longissimus muscles, which appear dark and hemorrhagic.

**Nutritional Myopathy.** Vitamin E/ selenium deficiency may cause a generalized myopathy in the skeletal muscle (Lannek and Lindberg 1975; Mortimer 1983; Nafstad and Tollersrud 1970; Trapp et al. 1970) but more likely manifests as mulberry heart disease or hepatois dietetica. Deficiency is more likely to occur when diets are constructed from plants that are grown in selenium-deficient soils or soils containing selenium antagonists. It may also follow ingestion of plants high in inhibitors of vitamin E, diets high in polyunsaturated fatty acids or copper, vitamin A deficiency, or possibly mycotoxins, which can destroy vitamin E or make it less available. Both vitamin E and selenium (together with vitamin C and pyruvate) act as antioxidants and are therefore extremely important in combating disease.

Swine seem less susceptible to nutritional muscular dystrophy of skeletal muscle than other domestic species. If it does occur, it is seen as paller in the skeletal muscles with white streaks (gritty, calcified myofibrils and muscle bundles) particularly in the longissimus dorsi. It is usually in pigs of 50–60 kg and is clinically seen as ataxia, stiff gait, staggering gait, weakness, paralysis, depression, anorexia, and recumbency prior to death. Chronic cases have lameness and shedding of hooves.

Macroscopically, the muscle is edematous and white, and histologically, there is loss of myocyte structure with vacuolation, fragmentation, and mineral deposition in individual fibers. The primary change is selective destruction of the type I fibers and lack of phosphorylase activity in the type II fibers (Ruth and van Fleet 1974). Gorham et al. (1951) described yellow fat disease where there was pale skeletal and cardiac muscles in animals that had been fed fish or fish products, and it was thought that these rations were deficient in vitamin E.

**Other Myopathies.** Pigs that have monensin toxicity have diarrhea, lethargy, dyspnea, myoglobinuria, and sudden death. Muscle enzymes particularly CPK and aspartate aminotransferase (AST-SGOT) are elevated and provide a method of diagnosis. The highly active muscles are more severely affected (diaphragm, thigh muscles, intercostal, longissimus, and the triceps), and these may show areas of paller corresponding to the areas of myodegeneration. Microscopically, there is hyaline necrosis, macrophage infiltration, and, if the ionophore is removed and they survive, evidence of muscle regeneration. The condition occurs when monensin is present in the diet at >100 ppm but also occurs if either salinomycin or tiamulin is also included in the diet (Kavanagh 1992; Miller et al. 1986; Morris
Asymmetric hindquarter syndrome is principally a disorder of slaughter weight pigs, although it was recognized as early as 2–3 months (Bradley and Wells 1978; Done et al. 1975). Viewed from behind, the affected leg has much reduced muscle mass, particularly in Mm adductors, semimembranosus, semitendinosus, and biceps femoris, and the other leg is larger. The discrepancy is due to a much reduced number of myocytes in the affected limb. The cause is not known.

Pietrain creeper syndrome was first described by Bradley and Wells (1978, 1980; Wells et al. 1980) and is a familial disease characterized by progressive muscular weakness. It starts at about 3 weeks and ends with permanent recumbency at about 12 weeks of age. It may be an autosomal recessive gene with about one-third to one-fourth of pigs affected in each affected litter. There is tremor, followed by collapse to sternal recumbency and then tremor stops. Progressively, there is an increased reluctance to stand, muscular weakness, loss of condition, and finally a creeping type of gait using flexed limbs.

There is a very low incidence of *Trichinella spiralis* (see Chapter 67) when there is a proper food inspection (Kapel et al. 1998). It has a predilection for the active striated muscles such as the diaphragm, intercostals, masseter, and ocular muscles. Occasionally, aberrant larvae are found in the brain and the meninges. The clinical signs include intense muscle pain, decreased weight gain, itching, loss of appetite, paralysis of the hindquarters, and stiff muscles. At postmortem examination, the encysted parasites are easily observed. They are found as inflammatory nodules surrounded by eosinophils. There is usually one larva in each cyst, but up to seven have been noted. It takes about 8–25 days for the cyst to develop following infection. Viable cysts are able to remain intact for years, but calcification begins gradually to destroy the larvae and capsules. The subject has recently been reviewed by Gottstein et al. (2009) and a description of a diagnostic test using PCR by Guenther et al. (2008).

*Taenia solium* may be the cause of “measly pork” where there are cysts in the heart, tongue, diaphragm, and generalized body muscles. *Sarcocystis* sp. can be found in any striated muscle. Each cyst is double walled and contains many spores given the name Rainey’S corpuscles. Most infections are asymptomatic, but there may occasionally be pyrexia, weakness in the loins, and posterior paralysis. At postmortem examination, there may be watery, light-colored, small white cysts in the muscle. Avargal et al. (2004) recently described the infection in wild boars.

Injection sites can be secondarily infected with opportunists, particularly if hygiene is poor, needles are reused, or injected animals are wet or soiled. The outcome of these infections depends on the nature of the contributing organisms. Most common infections are the usual bacteria (streptococci, staphylococci, *Arcanobacterium*) but may include gram-negative bacteria and, in a worst case scenario, *Clostridium* spp.

An injection administered in the wrong place may damage vital nerves, in particular, the sciatic nerve. This site is vulnerable with little protective muscle mass in the young piglet or crated sows. Injections in ham muscles are highly discouraged; the neck with less valuable muscle masses is the obvious choice as an injection site. *Clostridium septicum* (occasionally *Clostridium chauvoei*) can also infect traumatic sites and produce gross swelling, and there may be gas in the muscle and blackening of the site. It particularly affects the ventral abdomen, head, and ventral cervical regions. While usually sporadic in occurrence, epidemics of clostridial myositis and cellulitis can occur. *Bacillus anthracis*, although unusual in swine, can cause marked swelling, edema, and hemorrhage in subpharyngeal connective tissues. A specific myositis in association with *H. parasuis* has also been described in the masseter muscles, which results in the swelling of the head.

Muscle atrophy is not uncommon. Focal or extensive atrophy may follow nerve damage, disuse, senility, and undernutrition, and usually will require microscopic evaluation for diagnosis. Focal myopathy of individual muscle cells is seen under the microscope (Bradley and Wells 1978) and occurs in less than 1% of muscle cells. Wasting with generalized muscle atrophy is commonly associated with PCV2 or other specific diseases, but wasting can be linked to over 30 different disorders, so care still has to be made in making a specific diagnosis.

Myositis ossificans was described by Hulland (1974), and it is a generalized, familial disease in which bony lesions develop in the muscles adjacent to the spinal column, ribs, and tarsal bones at around 2 months of age. The muscle atrophy near the lesions and the cause are unknown.

Many pigs are born with or acquire hernias that involve muscle or connective tissues. These are natural openings that have not formed correctly. There are four possibilities for these: umbilical, inguinal or scrotal, perineal, and diaphragmatic, but only inguinal and umbilical are common. Umbilical hernias are generally related to umbilical infections acquired in the early postnatal period, perhaps exacerbated by trauma, whereas an inguinal hernia has good evidence for hereditary predisposition. Perineal hernia is likely of traumatic origin. Epidemics of diaphragmatic hernias have been reported (Schwartz 1991).

Unusual is muscular steatosis (Bradley and Wells 1978), which is found incidentally at slaughter. It is the replacement of muscles by fat. Inflammation of fat, steatitis, has been described by Kirby (1981).
Very old animals may have deposits of lipofuscin in the muscles, but often this can only be detected on histopathology and using special stains. Hypocalcemia in sows was described by Jennings (1985) in which there was muscle weakness and sudden deaths, and the sows took 1 week or more to farrow. Acute hypocalcemia can occur in growing swine related to dietary irregularities in calcium, vitamin D, and phosphorus in the ration. Affected pigs tremble, are weak and reluctant to move, become prostrate, and may be savaged by penmates.

**Postweaning: Bone**

Fractures found in several animals at the same time may be a result of accidental electrocution or outdoor lightning strike (Van Alstine and Widmer 2003). Lumbosacral vertebrae, as well as thoracic vertebrae, femur, neck of the femur, humerus, neck of the scapula, or pelvis, are the usual sites. Sometimes the fractures in the lumbosacral junction result in separation of the spinal cord and nerves and, if sublethal, cause posterior paresis, retroperitoneal and perirenal hemorrhage, and distension of urinary bladders from inability to urinate. Fractures were described in the outbreaks of osteoporosis (Douglas and Mackinnon 1993), which occurred in first-litter sows when they were moved from the farrowing quarters and involved the pelvis, spine, femur (Blowey 1994b), and other bones. It was considered multifactorial: early mating, rapid growth, high milking yield, large litters, and insufficient nutrients in the diet to provide both milk and sow growth. Fractures can also occur in late finishing stages, the result of improperly formulated rations. In this situation, electrical stunning at slaughter may increase the frequency of fractures in femurs and spine leading to hemorrhage and increased trimming of meat.

Kyphosis/lordosis is mentioned as a congenital abnormality but is also seen as “kinky back” lordosis or kyphosis associated with precocious behavior causing relaxation of the spinal ligaments (Done and Gresham 1998). Genetic selection for an extra vertebrae in the spinal column (Pearson and Done 2004) resulted in too much muscle weight for the skeleton. Hemivertebrae can be congenital or acquired. Kyphosis has also been related to metabolic bone disease in very young, rapidly growing pigs, perhaps as a consequence of metabolically compromised pigs and/or rachitic diets, particularly vitamin D deficiency (described below).

*Solanum malacoxylon*, widespread in South America, produces a natural vitamin D3 causing severe calcification of bones and soft tissues in range cattle and free-ranging pigs (Dobereiner et al. 1975), and was reproduced in pigs where it particularly affects tendons (Done et al. 1976). A variety of nutritional disorders associated with altered bone growth and subsequent deformity of the long bones and articular surface cartilages are seen in young growing pigs. In the adult, these changes are seen in abnormalities in the strength of the cortical bone (Wilkie 1959).

Excess vitamin A will cause short limbs, reduced bone growth and marrow, and metaphyseal growth plates that have closed prematurely. They also have thin cortices (Doige and Schoonoverdoord 1988). Inherited vitamin C deficiency was described by Jensen et al. (1983) in a group of 9- to 10-week-old pigs with radiodense metaphyses of the long bones and ribs, widening of the metaphyses, and lipping over the epiphyseal plates. There were also subperiosteal hemorrhages around the edge of the shafts of the long bones and histopathology revealed reduced osteoid in the growth plates. It is probably an autosomal recessive single gene (deletion mutation) leading to a deficiency of 1-gulonolactone oxidase.

Osteodystrophy has been described by Dobson (1969) associated with hypervitaminosis A in growing pigs (Thompson and Robinson 1989) (Tables 19.13 and 19.14).

**METABOLIC BONE DISEASES: RICKETS**

Hereditary rickets where there is no enzyme to convert D2 to D3 in the kidney has been described by Fox et al. (1985). Classic presentation of rickets from dietary causes is most frequently seen in pigs 2–6 months of age. A predominant sign is swollen joints, particularly carpal, humeral, elbow, and stifle joints. The clinical signs include a stunted, unthrifty appearance; lameness; fractured long bones; and paresis. In the young weaned growing pigs, there is a failure of mineralization of osteoid and cartilage matrix especially in the growth plates. At necropsy, the bones are pale and soft. The ribs, particularly, bend rather than snap under pressure and are radiolucent. Since the bones bend or fracture easily, there is often evidence of recent or healing fractures and calluses. The joint surfaces may be folded and growth plates are thickened and irregular. Hemorrhages occur in the cartilage or adjacent bone.

Key factors in rickets development include inadequate concentrations of calcium and/or phosphorus in the ration; inadequate concentrations of the active vitamin D3 (Pepper et al. 1978), since vitamin D2 is not well utilized by swine; improper balance of bioavailable calcium and phosphorus in the ration resulting in a Ca:P ratio greatly different from 1.2:1; inadequate Vitamin D3 supplementation or absence of sunlight in a dark environment; and excess iron in the diet. These are summarized in a recent case study (Madson 2010). Acute hypocalcemia in growing pigs may be induced by abrupt decrease of calcium in the diet, particularly if animals are already compensating because of a marginal plane of calcium, phosphorus, or vitamin D3 in the diet. Tremors and rapid death are the clinical signs of acute hypocalcemia.
Table 19.13. Diseases primarily affecting the bone

<table>
<thead>
<tr>
<th>Insult/Disease</th>
<th>Clinical</th>
</tr>
</thead>
<tbody>
<tr>
<td>Osteomyelitis*</td>
<td>Bacterial infections, abscesses</td>
</tr>
<tr>
<td>Fracture: trauma/nutritional*</td>
<td></td>
</tr>
<tr>
<td>Acute: hemorrhage, sharp edges</td>
<td>Crepitus, hemorrhage (long bones, ribs, vertebrae)</td>
</tr>
<tr>
<td>Chronic: calluses</td>
<td>Swollen, with fibrosis (ribs, long bones)</td>
</tr>
<tr>
<td>Electrocrution: lethal or sublethal*</td>
<td>Fractures (e.g., lumbosacral vertebrae, femur)</td>
</tr>
<tr>
<td>Osteochondrosis (<em>lates, manifesta, dissecans)</em></td>
<td>Primary effect on growth plates but can be manifested as joint disease</td>
</tr>
<tr>
<td>Epiphysiolysis*</td>
<td>(see text)</td>
</tr>
<tr>
<td>Apophysiolysis*</td>
<td>Physal separation, hemorrhage (related to osteochondrosis)</td>
</tr>
<tr>
<td>Metabolic bone disease*</td>
<td>Physal separation, hemorrhage (related to osteochondrosis)</td>
</tr>
<tr>
<td>Rickets—juvenile*</td>
<td>Variable, depending on age, stage, interactions of Ca, P, vitamin D3, and</td>
</tr>
<tr>
<td>Osteomalacia*</td>
<td>other dietary contributors or risk factors. Enlarged metaphyses; decreased</td>
</tr>
<tr>
<td>Osteoporosis*</td>
<td>bone strength; fractures; enlarged growth plates; acute hypocalcemia</td>
</tr>
<tr>
<td>Fibrous osteodystrophy</td>
<td>tremors; death; fractures; soft, brittle, or friable bones (see text)</td>
</tr>
<tr>
<td>Fluorine toxicity</td>
<td>Enlarged costochondral junctions</td>
</tr>
<tr>
<td>Copper deficiency</td>
<td>Fragile bones</td>
</tr>
<tr>
<td>Manganese deficiency</td>
<td>Limb deformity, decreased length</td>
</tr>
<tr>
<td>Congenital hyperostosis</td>
<td>Thickenened limb bones, heritable, fatal</td>
</tr>
<tr>
<td>Chondrodystrophic dwarf</td>
<td>Shortened bones</td>
</tr>
<tr>
<td>Excess vitamin A</td>
<td>Shortened bones</td>
</tr>
<tr>
<td>Congenital porphyria</td>
<td>Brown discoloration of bones</td>
</tr>
<tr>
<td>Solanum malacoxylon (vitamin D3 analog)</td>
<td>Excess vitamin D3, excess calcification of bones, soft tissues</td>
</tr>
<tr>
<td>Ascorbate deficiency (vitamin C)</td>
<td>Subperiosteal hemorrhages</td>
</tr>
<tr>
<td>CSF virus</td>
<td>Lines of discoloration</td>
</tr>
</tbody>
</table>

*aMost common.

Table 19.14. Some nutritional contributors to locomotor disease

<table>
<thead>
<tr>
<th>Insult/Disease</th>
<th>Clinical</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin D deficiency*</td>
<td>Rickets, enlarged joints, lameness, weak bones, brittle bones, fractures,</td>
<td>Interactions with calcium, phosphorus, phytase, sunlight, growth rate, and age can complicate identification of cause</td>
</tr>
<tr>
<td></td>
<td>“humpbacks”</td>
<td></td>
</tr>
<tr>
<td>Vitamin E (Se) deficiency*</td>
<td>Nutritional myopathy</td>
<td>Mulberry heart disease; see Chapter 14</td>
</tr>
<tr>
<td>Selenium deficient*</td>
<td>Nutritional myopathies</td>
<td></td>
</tr>
<tr>
<td>Hypoglycemia (suckling pigs)*</td>
<td>Coma, froth at the mouth, recumbent and paddling</td>
<td></td>
</tr>
<tr>
<td>Calcium and phosphorus*</td>
<td>Metabolic bone disease</td>
<td>See text (metabolic bone disease)</td>
</tr>
<tr>
<td>Biotin deficiency</td>
<td>Lameness, hoof lesions</td>
<td></td>
</tr>
<tr>
<td>Calcium—hypoalcalcemia</td>
<td>Abrupt onset of tremors, prostration, serum calcium low</td>
<td></td>
</tr>
<tr>
<td>Pantothentic acid</td>
<td>Incoordinated, goose step, dermatopathy</td>
<td>Mild colitis, dermatopathy</td>
</tr>
<tr>
<td>Niacin deficiency</td>
<td>Ataxia, paralysis, anemia, diarrhea</td>
<td>Malacia in the spinal cord, nonspecific colitis</td>
</tr>
<tr>
<td>Copper deficiency</td>
<td>Incoordination, paralysis, aortic rupture</td>
<td>Teague and Carpenter (1951), Follis (1955), Fletcher and Banting (1982), Pritchard et al. (1985)</td>
</tr>
<tr>
<td>Manganese deficiency</td>
<td>Weak, incoordination, lameness, bowed legs, reproductive effects</td>
<td></td>
</tr>
<tr>
<td>Magnesium deficiency</td>
<td>Hyperirritable, tetany</td>
<td></td>
</tr>
<tr>
<td>Nicotinamide deficiency</td>
<td>Paralysis, malacia in spinal cord</td>
<td></td>
</tr>
<tr>
<td>Pyridoxine</td>
<td>Ataxia, goose step, seizures</td>
<td>Feeding of swill and bakery wastes</td>
</tr>
<tr>
<td>Zinc deficiency</td>
<td>Parakeratosis, lameness</td>
<td>Brink et al. (1959)</td>
</tr>
<tr>
<td>Vitamin C deficiency</td>
<td>Joint hemorrhage</td>
<td></td>
</tr>
<tr>
<td>Vitamin A deficiency</td>
<td>Stillbirth, deformities</td>
<td></td>
</tr>
<tr>
<td>Newborn</td>
<td>Incoordination, blind</td>
<td></td>
</tr>
<tr>
<td>Growers</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*aMost common.
Rickets, osteomalacia, and osteoporosis are interacting forms of metabolic bone disease. Rickets and osteomalacia are the forms most likely to be observed in growing pigs, whereas osteomalacia and osteoporosis are more likely in adults (Table 19.15). These latter conditions have been described in diagnostic surveys of paretic or paralyzed sows (Doige 1982; Gayle and Schwartz 1980). Sows may become lame or unable to stand, and may have fractures (particularly of the humerus, femur, and vertebrae) and paraplegia. Crepitus can be heard and felt when clinically examined. At necropsy, most frequent sites for lesions are the proximal one-third of the humerus and proximal one-third of the femur. Comminuted spiral fractures extend from the metaphysis to the diaphysis. It is the result of an imbalance between bone formation (osteoblast activity) and bone resorption (osteoclast activity). There is usually calcium deficiency (Spencer 1979). Osteomalacia is, strictly speaking, bone softening. In late lactation, “downer sows” (Gayle and Schwartz 1980) are probably caused by fractures of the vertebrae, femurs and phalanges. Sows’ bones decalcify to mobilize calcium for milk production (usually in lactation [Doige 1979, 1982; Forsyth 1989]). Specific gravity of bone in these animals will be 1.018, whereas the normal is 1.022 (Spencer 1979). There is also a cortex to total area 0.2 or less compared with 0.3 in the normal cross section of the sixth rib. Prevention was reviewed by Gayle and Schwartz (1980).

In lactational osteoporosis (Spencer 1979), large amounts of calcium and phosphorus are preferentially resorbed from the skeleton to provide high levels of calcium and phosphorus in the milk. The bones are structurally normal but of lower mass. Complicating factors include absence of sunlight and periods of restricting exercise (sow stall, farrow crates), particularly for first-litter gilts that are still growing. Osteomalacia has been described (Doige 1982; Gayle and Schwartz 1980) as a result of deficiencies or imbalances of calcium and phosphorus and vitamin D but may also be as a result of the inability to consume sufficient food. Large quantities of nonmineralized osteoid develop, thereby weakening the bones. This is due to a higher secretion of parathyroid hormone in lactating sow.

Ankylosing spondylitis was identified by Grondalen (1974b,c) in culled sows and boars at abattoirs, but it is thought that the condition starts as early as the first year of life. Pigs have a painful lumbar region, may develop kyphosis, and waddle when walking or drag the hind feet. The cause is probably multiple effects of wear and tear, spine trauma, poor nutrition, genetics, arthritis of spinal joints, and so on (Grabell et al. 1962;
Gronda1en 1974b,c). Vertebrae may eventually fuse and alleviation occurs. Spondylosis results in bridging of the vertebrae with possible trapping of the vertebrae (Diige 1979, 1980a,b).

**LAMENESS AND BONE DISEASE**

Understanding lameness is central to a discussion of bones, muscles, and joints, realizing lameness is a clinical term applied to abnormalities of normal function. It has been described in piglets (Zoric 2010) largely associated with the feet. Lameness can involve the hoof, claw, foot, lower leg, thigh, shoulder, and the pelvis (apophysiolysis and epiphysiolysis). A variety of long-term conditions also contribute, such as osteochondrosis, arthrosis, osteomalacia, osteoporosis, fractures, and arthritis (Penny 1979; Wells 1984).

Leg weakness was first described by Funkquist (1929) in Swedish Landrace boars that were unable to stand to copulate, but leg weakness is now used to describe lameness in animals over 6 months of age (Walker et al. 1966). Comparing studies that report lameness without a clear definition of the term or measures used to evaluate lameness is problematic.

Recently, a U.S. study of the prevalence of lameness and claw lesions in two commercial sow herds by Sondeman et al. (2009) found less than 4% of sows with lameness. Heel erosions were the most common cause and overgrowth of dewclaws second. The osteochondrosis complex (OCD) is the most common cause of lameness in breeding pig animals (Dewey 1993; Grondalen 1974a; Hill et al. 1984a,b; Reiland 1975). However, a recent study (Ryan et al. 2010) has shown no relationship between lameness and OCD, but all the sows studied had evidence of OCD, particularly in the medial condyle of the humerus and anconel process of the ulna. In breeding herds, up to 100% of the animals may have foot problems (Penny 1979).

In summary, nearly all sows have some evidence of leg weakness, lesions, or lameness, which is a major cause of culling (Barnett et al. 2001). The reason for this is simple—These components of locomotion are the most influenced by genetics, nutrition (Nielsen et al. 1971), management, environment, and microorganisms and infection (Abiven et al. 1998; D’Allaire 1987; D’Allaire et al. 1987; Reiland 1975). Overfeeding can also result in too much weight and not enough bone to support the weight. In general, bone disease is indicated by lameness, pain on walking, and reluctance to stand.

Jensen and Toft (2009) divided leg disorders into three major groups: first, infectious arthritis (previously discussed); second, physical injuries; and third, osteochondrosis. If clinical enquiries have suggested that the problem lies in the skeletal component, it is unlikely that an accurate diagnosis will be made without gross and microscopic evaluations of the bones. Geriatric conditions are rarely encountered as pigs rarely live that long (Ryan et al. 2010).

**OSTEOCHONDROSIS**

Osteochondrosis is noninfectious, degenerative, generalized abnormal condition of the cartilage. It is usually progressive, shifting lameness affecting one or more limbs (Hill 1990a,b; Reiland 1975). Osteochondrosis occurred in 10% of breeding animals (Grondalen 1974b) and 40% of boars in a testing station (Reiland 1978b), 47% of culled sows in Canada (Dewey 1996), and 100% of commercial pigs by 6 months (Walker and Aherne 1987). In a recent study, the prevalence of osteochondrosis laterus (OCL) (lesions confined to the epiphyseal cartilage) and osteochondrosis manifesta (OCM) (lesions accompanied by delay in endochondral ossification) was about 65% (Busch et al. 2007), but OCD (cleft formation through the articular cartilage) was much lower at 7% (Busch et al. 2007) and 14% (Ytrehus et al. 2004).

OCD usually affects pigs from 6 to 20 weeks of age but will extend to 18 months. The growth plates that close last (see Table 19.2) are the ones that are most susceptible (medial condyles of the humerus and femur, ulna, costochondral junctions, and the sixth to eighth lumbar vertebrae).

Many terms have been used to describe the disease condition, including osteochondritis, osteoarthrits, DJD, arthropathy, arthritis, polyarthritis, and metaphyseal dysplasia to name but a few. Most of these titles are inaccurate because the condition has its origin in the growth cartilages and the bones are affected secondarily. It is, in fact, a dyschondroplasia affecting growth cartilages both in physeal and epiphyseal locations in most breeds of rapidly growing pigs, which results in cartilage and bone lesions (Olsson 1978).

The term osteochondrosis should be used to describe a group of syndromes that cause limb deformities or DJD in young, fast-growing pigs of either sex. Current consensus is that the effects of rapid growth (early excess weight) and lack of exercise on the developing DJD in young, fast-growing pigs of either sex. Current consensus is that the effects of rapid growth (early excess weight) and lack of exercise on the developing cartilage are major risk factors for OCD development. Historically important dates in the study of OCD and hereditary influences on growth rate are summarized in Table 19.16.

Dyschondroplasia should be used to describe the majority of lesions affecting growth plates, especially the physeal growth cartilages or physes and a few lesions involving the articular epiphyseal cartilage complex (AECC). Dyschondroplastic foci may undergo calcification and ossification, or alternatively, the chondrocytes die, and the necrotic chondrocytes and denatured matrix are removed by fibrous connective tissue that ossifies. Occasionally, features develop at the chondro-osseous interface with the metaphysis or within the calcified portion of the zone of hypertrophying
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replicated cells die and either there is failure of matrix production or formed matrix is disrupted. Clusters of chondrocytes often develop at the periphery of the lesion in an attempt to repair the lesion. The soft denatured cartilage is probably subjected to further damage during joint movement so that flaps, fissures, and craters develop. When the AECC is breached and subchondral bone is in contact with the joint space, the joint becomes painful and lameness develops. There is a consensus that vascular injury within cartilage canals is part of the pathogenesis (Bullough and Heard 1967; Carlson et al. 1986, 1989, 1990; Kincaid et al. 1985; Kincaid and Lidwall 1982; Visco et al. 1991; Woodard et al. 1987b). Without normal vascularization, there is no subsequent ossification. Diet and flooring appear to have no effect, but hereditary factors influencing growth rate are major factors.

Ytrehus (2004; Ytrehus et al. 2004, 2007) recently summarized the situation with regard to OCD and described it as being a premature regression of the blood supply to the epiphyseal growth cartilage, leading to ischemic necrosis of the cartilage canals. They suggest that there are three different manifestations of OCD. These are (1) OCL where there are foci areas of cartilage necrosis at the epiphyseal growth cartilage, which is not visible grossly but is on microscopic

Table 19.16. Historical contributions to the study of osteochondrosis

<table>
<thead>
<tr>
<th>Reference</th>
<th>Contribution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Funkquist (1929)</td>
<td>Swedish Landrace boars fail to copulate</td>
</tr>
<tr>
<td>Smith (1966)</td>
<td>Lesions weakly heritable</td>
</tr>
<tr>
<td>Thurley et al. (1967)</td>
<td>Complex etiology: exercise, rate of weight gain</td>
</tr>
<tr>
<td>Fell (1967)</td>
<td>Heritability—OCD found in pigs in the United States</td>
</tr>
<tr>
<td>Bjorklund and Svendsen (1980), Hill et al. (1984a, 1985b, 1990)</td>
<td>OCD occurs in pigs less than 15 days old, implication of heritable trait</td>
</tr>
<tr>
<td>Lundgren and Reiland (1970)</td>
<td>Applied “osteochondrosis” to a DJD of Swedish pigs</td>
</tr>
<tr>
<td>Grondaleen (1974d), Perrin et al. (1978), Nakano et al. (1979b)</td>
<td>No effect of growth rate</td>
</tr>
<tr>
<td>Grondaleen (1974a–i)</td>
<td>Implicated wide variety of factors: particularly lack of exercise, heritability</td>
</tr>
<tr>
<td>Dammrich and Unselm (1972), Grondaleen (1974b), Reiland (1975)</td>
<td>Implicated poor conformation</td>
</tr>
<tr>
<td>Reiland (1975)</td>
<td>Described OCD of spine in thoracolumbar region</td>
</tr>
<tr>
<td>Perrin and Bowland (1977)</td>
<td>Described effect of exercise</td>
</tr>
<tr>
<td>Reiland (1978c)</td>
<td>Heritability effect described</td>
</tr>
<tr>
<td>Reiland (1978c), Carlson et al. (1988b)</td>
<td>Growth rate during first 4 months influences OCD</td>
</tr>
<tr>
<td>Gouedegebuure et al. (1980), Reiland (1978c), Carlson et al. (1988a)</td>
<td>A reduced growth rate reduces incidence of lesions</td>
</tr>
<tr>
<td>Perrin et al. (1978)</td>
<td>Multiple effects on OCD</td>
</tr>
<tr>
<td>Reiland and Anderson (1979)</td>
<td>Crossbreeding experiments</td>
</tr>
<tr>
<td>Sather (1980, 1982)</td>
<td>Confinement increases OCD</td>
</tr>
<tr>
<td>Empel (1980)</td>
<td>Slow-growing pigs do not have OCD</td>
</tr>
<tr>
<td>Van der Wal et al. (1980)</td>
<td>Heritability—sites and severity vary within Landrace</td>
</tr>
<tr>
<td>Nakano (1981a)</td>
<td>Housing and leg weakness</td>
</tr>
<tr>
<td>Farnum et al. (1984)</td>
<td>Heritability—miniature pigs not affected</td>
</tr>
<tr>
<td>Hani et al. (1984), Lundeheim (1987)</td>
<td>Faster-growing pigs have more severe lesions</td>
</tr>
<tr>
<td>Lundieheim (1986)</td>
<td>Heritability of OCD—Landrace</td>
</tr>
<tr>
<td>Lundieheim (1987)</td>
<td>Heritability—feasibility of selection against OCD</td>
</tr>
<tr>
<td>Woodard (1987a,b)</td>
<td>Demonstrates effects on longitudinal bone growth</td>
</tr>
<tr>
<td>Jorgensen and Sorensen (1998)</td>
<td>Leg weakness and longevity</td>
</tr>
</tbody>
</table>

chondrocytes; cysts or clefts that contain blood persist and appear to stop the ossification front.

The lesions appear to develop when pigs are less than 1 month old when there is little muscle mass, which suggests that heavy musculature is not the prime cause but may exacerbate the disorder. OCD has been observed in pigs as young as 1 day old. However, in these pigs, the AECC and growth plates are proportionately thicker and possibly susceptible to stress (Hill et al. 1984b, 1990). This results in a thickening of part of the growth plate, which causes interference with metaphyseal growth. This in turn results in deformation of the bones, joints, and ultimately limbs. It is this distortion that may lead to incongruity of the joints with subsequent development of osteoarthritis.

In the past, many of the lesions affecting the AECC have been examined at a stage when DJD has become established. In these cases, the articular surface was advanced and subchondral bone was often exposed. However, examination of early lesions shows that the lesions are initiated as microscopic foci of chondrolysis at or near the interface of the articular cartilage and epiphyseal growth cartilage. The lesions may progress at this site and lysed cartilage persists in the deeper layers of the AECC, at the chondro-osseous interface, and within the bone of the epiphysis. The recently
Osteochondrosis Failure of endochondral bone formation leading to abnormal articular and physeal cartilage
Osteochondrosis *desiccans* (OCD) Cleft or fissures in articular cartilage that protrude into underlying bone; visible grossly; usually with altered gait or posture
Osteochondrosis *latens* Lesion confined to epiphyseal cartilage are visible microscopically but not grossly; may not be symptomatic
Osteochondrosis *manifesta* Lesions accompanied by delay in endochondral ossification visible by radiographic or gross examination; may or may not have clinical signs
Epiphyseolysis Separation of proximal femoral epiphysis
Apophysiolysis Bilateral separation of physis of ischiatic tuberosities; sitting with hind legs extended forward
Distribution of lesions Can be unilateral or bilateral and symmetrical
Joint locations More common in sites with later epiphyseal closure (more time to develop) including ulnar (distal), femur, humerus, and thoracolumbar vertebrae, costochondral junctions, glenoid of scapula
Joint surface Lesions particularly found on medial surfaces (greater weight bearing)
Primary lesions in cartilage and bone ends Lesions at bone ends include articular cartilage invaginated below level of surrounding cartilage; may be thick and yellow or thin and red (Grondalen 1974a; Reiland 1975); cartilage/bone border may be wrinkled (Reiland 1975); cartilage flaps or fragments (Grondalen 1974a; Nakano 1981b); fissures separating inner and other layers of cartilage (Grondalen 1974a); cartilage cysts; fractures between diaphysis and epiphysis; vascular lesions (Nemeth and van der Valk 1976; Visco et al. 1991)
Chronic lesions Shorter long bones; flared metaphyses; flattened femoral and humeral heads; premature closing of distal ulnar growth rate causing a varus deviation of the distal radius; fractured anconeal process and a more semicircular semilunar notch
Additional changes Increased synovial fluid; ruptured ligaments; hemorrhages in joint capsule; thickened joint capsule; villous joint lining (Grondalen 1974b; Nakano et al. 1982)

The pathology of osteochondrosis is summarized in Table 19.17. There is still much confusion as to whether there is an association between OCD and lameness (Brennan and Aherne 1986; Farnum et al. 1984; Fredeen and Sather 1978; Hill et al. 1984a,b, 1998; Jorgensen and Nielsen 2005; Jorgensen et al. 1995; Lundeheim 1987; Reiland et al. 1978a,b). Our considered opinion is that it is referable to the individual animal and depends entirely on the extent and severity of the lesion, the joints affected, the “meatiness” of the animal, and the age at which an animal is affected. The situation is complicated in many cases by secondary processes such as osteomyelitis, fractures, and damage to greater trochanter and tubercle (Blowey 1992, 1994a).

Epiphysiolysis and apophysiolysis are now considered to be part of the abnormalities of the AECC with fractures occurring at the weakened epiphyseal sites in the femur and tuber ischiadicum, respectively. These are summarized below. All of the conditions affecting the limbs are related to the growth patterns in the respective limb bones.

**LAMENESS IN SOWS**

A classification of lameness in sows was given by Blowey (1994a) in which he described nine main causes of leg weakness in sows. All of the more juvenile conditions can be seen in the adult until closure of the physeal plates. Rickets is seen from 8 weeks to physeal closure, osteomalacia from 8 weeks onward, OCD from 0 to 30 weeks, epiphyseal separation from 15 weeks to physeal closure, and spondylolysis in older sows or boars. Diagnosis of these conditions is achieved by ruling out other causes of lameness and then by confirming with a postmortem examination of culled sows.

**Epiphysiolysis**

Epiphysiolysis, a condition related to OCD, involves the proximal femoral epiphysis in which there is separation along the proximal femoral epiphysis. It generally occurs between 5 months and 3 years of age because epiphyses fuse at 3–7.5 age (Cunningham 1966; Duthie and Lancaster 1964; Grondalen 1974d; Nemeth and van der Valk 1976; Visco et al. 1991). The cause is a combination of excess tension in the hip joint across a weakened physeal region in the femur, which then separates. Usually, it is severe sudden onset lameness but occasionally insidious. Animals lie down, are unable to rise unless assisted, and usually eat and drink. It may be unilateral or bilateral, and manipulation reveals crepitation. It can be confused with fractures of the femur or spinal canal abscesses or lumbar sacral fractures. It may become a center of necrosis if secondary bacterial infection occurs.

**Apophysiolysis**

Apophysiolysis, a condition also related to OCD, is the bilateral separation of the ischiatic tuberosities along their physes. It has been recognized in young sows
(Done et al. 1979; Petterson and Reiland 1967; Van Alstine and Toben 1989). Most affected animals are heavily pregnant, and most dog sit with hind limbs forward and palpation elicits crepitus. It is associated with slippery floors excessively pulling the biceps femoris tendons from the tuber ischidicum. Unilateral lesions cause a moderate to severe lameness, but bilateral separation may prevent the sow from rising or walking (Done et al. 1979).

**Osteomyelitis**

Osteomyelitis is an uncommon problem but may result in lameness or pathological fractures of the vertebrae with compression of the spinal cord. It may follow septicemia or local progression as in tail biting abscession. It may be associated with apophasiolysis, with the marrow and metaphysis of a long bone or with pathological fractures. Sows are also very prone to arthritis, but in their case, it is often an extension from the septic feet (Reiland 1975) or other surface abrasions such as shoulder sores. It complicates spondylitis and may develop into osteoarthritis.

**Proliferative Osteitis**

Proliferative osteitis is described as proliferative osteitis of the femoral greater trochanter and medial epicondyle of the humerus (Blowey 1992), usually gilts after the first weaning. Affected animals are seen dog sitting and they rise with pain and discomfort, and the pathology is a hemolytic mass in the muscle.

**Fractures of the Bone**

Fractures can occur in all ages but are more common in gilts and sows postweaning. Piglets often have fractures if laid on by sows if they are hypoglycemic or weak. Older piglets may fracture bones when they are stuck in fences or equipment. Finishing pigs may fracture bones during transport (Vaughan 1977). An increased rate of fractures in a particular age group should elicit a broad investigation of risk factors, including dietary factors, housing and flooring effects, mechanical sources of trauma, transport trauma, and handling practices by caretakers.

**Arthrosis**

Arthrosis is sometimes called arthropathy, osteoarthrosis, or osteoarthritis and is a nonspecific degenerating condition of cartilage that develops in chronic joint disease (Palmer 1985). The incidence increases with age. Animals less than 18 months had 7% incidence, but for those over 18 months, the level was 82% (Reiland 1975). It is the result of instability resulting from osteochondrosis but the surface lesions in the joint fill with osseous repair tissue (Grondalen 1974b; Nakano et al. 1979a; Palmer 1985). Pathologically, the lesions include fibrillation of joint cartilage, ulceration of the articular surface, osteophyte produc-

tion, and thickened synovial membrane and joint capsule (Palmer 1985).

**Neoplasms**

Tumors are not common but include osteosarcoma of the maxilla occluding the nasal cavity (S. Done, personal observation), osteogenic sarcoma (Harcourt 1973), metastasis from malignant melanoma (Case 1964), congenital melanoma, and multiple myeloma (Rintisch et al. 2010). Fisher and Olander (1978) described a melanoma invading the cord, and a glioblastoma in the ventral cerebral cortex of a 6-month-old Yorkshire gilt while reporting a survey of swine tumors.

**Hoof and Claw**

A discussion of the diseases of the hoof and claw is found in Chapter 66.

**ACKNOWLEDGMENTS**

This chapter would not have been possible without the efforts of all the previous authors of these chapters in *Diseases of Swine* and in particular Jack Done, Ray Bradley, Mike Hill, and Cate Dewey. The Central Veterinary Laboratory at Weybridge (now the Veterinary Laboratories Agency-Weybridge) is a center for studies on muscle and nervous disorders of the pig started by Jack Done. He inspired colleagues including Ray Bradley, David Harding, David Thurley, Peter Ward, Gerald Wells, and Tony Wrathall, and they, along with Prof. J. E. T. Jones of the Royal Veterinary College, inspired us.

**REFERENCES**


FEMALE REPRODUCTIVE ANATOMY AND PHYSIOLOGY

Before attempting to control reproduction of pigs, a basic understanding of normal ovarian physiology and reproductive endocrinology is required. Ovarian follicular development is divided into phases; the gonadotropin-independent phase is the development of primordial follicles through to the end of the secondary follicle stage; follicles will be about 0.4 mm at that time. The gonadotropin-dependent phase is the development of the tertiary (antral) follicle growth from 0.4 mm to ovulation. However, swine differ from cattle in that bovine follicle growth is controlled by follicle-stimulating hormone (FSH) to the point of ovulation, while in pigs from 0.4 to 4 mm, it is controlled by luteinizing hormone (LH) (Driancourt et al. 1995). Knowledge of these phases has implications for when ovarian function is or is not amenable to exogenous control. Readers should refer to Almond et al. (2006) and Spencer and Bazer (2004) for further details of normal developmental anatomy and physiology.

CONTROL OF REPRODUCTION

Estrus Induction in Gilts

If done properly, boar exposure is an effective means of inducing early puberty in gilts. Adequate stimulation of estrus requires direct physical contact, while estrus detection may only need fence-line contact. If the efficacy of boar exposure appears inadequate, it is important to evaluate whether the rules of boar contact are being followed (Kirkwood 1999). The rules are related to age of gilts and boars as well as the contact and environment provided for gilts and boars.

Age of Gilts. Gilts must be at least 160 days of age with maximal response when contact starts at 180 days (Figure 20.1). There is evidence that gilts exhibiting a more rapid response to boar exposure are innately more fertile. The corollary is that gilts not pubertal within 28 days of boar contact are likely less fertile and should be culled. Indeed, gilts mated at an older age (presumably due to later puberty) are culled from the herd at a younger age.

Age of Boars. Boars must be old enough (i.e., at least 10 months of age). The stimulus value of a boar depends on his ability to produce sufficient stimulatory pheromones from his submaxillary salivary gland, which achieves adult proportions by 10 months of age. It is not necessary to use the oldest and smelliest boar, but one that is of sufficient stimulus value.

Period of Contact. Gilts should be in physical contact with the boar for at least 15 minutes per day to give time for all gilts to interact with the boar. If gilts are housed in large groups, use at least one boar per 12 gilts. Since a fertile mating at puberty is likely not desired, either the boar contact needs to be supervised or sterile boars (vasectomized or epididymectomized) should be used and mating allowed. Indeed, a sterile breeding at puberty will enhance fertility to a fertile breeding at the subsequent estrus, with a 5–10% increase in farrowing rate and a 0.5–1.5 increase in litter size.

Housing of Gilts and Boars. Housing of gilts should be at least 1 m away from potential stimulus boars, and preferably downwind. Gilts housed adjacent to boars will be stimulated to an earlier puberty but the estrus...
detection rate declines because some gilts will have stood previously and are refractory at the time of supervised boar contact.

**Location of Contact.** Gilts should be taken to the boar and not vice versa. In the event of poor estrus detection management, the use of a separate detection-mating area (DMA) should be considered.

**Other Considerations for Estrus Induction in Gilts.** Other variations, such as using different boars on different days or housing gilts with a boar continuously, will not usually improve the response over that obtained by daily contact with a single boar of at least 10 months of age. However, if the pubertal response is considered inadequate, consider twice daily boar exposure and/or rotating stimulus boars every 2–3 days. This is because some boars that have a low stimulus value can be compensated for by rotating. After puberty, boar exposure should continue at least every other day in order to promote a regular estrous cycle. In the absence of postpubertal boar contact, the maintenance of regular cycles was compromised and the interestrus intervals became prolonged and unpredictable (Table 20.1).

If appropriate boar exposure does not appear to be effective (e.g., as a component of seasonal infertility), hormonal treatment should be considered. Gonadotropin treatments include injection of 750–1000IU of equine chorionic gonadotropin (eCG) or a combination of 400IU eCG and 200IU of the LH analog, human chorionic gonadotropin (hCG) (PG600®, Intervet/Schering-Plough Animal Health, Summit, NJ). The eCG/hCG combination has proven more effective at inducing estrus in gilts than has eCG alone (73% vs. 15% estrus response; Manjarin et al. 2009), emphasizing the requirement for LH-like activity for follicular development. If a group of gilts are administered PG600 and the response is very poor (e.g., 10–15% exhibit estrus), it is likely that the gilts were already cyclic and their prior estrus missed. This can be confirmed by using a simple sow-side progesterone enzyme-linked immunosorbent assay (ELISA) (Althouse and Hixom 1999). Under these circumstances, a thorough review of estrus detection management is indicated.

When PG600 is administered to prepubertal gilts, up to 30% may not exhibit behavioral estrus and about 30% of those exhibiting behavioral estrus fail to cycle regularly. The etiology of the unpredictable responses is not known, but likely some gilts are too immature to respond to the hormones. Although gilt fertility will improve if bred at a subsequent natural estrus, it is generally recommended to breed gilts at the induced estrus because of the relative unpredictability of cyclic estrous behavior after gonadotropin injection. However, if experience on an individual farm indicates that gilts have a high regular return rate (e.g., >90%), then delaying breeding to the following natural estrus is the preferred option.

**Estrus Induction in Weaned Sows**

Longer wean-to-estrus intervals (i.e., WEIs > 5 days) make it difficult to meet breeding targets, reduce sow performance (Steverink et al. 1999), and increase the risk of early culling. The etiology of the unpredictable responses is not known, but likely some sows are too immature to respond to the hormones. Although gilt fertility will improve if bred at a subsequent natural estrus, it is generally recommended to breed gilts at the induced estrus because of the relative unpredictability of cyclic estrous behavior after gonadotropin injection. However, if experience on an individual farm indicates that gilts have a high regular return rate (e.g., >90%), then delaying breeding to the following natural estrus is the preferred option.

### Table 20.1. Effect of boar contact on gilt cyclicity

<table>
<thead>
<tr>
<th></th>
<th>Boar Contact</th>
<th>No Boar Contact</th>
</tr>
</thead>
<tbody>
<tr>
<td>R. Siswadi and P. E. Hughes (unpublished data)</td>
<td>4.9</td>
<td>3.0</td>
</tr>
<tr>
<td>Cycles in 100 days</td>
<td>6.0</td>
<td>3.0</td>
</tr>
<tr>
<td>Cycles &gt;25 days (%)</td>
<td>3.0</td>
<td>32.0</td>
</tr>
<tr>
<td>Philip et al. (1997)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gilts having three</td>
<td>97.0</td>
<td>66.0</td>
</tr>
<tr>
<td>cycles (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Interestrus interval (day)</td>
<td>20.5 ± 0.4</td>
<td>20.0 ± 2.3</td>
</tr>
</tbody>
</table>

Adapted from Philip et al. (1997) and Siswadi and Hughes (unpublished data).
when using eCG alone, higher doses (1000IU) may improve the response of primiparous sows. A more cost-effective use of gonadotropins would be to inject only those sows anestrus 7 days after weaning. Results from delayed treatment have been inconsistent (Manjarin et al. 2010). The response obtained will likely depend on the depth of anestrus, that is, the ability of the ovaries to respond to gonadotropic stimulation.

Controlling Time of Ovulation

Assuming good quality semen, the objective is to inseminate sows during the 24 hours before ovulation (Kemp and Soede 1997), although the optimal period is 12 hours if sperm are more than 48 hours old (Bennemann et al. 2005; Waberski et al. 1994). A short WEI is associated with a longer duration of estrus, and conversely, a long WEI is associated with a short duration of estrus. Ovulation tends to occur about 70% of the way through the estrous period and the net effect is that sows with short (3–5 days) WEI will tend to be late ovulators (estrous to ovulation >40 hours), while sows with long WEI will tend to be early ovulators (<24 hours).

Injection of gonadotropins induces a short WEI and longer duration of estrus (Knox et al. 2001). This effect can be used to advantage since research has indicated 10–20% improvements in farrowing rates when sows were bred to a hormone-controlled ovulation (Cassar et al. 2004, 2005). Time of ovulation is controlled by the surge of LH near the onset of estrus, and it is relatively simple to advance ovulation with exogenous hormones. Ovulation will occur in 85–90% of sows 42±2 hours after injection of hCG or 38±2 hours after GnRH or porcine luteinizing hormone (pLH) (Abad et al. 2007; Langendijk et al. 2000; Figure 20.2). If ovulation is predicted to occur at 38 or more hours after detection of estrus, such as following a gonadotropin-induced estrus, the injection of GnRH, pLH, or hCG will provide a high degree of predictability to the time of ovulation. If time of ovulation is known, then appropriate timing of insemination is simple, and sow fertility can be maintained even following single inseminations or when fewer sperm are inseminated.

An alternative strategy, assuming a high proportion of spontaneous estrus by day 4 after weaning, is to inject hCG or pLH 80–84 hours after weaning without the initial eCG or PG600 injection. The rationale is that these sows can be expected to be naturally late ovulating and so treatment at 80–84 hours will preempt the endogenous LH surge and therefore control time of ovulation. At 36–40 hours after injection, breed any sows exhibiting estrous behavior. This protocol has resulted in improved farrowing rates for bred sows (90% vs. 70%; Cassar et al. 2004), although only 87% of sows were bred. The fate of nonestrous sows was not determined. Most previous studies of controlled ovulation have employed sows, although farrowing rates between 88% and 99% have been observed for gilts receiving 1000IU eCG followed by GnRH at estrus detection (Dr. S. Calamanti, unpublished data).

From a herd management perspective, the ability to accurately predict time of ovulation allows for increased efficiencies in the breeding process. In addition to allowing for single inseminations and/or fewer sperm per dose, the labor requirements for estrus detection and inseminations are reduced. Furthermore, the ability to use fewer sperm in a single artificial insemination (AI), if associated with single-sire semen doses, will facilitate the selection for boar fertility. It has been suggested that when pooled semen is used, its fertility may be reduced by inclusion of a poor fertility boar and he will sire few piglets (Foxcroft et al. 2010). If pooled semen is to be used, all boars in the pool should have been previously selected for high fertility.

Estrus Synchronization

If gilts are known to be cyclic, the options for estrus control are limited to breed and abort, when females can be expected to return to estrus in 4–6 days with normal fertility, and the feeding of allyl trenbolone (Matrix® [Intervet/Schering-Plough Animal Health, Summit, NJ] or Regumate® [Merck Animal Health, Boxmeer, The Netherlands]). Injection of prostaglandin F2α (PGF2α) will not induce luteolysis before day 12 of the estrous cycle, so it is of little value in estrus control. Allyl trenbolone is an orally active progestagen, that is, it will mimic the biological activity of progesterone and so limits ovarian follicle growth to medium follicles (∼4 mm). Gilts should be individually fed so that they consume at least 15 mg/day because under dosing is associated with cystic follicles (Kraeling et al. 1981). Estrus suppression is only needed from the time of luteolysis. If cycle dates are known, costs can be minimized by feeding allyl trenbolone from 12 days after
estrus detection until 5 days before gilts are scheduled to be bred. Expect 90–95% of gilts to achieve estrus on days 4–8 after last feeding.

Allyl trenbolone is as effective for synchronizing estrus in weaned sows as in gilts. The WEI of primiparous sows may be prolonged and unpredictable, and they produce smaller subsequent litters. To counter this adverse effect on fertility, primiparous sows need a longer period after weaning for metabolic recovery. This can be achieved by skip-a-heat breeding, which may increase farrowing rates and is associated with larger subsequent litters (Clowes et al. 1994; Morrow et al. 1989). Unfortunately, skip-a-heat breeding results in an additional 21 nonproductive days (NPDs). The extra 21 NPDs has a penalty of a lost opportunity cost of 1.4–1.8 pigs, and it also does nothing for being able to predict numbers of sows available for breeding. Alternatively, the use of allyl trenbolone allows for less than 21 days of estrus suppression, is associated with a subsequent predictable occurrence of estrus, and may capture the benefits associated with skip-a-heat breeding (Morrow et al. 1989). Benefits from feeding allyl trenbolone may also be apparent in early weaned sows that may need a longer recovery period after weaning (Koutsotheodoros et al. 1998). Note that the first allyl trenbolone feeding must commence on the day of weaning (or before), and most sows (>85%) will likely be estrous 5–7 days after the last feeding.

Mating Management: Sperm Age, Dose, Site of Deposition, and Timing of Insemination

Using an AI catheter, \(2 \times 10^9\) sperm are deposited in the cervix at 24-hour intervals from detection of estrus. The utility of sperm deposition in the 24 hours before ovulation has been established (Kemp and Soede 1997). However, if sperm age exceeds 48 hours from collection, the optimal window for insemination is 12 hours before ovulation (Waberski et al. 1994). Catheter designs permitting sperm deposition into the uterine body allow for fewer sperm (\(1 \times 10^9\)) to be deposited without detriment to sow fertility (Watson and Behan 2002). As sperm age, there is generation of free radicals causing membrane peroxidation resulting in fewer fertile sperm in the semen dose (Am-in et al. 2010b). Interestingly, it has been observed that farrowing rates were maintained if aged sperm were deposited intrathecine (Am-in et al. 2010a).

Farrowing Induction

Problems during parturition result in anoxia, which may kill piglets or result in poor viability with increased neonatal mortality. Increased supervision of piglet delivery is indicated when stillbirths or neonatal mortality exceed target levels, with additional benefit to better supervise colostrum management. If farrowing is supervised, management procedures can be implemented to enhance litter performance. Split suckling involves removal of piglets at delivery to a warm crèche. After seven pigs are collected, they can be placed on the sow while subsequent pigs are placed in the crèche. When farrowing is complete, the two groups of pigs are swapped for 1–2 hours. Thereafter, the entire litter is placed with their sow.

Induction of farrowing should not be more than 2 days before the due date, and when calculating gestation length, measure from the day of last breeding, which is closer to the time of fertilization. The saccula phase of lung development is almost exponential in the pig fetus; at 100 days of gestation, no fetus has entered the saccula phase, but at term 113–117 days, the lungs are fully developed. A small error in timing of parturition can result in a major deficit in lung development. When induction is timed accurately, there will rarely be a problem of low-viability piglets. The fat content of colostrum from induced sows may be lower, but immunoglobulin content will not be affected (Jackson et al. 1995).

The administration of \(\text{PGE}_2\) or an analog is effective for induction of farrowing, but the period from treatment to parturition will vary. After injection, only 50–60% of treated sows are likely to farrow during the following working day. The costs of farrowing induction can be reduced by injection of \(\text{PGE}_2\) into the vulva at 50% (or even 25%) of the manufacturers’ recommended dose, which is equally as effective as an intramuscular (IM) injection at the full dose (Kirkwood et al. 1996). The efficacy of lower doses is the likely result of a locally high concentration due to the intimate interconnections of the genital vasculature and a reduction in the pulmonary first-pass effect. The injection is tolerated well by sows if given at the vulvar–cutaneous junction, where the vulva meets the skin using a 20 gauge × 0.5 in. needle (or smaller).

The injection of oxytocin approximately 20–24 hours after the injection of \(\text{PGE}_2\) causes a more rapid and synchronous onset of parturition, but also often may cause an interrupted farrowing. That is, a piglet is delivered but farrowing then stops, necessitating manual assistance. This may be due to pain associated with forced delivery through an incompletely dilated cervix, causing a release of epinephrine that binds to uterine receptors and stops contractions. Interestingly, if oxytocin was given after delivery of the first pig when the cervix was presumably fully dilated, an increase in dystocia still occurred and higher numbers of stillbirths were observed. Furthermore, the stillbirths were occurring among the first-born pigs of the litter rather than the norm, where stillbirths occur in the last few pigs of the litter (Table 20.2). Oxytocin caused powerful uterine contractions that traumatized umbilical cords causing fetal anoxia, as evidenced by more piglets being born with meconium staining. Taken together, we suggest that oxytocin not be used at farrowing except therapeutically in cases of slow farrowing. One method
shown to improve the predictability of farrowing is to split the dose whereby an injection of PGF<sub>2α</sub> is given in the morning and a second is given 6–8 hours later. With this technique, 84% of sows farrowed the next day during working hours compared with 56% of single-injected sows (Kirkwood and Aherne 1998).

### PREGNANCY DETECTION

Accurate diagnosis of pregnancy is essential to optimize the reproductive efficiency of the sow herd. There is no “perfect” pregnancy test, but technology has improved considerably.

Detection of estrus as a method of pregnancy diagnosis is based on the likelihood that nonpregnant sows will display estrous behavior within 18–24 days after breeding, but the overall accuracy of observation of estrus for pregnancy diagnosis varies considerably. Accuracy is improved if the sow's behavior is observed in the presence of a boar. Even with boars on the premises, field observations suggest that most producers identify only 50% of nonconceiving sows using this technique. False-positive diagnoses occur when sows are persistently anestrus due to cystic ovarian degeneration (COD), acyclic ovaries, or pseudopregnancy. Management factors that interfere with the detection of estrus include housing submissive sows in groups with dominant sows, attempting to detect estrous females in large groups, and assessing estrus without using boar exposure.

By 3 days after ovulation and throughout pregnancy, serum progesterone concentrations are greater than 5 ng/mL. Thus, serum progesterone is high in pregnant sows and gilts during the expected time of return to estrus and low (<5 ng/mL) in sows and gilts that failed to conceive. The interestrus interval of sows of varying parities ranges from 18 to 24 days, with a mean of 20–21 days. Therefore, the optimal time to obtain blood samples for progesterone determinations is from 17 to 20 days after mating. Serum progesterone concentrations for pregnancy diagnosis have reported sensitivity of 97%, but specificity ranged from 60% to 90% (Almond and Dial 1986, 1987; Larsson et al. 1975). False-positive tests occur in animals with delayed or irregular returns to estrus, pseudopregnancy, and COD.

A high proportion of fetal estrogens is secreted from the uterus into the maternal circulation as estrone sulfate. Serum estrone sulfate concentrations cannot be reliably determined until peak levels are reached between 25 and 30 days of gestation (Robertson et al. 1978). Levels decrease at 35–45 days with a second increase commencing at 70–80 days. Serum estrone sulfate concentrations of >0.5 ng/mL are indicative of pregnancy, whereas <0.5 ng/mL is suggestive of non-pregnant status. Using estrone sulfate as a pregnancy test, >97% sensitivity and >88% specificity were obtained when samples were collected between 25 and 30 days of pregnancy (Almond and Dial 1986). False-negative results were obtained in sows or gilts with a delayed rise in estrone sulfate concentrations or when sows and gilts have less than four pigs in a litter (Almond and Dial 1986).

If serum concentrations of PGF<sub>2α</sub> metabolite (PGFM) are low (<200 pg/mL) or undetectable between days 13 and 15 after mating, the sow can be assumed to be pregnant. The prostaglandin pregnancy test had approximately 90% sensitivity and 70% specificity (Bosc et al. 1975), but this method is not generally available and requires extensive laboratory procedures.

Pregnancy diagnosis by rectal palpation of the sow is reported as practical and highly accurate (Cameron 1977). This technique is based on the examination of the cervix and uterus, together with the palpation of the middle uterine artery to assess size, degree of tone, and type of pulse. The pelvic canal and rectum are often too small for the procedure to be used on gilts or low-parity sows. Despite the potential application of this technique, it has not gained popularity in North America.

Ultrasound techniques using Doppler instruments can detect fetal heartbeats or the pulsation of arteries. Approximately 50–100 pulses/min are detected in the uterine artery, while 150–250 pulses/min are evident in the umbilical arteries. The ultrasound waves are emitted by an abdominal probe positioned on the flank or in the rectum and received by transducers that convert them to audible signals. Sensitivity (>85%) and specificity (>95%) did not differ between the rectal and abdominal probes (Almond and Dial 1986), but optimal results were obtained at 29–34 days. False-positive results may occur when sows are tested during proestrus or estrus or when animals have active endometritis. False-negative diagnoses occur when examinations are conducted in a noisy environment or if feces become packed around the rectal probe.

Amplitude-depth ultrasound instruments (A-mode or pulse-echo) use ultrasound waves to detect the fluid-filled uterus. From approximately 30 days until 75 days after breeding, the overall accuracy in the determination of pregnancy is commonly >95%. False-negatives or inconclusive diagnoses increase from 75 days until farrowing due to changes in the allantoic

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**Table 20.2.** Effects of oxytocin (OT) after delivery of the first pig on farrowing performance

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>30 IU OT</th>
<th>40 IU OT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Live born</td>
<td>8.3</td>
<td>8.7</td>
<td>8.7</td>
</tr>
<tr>
<td>Stillborn (percent first four pigs)</td>
<td>0</td>
<td>70.8</td>
<td>40.0</td>
</tr>
<tr>
<td>Stillborn (percent from pig 9)</td>
<td>83.3</td>
<td>20.8</td>
<td>40.0</td>
</tr>
<tr>
<td>Dystocia (%)</td>
<td>5</td>
<td>10</td>
<td>20</td>
</tr>
</tbody>
</table>

Adapted from Alonso-Spilsbury et al. (2004).
fluids and fetal growth. The sensitivity and specificity vary between the different models of amplitude depth instruments (Almond and Dial 1986). Detection of a fluid-filled urinary bladder, pyometra, or endometrial edema yields a false-positive test. False-negative results were noted when animals were examined before 28 days of gestation or after day 80.

Real-time ultrasonography (RTU) can provide early and accurate pregnancy diagnosis in sows and gilts. The transducer of the RTU probe is placed against the flank of the animal, and the positioning is similar to other pregnancy detection devices. Pregnancy is based on the detection of distinct, fluid-filled vesicles in the reproductive tract. RTU is now a routine pregnancy detection method. On day 21 of gestation, the overall accuracy was 90% and 96% for the 3.5- and 5-mHz detection method. On day 21 of gestation, the overall accuracy was 90% and 96% for the 3.5- and 5-mHz probes, respectively (Armstrong et al. 1997). The technician, day of gestation, instrument, and probe (3.5 vs. 5 MHz and linear vs. sector) influence the accuracy of RTU. These sources of variation have much less impact when RTU is used at day 28, rather than day 21.

**DYSTOCIA**

Dystocia and other contributors to difficult farrowing (e.g., downer sow) are relatively frequent in sows. Knowledge of the normal process of parturition is essential to determine when there is a need to intervene. Swelling of the vulva occurs about 4 days prepurition. The mammary glands become more turbid and tense during the last 2 days before farrowing, with the mammary secretion being serous 48 hours prior to parturition and becoming milky within 24 hours of farrowing. Restlessness and nesting behavior usually start 24 hours prefarrowing but recede in the last hour before the first piglet. Intermittent abdominal straining occurs before the birth of the first piglet, but straining is usually mild thereafter except at the moment of expulsion. Farrowing is expected within 20 minutes when a viscid, blood-tinged secretion, often containing meconium, is observed at the vulva. Duration of parturition is usually less than 3 hours but ranges from 30 minutes to more than 10 hours; the average interval between the births of piglets is 15–20 minutes. Fetal membranes are expelled from 20 minutes to 12 hours after birth of the last piglet.

Signs of dystocia are anorexia, blood-tinged vulvar discharges, meconium without straining, straining without delivery of piglets, cessation of labor after straining, cessation of labor after delivery of one or more piglets, exhaustion of the sow, and foul-smelling and discolored vulvar discharge. Primary uterine inertia associated with a decreased contractile activity of the myometrium is uncommon in sows, whereas secondary uterine inertia is much more frequent and results from uterine and maternal exhaustion associated with fetal malpresentation or obstruction. The causes of dystocia are classified into two categories, maternal and fetal, depending on the origin. Arthur et al. (1989) reported the following causes of dystocia: uterine inertia without significant cause (37%), breech presentation (14.5%), obstruction of the birth canal (13%), simultaneous presentation of two fetuses (10%), downward deviation of the uterus (9%), and oversized fetuses (4%). In modern sow herds, dystocia may also occur as a result of the misuse of prostaglandin and oxytocin to induce or control farrowing.

Intervention for dystocia must be in a timely fashion. To optimize the success of intervention, sows should be observed at 30-minute intervals once parturition has commenced. At each observation, the number of piglets born and the time can be recorded to better assess the interval between the birth of piglets.

Correction of dystocia is achieved by manual examination of the vagina and cervix and removal of the obstruction or malpresented fetuses using strict hygiene, obstetrical gloves, and lubricant. Manual extraction is usually the safest technique to extract fetuses. Caution must be exercised when using forceps, blunt hooks, or cable snares due to the risk of trauma to the sow's reproductive tract. After removal of the malpresented pigs, the birth canal should be reexamined prior to the administration of oxytocin. High doses (>20 IU) of oxytocin may create a refractory period (3 hours) in which endogenous and exogenous oxytocin fails to stimulate contractions. Injectable antibiotics are warranted if sufficient contamination occurs. Intraterine infusions of antibiotics or iodine solutions are usually not effective in promoting uterine involution or preventing uterine infections.

Hemorrhage may occur postpartum as a result of uterine, vaginal, or vulvar lacerations. Lacerations of the vagina and vulva can be sutured externally, whereas severe uterine lacerations or uterine ruptures are difficult to repair without conducting a laparotomy. Oxytocin treatment promotes uterine contractions and may be beneficial with minor uterine lacerations. Hematomas of the vulva are resolved as the blood and fluid are resorbed; however, sharp projections in farrowing crates increase the danger of lacerating the hematomas.

**VAGINAL AND UTERINE PROLAPSE**

Prolapse is often seen shortly before, during, or up to several days after farrowing. Factors that have been reported to increase the risk of vaginal or uterine prolapse in sows are genetics, housing, physical trauma to the genital tract following parturition, older parity, nutrition, and effects of estrogenic mycotoxins.

**VULVAR DISCHARGES**

Individual cases of vulvar discharge rarely represent a major concern. In contrast, if 5–10% or more of a
Careful hand mating or AI procedures reduce the likelihood of breeding-inflicted vulvar lesions.

The presence of purulent vulvar discharge 10 or more days after breeding or estrus is suggestive of metritis or endometritis. The causative agent or agents likely enter the uterus during estrus, either by passive ascension or by active introduction by the boar during mating. Nonspecific endometritis results from infection by bacteria not considered as specific pathogens of the reproductive tract, including *Escherichia coli*, *Streptococcus* sp., *Staphylococcus* sp., *Arcanobacterium pyogenes*, *Proteus*, *Klebsiella*, and a variety of others.

Sows inseminated late during estrus (often the third AI) are more susceptible to discharge problems. During early metestrus, the protective mechanisms of high estrogen concentrations have dissipated, thereby rendering the animal susceptible to uterine infections. Furthermore, there is a strong correlation between serum progesterone concentrations and the development of endometritis (De Winter et al. 1992). Some multiple AI schemes may contribute to problems with endometritis by breeding after ovulation.

Endometritis also occurs following parturition as a result of dystocia, traumatic injury, abortion, and unhygienic manipulations. The likelihood of subsequent infertility is greater in sows that have a prolonged vulvar discharge following parturition, for example, more than 6 days (Waller et al. 2002). Pyometra, an acute or chronic suppurative inflammation of the uterus with accumulation of large quantities of pus, is rare in sows.

A purulent vulvar discharge with or without blood may be observed in cases of cystitis or pyelonephritis. This discharge usually contains mucus, is associated with urination, especially at the end of the stream, and is not related to the estrous cycle. To confirm urinary tract infections, urinalyses are performed on samples from affected animals, or collection and examination of urinary bladders and kidneys are performed at slaughter.

### Managing the Discharging Sow

Most discharging sows return to estrus shortly after the initial appearance of the discharge. Pregnancy rarely occurs when these animals are bred at this time. If economics and animal flow permit, these animals can be allowed to recycle one more time with the expectation that those that do not discharge at subsequent estrus will have conception rates similar to those of repeat breeders. The other option is to cull any animal with a discharge. Risk factors of hygiene and management procedures around farrowing, mating, and AI should also be evaluated.

Numerous treatment protocols have been attempted to resolve problems of discharging sows, but consistent efficacy is dubious. Medicated feed or injectable antibiotics are common treatments. Precise pathogens...
involved and their sensitivity to antibiotics are rarely known, so it is often difficult to assess the effectiveness of these treatments (Dial and MacLachlan 1988).

**CONGENITAL DEFECTS AND NEOPLASIA**

Defects of the female genital system are common and include cysts of the mesosalpinx; duplication of the vagina, cervix, or uterine horns; segmental or complete aplasia of the uterus, cervix, vagina, and vulva; and intersexuality (additional mention of congenital defects is found in other chapters). The aplasias, hypoplasias, and duplications appear to have genetic components that can be expressed in varying degrees in different individuals and may contribute to infertility and perhaps dystocia. Other defects of the female genital system include persistence of the hymen, ovarian aplasia, other ovarian defects, and hypoplasia or malformation of the nipples. The incidence of these conditions is low, and in most instances, the cause is unknown or suspected to be heritable.

Intersexuality is occasionally observed in pigs. True intersexes (hermaphrodites) have both testicular and ovarian tissues, whereas pseudohermaphrodites have gonads of one sex and other genital organs of the opposite sex. The external genitalia of intersex pigs are usually female in type. In most instances, the vulva appears normal, with a variable enlargement of the clitoris, but in some, the clitoris is greatly enlarged and the vulva is underdeveloped or prepuce-like. Some affected individuals show male behavior, while others show estrus and even become pregnant. Estrus, ovulation, and pregnancy are possible in true hermaphrodites. Reports of female pseudohermaphrodites in pigs are rare. Genetic analyses suggest that pig intersexuality is controlled multigenically.

Neoplasms of the genitalia in sows have not been studied intensively. Investigations of genital tracts at slaughter reveal a low prevalence of neoplasias. The most commonly observed neoplasms were leiomyoma, fibroma, cyst-adenoma, fibroleiomyoma, and carcinoma (Akkermans and van Beusekom 1984).

**DIAGNOSIS OF INADEQUATE REPRODUCTIVE PERFORMANCE**

Reproduction is an extremely complex process. It involves many highly specific biological functions and is influenced by innumerable factors grouped in headings of diet, housing, environment, genetics, disease, and management. Because many of the elements involved in reproduction are interrelated, one problem may give rise to others. Generally, studying records, astute clinical observation, pathological examination, and laboratory testing can implicate the source of reproductive failure (Dial 1990). Such failures can be grouped into six categories related to stages in the reproductive cycle: anestrus, estrus, ova production, fertilization, implantation, and maturation. The diagnosis of causes of infertility requires collection of reliable information and using it in a meaningful way (Muirhead and Alexander 1997).

**Reproductive Efficiency Targets**

Numerous systems are currently available commercially for assessing the biological performance of the breeding herd. Although there is variation in data entry, report format, and report content, all of the systems provide summaries of breeding, farrowing, and weaning information. Most provide either time-related or group reports for information relating to fertility, lactation performance, interval from entry or weaning to mating, and piglet weaning until weaning. Targets and the level at which a corrective intervention should be performed (interference levels) should be included in these production reports. The value of these parameters should be changed regularly as the herd performances change.

There are numerous risk factors or differential diagnoses for the different types of reproductive failure. Many can be incriminated or ruled out through examination of records. A diagnostic examination of environment, facilities, management, disease status, and nutrition may suggest one or several of them as a cause of reproductive failure; the diagnosis typically must be corroborated through the record analysis. For example, suboptimal total pigs born/litter may involve parity distribution, lactation length, WEI, season and ambient temperature, systemic reproductive disease, genetics, nutrition, and breeding management. Both record analysis and flow diagrams are extremely helpful to identify risk factors and explanation of reduced reproductive performance (Almond et al. 2006; Carr 2008).

Analysis of reproductive problems requires that the factors discussed above have been taken into account, then apply a systematic approach: (1) ensure that the failure to farrow is associated with a reproductive cause; (2) assess the timing of the returns to estrus; (3) determine whether the cause relates to the female, the male (natural and AI), or human error; (4) carefully observe, ideally using video recording, the whole mating process. Some failure-to-farrow problems are not specifically reproductive disease. For example, a farm with a water supply problem and high sow mortality associated with cystitis and pyelonephritis will experience a low farrowing rate since most sow deaths will be in gestation. To achieve an 87% farrowing rate, targets for nonfarrowing reasons are 10% returns to estrus, <1% abortions, <0.5% not in pig at moving to farrowing accommodation, 1% culled in-pig, and 1% died in-pig.

Within the returns to estrus category, the suggested target ratio of regular to irregular returns is 4:1. An understanding of early embryonic signaling provides
Boar Training
Mounting a collection dummy for successful semen collection requires two key, early training concepts: the collection area should focus the boar’s attention on the dummy sow upon entry into the pen, and boars must associate the collection area and process with a pleasurable experience.

Since the primary stimulus for initiation of the mounting reflex in boars is visualizing an immobile object that resembles another pig, the collection pen should be clean and free of extraneous items to allow the boar to focus on the collection dummy. Similarly, a well-used collection dummy and pen with a strong swine odor frequently provides olfactory stimulation, quickening the mounting process.

Attention span is short in young boars therefore mounting activity usually occurs within the first 5–7 minutes of a training session. If mounting or an interest in the collection dummy does not occur quickly, the probability is low that such behavior will occur in a current training session. During training, administration of PGF$_{2\alpha}$ (20 mg IM) to the boar 5–10 minutes prior to introduction to the collection pen may promote increased aggression and mounting of the dummy (Fonda et al. 1981).

Boars that associate pain or fear with the collection area or collection process are difficult to train. Unpleasant experiences during collection can reduce the sexual activity of trained AI boars. Avoidance of rough handling and loud noises during the training process is critical to a successful training program.

Boar Behavior
Immature boars are especially sensitive to behavioral conditioning through their environment. Tactile stimulation is influential in developing behaviors; young boars penned in isolation and deprived of tactile stimulation have a delay in puberty, poor sexual motivation, and decreased long-term mating performance. The types of human interaction condition boars for either approach or avoidance response as the boar matures. Once the boar is at stud, factors such as collection frequency, collection area, and boar preparation for semen collection can have a big impact on time to ejaculation and semen quality. Good stud management should allow sufficient time for collection of semen; rushing or “pushing” the boar can have negative repercussions on future semen collection sessions.
Table 20.3. Boar and laboratory management to minimize semen contamination

<table>
<thead>
<tr>
<th>Boar Preparation/Semen Collection</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Hair should be kept trimmed around the preputial opening.</td>
</tr>
<tr>
<td>2. Use double gloving, with the outer glove discarded after preparation of the boar, allowing for a clean gloved hand for grasping of the penis.</td>
</tr>
<tr>
<td>3. Disposable vinyl gloves or a hand disinfectant should be used during semen collection to minimize contamination of semen and reduce risk of cross-contamination between boars.</td>
</tr>
<tr>
<td>4. Clean preputial opening and surrounding area (if needed) with a single-use disposable wipe.</td>
</tr>
<tr>
<td>5. Manually evacuate preputial fluids prior to exteriorizing penis for semen collection.</td>
</tr>
<tr>
<td>6. Hold penis horizontally to minimize preputial fluid contamination of the semen and semen collection vessel.</td>
</tr>
<tr>
<td>7. Disrupt initial jets of an ejaculate (i.e., presperm fraction) and gel fraction from the semen collection vessel.</td>
</tr>
<tr>
<td>8. Dispose of rubber band and filter/gauze before passing collected semen to the laboratory for further processing.</td>
</tr>
</tbody>
</table>

Semen Processing/Laboratory and Barn Sanitation

1. Encourage single-use disposable products when economically feasible to minimize cross contamination.
2. If using reusable laboratory materials that cannot be heat/gas sterilized or boiled, clean initially using a laboratory grade detergent (residue free) with water, followed by a distilled water rinse, and lastly, a 70% alcohol (nondenatured) rinse. Allow enough time and proper ventilation for complete evaporation of residual alcohol. Rinse with semen extender prior to their first use of the day.
3. Disinfect countertops and contaminated lab equipment at the end of the processing day with a moist residue-free detergent and rinse.
4. Floor should be mopped at the end of the day with a disinfectant (e.g., phenolic or formalin product).
5. Break down bulk products into smaller, daily use quantities immediately after opening.
6. Ultraviolet lighting can be installed to aid in sanitizing reusables and lab surfaces; however, safety precautions need to be integrated to prevent exposure of personnel.
7. Boar housing should be on a maintenance schedule that minimizes buildup of organic material and surface moisture.
8. The semen collection area and collection dummy should be thoroughly cleaned and disinfected at the end of the collection day.

Table 20.4. Minimum requirements for use of fresh boar semen for artificial insemination

<table>
<thead>
<tr>
<th>semen Variable</th>
<th>Descriptor/Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Appearance</td>
<td>Milky to creamy consistency</td>
</tr>
<tr>
<td>Color</td>
<td>Gray-white to white</td>
</tr>
<tr>
<td>Gross motility</td>
<td>≥70% (if used by 48 hours)</td>
</tr>
<tr>
<td>(unextended)</td>
<td>≥80% (if used after 72 hours)</td>
</tr>
<tr>
<td>Abnormal morphology</td>
<td>&lt;25% (includes cytoplasmic droplets)</td>
</tr>
<tr>
<td>Cytoplasmic droplets</td>
<td>&lt;15% (proximal and distal droplets)</td>
</tr>
</tbody>
</table>


Table 20.3

Semen Collection

The most common method of boar semen collection is the gloved-hand technique (Althouse 2007), but newer technologies incorporating an integrated collection dummy that allows for hands-free (automatic) semen collection are gaining popularity. With either technique, digital pressure is applied to the boar’s glans penis to stimulate erection and ejaculation. It is common for pressure on the end of the penis to be relaxed slightly after an erection has occurred in order to facilitate ejaculation. Routine management practices such as removal of preputial fluid prior to collection and clipping sheath hairs can greatly aid in reducing the risk of contamination of semen during collection. Further techniques for minimizing contamination of swine semen during collection and processing are outlined in Table 20.3. Surgical techniques such as a preputial diverticulectomy may also be used to reduce contamination of the boar ejaculate (Althouse and Evans 1994).

Semen Evaluation

To date, the development and application of a single in vitro test, which can be routinely performed on freshly collected ejaculates to determine quality (e.g., fertility potential), has yet to be discovered. Many of the current in vitro tests performed at boar studs, however, do have the ability to diagnose ejaculates of overtly poor quality. Standard tests utilized in the swine AI industry today include visual inspection, gross sperm motility, sperm morphology, sperm concentration, and ejaculate volume. Minimum requirements for use of fresh boar semen for AI are listed in Table 20.4. Additional tests examining sperm cell viability and functionality exist but are currently of limited use in commercial settings due to expense and/or time to perform.

Visual Assessment. After collection, each ejaculate should be visually inspected for opacity and color. Normal ejaculates will be gray-white in color with a milky opacity. Ejaculates that exhibit an abnormal color (e.g., brown, red, yellow) are usually indicative of contamination with pus, blood, or urine and thus should not be further considered for AI use.

Motility and Morphology of Spermatozoa. The percentage of sperm cells exhibiting progressive motility is the most common measurement recorded during semen evaluations. Motility assessment can be done either by subjective estimation using a microscope (200 or 400 magnification) or objectively with a computer-assisted semen analysis (CASA) system. In general,
motility is best used as an estimator of sperm cell viability rather than of fertility. In the boar, it is common to obtain motility scores of 80% or greater. Previous work has suggested that ejaculates exhibiting motilities of 60% or higher in AI programs should not compromise herd reproductive performance (Flowers 1997).

Both dry-mount and wet-mount techniques are used in the swine industry for assessing sperm morphology. Dry-mount techniques utilize a contrast stain to accentuate the outline of the sperm and examine it microscopically under oil immersion. With wet-mount techniques, sperm morphology is performed on a monolayer of immobilized sperm using a microscope or CASA system with its own internal contrast (e.g., phase- or differential-interference contrast). Protocols for accurately estimating the percentage of morphologically normal spermatozoa in an ejaculate have been published (Kuster et al. 2004). Normal boar ejaculates should exhibit less than 20%–25% abnormal sperm (Althouse 1998).

Sperm Concentration and Total Number of Spermatozoa. Sperm concentration in a gel-free ejaculate is most commonly determined by measuring sample opacity using a photometer or, alternatively, by quantitating cells using CASA. Raw boar semen is normally too opaque to measure with either of these devices, so it is common to determine concentration on samples diluted with an isotonic solution using calibrated pipettes. It is important that the photometer be calibrated specifically for boar semen, and that validated boar semen parameters are imputed for the CASA. Accuracy of either of these systems for determining sperm concentration is via hemacytometry but is not routinely used in studs due to the tediousness and length of time needed to perform accurate counts.

Estimation of ejaculate volume is needed in order to determine total sperm numbers. Ejaculate volume is determined by weighing the ejaculate, with the assumption that a 1 g weight is equal to a 1 mL volume. Ejaculate volume is then multiplied by sperm concentration to determine total sperm numbers.

Other Fertility Tests. In addition to the mechanical requirements associated with egg penetration, spermatozoa must also undergo biochemical, metabolic, and molecular changes to acquire fertilizational competence. Published tests assessing sperm-binding ability, hypo-osmotic swelling, osmotic resistance, membrane/organelle assays, and selected biochemical and molecular assays of sperm or seminal plasma have been promoted. Their utility within the swine industry remains to be determined.

Semen Processing

Semen Extenders. Semen extenders provide nutritional and metabolic support for stored semen. Glucose is the predominant energy source; electrolytes assist in the regulation of osmotic pressure; buffers are involved in neutralization of metabolic wastes and maintenance of pH; and antimicrobials are added to control for any bacterial contamination. Fertility decreases as the length of storage increases. The rate at which fertility decreases during storage is primarily a function of the ingredient combination used in the given semen extender. Several different factors determine the longevity of stored semen, including individual boar characteristics, particular semen extender, sperm concentration, and storage temperature. An example of the interaction between individual boar characteristics and semen extender type on sperm cell longevity is illustrated in Figure 20.5. In this example, the viability of semen in storage was optimized by use of one semen extender for one boar and a different extender for a second boar. The data indicate that problems with maintenance of semen viability in vitro may be related to an incompatibility between components of the semen extender and seminal fluids of an individual boar. If such incompatibilities exist, then using different semen extenders may solve the problem. With any

20.5. Changes in viability of spermatozoa over time for two littermate boars. Spermatozoa from both boars were stored in three different semen extenders. Top panel: Data from boar A. Bottom panel: Data from boar B. Pooled standard errors for A and B were 7.3% and 8.2%, respectively.
extender, upper limitations exist with respect to the concentration of sperm which can be maintained over the shelf life of the product.

**Semen Extension Procedures.** A majority of semen extenders are commercially produced and come in powdered form. Extenders should be reconstituted using purified (Clinical and Laboratory Standards Institute [CLSI]/College of American Pathologists [CAP] reagent grade type 1) water. Once reconstituted, it is important to allow sufficient time for the stabilization of the pH and osmolarity of freshly prepared extender before it is mixed with semen. The pH of most semen extenders equilibrates 45–60 minutes after preparation.

Minimization of the temperature between the extender and raw semen is necessary to maintain high sperm viability during the dilution process. This is accomplished by monitoring the temperature of both the extender and the semen and adjusting the temperature of the extender to within 2°C of the semen. With extended swine semen used for AI, final sperm concentrations of 25–65 million/mL are used in a 65- to 80-mL dose volume. Once extended, doses of semen are cooled to and then stored at 15–18°C. Both single-sire and pooled semen from different boars are used via AI in the swine industry. Due to low fecundity rates, frozen-thawed boar semen is not commonly used in current swine breeding programs.

**Breeding Soundness Examination**

Breeding soundness examinations (BSEs) are often not conducted on boars that are used for natural service because producers often rely on conception or farrowing rates as an indicator of boar fertility. The use of heterospermic matings interferes with attempts to identify subfertile boars by examination of farrowing records. Other factors influence farrowing rates and litter size; therefore, it is difficult to identify problem boars solely by record analysis.

Natural-service boars are selected for BSE when subfertility is suspected or following disease or injury, especially if the testes or penis is involved. Clinical history should include the boar’s previous libido and mating ability, previous injuries, illnesses, vaccinations, treatments, and information regarding litter size and farrowing rates in females inseminated by the boar.

The body condition, general health, skeletal conformation, and soundness should be assessed. Soundness examination should determine if any innate problems of the boar exist, which may interfere with his ability to approach, to mount, and to successfully breed/ejaculate. Physical examination of the external genitalia should include examination and palpation of the scrotum, testes, epididymides, and prepuce. Testes enlarge up to approximately 18 months of age in the boar; mature boars should have a minimum size of 6.5 × 10 cm (W × L). Abnormalities, such as abscesses, wounds, trauma, or scars on the scrotum, should be recorded and the preputial fluid should be examined for purulent material or blood.

The gloved-hand technique is the most practical method to collect semen from natural-service and AI boars. Natural-service boars are exposed to a sow in standing estrus and are allowed to mount, and the penis is grasped and diverted before penetration. At this point, the procedures for semen collection are similar to those used for routine collection from AI boars. A complete semen evaluation includes determining the total number of sperm cells and estimating the viability and fertility of sperm cells. There are few differences between semen assessment for BSE and AI, but for natural-service boars, one should consider the following: (1) a clean ejaculate has little odor compared with the distinctive odors of ejaculates that are contaminated with preputial fluid, purulent material, or urine; and (2) boars ejaculate 150–250 mL of semen with a range from 50 to 500 mL, but boars without previous experience with gloved-hand collection may not provide a complete ejaculation. Semen obtained should be evaluated using the techniques outlined above.

**Effects of Heat Stress and Fever**

Common causes of poor semen quality include stress and overuse. Overheating or heat stress is the most common cause of abnormal semen. Heat stress disrupts spermatogenesis, resulting in decreases in sperm motility, increases in morphologically abnormal sperm, and a possible decrease in total sperm output. These ejaculate alterations first appear at 10–14 days postinsult and can remain for several weeks (Figure 20.6). The boar's extender, upper limitations exist with respect to the concentration of sperm which can be maintained over the shelf life of the product.

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environment should be kept below 27°C at 50% relative humidity to avoid heat stress.

Infectious diseases, vaccinations, wounds, bruising, and lacerations to the testes or scrotum can raise the boar’s core body temperature or the temperature of the injured organ. Because the scrotum plays a role in regulating the temperature of the testes, any defect or abnormal condition of the scrotal area may also have an adverse effect on the production of sperm cells. In general, changes in semen quality after infection are similar to those that occur after boars are exposed to heat stress conditions. To reduce the effects of systemic illness, fever should be kept below 39.5°C using medication.

**Localized Infections.** Epididymitis often causes swelling of the testis and scrotum. An epididymal infection lowers fertility and should receive immediate attention. Fever, infection, orchitis, or inflammation of the scrotum can cause testicular degeneration of boars by causing degeneration of the germinal epithelium of the seminiferous tubules, usually diagnosed with a biopsy. Eventually, the testicular tissue will atrophy, resulting in flaccid testicles, some of which may become firm if fibrous growths or calcium deposits accumulate. Although the boar’s libido is unaffected, the ejaculate will have reduced sperm numbers, sperm motility will be poor, and a high percentage of sperm will be abnormal. If an 8-week rest period does not improve the condition and spermogram, these boars should be culled.

Pathogens that can localize within the testicular parenchyma and disrupt spermatogenesis include *Brucella, Chlamydia*, Japanese B encephalitis virus, and rubulavirus. Erysipelas, mycoplasmosis, swine influenza, and other infectious diseases that elicit a febrile response can indirectly disrupt spermatogenesis. Lastly, foot-and-mouth disease virus, hog cholera virus, *Leptospira*, pseudorabies virus (PRV), porcine parvovirus (PPV), porcine circovirus, swine vesicular disease, and porcine reproductive and respiratory syndrome virus (PRRSV) can be shed in the semen of infected boars, potentially allowing disease to spread into the sow breeding herd.

**DIAGNOSIS OF ABORTION AND REPRODUCTIVE DISEASES**

The corpus luteum (CL) provides progesterone to maintain pregnancy, and the abrupt loss of CL will terminate pregnancy. The release of cortisol by distressed sows and/or fetuses can initiate a hormonal cascade culminating in prostaglandin release from the pregnant uterus and luteolysis leads to irreversible parturition. Hence, abortion can result from interplay of endocrine systems and dam–fetus pathophysiology, and can be influenced by infectious or noninfectious insults.

Pseudopregnancy occurs when corpora lutea are maintained beyond the expected time of luteolysis and the absence of viable fetuses. Pseudopregnancy can be induced by treating gilts or sows with estradiol beginning on day 11 or 12 of an estrous cycle. Short-term treatment (i.e., treatment on days 11 and 12) will induce short pseudopregnancy (extend gestation length several days to several weeks) in most animals, but longer-term treatment (treatment for 8–9 days beginning on day 11 or 12) is required to induce consistent long (greater than several weeks) pseudopregnancy. Corn contaminated with zearalenone from *Fusarium rosecum* may cause either short- or long-term pseudopregnancy. The presence of mummified fetuses, with or without a few live fetuses, can extend gestation length by a few days.

The perception that infectious abortion is an acute process is generally misleading. A brief generalized pathogenesis of porcine abortion illustrates several key features of most infectious agents of abortion: damage viremia/bacteremia (days 1–2) → local tissue replication (days 2–7) → in utero spread between fetuses → abortion (day 14). In swine, the most common mechanisms of infectious abortion are infection of the fetoplacental unit or the systemic effects of maternal illness. Clinical signs observed and the selection of appropriate specimens and diagnostic tests will differ depending on the mechanism of abortion. In abortions due to fetoplacental infection, (1) the dam will typically have seroconverted at the time of abortion, (2) the dam is generally no longer viremic/bacteremic, and (3) the organism can typically be detected in the placenta or in one or more of the aborted fetuses. Therefore, diagnostic efforts should focus on the placenta, fetuses, and seroconversion of dams. In contrast, abortion due to maternal illness generally occurs during the acute phase of the disease process, at which time (1) sows typically exhibit signs of systemic illness, (2) sows have not seroconverted to the causative infectious agent, (3) there is the potential to detect the agent in specimens (serum, nasal swab, tonsil scraping, tissues) from the sow, and (4) fetal infection is unlikely. Therefore, examination of aborted fetuses from acute maternal illness is generally unrewarding and diagnostic efforts should focus on the sow.

The first step in investigating an abortion outbreak is to assess whether aborting sows have clinical signs of systemic illness either at or near the time of abortion. If the aborting dam is clinically ill, then specimens from both the dam and fetuses should be submitted for diagnostic tests. The nature of the sow’s illness dictates the appropriate sampling strategy. Swine influenza virus (SIV), for example, can cause sows to be febrile, depressed, anorexic, and have respiratory signs including cough. Since SIV does not cause viremia,
abortion is thought to result from maternal illness and stress, so examination of fetuses will be unrewarding. A diagnosis is established by detecting the virus in nasal swabs of sick sows or demonstrating seroconversion in paired serum samples.

When abortion results from fetal infection, the agent can generally be detected in fetal tissues and/or placentae if sufficient numbers are examined. A number of studies have demonstrated that during the period of maternal bacteremia or viremia, typically only a few to several fetuses become infected, which may be followed by in utero spread from fetus to fetus (Nielsen et al. 1991). The consequence is that not all fetuses in a litter are infected at the time of abortion; hence, diagnosis of cause can be missed if only a few fetuses are evaluated. The number of fetuses to sample varies by agent, strain of the agent, and stage of gestation. A good general recommendation is to sample four to six fetuses per litter from at least three litters. This is based on a calculation to achieve 90–95% confidence that at least one infected fetus is represented in a submission, assuming a fetal infection rate of 50% and a litter size of 12 (Benson et al. 2002). For most diagnostic tests, pooling of samples from several fetuses generally has limited impact on diagnostic sensitivity, especially when tests such as polymerase chain reaction (PCR) are used.

The stage of gestation, large litter size, and in utero spread of pathogens influence the clinical presentation of reproductive disease with fetoplacental infection. Infection of a fetus can result in a range of outcomes, including embryonic death and resorption, fetal death and mummification, abortion of fresh or autolyzed fetuses, stillbirth, or weak-born offspring with increased neonatal mortality (Christianson 1992). Infection early in gestation may lead to embryonic death and resorption of some but not all fetuses, resulting in decreased litter size. Progressive in utero spread of the pathogen between fetuses may lead to fetal death and mummification, with variation in mummy size. Fetuses infected after 70 days of gestation can mount an immune response to a number of agents and may be born live but seropositive to the pathogen (Nielsen et al. 1991). Clinically, this manifests as variably sized mummified fetuses with a few apparently normal, live-born piglets.

**Porcine Abortion: Serology**

Since most infectious abortions are the result of fetal infections, which occur ≥14 days after the dam became infected, antibody is often demonstrable in the dam at the time of abortion. In this situation, paired serum samples are of limited diagnostic value because significant titer changes will not be detected 2 weeks postabortion. The absence of antibody in dam serum may help rule out a particular cause. In contrast, a sow aborting due to acute systemic illness will not yet have seroconverted at the time of abortion, and acute and convalescent samples will demonstrate a rise in titer in relation to the abortion. Both scenarios can occur simultaneously within a herd.

Sows that are systemically ill likely have acute infection; hence, both acute and convalescent serum samples should be collected. The acute serum sample can also be used for direct diagnosis of systemic agents such as PRRSV, detectable by PCR.

A single postabortion serum sample may be useful in cases where antibody is present for an agent not thought to be present herd (e.g., PRRSV) or when very high titers are encountered and unexpected, such as to *Leptospira* in animals not recently vaccinated. Titers to endemic agents such as PPV, porcine circovirus type 2 (PCV2), or vaccine antigens are difficult to interpret without paired or sequential testing, and at best are indirect evidence for causation.

Serology performed on fetal fluids (e.g., thoracic fluid) is not reliable. There is strong propensity for nonspecific positive reactions for a multitude of agents in fluids from aborted porcine fetuses.

**Diagnosis of Infectious Abortion**

Abortions represent a conspicuous contrast to most other disease diagnostic investigations where submission of quality samples from representative, acutely affected, untreated animals results in a diagnosis in a high percentage of cases. Diagnostic laboratory records commonly report a high percentage of abortion diagnoses as “idiopathic,” that is, no evidence (gross or microscopic lesions, no agents detected) for infectious causation (Table 20.5).

Diagnosis of embryonic and fetal death is challenging due to the broad array of processes that have been associated with in utero mortality, including genetic, hormonal, nutritional, toxic, traumatic, metabolic, hypoxic, and infectious causes (Christianson 1992). Even with excellent specimens and tests, a definitive diagnosis in only one-third of abortion cases suggests many are not infectious and reinforces the need for scrupulous and complete clinical investigation prior to initiating diagnostic testing. Submission of specimens from sporadic or individual abortions is unlikely to

<table>
<thead>
<tr>
<th>Type of Cause</th>
<th>Number of Cases</th>
<th>Percent of Abortions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Idiopathic</td>
<td>942</td>
<td>67</td>
</tr>
<tr>
<td>Infectious</td>
<td>409</td>
<td>30</td>
</tr>
<tr>
<td>Viral</td>
<td>318</td>
<td>23</td>
</tr>
<tr>
<td>Bacterial</td>
<td>89</td>
<td>6.4</td>
</tr>
<tr>
<td>Fetoplacental inflammation, undetermined cause</td>
<td>40</td>
<td>3</td>
</tr>
</tbody>
</table>

Table 20.5. Diagnostic results on 1396 porcine abortion cases submitted to the Iowa State University Veterinary Diagnostic Laboratory from 1/2003 to 1/2010.
yield diagnostic results of herd significance. Potential epidemiological indicators of contagious abortions include (1) current or previous illness in pregnant animals (fever, depression, off feed), which may have occurred 1–4 weeks prior to the onset of abortions; (2) abortion in specific subpopulations (newly introduced animals, gilts, etc.); (3) clusters of abortions; (4) progressive spread throughout the barn; and (5) abortion in unvaccinated animals.

**General Tissue Sampling Guidelines**

Lists of the preferred specimens to submit for routine porcine abortion diagnostics (Table 20.6) should represent at least four to six fetuses per litter. Submission of chilled intact fetuses is also acceptable, especially if fetuses are cooled and shipped in such a way as to minimize further autolysis. Specimens should include placenta (chorioallantois) because the presence or absence of placental lesions can assist in categorizing the abortion as infectious or noninfectious and also aid in interpreting the significance of bacteria isolated from fetal tissues. Fetuses that die in utero and dehydrate in the absence of bacterial infection are said to be mummified, a common feature of viral-induced reproductive disease. The crown-rump length of fetuses/mummies should be measured to obtain an estimate of fetal age at the time of in utero abortion or death; a reasonable estimate of age in days is by measuring the crown-rump length (millimeter), dividing by 3, and adding 21.

Histopathology is a very useful tool for routine diagnostic testing by establishing a likely causative role for a detected organism and determining if inflammatory or necrotizing lesions suggest the presence of an infectious abortion where an organism is not detected by laboratory testing. If the epidemiology suggests an infectious process, lesions are present, and an agent is not detected on initial testing, then diagnostic testing should be expanded in an attempt to identify less common infectious agents.

**Viruses Affecting Reproduction**

In contrast to other domestic species, virus infections are clearly the most common infectious cause of reproductive wastage. PRRSV currently accounts for nearly 60% of infectious abortions in the United States (Table 20.7). Infection of the fetoplacental unit is common, although maternal illness can be the primary (e.g., SIV) or contributing cause of abortion with a number of viruses (e.g., PRRSV).

Although a large number of viruses have been associated with reproductive disease in swine, PRRSV, PCV2, and PPV are by far the most common in North America. Other abortifacient viruses indigenous to the United States include SIV, encephalomyocarditis virus (EMCV), porcine enteroviruses (PEVs), porcine teschoviruses (PTVs), bovine viral diarrhea virus (BVDV)/border disease virus (BDV), swine vesicular disease, porcine reproductive and neurological syndrome (PRNS) virus, porcine cytomegalovirus (PCMV), and, in feral pigs, PRV. In some countries, reproductive disease occurs as a result of infection with Japanese encephalitis virus (JEV), classical swine fever (hog cholera; CSF), African swine fever, Menangle virus, and La Piedad-Michoacan virus (blue eye).

**Diagnosis of Viral Abortion**

Gross lesions that may indicate viral-induced reproductive disease include an increased number of mummified fetuses, the presence of fetal anomalies in multiple

---

**Table 20.6.** Fetal tissue sampling guidelines in cases of porcine abortion

<table>
<thead>
<tr>
<th>Formalin-fixed Tissues</th>
<th>Fresh-chilled Tissues</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brain</td>
<td>Brain</td>
</tr>
<tr>
<td>Heart*</td>
<td>Heart</td>
</tr>
<tr>
<td>Lung</td>
<td>Lung</td>
</tr>
<tr>
<td>Liver</td>
<td>Liver</td>
</tr>
<tr>
<td>Spleen</td>
<td>Spleen</td>
</tr>
<tr>
<td>Kidney</td>
<td>Kidney</td>
</tr>
<tr>
<td>Placenta</td>
<td>Placenta</td>
</tr>
<tr>
<td>Thymus</td>
<td>Fetal thoracic fluid*</td>
</tr>
<tr>
<td></td>
<td>Fetal stomach contents*</td>
</tr>
<tr>
<td></td>
<td>Serum ± nasal swab, other specimens from aborting sows</td>
</tr>
</tbody>
</table>

*Heart is a target tissue for PCV2 and EMCV in the fetus. Thymus is valuable for PRRSV immunohistochemistry (IHC).

Fetal thoracic fluid is easily pooled and is equivalent to tissue pools for PRRSV PCR. It is also the preferred sample to detect fetal seroconversion.

Fetal stomach contents are a minimally contaminated specimen for bacterial culture and can easily be harvested by puncturing a syringed needle through the wall of the stomach and aspirating.

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**Table 20.7.** Common agents detected in 409 porcine cases classified as infectious abortions at Iowa State University Veterinary Diagnostic Laboratory from 1/2003 to 1/2010

<table>
<thead>
<tr>
<th>Agents</th>
<th>Number of Diagnoses (N = 409)</th>
<th>Percent of Infectious Abortions</th>
</tr>
</thead>
<tbody>
<tr>
<td>PRRSV</td>
<td>232</td>
<td>57</td>
</tr>
<tr>
<td>PCV2</td>
<td>52</td>
<td>13</td>
</tr>
<tr>
<td>PPV (mummified fetuses)</td>
<td>34</td>
<td>8.3</td>
</tr>
<tr>
<td>Leptospira*</td>
<td>24</td>
<td>5.9</td>
</tr>
<tr>
<td>Streptococcus sp. (Streptococcus suis most common)</td>
<td>12</td>
<td>2.9</td>
</tr>
<tr>
<td>E. coli</td>
<td>11</td>
<td>2.7</td>
</tr>
<tr>
<td>A. pyogenes</td>
<td>6</td>
<td>1.5</td>
</tr>
<tr>
<td>Staphylococcus sp. (S. hyicus most common)</td>
<td>5</td>
<td>1.2</td>
</tr>
<tr>
<td>Salmonella sp.</td>
<td>2</td>
<td>0.5</td>
</tr>
</tbody>
</table>

*The number of Leptospira abortions is artificially elevated as 71% of the cases (17/24) were from a single large outbreak.
litters (e.g., cerebellar hypoplasia, arthrogryposis), segmental umbilical cord hemorrhage and edema (PRRSV), multiple pinpoint foci of necrosis (PRV), and gross lesions indicative of heart failure (PCV2, EMCV). Microscopic lesions are valuable indicators of viral abortion and are present more frequently than gross lesions. Microscopic lesions that may signal viral involvement include nonsuppurative encephalitis (PRRSV, EMCV, PRV, PEV/PTV, BV/DV-BDV), myocarditis (PRRSV, EMCV, PCV2), interstitial pneumonia (PRRSV, PCV2, PRV), and necrotizing lesions in multiple tissues (PRV).

Preferred fetal tissues for laboratory testing may vary, but submission of tissues as described in the general sampling guidelines should be adequate for all viral agents. Fetuses with autolysis or desiccation (mummified) are common with viral infections but of minimal value for virus isolation. However, viral antigen and/or nucleic acid may still be detectable by fluorescent antibody test (FAT) or PCR, especially for PPV or PCV2. Virus isolation is best performed on fresh dead fetuses, weak-born piglets, or specimens from sick sows.

PCR tests provide rapid results and are sensitive, specific, and less impacted by autolysis. However, the exquisite specificity may not detect closely related strains (e.g., PRRSV), may not be optimized for particular specimen types, and does not offer broader capabilities of virus isolation. Discarding conventional viral isolation techniques in favor of specific methods is partially responsible for the current dearth of information on the diagnostic frequency of less common viral causes of abortion. When routine testing for viruses is negative, virus isolation should be considered, particularly if fetal anomalies, mummification, interstitial pneumonia, myocarditis, encephalitis, or necrotizing lesions have been identified.

**Bacterial and Fungal Abortion**
A wide variety of bacteria and fungi have been isolated from the products of porcine abortion (Eustis et al. 1981; Kirkbride and McAdaragh 1978) but are not commonly diagnosed as herd problems. Maternal bacteria leading to infection of the fetoplacental unit is considered to be the most common mechanism of bacterial abortion in swine, although the physiological effects of acute bacterial septicemia can also result in abortion. Fungi are only reported to account for roughly 0.3% of porcine abortions (Kirkbride and McAdaragh 1978). Bacteria isolated from aborted fetuses can be broadly divided into three categories: (1) primary contagious causes of abortion (abortion is the principal sign of infection), (2) secondary contagious causes of abortion (abortion as a sequel to maternal infection), and (3) noncontagious bacterial causes of abortion. The herd significance of various bacterial species differs considerably.

Infectious is not synonymous with contagious when considering bacterial abortion in swine. The majority of bacteria isolated from aborted porcine fetuses are organisms that are not considered to be contagious pathogens of adult swine (e.g., *E. coli, A. pyogenes, Staphylococcus aureus*). As such, these bacteria are typically isolated from individual animal and sporadic abortions, and are of limited significance at the herd level.

*Leptospira* and *Brucella suis* are the primary, abortion-causing bacteria of swine. *Leptospira*-associated reproductive disease in pigs is currently uncommon because of the management practices, which bolster immunity (vaccination) or diminish exposure to the organism (eliminate access to wildlife reservoirs and untreated surface waters). Regulatory programs based on serological testing and removal of seroreactive herds have lead to the virtual elimination of *B. suis* from domestic populations in the United States, northern Europe, and Australia. However, there is the potential for reintroduction of *B. suis* into commercial populations from feral swine.

Contagious bacteria of adult swine capable of causing a bacteremia and outbreaks of abortion include *Erysipelothrix, Actinobacillus suis*, and *Salmonella* sp. (Mauch and Bilkei 2004). The herd significance depends on both the pathogenic potential of the organism and the level of herd immunity. Management changes intended to produce high-health pigs may result in breeding stock that lack immunity to common contagious pathogens of nursery and grow–finish pigs; for example, naïve gilts may suffer outbreaks of bacteremia/septicemia with subsequent abortion due to agents that have historically caused disease in younger swine. If the significance of a potentially contagious bacterium isolated from fetal tissues (e.g., *Staphylococcus hyicus*) is unclear in an abortion outbreak, multiple aborted litters should be evaluated to determine if the organism is consistently present before ascribing herd significance or primary cause.

**Diagnosis of Bacterial Abortion**
Gross lesions indicative of bacterial abortion are uncommon but may include fetal icterus (*Leptospira*); a scant fibrinous exudate on the surface of abdominal or thoracic viscera; or gross placental hemorrhage, necrosis, or exudates. Microscopic lesions are identified more commonly than gross lesions and are most consistently observed in the placenta (68%) and lungs (62%). Fresh and formalin-fixed placentas should be routinely examined.

Preferred fetal tissues for bacterial culture are lungs and stomach contents; kidney is useful to assess for *Leptospira*. Interpreting the role of bacteria isolated from the placenta is problematic because of maternal and environmental contamination, hence the need for microscopic examination for placentitis.
Toxins Associated with Abortion and Infertility

Abortion and infertility associated with exposure to toxins can impact multiple animals in breeding herds and, to a degree, mimic contagious abortions. The two most common toxins associated with reproductive wastage in swine are carbon monoxide and zearalenone.

Carbon monoxide (CO) poisoning is usually the result of inadequate combustion of fuels in heated facilities. Cherry-red discoloration of fetal subcutaneous tissue, muscle, abdominal, and thoracic viscera is a hallmark for carbon monoxide poisoning, confirmed by detection of carboxyhemoglobin levels >2% in fetal thoracic fluid (Carson 1990).

Zearalenone is an estrogenic mycotoxin, which, despite the structural dissimilarity to steroidal estrogens, functions as a weak estrogen, binds receptors for estradiol-17β, and promotes signs of hyperestrogenism (Meerdink 2004). Nongravid sows exposed early in the estrous cycle develop persistent estrus, whereas exposure in midcycle leads to anestrous and pseudopregnancy. Abortion and stillbirth are not features of zearalenone toxicosis (Kirkbride and McAdaragh 1978). A summary of infectious and toxic causes of reproductive wastage in swine is presented in Table 20.8.

Table 20.8. Infectious and toxic diseases causing abortion, stillbirth, and mummification in swine

<table>
<thead>
<tr>
<th>Disease</th>
<th>Signs in Sow</th>
<th>Reproductive Manifestations</th>
<th>Gross Fetal Lesions</th>
<th>Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>PRRSV</td>
<td>Mild depression, anorexia, fever</td>
<td>Late-term abortion, stillbirths, weak-born pigs</td>
<td>Meconium staining of fetal skin, umbilical cord edema and segmental hemorrhage, perirenal edema, mesocolonic edema</td>
<td>PCR on pooled fetal thoracic fluid from four to six fetuses per litter; PCR on dam serum if acutely ill at time of abortion</td>
</tr>
<tr>
<td>PCV2</td>
<td>None</td>
<td>Weak births, stillbirths, fetal mummification</td>
<td>Dilated and hypertrophied heart; fluid in the body cavities; enlarged, congested liver</td>
<td>Immunohistochemistry (IHC), PCR on fetal heart</td>
</tr>
<tr>
<td>Parvovirus (PPV)</td>
<td>None</td>
<td>Embryonic death and resorption (small litter size), fetal mummification</td>
<td>Fetal mummification (3- to 16-cm crown-rump length)</td>
<td>FAT/PCR mummified fetuses (lungs); fetal serology on stillborn fetuses</td>
</tr>
<tr>
<td>Pseudorabies virus (PRV)</td>
<td>Generally none</td>
<td>Embryonic death, fetal mummification, abortion, stillborn, and weak-born pigs</td>
<td>Multifocal, random, pinpoint white foci (necrosis) in liver, spleen, and possibly lungs</td>
<td>FAT, PCR, virus isolation (VI) on lungs, liver, spleen, brain, and kidneys</td>
</tr>
<tr>
<td>Influenza virus A (SIV)</td>
<td>Fever, lethargy, coughing, dyspnea</td>
<td>Infertility, decreased litter size, abortion, and stillbirths</td>
<td>None</td>
<td>PCR, VI, or antigen capture ELISA on nasal swabs/fresh lungs from aborting sows; acute and convalescent serum samples</td>
</tr>
<tr>
<td>Encephalomyocarditis virus (EMCV)</td>
<td>None</td>
<td>Decreased farrowing rates, abortion, mummification, stillbirths, weak-born pigs</td>
<td>Chalky white foci in the heart, hydrothorax, hydropericardium, ascites</td>
<td>PCR on tonsil, kidneys, spleen, lungs, placenta</td>
</tr>
<tr>
<td>Enterovirus, teschovirus</td>
<td>None</td>
<td>Infertility, embryonic death, mummification, stillbirths, neonatal mortality</td>
<td>None</td>
<td>PCR on fetal lungs; fetal serology</td>
</tr>
<tr>
<td>Classical swine fever</td>
<td>Fever, reduced feed intake, depression, ataxia, conjunctivitis, constipation, cachexia, and cutaneous erythema</td>
<td>Embryonic death and resorption, abortion, mummification, stillbirths, fetal malformations, and increased neonatal mortality</td>
<td>Ascites, widespread petechiation, pulmonary hypoplasia, malformations, micrognathia, cerebellar hypoplasia, microcephaly</td>
<td>PCR on tonsil, kidneys, spleen, lungs, and placenta</td>
</tr>
<tr>
<td>Bovine viral diarrhea (BVD)/border disease virus (BDV)</td>
<td>None</td>
<td>Poor conception rates, small litters, abortions, stillbirths, congenital anomalies, increased neonatal mortality</td>
<td>Cerebellar hypoplasia</td>
<td>VI, FAT, PCR on brain, spleen, lungs, and kidneys</td>
</tr>
</tbody>
</table>

(Continued)
### Table 20.8. (Continued)

<table>
<thead>
<tr>
<th>Disease</th>
<th>Signs in Sow</th>
<th>Reproductive Manifestations</th>
<th>Gross Fetal Lesions</th>
<th>Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Porcine reproductive and neurological syndrome (PRNS)</td>
<td>None</td>
<td>Infertility, abortion</td>
<td>None</td>
<td>VI, FAT tonsil, spleen, and lymph node</td>
</tr>
<tr>
<td>Japanese encephalitis virus (JEV)</td>
<td>None</td>
<td>Abortion, fetal mummification, stillborn or weak piglets</td>
<td>Subcutaneous edema, hydrocephalus, cerebellar hypoplasia, bicavitary effusions, serosal petechia</td>
<td>VI, FAT, IHC, PCR brain, liver, spleen, lungs, and placenta; fetal serology</td>
</tr>
<tr>
<td>Porcine cytomegalovirus (PCMV)</td>
<td>None</td>
<td>Decreased litter size, fetal mummification, stillbirths, neonatal mortality</td>
<td>Interlobular pulmonary edema</td>
<td>VI, FAT, PCR; fetal lungs</td>
</tr>
<tr>
<td>Rubulavirus (La Piedad-Michoacan virus)</td>
<td>Transient anorexia, occasional corneal opacity</td>
<td>Infertility, stillbirths, fetal mummification, infrequent abortion, reduced number of live-born piglets, stillborn fetuses often with congenital anomalies</td>
<td>Fetal mummification</td>
<td>VI, FAT, PCR on brain, lungs, liver, and placenta</td>
</tr>
<tr>
<td>Menangle virus</td>
<td>None</td>
<td>Infertility, abortion, stillbirths, abortion, stillbirths, and weak-born pigs</td>
<td>Malformations including arthrogryposis, brachygnathia, kyphosis, pulmonary hypoplasia and a variety of central nervous system (CNS) abnormalities</td>
<td>VI brain, lungs, and heart; serology</td>
</tr>
<tr>
<td>Leptospira</td>
<td>Transient fever, anorexia, and depression</td>
<td>Infertility, fetal mummification, abortion, stillbirths, and weak-born pigs</td>
<td>Occasional fetal jaundice</td>
<td>FAT, IHC, PCR on fetal kidneys</td>
</tr>
<tr>
<td>Brucella suis</td>
<td>None</td>
<td>Infertility, abortion, stillbirth, and weak-born pigs</td>
<td>Placentitis</td>
<td>Culture fetal stomach content, liver, lungs, and pericardial fluid</td>
</tr>
<tr>
<td>Chlamydia sp.</td>
<td>None</td>
<td>Abortion, infertility</td>
<td>None</td>
<td>IHC, PCR, antigen capture ELISA on placenta, liver Carboxyhemoglobin levels on fetal thoracic fluid</td>
</tr>
<tr>
<td>Carbon monoxide</td>
<td>Typically none</td>
<td>Abortions, stillbirths, weak-born piglets</td>
<td>Cherry-red discoloration of subcutaneous tissue, muscle, abdominal, and thoracic viscera</td>
<td></td>
</tr>
<tr>
<td>Zearalenone</td>
<td>Infertility, mammary enlargement, swelling of vulva, persistent estrus, anestrus, pseudopregnancy</td>
<td>Infertility, embryonic death, small litter size, small pigs, piglets with hyperestrogenism</td>
<td>None</td>
<td>Feed analysis</td>
</tr>
</tbody>
</table>

**REFERENCES**


INTRODUCTION

In most swine-producing areas, large groups of pigs are housed under intensive conditions, often in geographical regions with a dense pig population. High stocking density in a closed environment facilitates transmission of airborne pathogens within a herd (Buddle et al. 1997; Donham 1991) and between herds (Christensen et al. 1993; Stark et al. 1992). Worldwide, respiratory diseases continue to cause substantial losses to the swine industry (Bak et al. 2008; Dee et al. 1997; Garner et al. 2001; Grandia et al. 2010; Jager et al. 2010; Neumann et al. 2005; Pejsak et al. 1997; Sales et al. 2010). Financial losses are often due to increased mortality; decreased weight gain; increased feed costs; increased condemnation at slaughter; and increased costs for treatments, vaccination, and labor. Consequently, respiratory diseases are regarded as some of the most serious disease problems in modern swine production.

STRUCTURE OF THE NORMAL RESPIRATORY SYSTEM

The mature respiratory tract is composed of the nasal cavity, nasopharynx, larynx, the tubular conducting airways including trachea, intra- and extrapulmonary bronchi, bronchioles, and the gas exchange system including terminal bronchioles and alveoli where gas exchange occurs. The lungs and chest cavity are covered in a thin, translucent pleural membrane.

There are two separate blood-conducting systems in the lungs. The pulmonary artery system supplies venous blood from the right ventricle to the capillary plexus surrounding the pulmonary alveoli. The intimate association of this large vasculature network and the large airspace of the alveoli can serve as a portal of entry for pathogens into the body. The supporting structures around the trachea, bronchi, and bronchioles are vascularized with blood from the bronchial tree.

Conducting Airways

The nasal cavity is divided longitudinally by a cartilaginous nasal septum. Two turbinate bones divide each of the two halves of the nasal cavity into three meatuses: dorsal, middle, and ventral (Figure 21.1). The length of the nasal cavity varies between different breeds.

The trachea is relatively short and divides posteriorly into two primary bronchi, one for the left lung and one for the right lung. A separate, small bronchus branches from the trachea leading to the right cranial (apical) lobe. Bronchi branch into the left cranial (apical) lobe, left and right middle (cardiac) lobes, left and right caudal (diaphragmatic) lobes, and the right accessory (intermediate) lobes as outlined in Figure 21.2. The finest branches of the tubular system are the bronchioles, each dividing into alveolar ducts and alveoli.

The vestibular region of the nasal cavity is lined with stratified squamous epithelium. The rest of the nasal cavity is lined by ciliated pseudostratified epithelium with mucus-producing goblet cells. This ciliated epithelium is covered by a bilayered mucus coating produced by the goblet cells and mucosal glands, and this epithelium continues through the pharynx, larynx, trachea, and bronchi. As the bronchioles approach the alveoli, the epithelium is reduced in height, becoming more squamous. Alveoli are lined by very flat, single-layered epithelial cells (type I pneumocytes) and by a small percentage of cuboidal epithelial cells (type II pneumocytes). Type II pneumocytes produce...
FUNCTION AND DEFENSE OF THE NORMAL RESPIRATORY SYSTEM

Gas exchange occurs in the pulmonary alveoli. Each breath renews only a small portion of the total alveolar air volume. In the resting pig, 10–15% of the alveolar air is exchanged per inspiration. The normal respiratory rate (breaths/minute) varies according to age of the animal (piglets and growing pigs, 25–40; finishing pigs, 25–35; sows in gestation, 15–20).

The mucosal surface of the respiratory tract provides a critical interface between the pig and its environment. The alveolar surface area is very large (in humans more than 100 m²). This large surface area is continuously exposed to the environment through inspired air. So, the respiratory tract is equipped with a potent and

Table 21.1. Relative weights of lung lobes as percentages of total lung weight in 90- to 100-kg pigs

<table>
<thead>
<tr>
<th>Study</th>
<th>N</th>
<th>Left Lung Lobes</th>
<th></th>
<th>Right Lung Lobes</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Cranial Middle Caudal</td>
<td></td>
<td>Cranial Middle Caudal Accessory</td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>11</td>
<td>7 7 32</td>
<td></td>
<td>12 6 34</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>20</td>
<td>5 7 32</td>
<td></td>
<td>6 9 36</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>13</td>
<td>5 6 29</td>
<td></td>
<td>11 10 34</td>
<td></td>
</tr>
</tbody>
</table>

Note: A = Morrison et al. (1985); B = Heilmann et al. (1988); C = W. Christensen (unpublished data). Rounding numbers caused a total percentage of 101%. Adapted from the previous edition of Diseases of Swine (Sorensen et al. 2006).

N', number of pigs examined in each study.
specialized defense system. Important components of the respiratory defense are listed in Table 21.2.

### Conditioning and Filtering by the Conducting Airways

Air is well filtered and properly conditioned before it reaches the alveoli. Inspired air is warmed by the extensive capillary network superficially located in the nasal mucosa and is moistened by the mucus bilayer. Most inspired particles are trapped in the mucus layer over of the nasal, pharyngeal, laryngeal, and tracheal epithelium. High air velocity in nasal cavity coupled with marked air turbulence around nasal turbinates results in impaction of larger airborne particles (>30 µm) onto the mucus bilayer lining the nasal mucosa. Particles greater than 10-µm diameter mostly removed before reaching the bronchial tree (Baskerville 1981). Particles trapped in the mucus are handled by the mucociliary clearance mechanism. The ciliary carpet in the bronchi and bronchioli gives rise to a continuous flow of mucus toward the pharynx. The rhythmic beating of cilia results in a mucus flow of about 4–15 mm/min (Done 1988). Like the mucus from the nasal cavity, mucus from the trachea and bronchi is delivered to the pharyngeal cavity and subsequently swallowed. Particles less than 5 µm in diameter are able to reach the alveoli. The primary defense against particulates in the alveolus is the alveolar macrophages.

### Phagocytes

Alveolar macrophages remove foreign material that escapes the mucociliary defense mechanism. Non-pathogenic particles and microbes are handled by simple phagocytic activity and are removed in the mucus flow or by the lymphatic system. Pathogenic microorganisms are neutralized with the aid of secretions such as lysozyme, interferons, proteolytic enzymes and enzyme inhibitors, opsonins, lactoferrins, complement factors, oxygen radicals and free radical scavengers, and specific immunoglobulins in the mucus. Neutrophils, with phagocytic activity and a potent array of pathogen-killing enzymes, are recruited from the blood into the alveolus and assist the macrophages in the phagocytic activity. In healthy pigs, the normal ratio between the cellular elements in the bronchoalveolar mucus is 70–90% alveolar macrophages, 5–18% lymphocytes, 4–12% neutrophils, and up to 5% eosinophilic granulocytes (Jolie et al. 2000; Neumann et al. 1985). The phagocytic cell system also comprises intravascular macrophages, which in the pig are particularly numerous in the pig lungs (Bertram 1985; Ohgami et al. 1989).

If the invading agents are not neutralized by the alveolar macrophages, the activity of the phagocytes is highly accelerated and inflammation or tissue damage can result. Proinflammatory cytokines produced by macrophages play an important role in porcine respiratory disease by coordinating and activating the adaptive immune response, which enables the host to eliminate pathogens (Thanawongnuwech et al. 2004). This adaptive immune response ideally ends in cell-mediated immunity and local and systemic production of specific antibodies.

### Immunoglobulins

The production of specific antibodies is crucial in the respiratory immune defense. Immunoglobulin A (IgA) is the predominant antibody in the mucus of the conducting airways. IgM antibodies are potent proteins released in the early immune response, particularly in the newborn pig. IgG antibodies, which originates in the blood, are the predominant immunoglobulin in the mucus of the lower respiratory tract near the alveoli. Immunoglobulins in the mucus carpet act primarily to prevent the initial establishment and penetration of pathogens. IgE antibodies contribute to the immune response against parasites such as lungworms (Metastrongylus sp.) and migrating ascarid larvae.

### Cell-mediated Immune Response

Immunity is divided into a humoral immune response, in which the immunoglobulins play an essential role, and a cell-mediated immune (CMI) response based on antibody-independent components. However, immunity cannot be distinctly separated into these two parts, because many mechanisms are closely linked and dependent on each other. Generally, CMI is identified with cytotoxic T cells, natural killer (NK) cells, activated macrophages, and cells mediating antibody-dependent cytotoxicity. The CMI response not only is particularly important in viral infections, such as influenza, porcine reproductive and respiratory syndrome (PRRS), porcine circovirus type 2 (PCV2) infection, and

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Table 21.2. Physical, humoral, and cellular defense mechanisms in the respiratory tract

<table>
<thead>
<tr>
<th>Physical/chemical</th>
<th>Humoral components</th>
<th>Cellular</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hair in the nostrils</td>
<td>Mucus contents and multiple types of mucus flow or by the lymphatic system.</td>
<td>Alveolar macrophage</td>
</tr>
<tr>
<td>Turbinates (tortuous airflow)</td>
<td>Physical/chemical</td>
<td>Intravascular macrophage</td>
</tr>
<tr>
<td>Ciliated epithelium (coordinated movement of particles)</td>
<td>Humoral components</td>
<td>Neutrophils</td>
</tr>
<tr>
<td>Mucus (adhesiveness and contains nonspecific lysozymes, interferons, opsonins, lactoferrins, α1 antitrypsin)</td>
<td>Mucus (adhesiveness and contains nonspecific lysozymes, interferons, opsonins, lactoferrins, α1 antitrypsin)</td>
<td>Monocytes</td>
</tr>
<tr>
<td>Interleukins, complement factors, antibodies, etc.</td>
<td>Interleukins, complement factors, antibodies, etc.</td>
<td>Eosinophils</td>
</tr>
<tr>
<td>Adapted from the previous edition of Diseases of Swine (Sorensen et al. 2006).</td>
<td>Adapted from the previous edition of Diseases of Swine (Sorensen et al. 2006).</td>
<td>Plasma cells</td>
</tr>
<tr>
<td>T lymphocytes (helper lymphocytes, suppressor lymphocytes, cytotoxic lymphocytes, natural killer cells)</td>
<td>T lymphocytes (helper lymphocytes, suppressor lymphocytes, cytotoxic lymphocytes, natural killer cells)</td>
<td>T lymphocytes (helper lymphocytes, suppressor lymphocytes, cytotoxic lymphocytes, natural killer cells)</td>
</tr>
</tbody>
</table>
pseudorabies virus (PRV, Aujeszky’s disease), but is also assumed to play an important role in *Mycoplasma hyopneumoniae* infection (Fort et al. 2008; Lowe et al. 2005; Maes 2010; Tsai et al. 2010).

Toll-like receptors are pathogen-associated molecular recognition receptors responsible for signaling intrusion by pathogens. These receptors are present on porcine NK cells and appear to perform an important role in innate and adaptive immunity to viral pathogens affecting pigs (Toka et al. 2009). Surface proteins belonging to the swine leukocyte antigen (SLA) gene complex or major histocompatibility complex (MHC) play a significant role in the cellular and humoral immune response (Mallard et al. 1989; Swindle 2007). Genetic variability of the SLA complex has been demonstrated in different breeds of pigs (Vaiman et al. 1979).

Since placentas of pregnant sows are impermeable to immunoglobulin passage, the neonates are born without antibodies. Although immunocompetent, piglets are unable to rapidly develop an active immune response to protect them against respiratory diseases. Their survival depends on the passive acquisition of maternal immunity including at least three components: (1) systemic humoral immunity, transmitted through colostrum; (2) mucosal humoral immunity, transmitted through milk; and (3) cellular immunity, transmitted via maternal immunocompetent cells present in mammary secretions (Salmon 2000).

Piglets are capable of intestinal absorption of intact immunocompetent maternal lymphocytes from colostrum (Bandrick et al. 2006; Tuboly and Bernath 2002; Tuboly et al. 1988). Williams (1993) showed the distribution of immune cells to the liver, lungs, lymph nodes, spleen, and gastrointestinal tissue of the piglets by 24 hours postfeeding.

Mucosal immunity plays an important role in respiratory protection, suggesting that aerosol-based vaccines may have value against respiratory diseases. Antigenically intact macromolecules can move from the lumen of the respiratory tract through the epithelium into the blood during the first days of life and also to a considerable degree in older pigs (Folkesson et al. 1990). Mucosal lymphoid tissue also plays a role in antigen uptake. Vaccination experiments with PRV in young piglets with passively acquired antibodies indicate that the systemic immune mechanism can be bypassed and a local immune response obtained by application of antigen on the respiratory epithelium (Schlesinger et al. 1990). Nielsen et al. (1990) obtained good immunological protection against *Actinobacillus pleuropneumoniae* with local application of antigens in the respiratory tract. However, local and systemically induced mucosal immunity using subunit vaccines has had variable success in protecting against the *M. hyopneumoniae* challenge (Maes 2010; Murphy et al. 1993; Ogawa et al. 2009). Additional research will be needed to determine if aerosol-based vaccines will be a viable practice option.

**PRIMARY AND SECONDARY PATHOGENS IN THE LUNGS**

The upper respiratory tract is the natural habitat for many potential pathogens, including viruses, mycoplasmas, chlamydias, and many other bacteria. The commensal flora may have a favorable competitive effect for their host by outnumbering pathogenic agents. There is no distinct division between commensals and potentially pathogenic microorganisms. Different studies categorize the same microorganism as either commensal or potentially pathogenic. For example, *Mycoplasma flocculare, Mycoplasma hyorhinis*, and *Haemophilus parasuis* belong to a group of microorganisms that can regularly be isolated in the upper respiratory tract of healthy pigs.

Bacterial flora of live, healthy pigs that are considered potential or secondary pathogens includes *M. hyorhinis*, streptococci (nonhemolytic, alpha-hemolytic), staphylococci, *Escherichia coli*, *Klebsiella*, *Arcanobacterium pyogenes*, *Bordetella bronchiseptica*, *H. parasuis*, and V factor-dependent *Pasteurellaceae* (Amass et al. 1994; Castryck et al. 1990; Gaemperli et al. 1990; Hansen et al. 2010; Lambotte et al. 1990; Moller and Kilian 1990). *Haemophilus parasuis* and *M. hyorhinis* behave as unimportant normal flora in immunocompetent pigs. When pulmonary defenses are compromised, these potential pathogens flourish resulting in severe systemic disease such as polyserositis, polysynovitis, and meningitis (Nielsen and Danielsen 1975). Disease outbreaks can occur in small, isolated herds that are naïve to certain pathogens such as herds with very early weaning or strict separation between animals of different ages, and in specific pathogen-free (SPF) herds established originally from cesarean-derived piglets (Nielsen and Danielsen 1975; Smart et al. 1989).

Some primary pathogens such as *A. pleuropneumoniae* and *M. hyopneumoniae* may be common at herd level but are relatively seldom isolated from healthy individuals (Castryck et al. 1990; Friis 1974). Their presence may be more commonly associated with subclinical infection more often than clinical disease (Regula et al. 2000; Rohrbach et al. 1993).

Primary and secondary bacterial pathogens behave differently, especially during the compromised period between waning passive and protective active immunity, for several reasons. First, *A. pleuropneumoniae* and *M. hyopneumoniae* have a higher pathogenicity than *H. parasuis* and *M. hyorhinis*. Second and very importantly, *H. parasuis* and *M. hyorhinis* infect the nasal and tracheobronchial epithelium very early in the piglet’s life (Ross 1984). This might facilitate a gradual development of active immunity under the cover of humoral colostral antibodies, which is beneficial to the host.
Also, the lesions induced by secondary pathogens like *H. parasuis* and *M. hyorhinis* occur in sites (pleura, pericardium, peritoneum, meninges, and joints) outside the respiratory tract. There is a clear physical barrier that exists between the infection site and the effector site of the microbe. In contrast, primary pulmonary pathogens like *A. pleuropneumoniae* and *M. hyopneumoniae* are easily delivered to the lungs directly from the environment or from the nasal and tonsillar epithelium.

Strain variation can influence disease outcome. Strains of low pathogenicity may generate protective antibodies against closely related but more pathogenic strains, as demonstrated for *A. pleuropneumoniae* (Nielsen et al. 1990). Also, pig breeds likely vary in genetic susceptibility to some primary respiratory pathogens (Hoeltig et al. 2010; Probst and Hoeltig 2010).

The constant presence of potential pathogens that are unlikely to be permanently excluded from the herd and that behave as secondary pathogens, like *H. parasuis*, *M. hyorhinus*, *Streptococcus suis*, or *Pasteurella multocida*, may be quite acceptable for the herd. However, the presence of primary pathogens that behave like *A. pleuropneumoniae* and *M. hyopneumoniae* can increase the risk of serious recurrent disease.

### Interaction between Infectious Agents

The term porcine respiratory disease complex (PRDC) was coined to emphasize the complexity of events leading to pneumonia, including the viral and bacterial pathogens as well as the environmental, management, and genetic factors (Brockmeier et al. 2002). PRDC is seldom the result of an infection with only one pathogen. Multiple pathogens are usually involved in respiratory disease (Brockmeier et al. 2001; Hansen et al. 2010; Palzer et al. 2008). Viruses and *Mycoplasma* sp. have long been known to predispose pigs to more severe bacterial infections (Brockmeier et al. 2008; Ellis et al. 2008; Harms et al. 2002; Kubo et al. 1995; Lai et al. 1986; Loving et al. 2010; Maes 2010; Pol et al. 1997; Scatozza and Sidoli 1986; Thacker et al. 1999, 2001; Van Reeth and Pensaert 1994; Yagihashi et al. 1984). Table 21.3 lists some common agent–agent interactions found in swine respiratory outbreaks.

Pathogens involved in PRDC vary tremendously between countries, regions, and farms (Thacker et al. 2010). Primary pathogens, such as respiratory epithelium-damaging viruses (influenza) predispose the pig to secondary invaders by lowering the local and systemic defense mechanisms of the host. Generally, primary pathogenic agents are viruses or mycoplasmas, and secondary invaders are bacteria. The most common viral agents involved with PDRC include porcine reproductive and respiratory syndrome virus (PRRSV), classical swine fever (CSF), swine influenza virus (SIV), PRV, and PCV2 (Brockmeier et al. 2002; Thacker et al. 2010). Torque teno virus 2, a relatively newly identified virus, may facilitate the development of PCV2-induced postweaning multisystemic wasting syndrome (Ellis et al. 2008) and perhaps causes an interstitial pneumonia, hence may play a role in PDRC (Krakowka and Ellis 2008; Lang et al. 2010). In addition to the typical SIVs affecting pigs (H1N1, H1N2, H3N2), a novel or human pandemic A/H1N1 influenza virus that was recognized worldwide in 2009 can cause gross and microscopic lesions in pigs that are similar to the typical influenza viruses affecting pigs (Capuccio et al. 2010; Kim et al. 2010; Lange et al. 2009; Smith et al. 2009; Valheim et al. 2010; Williamson et al. 2010). In the nasal cavity, *B. bronchiseptica* infection can be a predisposing cause for the invasion and replication of toxigenic strains of *P. multocida* (Pedersen and Barfod 1981), which is important in the pathogenesis of progressive atrophic rhinitis.

In general, combined infections are more severe than individual infections. However, pathogens can influence each other in interesting ways. Van Reeth and Pensaert (1994) demonstrated that clinical disease was more severe in pigs coinfected with SIV and porcine respiratory coronavirus (PRCV) than pigs infected with SIV or PRCV alone. Interestingly, prior infection with PRCV decreased the replication and shedding of SIV. SIV replication was likely inhibited by high interferon levels induced by PRCV.

### RESPIRATORY PATHOLOGY

Respiratory lesions can be categorized into three main disease entities: rhinitis, pneumonia, and pleuritis. Pneumonia (inflammation of the lungs) can be categorized based on gross morphological patterns to help predict some causes (viral, bacterial, and parasitic) of pneumonia (López 2007). Figure 21.1 illustrates the grossly identifiable morphological patterns of pneumo-

<table>
<thead>
<tr>
<th>Diseases</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bordetella bronchiseptica, PRRSV, Pasteurella multocida</td>
<td>Brockmeier et al. (2001)</td>
</tr>
<tr>
<td>SIV, Bordetella bronchiseptica</td>
<td>Loving et al. (2010)</td>
</tr>
<tr>
<td>Mycoplasma hyopneumoniae, PCV2</td>
<td>Opriessnig et al. (2005)</td>
</tr>
<tr>
<td>PRCV, Bordetella bronchiseptica</td>
<td>Brockmeier et al. (2008)</td>
</tr>
<tr>
<td>Torque teno virus, PCV2</td>
<td>Ellis et al. (2008)</td>
</tr>
<tr>
<td>SIV, Haemophilus parasuis,</td>
<td>Mousing (1991)</td>
</tr>
<tr>
<td>Actinobacillus pleuropneumoniae</td>
<td></td>
</tr>
<tr>
<td>PCV2, SIV, Mycoplasma</td>
<td>Dorr et al. (2007)</td>
</tr>
<tr>
<td>hyopneumoniae</td>
<td></td>
</tr>
<tr>
<td>Various viruses and various bacterial pathogens</td>
<td>Hansen et al. (2010)</td>
</tr>
</tbody>
</table>

PRRSV, porcine reproductive and respiratory syndrome virus; PCV2, porcine circovirus type 2; PRCV, porcine respiratory coronavirus; SIV, swine influenza virus.

Table 21.3. Some studies demonstrating multiple pathogen infections in swine
21.3. Schematic diagram of the patterns of pneumonia in swine. (A) Normal lungs; (B) suppurative bronchopneumonia with patchy cranioventral consolidation and accentuated lobular pattern; (C) fibrinous/necrotizing bronchopneumonia cranioventral consolidation and pleural fibrin; (D) interstitial pneumonia with noncollapsing lungs and faint rib imprints in all lobes; (E) embolic pneumonia with variably sized nodules scattered in many lobes.

Rhinitis and Tracheitis

Inflammation of the nasal mucosa is common in young animals. Many agents that affect the ciliated nasal epithelium can also affect the tracheal ciliated epithelium. Table 21.5 lists some common causes of rhinitis and sneezing in pigs. The cause is often infectious (pseudorabies disease virus, influenza virus, cytomegalovirus, B. bronchiseptica, toxigenic strains of P. multocida, M. hyorhinis), but air pollution such as elevated ammonia and dust in the air can initiate mucosal inflammation. Pasteurella multocida can colonize even a slightly damaged mucosa and induce progressive atrophic rhinitis with permanent distortion and shrinkage of turbinates (see Figure 21.4). The structural changes in atrophic rhinitis are the result of altered turbinate bone metabolism (Foged et al. 1987). While presumed to have an effect on growth rate, the economic impact associated with atrophic rhinitis is uncertain (Dumas et al. 1990; Rissing et al. 2002; Scheidt et al. 1990; Straw and Ralston 1986; Wilson et al. 1986).

A cilia-associated respiratory (CAR) bacillus has been identified in the trachea of pigs with other respiratory diseases, but its role as a pathogen remains unclear (Nietfeld et al. 1995, 1999).

Bronchopneumonia

Bronchopneumonia is a frequent lesion of the lungs in growing pigs and is characterized by cranioventral consolidation of the lungs. Bronchopneumonias are subclassified as suppurative (lobular) and fibrinous (lobar) bronchopneumonias by pathologists. Mycoplasma hyopneumoniae and/or respiratory viruses with secondary bacteria are the main causes of bronchopneumonia in
Table 21.4. Classification of pneumonias based on morphology

<table>
<thead>
<tr>
<th>Type of Pneumonia</th>
<th>Route of Pathogen Entry</th>
<th>Distribution of Lesions</th>
<th>Texture of the Lungs</th>
<th>Grossly Visible Exudate</th>
<th>Disease Example</th>
<th>Respiratory Signs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Suppurative bronchopneumonia (lobular)</td>
<td>Aerogenous</td>
<td>Cranioventral consolidation</td>
<td>Firm</td>
<td>White to green exudate in bronchi</td>
<td>Mycoplasma hyopneumoniae and secondary bacteria, Metastrongylus sp.</td>
<td>Deep productive cough</td>
</tr>
<tr>
<td>Fibrinous bronchopneumonia (lobar)</td>
<td>Aerogenous</td>
<td>Cranioventral consolidation with pleuritis</td>
<td>Very firm</td>
<td>Fibrin in the lungs and often on pleura</td>
<td>Actinobacillus pleuropneumoniae, Bordetella in baby pigs</td>
<td>Deep productive cough</td>
</tr>
<tr>
<td>Interstitial pneumonia</td>
<td>Aerogenous or hematogenous</td>
<td>Diffuse</td>
<td>Elastic with rib imprints</td>
<td>Not visible, trapped in alveolar septa</td>
<td>Viruses (SIV, PRRSV, PRCV, PCV2, TTV), ascarid migration, gram-negative septicemia, acute gas exposure</td>
<td>Hacking cough (SIV) or no cough with rapid breathing</td>
</tr>
<tr>
<td>Embolic pneumonia</td>
<td>Hematogenous (septic emboli)</td>
<td>Multifocal</td>
<td>Nodular</td>
<td>Purulent foci surrounded by hyperemia, abscesses or granulomas</td>
<td>Streptococcus, Actinobacillus suis, other pyogenic bacteria, tuberculosis</td>
<td>Often no cough or mild productive cough</td>
</tr>
</tbody>
</table>

*Secondary bacteria commonly associated with mycoplasmosis include Streptococcus suis, Pasteurella multocida, Staphylococcus aureus, and Arcanobacterium pyogenes.

SIV, swine influenza virus; PRRS, porcine respiratory and reproductive syndrome virus; PRCV, porcine respiratory circovirus; PCV2, porcine circovirus type 2; TTV, torque teno virus.

Table 21.5. Respiratory disease entities and agents associated with sneezing

<table>
<thead>
<tr>
<th>Disease</th>
<th>Agent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rhinitis</td>
<td>Bordetella bronchiseptica, cytomegalovirus, influenza virus, pseudorabies virus, hemagglutinating encephalomyelitis virus, dust, ammonia</td>
</tr>
<tr>
<td>Atrophic rhinitis</td>
<td>Bordetella bronchiseptica and toxigenic Pasteurella multocida</td>
</tr>
</tbody>
</table>

Adapted from the previous edition of Diseases of Swine (Sorensen et al. 2006).

growing pigs. Microscopically, small conducting airways and alveoli are filled with exudate. The affected portions of the lungs are very firm and, sometimes, friable. Cranioventral distribution of bacterial pathogens might be due to less effective defense mechanisms in this region and gravitational influence impeding clearance of exudate.

Suppurative bronchopneumonia is the most common lesion with M. hyopneumoniae infection. In uncomplicated mycoplasmal pneumonia, the cranioventral lung contains scattered purple lobules that are often more collapsed than normal lung tissue. The lesions are clearly demarcated from adjacent normal tissue by a sharp line following the interlobular septa. Whitish exudate may be expressed from the airway openings of affected tissue. Lesions with secondary bacterial infections may change to a more gray-to-green color, and the consistency will be firmer due to formation of fibrous tissue. Lungworms (Metastrongylus sp.) usually fill small airways and will grossly resemble a mild bronchopneumonia often affecting the edges of multiple lung lobes, even the caudal lobes.

Fibrinous/necrotizing bronchopneumonia often involves whole lung lobes that are very firm, turgid and often have fibrin on the pleural surface. Severe bacterial infections with pyogenic potential and some gram-negative bacteria can result in marked vascular damage leading to fibrin exudation. Affected tissue is frequently raised above the adjacent area and crosses interlobular septae. Actinobacillus pleuropneumoniae infection often causes a fibrinous pneumonia with pleuritis. Fibrinous/necrotizing pneumonia is often called pleuropneumonia since the pleural surface of affected lung tissue is involved in many cases. In severe cases, A. pleuropneumoniae infection can also affect caudal lobes, often with large hemorrhages. Severe cases may result in adhesions between the lungs and the thoracic wall.

**Interstitial Pneumonia**

Interstitial pneumonia is a condition where the inflammatory processes are focused on the alveolar walls causing diffuse alveolar damage. In contrast to bronchopneumonia, interstitial pneumonia is widely distributed throughout the lungs. Lung lobes are often uniformly involved, do not collapse normally, and have a rubbery consistency. Often, rib imprints are present on pleural surfaces. Interstitial pneumonia is
most often caused by viral infections and gram-negative bacterial septicemias (Salmonella, H. parasuis, etc.). When gram-negative bacteria cause interstitial pneumonia, the lungs are also edematous, reddened, and heavy with widened interlobular septa. Migration of Ascaris suum through the lungs results in small hemorrhages in many lobes and, if sufficiently severe, will resemble an interstitial pneumonia.

**Embolic Pneumonia**

Embolic pneumonia is identified as randomly scattered discolored firm foci in multiple lobes caused by hematogenically disseminated bacteria from lesions somewhere else in the body. Typically, this type of pneumonia starts as tiny necrotic foci surrounded by hemorrhagic zones. These zones may progress to circumscribed lesions with a central area of necrosis and exudation representing an abscess or granuloma. While most of the lung abscesses in pigs are due to pyogenic bacteria, mycobacteriosis (Mycobacterium tuberculosis complex and Mycobacterium avium complex) is a recognized cause of nodular lesions in the lungs and thoracic lymph nodes in some parts of the world (Gomez-Laguna et al. 2010; Mohamed et al. 2009). Abscesses reaching the pleural surface may rupture leading to focal pleuritis and/or adhesions.

**Healing of Pneumonia**

The healing period depends greatly on the agents involved. Uncomplicated viral diseases, such as swine influenza, cause a bronchiolitis and mild interstitial pneumonia that can heal in 2–3 weeks with no visible lesions remaining in the lungs. Bronchopneumonia usually takes much longer to heal and may result in lung fibrosis with pleural adhesions to the chest wall. In SPF pigs inoculated with M. hyopneumoniae, lung lesions were healed after 2 months, but pleural adhesions and fissures persisted for more than 3 months (Kobisch et al. 1993; Sørensen et al. 1997). By comparing the time of seroconversion with lung lesions at slaughter, Wallgren et al. (1994) estimated that active mycoplasmal lesions lasted 12 weeks. Pattison (1956) identified lesions of pneumonia up to 25 weeks after inoculation with M. hyopneumoniae, presumably due to secondary bacterial infection.

**Pleuritis**

Haemophilus parasuis, S. suis, M. hyorhinis, and Actinobacillus sp. can cause an acute fibrinous pleuritis (and polyserositis) with or without frank pneumonia. Fibrous adhesions between the lung and chest wall is one outcome of fibrinous pleuritis and one of the most frequent lesions observed in slaughter swine. These firm adhesions may take months to develop from the more acute pleuritis associated with some pneumonias (Christensen 1984). Enoe et al. (2002) demonstrated that herds seropositive for A. pleuropneumoniae serotype 2 and M. hyopneumoniae had chronic pleuritis in 51% and 29% of the pigs at slaughter, respectively.

**TRANSMISSION**

Respiratory disease in pigs is a complex, multifactorial disease condition associated with respiratory pathogens that are often found in combination within pneumonic...
lungs (Palzer et al. 2008). Most bacterial pathogens identified are opportunistic invaders and cause disease when host pulmonary defense mechanisms are damaged. Some bacterial and many viral pathogens are primary pathogens that can cause serious disease on their own. Mixed infections involving primary and opportunistic pathogens result in the greatest economic losses. Respiratory disease results from a breakdown in pulmonary defense and should be evaluated at the level of the lungs, the pig, the farm, and the environment. The spread of respiratory diseases from herd to herd involves two distinct mechanisms. First, respiratory pathogens may be disseminated through infectious contacts (purchase of pigs, incoming and outgoing vehicles, birds, rodents, persons, etc.). Flies can become preys (Heyen et al. 2003). PRRS (Dee et al. 2010; Mortensen et al. 2002), and PRV (Mortensen et al. 1990). Airborne transmission between small pig units at close range can be experimentally induced with PRRSV, A. pleuropneumoniae, B. bronchiseptica, and M. hyopneumoniae (Brockmeier and Lager 2002; Kristensen et al. 2004a,b; Stark et al. 1998; Torremorell et al. 1997). The typical pattern of simultaneous influenza outbreaks in many herds is highly suggestive of the airborne transmission of this infection. In Danish epidemics, closed SPF herds are infected with influenza virus just as frequently as conventional neighboring herds. Epidemics in Brittany also seem to follow an airborne transmission (Madec et al. 1982). Factors affecting the risk of a herd receiving an airborne infection are increasing herd size (Anderson et al. 1990; Flori et al. 1995; Mortensen et al. 1990), short distances between herds and large-sized neighboring herds (Flori et al. 1995; Stark et al. 1992), high regional pig density (Stark et al. 1992), and herds infected concurrently with A. pleuropneumoniae (Anderson et al. 1990).

Viruses can be labile and susceptible to varied environmental conditions (Jacobs et al. 2010; Van Alstine et al. 1993). However, airborne spread of disease between herds may be facilitated by several meteorological factors—most significantly, the direction and velocity of the prevailing winds. Factors such as cloud cover, turbulence, and topography are also important. Overcast skies, night (when the turbulence is often low), and high relative humidity facilitate airborne transmission (Gloster et al. 1981).

**Airborne Transmission of Respiratory Diseases between Herds**

Respiratory pathogens in swine that can be transmitted over distances up to several kilometers include M. hyopneumoniae (Dee et al. 2010; Goodwin 1985; Stark et al. 1992), PRCV (Henningsen et al. 1988), foot-and-mouth disease virus (Gloster et al. 2003), PRRS (Dee et al. 2010; Mortensen et al. 2002), and PRV (Mortensen et al. 1990). Airborne transmission between small pig units at close range can be experimentally induced with PRRSV, A. pleuropneumoniae, B. bronchiseptica, and M. hyopneumoniae (Brockmeier and Lager 2002; Kristensen et al. 2004a,b; Stark et al. 1998; Torremorell et al. 1997). The typical pattern of simultaneous influenza outbreaks in many herds is highly suggestive of the airborne transmission of this infection. In Danish epidemics, closed SPF herds are infected with influenza virus just as frequently as conventional neighboring herds. Epidemics in Brittany also seem to follow an airborne transmission (Madec et al. 1982). Factors affecting the risk of a herd receiving an airborne infection are increasing herd size (Anderson et al. 1990; Flori et al. 1995; Mortensen et al. 1990), short distances between herds and large-sized neighboring herds (Flori et al. 1995; Stark et al. 1992), high regional pig density (Stark et al. 1992), and herds infected concurrently with A. pleuropneumoniae (Anderson et al. 1990).

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**OTHER FACTORS INFLUENCING RESPIRATORY DISEASE**

**Number of Individuals in a Herd, Airspace, or Room**

Climate
Goodall et al. (1993) combined data from abattoirs with meteorological data and found a correlation between the number of condemnations due to pneumonia and air temperature. A higher incidence of pneumonia occurs in pigs during the cold seasons than during the summer months, indicating that climatic factors can influence the respiratory diseases on a farm (Bille et al. 1975; Maes et al. 2001a).

Airflow
A high exchange rate of air often causes local drafts and chilling of animals. A sudden chilling by drafts may predispose pigs to respiratory infections. In weaned pigs subjected periodically to drafts, Scheepens (1996) reported a higher frequency of sneezing and coughing than in controls. Subjecting controls and pigs with atrophic rhinitis to similar climatic stress caused an 8-day difference in days to slaughter, compared with a 3-day difference in the two groups of pigs when not exposed to adverse climatic conditions. Flesja et al. (1982) found that solid-sided pens were associated with a reduction in incidence of pneumonia, presumably by preventing drafts. According to Kelley (1980), cold drafts and wide temperature differentials stress the immune mechanisms, thus increasing susceptibility to disease. This is confirmed in weaned pigs, where exposure to cold drafts significantly reduced immune response (Scheepens et al. 1988). Prolonged cold stress in suckling piglets experimentally infected with *P. multocida* resulted in lowered levels of serum IgG, lowered phagocytic activity of the polymorphonuclear granulocytes, and delayed local cellular reaction in the lungs of some of the piglets compared with controls. However, the course of infection, the clinical response, and post-mortem findings were not influenced by cold stress (Rafai et al. 1987).

Air Pollution
Stocking density and poorly balanced air recirculation systems can profoundly affect air quality and the ability of air handling system to clear airborne contaminants (Meyer and Manbeck 1986; Nicks et al. 1989; Wathes 1983). High concentrations of ammonia in the air may be detrimental to respiratory health. Ammonia in concentrations of 50–100 ppm particularly interferes with normal mucociliary function (Curtis et al. 1975a; Neumann et al. 1987). Under normal conditions, the ammonia concentration in pig pens does not exceed 20 ppm. Clark et al. (1993) did not find that ammonia concentrations of 18 ppm influenced development of mycoplasmal pneumonia. Nevertheless, epidemiological studies have revealed the highest incidence of respiratory disease in herds with the highest ammonia concentrations in the air (Pointon et al. 1985). Also, when pigs were given the choice of moving between fresh air and air containing 100 ppm ammonia, they preferred the fresh air (Smith et al. 1996). Donham (1991) found several air contaminants, such as dust, ammonia, and microbes, correlated with pneumonia and pleuritis in swine and has proposed maximal safe concentrations on the basis of dose–response correlation to swine health and human health problems: dust 2.4 mg/m³, ammonia 7 ppm, endotoxin 0.08 mg/m³, total microbes 10⁵ CFU/m³, and carbon dioxide 1540 ppm. However, some studies did not demonstrate a relationship between dust and respiratory disease (Curtis et al. 1975b; Gilmour 1989; Jansen and Feddes 1995).

Concurrent Disease: Enteritis
As might be expected, herds with diarrhea (transmissible gastroenteritis virus, rotavirus, and others pathogens affecting the intestines) have higher rates of respiratory disease. This is presumably because overall herd health disease resistance and immunity are decreased (Allan and Ellis 2000; Jørgensen 1988; Marois et al. 1989; Svensmark et al. 1989).

Gender
From surveillance of slaughter swine at Danish abattoirs, the incidence of pneumonia and pleuritis in castrated males is 10% higher than in females (Kruijf and Welling 1988). Andreasen et al. (2001) found larger pleural lesions in castrated males than females at the time of slaughter. Castration may have been responsible for the differences through stress, bacterial exposure through castration sites, or hormonal changes.

Heredity
Respiratory diseases can be influenced by heredity. In genetically selected obese swine, the phagocytic functions of pulmonary alveolar macrophages were found significantly more effective than those from genetically selected lean swine (Caruso and Jeska 1990). Clinical observations in a herd having purebred Hampshires and Yorkshires revealed a much lower level of respiratory diseases in Hampshire than Yorkshire pigs (Lundeheim and Tafvelin 1986). The same investigators examined 45,000 slaughter pigs consisting of Hampshire, Landrace, and Yorkshire crosses. The Hampshire crosses had a significantly lower incidence of pneumonia and pleuritis than the other crosses. The susceptibility to atrophic rhinitis is greater in Yorkshire pigs than in Landrace pigs (Lundeheim and Tafvelin 1986; Smith 1983; Straw et al. 1983). In a challenge study, Hampshire pigs were more resistant to *A. pleuropneumoniae* infection than three other breeding lines (Hoeltig et al. 2010). Breed differences in susceptibility to PCV2 infection were demonstrated between Landrace and Duroc pigs (Opriessnig et al. 2006). Ruiz et al. (2002) identified different patterns of colonization of *M. hyopneumoniae* between pigs sired by three different boars, suggesting a possible genetic effect. Early studies suggest many genetic markers may be useful for DNA-based breeding.
selection to increase resistance to lung infections (Hoeltig et al. 2010; Probst and Hoeltig 2010).

**MONITORING AND DIAGNOSIS OF RESPIRATORY DISEASE**

Definitive diagnosis of respiratory diseases is based on a combination of history, clinical observations, necropsies, gross and microscopic lesion evaluations, and laboratory tests, and can include slaughter checks. A clinical diagnosis can only be tentative, since visible signs from the respiratory system may be the result of dysfunction of other organs. Necropsy and gross observation of lesions are invaluable for initiating diagnostic investigation as summarized by Andrews (1986). Also, lesions in the respiratory system such as chronic pneumonia and pleuritis may have no clinical signs. Table 21.6 summarizes some historical and herd factors with detrimental effects on the respiratory system.

**Table 21.6.** Herd factors with detrimental effects on the respiratory system

<table>
<thead>
<tr>
<th>Factors</th>
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<tbody>
<tr>
<td>Production system</td>
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<tr>
<td>Large herd size</td>
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<tr>
<td>High stocking density</td>
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<tr>
<td>Conventional health system (not SPF or minimal disease production system)</td>
</tr>
<tr>
<td>Introduction of animals from herds with unknown or low health status</td>
</tr>
<tr>
<td>Continuous flow of animals through facilities (no movement of pigs in batches)</td>
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<tr>
<td>Use of genetically predisposed breeds</td>
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<tr>
<td>Use of purebreds instead of crossbreds</td>
</tr>
<tr>
<td>Housing</td>
</tr>
<tr>
<td>Badly insulated and ventilated facilities (causing improper temperature regulation and air exchange, drafts)</td>
</tr>
<tr>
<td>Insufficiently divided facilities combined with housing of differently aged animals in the same airspace</td>
</tr>
<tr>
<td>Pens divided without solid separations</td>
</tr>
<tr>
<td>Large grower or finishing rooms (containing more than 200–300 pigs)</td>
</tr>
<tr>
<td>Slatted floors</td>
</tr>
<tr>
<td>Nutrition</td>
</tr>
<tr>
<td>Insufficient caloric intake</td>
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<tr>
<td>Improper content of macro- and microelements in feed</td>
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<tr>
<td>Presence of nonrespiratory pathogens</td>
</tr>
<tr>
<td>Nonrespiratory diseases such as colibacilosis, intestinal viruses, dysentery</td>
</tr>
<tr>
<td>Parasites such as mange, ascarids</td>
</tr>
<tr>
<td>Management deficiencies</td>
</tr>
<tr>
<td>Insufficient control of climate</td>
</tr>
<tr>
<td>Poor monitoring of signs of disease</td>
</tr>
<tr>
<td>Incorrect preventive measures</td>
</tr>
<tr>
<td>Poor caretaking of sick animals (isolation, treatment)</td>
</tr>
<tr>
<td>Random distribution of piglets between sows</td>
</tr>
<tr>
<td>Multiple moving and mingling of pigs during growth period</td>
</tr>
<tr>
<td>Poor hygiene</td>
</tr>
<tr>
<td>Poor biosecurity</td>
</tr>
</tbody>
</table>

Adapted from the previous edition of *Diseases of Swine* (Sorensen et al. 2006).

**Monitoring Respiratory Disease**

The purpose of monitoring respiratory diseases is to identify clinical and subclinical diseases that can affect production. One goal of a health status monitoring program is to detect infection and intervene before it has a significant economic impact. Farm inspections, routine slaughter checks, serosurveys, and postmortem examinations of dead or euthanized animals are often the basis for diagnosing and estimating the severity of respiratory disease. Large-scale data collection and evaluation on the respiratory health of slaughter swine are routinely carried out in some countries (Mousing 1986; Rautiainen et al. 2001).

National herd health monitoring programs may use slaughter inspections as one tool for monitoring the respiratory health of a herd. Different health improvement surveillance programs may have specific methods required for sectioning and evaluating the respiratory tract. Slaughter checks of market weight pigs can help determine chronic lung lesions or respiratory diseases that are active in the late stages of production. Labor concerns, including the fast line speed of modern slaughter facilities, have made slaughter inspections less practical and less common than a decade ago. Cross-sectional and longitudinal serology and, sometimes, nasal swabs have increasingly replaced slaughter checks to determine the incidence, prevalence, and location of specific pathogens on farms (Andreasen et al. 2000, 2001; Chiers et al. 2002; Maes et al. 2001b). Serosurveys may be more effective than slaughter checks to detect subclinical infections that may affect some production parameters (Regula et al. 2000). Oral fluids (saliva and mucosal transudate) obtained from cotton ropes suspended in pens has been successfully used to detect agents and antibodies to some respiratory pathogens (Kittawornrat et al. 2010; Prickett et al. 2008; Strait et al. 2010). Meat juice (fluid recovered from frozen and thawed muscle from slaughtered pigs) has also been used to detect antibodies to PRRS, PRV, and *A. pleuropneumoniae* type 2 (Molina et al. 2008; Wallgren and Persson 2000). These minimally invasive techniques may be easy and efficient ways to monitor for some respiratory diseases.

**General Concepts of Examination**

**Slaughter Checks: Examining the Snout for Atrophic Rhinitis.** Slaughter checks for atrophic rhinitis are usually performed by examining a transverse section of the snout (see Figures 21.1 and 21.4). Optimal results are obtained if the cut is placed between premolars 1 and 2 (Martineau-Doizé et al. 1990). Several methods of scoring atrophic rhinitis have been used (Bäckström et al. 1985; Straw et al. 1983). These methods are based on subjective and visual assessment of structures. Results from different slaughter checks should be compared with caution, as demonstrated by D’Allaire et al. (1988). Comparisons should be performed by the same...
experienced observer using the same scoring system. A morphometric technique described by Collins et al. (1989) and modified by Gatlin et al. (1996) yielded highly reproducible results, but may be less practical for field use.

**Slaughter Checks: Examining Thoracic Viscera (Plucks).** The progression and regression of pneumonia in growing pigs are highly dependent on the type and severity of pneumonia (Morrison et al. 1985; Noyes et al. 1988; Wallgren et al. 1994). Slaughter examinations of the respiratory tract may not detect subclinical diseases or diseases affecting young pigs that heal without scarring (Regula et al. 2000).

Careful slaughter checks of the thoracic viscera cannot normally be performed at the slaughter line. The material has to be transferred to an appropriate place for a thorough visual examination and palpation. The percentage of pneumonia is usually based on the percentage of lung surface that is abnormally firm and discolored (Bollo et al. 2010; Clark et al. 1993; Mousing and Christensen 1993; Sorensen et al. 2006; Thacker et al. 1999, 2010). Lesions are sketched onto a standard diagram of the lungs followed by image analysis of the diagrams to determine the overall lung score. Some slaughter-check diagrams include more detailed information about the lungs, such as the type of pneumonia, pleuritis, and adhesions, plus evaluations of the liver and heart (Christensen et al. 1999). Visual-only evaluations of digital images of the lungs obtained at slaughter may be an alternative to drawings (Baysinger et al. 2010). In most situations, slaughter checks should be performed on at least 30 pigs (with similar or known age if possible) to get a sufficient sample and thus a reliable picture of the herd problem (Straw et al. 1989).

**Laboratory Tests**
A wide range of serological, isolation, and molecular tests are available to practitioners to detect the presence of and exposure to respiratory pathogens in swine. As diagnostic tests get increasingly more sensitive, a case-by-case interpretation of the biological significance of the results is critical. Laboratory tests for specific respiratory pathogens will be covered under their specific chapters.

**CONTROL OF RESPIRATORY DISEASES**
Pathogenic microorganisms are involved in all important respiratory diseases, and in practice, it can be very difficult to protect pig herds from exposure. The primary principles for herd level control of respiratory diseases are to eliminate or reduce pathogen load (diminish the infection pressure) and to maximize the pig’s respiratory defense mechanisms (Sorensen et al. 2006). Approaches to eradicate or reduce pathogens must be tailored to the unique circumstances of each farm, region, and country, and regulatory considerations will influence these approaches. University, government, and industry resources are readily available to help guide eradication and pathogen reduction efforts. There are useful websites that include information on national and local prevention and control programs and biosecurity.

Methods used to reduce or eliminate respiratory pathogens vary widely and will be covered under individual pathogen chapters. Briefly, some methods include depopulation/repopulation or modified depopulation techniques, segregated early weaning, strict age segregation especially with multiple-site separation of ages, medicated early weaning, test with removal of sick/infected pigs, and strategic vaccination and medication (Dee et al. 2001; Dritz et al. 1996; Larsen et al. 1990a,b; Pejsak and Truszczynski 2006; Rautiainen et al. 2001; Sorensen et al. 2006; Stegeman et al. 1994; Van Oirschot et al. 1996).

Once pathogen load or prevalence is low, many factors can influence host/pathogen balance. Table 21.6 lists some herd factors that can be detrimental to the respiratory system of pigs and affect pig health.

Biosecurity is paramount to sustaining healthy pigs (Amass 2005a,b). Strict hygiene and disinfection controls over vehicles, equipment, and personnel entering the farm will help reduce the reintroduction of pathogens and minimize transmission between herds. Barn workers should understand that some bacterial and viral diseases may spread from humans with respiratory illnesses to healthy pigs (Keenliside et al. 2010; Nielsen and Frederiksen 1990).

Breeding stock should be quarantined, tested, and treated or vaccinated appropriately for respiratory pathogens prior to introduction to the herd.

Some other factors that are important in maintaining a clean herd include personnel training to identify the earliest signs of respiratory disease, proper nutrition, reduction of nonrespiratory pathogens, separating pigs with solid barriers between different age groups if housed in the same airspace, maintaining appropriate stocking density to avoid overcrowding, maintaining proper temperature and airflow in the barn, minimizing ammonia and dust in the air, and ensuring appropriate sanitation between groups of pigs.

**REFERENCES**


ANATOMY

The kidneys of swine are bean-shaped, generally smooth on the surface, and brown. They are elongated, flattened dorsoventrally, and at least twice as long as wide. At the middle of the medial border of each of them is an indentation, the hilus of the kidney, where the vessels, nerves, and ureter communicate with the organ. The kidneys are located ventral to the psoas muscles at the level of the first four lumbar vertebrae. Their relative location is slightly asymmetrical, but contrary to what is observed in many other species, the left kidney of most individuals is often situated cranially to the right one; the extremity of the cranial pole of the former may reach the last intercostal space. In the adult, the ratio of the combined weight of the kidneys to that of the body is about 0.50–0.66% (Sisson 1975).

The kidneys are enveloped by a rather thin fibrous capsule that can be easily peeled off. In a kidney section, the relative surface occupied by the cortex and the medulla is readily apparent (Figure 22.1). Pigs have multipyramidal or multilobar kidneys but without the external lobation typically found in the bovine species. The medullary portion of each lobe is called a pyramid; some are simple, whereas others are compound, that is, formed by the fusion of two or more primitively separate pyramids. The pale apical portion of a pyramid, called the papilla, projects into the renal pelvis or its ramifications; these latter are referred to as calyces. Papillae of simple pyramids are generally narrow and conical, whereas those of compound pyramids, often located in the area of the renal poles, are broad and flattened. There are 8–12 papillae per kidney. Collecting ducts of the kidneys have their openings at the tips of the papillae.

The ureters, which are continuous with the renal pelvis, leave the kidneys in a sharp caudal curve. They ultimately reach the dorsolateral sides of the bladder neck area, penetrating its muscular coat at almost the right angles, and pass obliquely through the submucosa, raising the mucosa slightly before ending at the ureteric orifices. In newborn piglets, the length of the portion of the ureter running beneath the bladder mucosa is about 5 mm, whereas it reaches a mean length of about 35 mm in the adult (Carr et al. 1990). The intravesical portion of the ureters acts as a valve that prevents vesicoureteral reflux of urine.

The urinary bladder of the pig is large and has a long neck. When full, it lies well down into the abdominal cavity. The bladder is supported by one median (ventrally located) and two lateral ligaments. The urethra of the adult female is about 7–8 cm long, and its external ostium is located ventrally, at the junction of the vagina and vestibule; beneath it is a small depression, the suburethral diverticulum. In the male, the urethra opens into a slit-like structure at the tip of the penis.

PHYSIOLOGY

Histophysiology

The kidney is involved in many vital functions: the elimination of waste products from the body, the conservation of water, and the regulation of the acid–base balance and electrolyte composition. In addition, it has an endocrine function: It produces a variety of hormones, including erythropoietin, renin, prostaglandins, and vitamin D₃.
Most of these functions are achieved by a multitude of microscopic anatomical structures called nephrons, which, all together, form the bulk of the renal parenchyma. The kidney of a pig contains well over 1 million nephrons.

Newborn piglets have immature kidneys, and nephrogenesis continues during the first 3 months of life (Friis 1980). The nephron, the functional unit of the kidney, consists of a renal corpuscle, proximal tubule, loop of Henle, and distal tubule. The renal corpuscle comprises the glomerulus, a tuft of arterial capillaries, and Bowman’s capsule. The first mechanism used to accomplish renal function is glomerular filtration. The volume of plasma filtered depends essentially on the renal perfusion, blood pressure, and integrity of the glomerulus itself. The glomerular filtrate is an ultrafiltrate of blood plasma that contains water, glucose, salts, ions, amino acids, and small amounts of protein of low molecular weight.

The glomerular filtrate entering the tubular components of the nephron is profoundly modified by various processes of absorption and secretion that are, at least in part, governed by the needs of the animal. A significant part of these changes takes place in the proximal tubule, a segment of the nephron lined by well-developed and metabolically very active epithelial cells. At that site, for example, 100% of the filtered glucose (in a normoglycemic animal) is reabsorbed by an active transport mechanism, and many other substances, such as water, sodium, amino acids, albumin, and bicarbonate, are also reabsorbed in significant amounts (Banks 1993).

Pigs are distinctive in that they reabsorb very few urates from glomerular filtrate compared with most other species. Tubular secretion of various endogenous and exogenous compounds complements the clearance of substances that are filtered at the glomerulus.

The filtrate finally enters the collecting ducts, where it may be further concentrated. Under normal conditions, urine expelled into the pelvis is not further modified as it travels through the rest of the lower urinary tract, the mucosa of which is lined with a transitional epithelium often referred to as urothelium.

### Urine

The volume of urine produced daily depends on several variables, including diet, fluid intake, ambient temperature and humidity, and the size and weight of the animal. Accurate data on the normal ranges of the amount of urine excreted per day in pigs are limited. Salmon-Legagneur et al. (1973) reported a mean urinary output of 9 and 5.3 L/day in gestating and lactating sows, respectively. Other factors, such as the water distribution system used, may also affect the production of urine if they influence drinking behavior.

The mean specific gravity of urine in adult swine is about 1.020 (plasma > 1.010), one of the lowest found in domestic animals (Ruckebusch et al. 1991). Young animals have even lower values. Specific gravity of urine is usually inversely related to urine volume. Urinary pH is usually between 5.5 and 7.5. It is influenced by the metabolism and the composition of the feed; starvation or a high protein intake lowers urinary pH. Urinary infection with urea-splitting bacteria (e.g., *Actinobaculum suis*) may result in a significant alkalization of urine.

As mentioned previously, the small amount of protein that passes through the glomeruli is, for the most part, reabsorbed by the proximal tubules, so normally, no protein is detected in urine by usual methods. The presence of proteinuria may be of diagnostic significance and must be interpreted in conjunction with the specific gravity. Significant proteinuria is associated with various renal diseases, such as glomerulonephritis (GN) (increased permeability to protein), tubular necrosis (decreased reabsorption of protein), and pyelonephritis (inflammation), and with lower urinary tract inflammation. The presence of protein in the urine is, however, not always pathological, because transient physiological or functional proteinuria also occurs in some instances. Proteinuria is observed in baby pigs in their first few days of life, their glomeruli being permeable to colostral protein found in high levels in the blood. Transient proteinuria may also occur following excessive physical activity (e.g., transport) or intense stress or when excessive amounts of protein are ingested.

The color of urine is usually yellow to amber depending on the concentration of urochromes. Abnormal coloration of urine is observed with some underlying urinary tract diseases. Urine sediment examination is very informative because it may reveal findings of diagnostic significance (e.g., in the case of cystitis–pyelonephritis).
Impaired Renal Function
In some pathological situations, renal function is impaired so much that renal failure ensues. Renal failure may have a prerenal (e.g., any condition that reduces renal blood flow), postrenal (e.g., obstructive uropathy), or primary renal origin (e.g., extensive renal parenchymal disease).

Renal failure can cause metabolic acidosis, electrolytic imbalances, and intravascular accumulations of various metabolic waste products, including blood urea nitrogen (BUN) and creatinine. Determination of both BUN and serum creatinine concentration may be used to assess the renal function. The serum creatinine concentration is a more accurate index of the glomerular filtration rate than is the BUN because it is less dependent on nonrenal factors. Concentrations of BUN may rise in animals with high dietary protein intake or in any conditions resulting in increased protein catabolism. Friendship et al. (1984) reported normal-range values for BUN and serum creatinine in weaned and feeder pigs, gilts, and sows. In sows, for example, the mean BUN concentration has been reported as 5.3 mmol/L \((n = 102)\) (Friendship et al. 1984) and 5.0 mmol/L \((n = 120)\) (McLaughlin and McLaughlin 1987), whereas the mean serum creatinine concentrations reported in the same two studies are 160 and 186 µmol/L, respectively.

DEVELOPMENTAL ANOMALIES
Anomalies of development occur in all body systems, and the urinary tract is no exception. These anomalies may involve the kidneys as well as the lower urinary tract. Many of these conditions in swine are relatively rare and of little economic significance. Few of these malformations in pigs are common, and only rarely are they associated with clinical signs. In some instances, developmental anomalies have been shown to be inherited.

Malformations of the Kidneys
Most of the well-characterized renal anomalies of development occurring in domestic animals have also been documented in pigs. Depressions of the external surface and partial persistence of fetal lobation of the kidneys have been reported to be relatively common in Norwegian slaughtered pigs (Jansen and Nordstoga 1992). Unilateral renal agenesis (aplasia) occurs sporadically in pigs and is relatively common compared with other domestic animals (von Höfliger 1971). Bilateral renal agenesis is incompatible with life and would be encountered in the fetus or stillborn piglet. Cases of bilateral renal agenesis and renal hypoplasia have been described in pigs and linked to a genetic cause (Cordes and Dodd 1965; Mason and Cooper 1985). Malposition of the kidneys (renal ectopia) is not rare and is often characterized by caudal displacement of one kidney, often the left, to the pelvic area (Sisson 1975). Duplication of one kidney has been observed on a number of occasions in pigs (Nieberle and Cohrs 1967). Horseshoe kidney, rarely observed in swine, is a condition in which the kidneys have fused at either the cranial or the caudal poles, resulting in a horseshoe-shaped organ (Nieberle and Cohrs 1967). Renal dysplasia, a disorganized development of renal parenchyma due to anomalous differentiation, is also relatively rare (Maxie and Newman 2007).

Congenital renal cysts are frequently seen in the kidneys of various species but are more common in swine (Figure 22.2). The presence of one or a few cysts in the kidney, often referred to as simple renal cysts, is a condition in which the kidneys have fused at either the cranial or the caudal poles, resulting in a horseshoe-shaped organ (Nieberle and Cohrs 1967). Renal dysplasia, a disorganized development of renal parenchyma due to anomalous differentiation, is also relatively rare (Maxie and Newman 2007).

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sizes of cysts, ranging from one extreme to the other. Further investigation showed that affected animals were the progeny of a Landrace boar. The disease was found to be inherited as an autosomal dominant trait, the number of cysts being determined by polygenic inheritance (Wijeratne and Wells 1980).

**Malformations of the Lower Urinary Tract**
Developmental anomalies of the lower urinary tract appear to be rare in swine, and like those found in the kidneys, their true prevalence is unknown. Cases of duplication of the ureter (Benko 1969), persistent urachus (Weaver 1966), and congenital ureteral occlusion (Nieberle and Cohrs 1967) have been reported in pigs.

**CIRCULATORY DISTURBANCES**
Circulatory disturbances may occur in the urinary tract as well as in any other tissues of the body. Some of these disturbances of the circulation produce lesions that may be of diagnostic significance on postmortem inspection.

**Hemorrhage**
Hemorrhages, in the form of widespread petechiae or less commonly ecchymoses, may be found in any part of the kidney or lower urinary tract in various septicemic illnesses (Figure 22.3). Bacterial infections commonly associated with these lesions include septicaemia due to salmonellae, streptococci, *Erysipelothrix rhusiopathiae*, and *Actinobacillus* spp. These lesions are often seen in acute cases of classical swine fever and African swine fever and in other viremic diseases such as cytomegalovirus infection (Orr et al. 1988). Petechiation of the renal cortex is also observed occasionally in acute GN, in some acute intoxications, and in electrocuted animals. Noteworthy is that neonates normally have dilated, congested glomerular tufts within an otherwise normal renal cortex that erroneously can be interpreted as petechiae.

Larger intrarenal or subcapsular hemorrhages are usually caused by trauma, necrosis, or clotting defect, including poisoning by anticoagulant rodenticides. They may also occur in some cases of disseminated intravascular coagulation. Widespread hemorrhages in almost all body systems, including the urinary tract, are a striking pathological feature in suckling piglets with isoimmune thrombocytopenic purpura due to passively transferred antiplatelet antibody from the dam's colostrum (Andersen and Nielsen 1973; Dimmock et al. 1982). In this relatively common disease, affected piglets appear normal at birth but eventually die between 1 and 3 weeks of age from hemorrhagic diathesis.

**Infarction**
Renal infarcts, infrequently found in the kidneys of swine, are localized areas of ischemic coagulative necrosis produced by the occlusion of the renal artery or one of its tributaries. The localization and size of the infarct reflect the area vascularized normally by the involved blood vessel. The occlusion is usually due to thrombosis or to aseptic emboli (the consequences of septic emboli are discussed under embolic nephritis). In some instances, infarction of the renal parenchyma is primarily associated with renal vasculitis (Jansen and Nordstoga 1992), including polyarteritis nodosa (Nieberle and Cohrs 1967).

Bilateral renal cortical necrosis is seen on rare occasions in pigs and is considered to be the result of an infarct of a major part of the cortex of both kidneys (Häni and Indermühle 1980). The reaction is characterized by disseminated intravascular coagulation with a marked tropism for the small arterial blood vessels of the renal cortex. The etiopathogenesis of these lesions remains unclear but the condition has been associated with septicemia, endotoxia, and hemorrhagic shock due to bleeding gastric ulcers.

**GLOMERULAR DISEASES**
Renal diseases that involve primarily the glomeruli include amyloidosis and GN. Renal amyloidosis has been rarely reported in pigs (Jakob 1971; Maxie and Newman 2007). GN represents an important category of renal diseases in animals, and in recent years, this condition has been recognized with increasing frequency in swine.

**Glomerulonephritis**
Inflammatory changes in renal glomeruli may take place via a number of mechanisms, including immunological, thrombotic, toxic, and as yet uncharacter-
ized mechanisms. Most cases of glomerulonephritis (GN) in humans and animals are thought to be immune mediated.

The main types of glomerular immunological injury recognized are trapping of circulating immune complexes (antigen–antibody complexes), in situ immune complex formation, activation of the alternate pathway of complement, and cell-mediated processes (Spargo and Taylor 1988). Because immunoglobulins and complement components are frequently found in inflamed glomeruli, antibody-mediated injury has received the most attention. In veterinary medicine, the commonly used classification for the various morphological types of immune-mediated GN are membranous, proliferative, membranoproliferative, and exudative.

Although the pathogenesis of GN is now reasonably well understood, knowledge of the etiology or triggering event is still unknown in most cases (idiopathic immune-mediated GN). Theoretically, GN may be triggered by a variety of factors including drugs, chemicals, food allergens, endogenous antigens, and infectious agents (Drolet et al. 1999).

GN is not often diagnosed in swine but does occur occasionally as a sporadic event (Bourgault and Drolet 1995; Maxie and Newman 2007; Nieberle and Cohrs 1967; Slauson and Lewis 1979). It has also been reported as a sequel to chronic infectious diseases such as classical swine fever, African swine fever (Choi and Chae 2003; Hervas et al. 1996; Martin-Fernandez et al. 1991), systemic cytomegalovirus infection (Yoshikawa et al. 1988), and group A streptococcal abscesses (Morales and Guzman 1976). In these cases, the resulting GN appears to be caused by the presence of glomerular immune complexes in which the antigen is related to the agent responsible for the underlying disease (Slauson and Lewis 1979). Nutritionally induced GN has also been reported on a few occasions in pigs. Ingestion of a protein-rich by-product (Elling 1979) and ingestion of “smut fungus” contained in the feedstuff (Müller 1977) have been incriminated.

An inherited renal disease, classified morphologically as membranoproliferative GN type II, has been described in Yorkshire piglets from Norway (Jansen 1994). This familial disease is not associated with the presence of intraglomerular immune complexes but is rather caused by an autosomal recessive deficiency of the complement inhibitory protein “factor H” (Jansen et al. 1995). Deficiency of factor H ultimately provokes activation of the alternate pathway of complement, with subsequent massive deposition of complement in renal glomeruli, intramembranous dense deposits, and mesangial hypercellularity. This disease in Norwegian Yorkshire pigs represents a promising animal model for the study of membranoproliferative GN type II (dense deposit disease) in humans (Jansen et al. 1998).

In many species, generalized or focal GN is also observed in some cases of systemic vasculitis (mainly immune-mediated vasculitis). In pigs, the best example of this certainly is porcine dermatitis and nephropathy syndrome (PDNS). The condition, first described in the United Kingdom in 1993 (Smith et al. 1993; White and Higgins 1993), has been subsequently observed in most parts of the world. The disease affects nursery and growing pigs and, less commonly, breeding animals (Drolet et al. 1999). The prevalence of the syndrome in affected herds is usually less than 1% although higher prevalences have been detected in the United Kingdom and other countries, with case mortality in affected herds ranging from 0.25% to over 20% (Segalés et al. 2005).

Affected animals present a systemic necrotizing vasculitis with marked tropism for the skin and kidneys (Hélie et al. 1995; Smith et al. 1993; Thibault et al. 1998). Vascular lesions in the skin provoke a conspicuous dermatopathy (see Chapters 17 and 26). Kidney lesions in acute cases may include exudative and occasionally necrotizing GN and vasculitis. Vascular lesions in other tissues vary considerably in frequency and distribution in individual pigs (Thomson et al. 2002). In some atypical cases, there may be cutaneous lesions without renal lesions and vice versa (Figure 22.4). An animal with GN without any other vascular lesions elsewhere should probably not be diagnosed as PDNS since other glomerular diseases not related to this syndrome are known to occur in pigs.

In PDNS, the glomerular and systemic vascular damages are thought to be immune mediated, possibly through a type III hypersensitivity reaction, which is characterized by deposition of antigen–antibody aggregates or immune complexes within these sites (Hélie et al. 1995; Sierra et al. 1997; Smith et al. 1993; Thibault et al. 1998; Thomson et al. 2002; Wellenberg et al. 2005).

22.4. A white sow affected with PDNS. Note multifocal to coalescent erythematous macules and typical caudal distribution.
2004). Over the years, several bacterial and viral pathogens, including porcine reproductive and respiratory syndrome (PRRS) virus and porcine circovirus type 2 (PCV2), have been incriminated as possible etiologies for PDNS (Opriessnig et al. 2007). Observations from the last decade point toward an association between PCV2 and PDNS. The precise way by which this virus promotes directly or indirectly the development of PDNS remains unknown at this time. Recently, it has been shown that animals with PDNS often have relatively low PCV2 viral loads (Olvera et al. 2004) but very high PCV2 antibody titters (Wellenberg et al. 2004).

Other examples of glomerular disease in swine have been reported in the literature in recent years (Carrasco et al. 2003; Jansen and Nordstoga 1992; Pace et al. 1998; Shirato et al. 1984, 1995; Tamura et al. 1986; Yoshie 1991).

Although several of the previously described glomerulonephropathy types are associated with an underlying disease process (chronic infections, genetic defect, systemic vasculitis, etc.) that gives insight into the likely etiology of the condition, many spontaneous cases of GN, however, remain idiopathic (primary idiopathic GN) (Bourgault and Drolet 1995; Shirato et al. 1986; Slauson and Lewis 1979).

The clinical significance of GN is highly variable, with a spectrum ranging from a subclinical condition to a fulminating and rapidly fatal disease. Shirato and coworkers (1986) found deposition of immune complexes containing immunoglobulin G (IgG) and third-complement component (C3) in the glomerular mesangium of most of the 100 normal slaughtered swine they examined. The mesangioproliferative GN, disclosed only upon microscopic examination of the kidneys, was seemingly not associated with clinical disease.

On the other end of the clinical spectrum, the proliferative and exudative GN observed in most of the cases of spontaneous GN reported by Bourgault and Drolet (1995) was found to be responsible for the death of at least one-third of the pigs affected. The hereditary GN of Norwegian Yorkshire pigs appears to be invariably fatal; affected piglets die of renal failure within 11 weeks of birth (Jansen et al. 1995). Mortalities are also commonly recorded in pigs affected with PDNS (Hélie et al. 1995; Kavanagh 1994; Segalés et al. 1998; Smith et al. 1993; Thomson et al. 2002; White and Higgins 1993). In this latter condition, the survival of the affected pigs depends on the extent and severity of the vascular lesions in the internal organs, particularly within the kidneys.

Unlike most other domestic animals affected with GN (with the exception of the familial glomerulopathies), pigs appear to be affected at a relatively young age. The condition has been described most commonly in weaned and feeder pigs (1.5–6 months old), occasionally in breeding pigs, and rarely in nursing piglets. Clinical signs, when present, may include anorexia, lethargy, unwillingness to move, subcutaneous edema, rapid loss of condition, and death.

GN is rarely recognized clinically because most of the signs are nonspecific, and analysis of urine and blood from an individual is rarely considered of practical value in the herd medicine approach applied in our large units of production. Pigs affected with PDNS are often more easy to detect clinically because of the presence of hemorrhagic and necrotizing skin lesions, mainly located on the hind limbs and perineal area (Segalés et al. 2003). Pigs with GN may show concomitant hypoproteinemia, hypoalbuminemia, and persistent proteinuria, which are highly suggestive of a protein-losing glomerulopathy. The urine protein/creatinine ratio may also be increased (Hélie et al. 1995). Proteinuria, hematuria, and pyuria, which are usually compatible with lower urinary tract lesions, may also occur in severe types of GN—namely, in acute exudative GN. Blood of affected animals may also reveal elevated urea and creatinine levels suggestive of renal failure (Drolet et al. 1999; Hélie et al. 1995; Jansen et al. 1995; Thomson et al. 2002; White and Higgins 1993).

Gross lesions of GN may be absent, subtle, or very conspicuous. The appearance of the kidneys will depend largely on the severity of the glomerular lesions and the stage (acute vs. chronic) of the disease process. In acute GN, the kidneys may be slightly to markedly enlarged, pale, and edematous, often with cortical petechiation (Figure 22.5). The most important differential diagnoses to consider for such acutely affected kidneys are various bacterial sepsisemia (E. rhusiopathiae, Actinobacillus suis, Salmonella choleraesuis), acute viremia (classical swine fever, African swine fever, cytomegalovirus (CMV), and other viral infections).
are especially susceptible to damage caused by prolonged ischemia or nephrotoxins, the two main causes of this type of nephropathy.

**Ischemic Tubular Necrosis**

Ischemic tubular necrosis is generally the result of a severe and prolonged period of hypotension associated with shock of endotoxic, hypovolemic, cardiogenic, or neurogenic origin (Maxie and Newman 2007). These renal lesions are potentially life threatening, and the clinical signs of the resulting renal failure are often hidden by the marked systemic effects of the primary disease responsible for the state of shock.

**Nephrotoxic Tubular Necrosis**

Nephrotoxic tubular necrosis has been documented in domestic animals in association with a wide variety of exogenous natural and synthetic compounds. These toxic substances may affect tubular function and ultimately cause cellular damage by several mechanisms, including metabolic alterations affecting cellular respiration, interference with the tubular transport system, and damage to specific organelles (Brown and Engelhardt 1987).

Plants, mycotoxins, antimicrobial drugs, heavy metals, ethylene glycol, and some other industrial compounds are potential nephrotoxins in swine. Some of these toxic products are covered in Chapters 69 and 70. Many plants are nephrotoxic to animals, especially ruminants. Several species of pigweed, particularly redroot pigweed (*Amaranthus retroflexus*), may cause acute renal failure in pigs when ingested. The disease occurs in summer and early fall, corresponding to the months in which animals may have access to the plants. The onset of clinical signs usually occurs about a week after the ingestion. Characteristic signs include weakness, trembling, and incoordination, rapidly progressing to posterior paralysis and sternal recumbency, and finally to death (Osweiler et al. 1969).

Fungi of some species of *Aspergillus* and *Penicillium* produce nephrotoxins that can contaminate grains used as feedstuff. Ochratoxin A and citrinin are the most common nephrotoxic mycotoxins. Monogastric animals, particularly pigs, may develop significant disease when moldy feed containing ochratoxin A is ingested. Acute clinical signs are relatively rare; a subacute to chronic wasting disease is more commonly associated with this poisoning (Osweiler et al. 2003).

**TUBULAR DISEASES**

Renal diseases characterized primarily by degenerative changes affecting the tubular epithelial cells of the nephrons may occur under certain circumstances. In these cases, the epithelial lining cells of the tubules may undergo degeneration, followed by necrosis and sloughing of the cells.

Acute tubular necrosis, often called nephrosis, represents an important cause of acute renal failure in animals (Figure 22.6). The epithelial cells of the proximal tubules, because of their high metabolic activity,
health status (dehydration, shock, preexisting renal disease) of the animal.

Ethylene glycol is another potential cause of poisoning in pigs. This product, found in high concentration in many antifreeze solutions, is not toxic per se, but once it is ingested and absorbed from the gastrointestinal tract, a proportion is enzymatically oxidized in the liver and successively transformed into several nephrotoxic compounds and finally to oxalate. Poisoning occurs in swine with the ingestion of 4–5 mL of ethylene glycol/kg of body weight (Carson 2006). Pigs may be poisoned when they have accidental access to antifreeze solution expelled during engine maintenance or from the plumbing systems in which these products are used to prevent freezing.

Many metallic compounds are nephrotoxic, including inorganic mercury, arsenic, cadmium, lead, thallium, and bismuth. Cases of poisoning with these products are relatively rare in pigs.

Gross renal lesions observed in acute cases of nephrotoxic tubular necrosis are not always conspicuous, but the kidneys may appear slightly swollen, pale, and moist (Figure 22.6). In pigweed (A. retroflexus) poisoning, these renal lesions are often accompanied by a marked perirenal edema that may contain blood and possibly by serous effusions elsewhere in the body (Osweiler et al. 1969). In severe acute tubular necrosis, death from acute renal failure may ensue. Animals that survive the acute phase of the disease either recover or develop progressive fibrosis of the kidneys that may or may not lead to chronic renal failure. This chronic evolution appears relatively common in pigs with ochratoxin A toxicosis (Cook et al. 1986; Krogh 1977; Rutqvist et al. 1978).

Histologically, acute tubular necrosis is generally characterized by swelling and necrosis of the lining epithelial cells of the proximal and distal tubules, the presence of granular casts in the tubular lumen, dilated tubules, and mild interstitial edema. The presence of large numbers of calcium oxalate crystals within tubules is a characteristic finding in ethylene glycol poisoning. Kidneys of animals surviving the acute toxic insult show evidence of epithelial regeneration and eventually, at least in some cases, interstitial fibrosis accompanied by focal loss of nephrons and a mild interstitial inflammatory infiltrate.

Since specific therapies for most of these toxicoses is virtually lacking, treatment of affected animals is essentially supportive and symptomatic. When a specific nephrotoxin is suspected, action should be taken to ensure that the offending toxin is rapidly withdrawn or that the pigs are immediately removed from the source of the intoxication. Practical measures can be taken to prevent intoxication by some of these nephrotoxins. Proper drying and storage of grain, for example, is one of the best methods of preventing mycotoxicosis, such as from ochratoxin A.

**Tubulointerstitial Diseases**

Tubulointerstitial diseases include a relatively large group of conditions characterized primarily by interstitial inflammation and tubular damage, namely, interstitial nephritis (such as that occurring from leptospirosis), embolic nephritis, and pyelonephritis (one of the most significant urinary tract diseases in swine). Immunologically mediated tubulointerstitial disease as occurs in humans has only rarely been documented in domestic animals.

**Interstitial Nephritis**

Leptospirosis is probably one of the best known causes of interstitial nephritis in pigs. Many serovars of *Leptospira* spp.—those of the serogroups Pomona, Tarassovi, and Australis, for which pigs act as maintenance hosts—cause significant disease in swine, most notably linked with reproductive problems, including infertility, abortion, and birth of weak or dead piglets. The pathogenesis of the disease involves the penetration by the leptospires of mucosal surfaces or skin, a bacteremia of a few days that lasts until the beginning of the humoral immune response, and the preferential localization and persistence of the organisms at sites physically protected from antibodies, such as in the ocular vitreous humor, the cerebrospinal fluid, the genital tract, and the lumen of the renal proximal tubules (Prescott 2007). The passage of the leptospires from the bloodstream to the interstitial tissue of the renal parenchyma and finally to the tubular lumen elicits multifocal lesions of interstitial nephritis (Cheville et al. 1980).

The severity of the interstitial nephritis varies and ranges from grossly undetectable to extensive lesions, particularly when serovars of the Pomona serogroup are involved. The lesions are randomly distributed and appear as poorly circumscribed whitish foci of various shapes and sizes, becoming confluent in severe cases. Histologically, these foci correspond to the infiltration of lymphocytes, plasma cells, and macrophages in the interstitial tissue, along with some degenerative changes of the surrounding nephrons. In chronic cases, interstitial fibrosis occurs.

In most cases, these lesions are not extensive enough to cause renal failure, so the generally asymptomatic animal may shed the leptospires in urine for a relatively long period of time and become an important source of contamination of the premises. With time, the leptospiruria becomes less intense and intermittent, but it has been reported to occur for up to 2 years in some cases (Mitchell et al. 1966).

The association between lesions of interstitial nephritis in pigs and the detection of leptospires within these kidneys is highly variable among studies (Baker et al. 1989; Boqvist et al. 2003; Hunter et al. 1987; Jones et al. 1987; McErlean 1973). Factors that may influence these results include the serovar of *Leptospira* spp.
Multifocal lesions of interstitial nephritis also occur in swine with other bacterial (see the section on “Embolic Nephritis”) and viral hematogenous infections. Although in most of these cases, the lesions do not impair renal function, they are of diagnostic significance because they are suggestive of a systemic disease.

Lesions of interstitial nephritis caused by systemic viral infections are often disclosed only upon microscopic examination and are characterized by the presence of foci of nonsuppurative inflammation. Viral infections that may produce these lesions include cytomegalovirus (Kelly 1967), adenovirus (Nietfeld and Leslie-Steen 1993; Shadduck et al. 1967), PRRS virus, and possibly others. Multifocal lesions of nonsuppurative interstitial nephritis have been reproduced experimentally in PRRS virus-infected piglets examined 2–3 weeks postinoculation (Cooper et al. 1997; Rossow et al. 1995). These lesions are found in the renal cortex as well as in the medulla. Similar renal lesions are also frequently found in naturally infected pigs.

Gross lesions of multifocal interstitial nephritis, often called white-spotted or white-dotted kidneys, represent a common cause of kidney condemnation at the slaughterhouse in some areas (Drolet et al. 2002). Lesions generally appear either as few randomly distributed or as numerous widely disseminated whitish foci, 1–3 mm in diameter (Figure 22.8). These lesions, although more numerous in the cortex, may also be found in the medulla. The finding of such renal lesions when performing a necropsy strongly suggests the possibility of a septicemia. In swine, infections with Actinobacillus suis, Streptococcus...
BODY SYSTEMS

is primarily bacterial. Ascending infection of the sterile portions of the urinary tract may lead to cystitis and pyelonephritis.

The cystitis–pyelonephritis complex has been documented as a leading cause of mortality in sows (D’Allaire and Drolet 2006). Porcine cystitis–pyelonephritis has been reported throughout the world, and the increased incidence appears to be correlated with changes in management, particularly the adoption of confinement housing for gestating sows.

A wide variety of bacteria have been isolated from cases of porcine cystitis and pyelonephritis, including *E. coli*, *A. pyogenes*, *Streptococcus* spp., and *Staphylococcus* spp. (Carr and Walton 1993). These endogenous and opportunistic organisms typically inhabit the lower urinary tract and are often referred to as being responsible for “nonspecific” urinary tract infections, which
are reviewed in their respective chapters. *Actinobaculum suis*, a specific urinary pathogen, is also an important cause of ascending urinary infection in swine. Infection with *Actinobaculum suis* frequently results in elevated sow mortality, and *Actinobaculum suis* has been isolated, either alone or in combination with other bacteria, from nearly half of the reported cases of cystitis and pyelonephritis reviewed by Carr and Walton (1993).

The pathogenesis of the cystitis–pyelonephritis complex begins with the infection of the lower urinary tract. As normal voiding of urine is one of the main mechanisms involved in maintaining sterility of the bladder, any factor that causes stasis of urine (decreased water intake/decreased urination) should be considered important in the development of this condition (Maxie and Newman 2007).

As the popularity of confinement gestation housing has risen, so has the incidence of urinary tract infections. Problems frequently encountered in confinement facilities are the reduced availability of water, increased fecal contamination of the perineal area, excessive weight gain, and leg injuries, all of which result in a reduction in the frequency of urination and enhanced bacterial establishment in the urogenital tract. Advanced-parity sows are considered more susceptible to ascending infections possibly in part because older animals are more prone to obesity, limb injuries, and lack of exercise (D’Allaire and Drolet 2006).

Once infection is established into the bladder, probably the most significant mechanism in causing renal infection (pyelonephritis) is vesicoureteral reflux. Deformation of the intravesical portion of the ureter and of ureteric orifices, as it may occur in sows with cystitis, may facilitate this retrograde flow of infected urine up the ureters and into the kidney (Maxie and Newman 2007).

Clinical signs associated with infections of the urinary tract vary according to the severity and the phase of the disease. Many cases of urinary tract infection are asymptomatic, not associated with significant lesions and disclosed only upon examination of urine. At the other end of the spectrum, in acute and severe cases of cystitis–pyelonephritis, affected animals may be found dead, probably from acute renal failure. Symptomatic animals are usually afebrile and may show anorexia, hematuria, and pyuria. The urine is often reddish brown in color with a strong odor. With *Actinobaculum suis* infection, urinary pH may increase from normal values of 5.5–7.5 up to 8–9 due to the cleavage of urea into ammonia through the use of urease enzyme of the bacteria. Animals that survive the initial infection frequently experience weight loss and reduced productivity secondary to end-stage renal disease, resulting in premature removal from the breeding herd.

Grossly, the inflammatory reaction on the mucosal surface of the bladder may be catarrhal, hemorrhagic, purulent, or necrotic, and the bladder wall may be thickened. Struvites can also be found in the lumen. The ureters, often filled with exudate, may increase to as much as 2.5 cm in diameter.

Unilateral or bilateral pyelonephritis or pyelitis is the primary lesion detected in the kidneys. The pelvic region, frequently distended with blood, pus, and foul-smelling urine, often shows irregular ulceration and necrosis of the papillae. These supplicative lesions may eventually extend irregularly through the renal medulla and even into the cortex, causing exophytic and discolored deformations of the renal surface. These foci of cortical inflammation, when present, seem to occur more frequently at the renal poles. Compound papillae that are mainly located in these latter areas are considered more susceptible to intrarenal reflux of septic urine because of the inability of their papillary ducts to close under intrapelvic pressure (Carr et al. 1991; Ransley and Risdon 1974). In long-standing cases of pyelonephritis, fibrosis ultimately replaces inflammation (Figure 22.11).

Microscopically, necrotizing ureteritis and pyelitis with accumulation of bacterial colonies can be seen, along with epithelial hyperplasia, desquamation of superficial epithelial cells, and goblet cell metaplasia with intraepithelial cyst formation (Woldemeskel et al. 2002). Renal tubules may contain protein casts, bacteria, and purulent exudate. The interstitium contains mononuclear inflammatory cells, neutrophils, and possibly some fibrosis. Examination of the ureteric valves may reveal inflammation, necrosis, and fibrosis.

Presumptive diagnosis of cystitis and pyelonephritis in live animals is best achieved when frequent micturition of bloodstained and cloudy urine can be observed. Examination of the urine sediments may also be very informative, because it may reveal the presence of inflammatory cells, erythrocytes, granular renal casts, bacteria, and crystals (Carr and Walton 1992). Blood concentrations of urea and creatinine may indicate
renal failure. Due to the striking gross lesions, confirmatory diagnosis of the condition is usually not difficult.

Determination of urea concentration in ocular fluids can be a useful aid in diagnosing cystitis–pyelonephritis in dead animals, particularly when a complete necropsy is not possible or when it is difficult to ascertain that the lesions found in the urinary tract are responsible for death (Drolet et al. 1990). A significantly higher aqueous humor urea concentration was found in sows dead of cystitis–pyelonephritis (45–92 mmol/L) than in those dead of other causes (9–10 mmol/L) (Arauz and Perfumo 2000; Chagnon et al. 1991).

Treatment of urinary tract infections may be successful if the correct antibiotic is administered early in the course of the disease. Detailed information on antibiotherapy is addressed in the section on “Nonspecific Urinary Tract Infection” of Chapter 53.

Prevention of urinary tract infections should include the maintenance of a high degree of hygiene during breeding and parturition, as well as throughout the gestation period. Facilities need to be properly designed to reduce the spread of pathogens within the breeding herd and allow efficient removal of feces from the environment. It is recommended that free-choice water be available at all times to reduce the possibility of water deprivation and its sequels. Because a higher degree of urinary tract infection can be seen in older sows, proper culling procedures are important to ensure that an optimal parity distribution is maintained within the breeding herd.

NEOPLASIA

Neoplasms are infrequent in pigs because of the low average age of the population. However, those most often recorded have been from young animals (Nielsen and Moulton 1990). Tumors of the urinary tract in swine involve mainly the kidneys. Neoplasms of the lower urinary tract, although they have been reported (Nieberle and Cohrs 1967), are generally considered exceedingly rare.

Embryonal nephroma, also named nephroblastoma, is one of the most common neoplasms of swine and is certainly the most common primary renal tumor observed in this species, although its relative prevalence varies from one region to another. As its name implies, this neoplasm appears to originate from the embryonic renal blastema. The tumor arises from the kidney or, rarely, from the perirenal tissues (probably from remnants of embryonic renal tissues). Affected animals are typically young, and most of them reach market age without significant clinical signs, the tumor being discovered at postmortem inspection. Embryonal nephroma is most commonly found as a single mass involving one kidney, but it may be multiple or bilateral (Nielsen and Moulton 1990). The tumor, which can reach impressive size, often appears firm, pale, and nodular. Metastasis infrequently occurs in swine compared with other mammals affected with embryonal nephroma. Histologically, this tumor is very peculiar and resembles disorganized embryonic renal tissue. The primitive tissue from which it arises is pluripotent and accounts for the presence of neoplastic epithelial and mesenchymal elements simultaneously observed within the tumor. Hayashi et al. (1986) classified porcine nephroblastomas into four types according to their contents: nephroblastic, epithelial, mesenchymal, and miscellaneous. Only a few of the nephroblastic tumors described in their case series had metastasized.

Other primary renal tumors are believed to be uncommon in swine. Renal carcinomas have been occasionally reported (Anderson et al. 1969; Sandison and Anderson 1968). Neoplasms originating from the renal pelvis are very rare (Vitovec 1977).

Secondary renal involvement may occur with some multisystemic or generalized cancers such as the malignant lymphoma (lymphosarcoma). In pigs, this relatively common neoplasm occurs predominantly as multicentric and thymic forms. In advanced cases of multicentric and thymic lymphomas, which involve primarily the lymph nodes and the thymus, respectively, infiltration of liver, spleen, kidneys, and other organs may occur. Renal involvement is diffuse or more often nodular so that the organs appear either enlarged and pale or dotted with pale nodules often protruding from the cortical surface (Figure 22.12). In the course of the disease, some animals may develop a leukemic phase. Renal lesions in some of these cases appear rather hemorrhagic (Marcato 1987; Stevenson and DeWitt 1973) and may be confused with some systemic infectious diseases (Figure 22.13). The precise pathogenesis of these latter lesions is uncertain but may involve either a coagulation defect or a phenomenon of acute infarction caused by the presence of intravascular neoplastic cells.
from postrenal uremia. Ruptured bladder may also occur in some cases. Treatment of pigs with obstructive urolithiasis is theoretically feasible but is generally not considered cost-effective.

The bladder of sows sometimes contains yellowish sediments that do not seem to be of clinical significance. On postmortem examination, such sediments, admixed with desquamated epithelial cells, may give the false impression of a cystitis because of the turbidity of the urine. Infection-induced calculi are also observed occasionally in sows with cystitis and pyelonephritis (Figure 22.14).

Uric acid and urate uroliths are frequently found in the kidneys of newborn piglets (Figure 22.15). These

**MISCELLANEOUS CONDITIONS**

**Urolithiasis**

Urolithiasis is the presence of calculi, or uroliths, in the urinary passages. Uroliths are macroscopic mineral (polycrystalline) concretions that may contain small quantities of organic material; the term crystalluria is used for abnormal microscopic crystalloid precipitates in urine. The mineral composition of calculi found in pigs and their relative importance have not been extensively studied. Nevertheless, various types of calculi can be found, including calcium carbonate, calcium apatite (calcium phosphate), struvite (magnesium ammonium phosphate hexahydrate), and uric acid and urate. Factors known to predispose to the formation of uroliths include the diet, urinary pH, reduced water intake, urinary stasis, and preexisting urinary tract diseases.

Swine are rarely afflicted with urolithiasis in comparison to other domestic animals. The condition is sporadically found in pigs of all ages and is also occasionally observed as incidental findings in slaughtered pigs. Outbreaks of obstructive urolithiasis have been reported on some occasions (Inoue et al. 1977; Sim 1978; Smyth et al. 1986). In these outbreaks, which involved weaned and feeder pigs as well as breeding animals, the predisposing cause for the condition was not elucidated. Animals affected with obstructive urolithiasis may demonstrate decreased appetite, oliguria or anuria, abdominal distension and pain, and death from postrenal uremia. Ruptured bladder may also occur in some cases. Treatment of pigs with obstructive urolithiasis is theoretically feasible but is generally not considered cost-effective.

The bladder of sows sometimes contains yellowish sediments that do not seem to be of clinical significance. On postmortem examination, such sediments, admixed with desquamated epithelial cells, may give the false impression of a cystitis because of the turbidity of the urine. Infection-induced calculi are also observed occasionally in sows with cystitis and pyelonephritis (Figure 22.14).

Uric acid and urate uroliths are frequently found in the kidneys of newborn piglets (Figure 22.15). These
often appear as fine orange precipitates in the medulla and pelvis. This peculiar form of urolithiasis is observed mainly in piglets that have no access to the sow’s milk (which contains both fluids and nutrients) or are afflicted by a debilitating disease associated with anorexia and diarrhea (e.g., transmissible gastroenteritis), thus contributing to dehydration. Accelerated catabolism of tissue proteins and purines to supply energy needs and decreased kidney function related to dehydration are responsible for the high levels of blood urea and uric acid found in these piglets. The excess solute, poorly reabsorbed from the glomerular filtrate, is ultimately deposited in the inner medulla and pelvis (Maxie and Newman 2007).

Hydronephrosis
Distension of the renal pelvis and calyces with urine, associated with progressive atrophy of the kidney parenchyma, is the hallmark of hydronephrosis, which is uncommon and sporadic in swine. The pathogenesis of this condition always involves some form of obstructive impediment to the normal passage of urine within the lower urinary tract, anywhere from the pelvis to the distal urethra. The causes of the obstruction include urinary calculi, exudate within urinary passages, ureteral kinking, focal external compression (abscesses, tumors), and posttraumatic or postinflammatory strictures.

Severe unilateral hydronephrosis (Figure 22.16) may develop unnoticed since the remaining kidney, if normal, may compensate adequately. In these cases, the affected kidney shows extensive dilatation of the pelvis and calyces at the expense of the renal parenchyma, which may appear as a thin layer of cortical tissue. Depending on the location of the obstruction, hydroureter may also develop. In long-standing cases, the kidney may be virtually transformed into a large fluid-filled sac delimited by a severely distended renal capsule. These extreme lesions may take months to develop. Since stagnation of urine predisposes to infection, the urine may be transformed into a purulent exudate in some instances. In cases of bilateral hydronephrosis, affected animals usually die from uremia before renal lesions get fully developed.

Parasitic Infections
The pig is the final or intermediate host of a number of parasitic helminths (see Chapter 67). Compared with some other body systems, the urinary tract is the niche of very few of these parasites. Renal infections with Dioctophyma renale, the giant kidney worm, and with larval stages of certain tapeworms may occur on rare occasions. The most significant helminth with tropism for the urinary system of swine is the nematode Stephanurus dentatus, the so-called kidney worm of swine.

Stephanurus dentatus is a widely distributed strongylid worm and is most prevalent in warm climates, including the southern United States. In endemic areas, this parasitic infection may have significant economic impact since it is associated with deaths, retarded growth, decreased feed efficiency, and condemnations at the abattoir (Batte et al. 1960). Larvae of this nematode need moisture and shade for optimal survival, so pigs raised on soil in this type of environment are the most prone to the disease. Infective larvae penetrate the skin or are ingested by the pigs. Transplacental fetal infection is also possible (Batte et al. 1966).

After being introduced into the host, the larvae molt and migrate to the liver, where they remain for several months, causing severe hepatic damage and inflammation. The presence of the parasite within the hepatic parenchyma is often responsible for extensive liver condemnation in some herds (Hale and Marti 1983). Eventually, some larvae escape from the liver and migrate to the abdominal cavity and potentially to various ectopic sites, eliciting a severe inflammatory reaction. To complete the cycle, some adults establish themselves in the perirenal tissues or, more rarely, within the kidney. At that site, the worms, measuring about 3 cm in length, are found in cystic inflammatory nodules that communicate with either the pelvis or the ureter in order to shed their eggs successfully into urine. The prepatent period in most cases is at least 9 months, and adults may shed ova in urine for over 2 years (Batte et al. 1960, 1966). Preventive and curative measures for the control of parasites are addressed in detail in Chapter 67.

Other Conditions
Mineralization of the kidneys occurs in swine with acute vitamin D toxicosis. This poisoning is usually observed when excessive amounts of vitamin D$_3$ are
Mucinous metaplasia of the epithelial cells lining the renal pelvis, ureter, and urinary bladder is occasionally observed in pigs. This rather nonspecific lesion, of uncertain pathogenesis, has been reported in pigs with various conditions, including exudative epidermitis, *E. coli* enteritis, classical swine fever, and suppurative arthritis (Brobst et al. 1971), as well as in urinary tract infections.

Ossification of the renal pelvis of unknown etiology has been reported in slaughtered pigs (Bundza 1990).

**DIFFERENTIAL DIAGNOSIS**

Main differential diagnoses based on gross renal findings are presented in Table 22.1.

<table>
<thead>
<tr>
<th>Gross Findings</th>
<th>Differential Diagnosis</th>
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<tbody>
<tr>
<td>Normal size kidneys with widespread petechiae</td>
<td>• Bacterial septicemia (Figure 22.3)</td>
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<tr>
<td></td>
<td>• Acute viremic diseases: classical swine fever (CSF), African swine fever (ASF), cytomegalovirus</td>
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<tr>
<td></td>
<td>• Electrocuton</td>
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<tr>
<td></td>
<td>• Some intoxications</td>
</tr>
<tr>
<td>Enlarged and edematous kidneys with widespread petechiae</td>
<td>• Acute glomerulonephritis(^a) (GN) including PDNS (Figure 22.5)</td>
</tr>
<tr>
<td>Enlarged, pale, and edematous kidneys</td>
<td>• Acute GN(^b)</td>
</tr>
<tr>
<td></td>
<td>• Acute tubular necrosis: various toxic agents (Figure 22.6)</td>
</tr>
<tr>
<td>Multiple renal hemorrhages (larger than petechiae)</td>
<td>• Trauma</td>
</tr>
<tr>
<td>One or both kidneys with red or white foci of necrosis (often wedge-shaped and cortical)</td>
<td>• Necrosis</td>
</tr>
<tr>
<td>Kidneys with patchy palor or obvious white foci within renal parenchyma</td>
<td>• Clotting defects: anticoagulant poisoning, isoimmune thrombocytopenia</td>
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<tr>
<td>Kidneys with multiple microabcesses</td>
<td>• Acute or subacute infarcts: thrombosis, vasculitis, emboli</td>
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<tr>
<td>One or both kidneys with exudate within dilated pelvis and possibly papillary necrosis and asymmetric foci of parenchymal suppurative inflammation</td>
<td>• Interstitial nephritis: circovirus, leptospirosis, undetermined cause (Figures 22.7 and 22.8)</td>
</tr>
<tr>
<td>Kidneys with diffusely pale, firm, and fibrotic with generalized fine granularity of the capsular surface</td>
<td>Note: kidneys may also be enlarged with circovirus</td>
</tr>
<tr>
<td>One or both kidneys with large irregular fibrous bands with intervening areas of normal parenchyma; dilation of pelvis and deformities of papillae</td>
<td>• Suppurative embolic nephritis: <em>Erysipelothrix rhusiopathiae</em>, <em>Actinobacillus suis</em> (Figure 22.9)</td>
</tr>
<tr>
<td>One or both kidneys with large irregular fibrous bands with intervening areas of normal parenchyma; normal pelvis</td>
<td>• Acute pyelonephritis: various bacteria including <em>Actinobaculum suis</em></td>
</tr>
<tr>
<td>One or both kidneys with one to several fluid-filled cysts that do not communicate with the pelvis</td>
<td>Note: often accompanied with ureteritis and cystitis</td>
</tr>
<tr>
<td>One or both kidneys with dilation of the pelvis and calyces associated with atrophy and cystic enlargement of the kidney</td>
<td>• Chronic GN(^b)</td>
</tr>
<tr>
<td>One or both kidneys with multiple exophytic nodules of various sizes</td>
<td>• Chronic tubular necrosis: some toxic agents including ochratoxin A</td>
</tr>
<tr>
<td></td>
<td>• Chronic generalized interstitial nephritis (uncommon in pigs)</td>
</tr>
<tr>
<td></td>
<td>• Chronic pyelonephritis (Figure 22.11)</td>
</tr>
<tr>
<td></td>
<td>• Chronic infarcts (septic or not)</td>
</tr>
<tr>
<td></td>
<td>• Renal dysplasia (rare)</td>
</tr>
<tr>
<td></td>
<td>• Congenital renal cysts (Figure 22.2)</td>
</tr>
<tr>
<td></td>
<td>• Hydronephrosis(^b) (obstructive nephropathy) (Figure 22.16)</td>
</tr>
<tr>
<td></td>
<td>• Lymphoma (often bilateral) (Figures 22.12–22.13)</td>
</tr>
<tr>
<td></td>
<td>• Embryonal nephroma (often unilateral)</td>
</tr>
<tr>
<td></td>
<td>• Other neoplasms (rare)</td>
</tr>
</tbody>
</table>

\(^{a}\)Acute renal failure possible.

\(^{b}\)Chronic renal failure possible.
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Overview of Viruses

Kyoung-Jin Yoon

VIRUS, VIRION, AND VIRUS STRUCTURE

Viruses are unicellular, intracellular parasites with characteristics very distinct from other known unicellular microorganisms (Table 23.1). An important property common to all viruses is their requirement to replicate within living cells. Indeed, they rely on the host cell to provide many of the resources required for their replication. Historically, the existence of viruses was established when it was shown that exposure to “filterable” (bacteria-free) material could cause disease. Filtration through small-sized pores (<300 nm) is still used to separate viruses from bacteria and other microorganisms.

A “virion” is an intact infectious virus outside a living cell (Figure 23.1). A virion contains RNA or DNA for encoding the proteins responsible for its replication and/or structure. The genome can be single stranded or double stranded, can be linear or circular form, and can contain one or more segments. A protein coat (“capsid”) protects the viral genome, and these two components together form the nucleocapsid. The nucleocapsid is sometimes covered by an outer structure (“envelope”), which is acquired as the nucleocapsid passes (“buds”) through a cell membrane, that is, cytoplasmic, intracytoplasmic, or nuclear membrane. Due to the nature of the process, the viral envelope is structurally identical (lipid bilayer) to the cell membrane from which it was derived, but an enveloped virion also has additional proteins on the surface of the envelope. Those proteins are encoded by the viral genome, not the host cell genome, and are attached to the matrix protein (or the tegument in the case of herpesviruses) located between the envelope and the nucleocapsid. These envelope proteins, sometimes called envelope glycoproteins, envelope-associated proteins, or peplomers, play a critical role in the process of virus infection and replication. For example, they are involved in virus attachment (receptor binding), membrane fusion and uncoating, and release of progeny virus (receptor destruction).

Virions are assembled from newly synthesized components in either the cytoplasm or nucleus of the host cell. Once assembled, they exit the cell via budding, membrane fusion, or cell lysis. Some virions include a small amount of a nonstructural protein (viral DNA or RNA polymerase) used at the initiation of the next cycle of replication to produce an intermediate replicating form of the viral genome. Alternatively, viruses may encode a polymerase or replicase in the genome, along with the replicative proteins that will be expressed at the initiation of the virus replication cycle.

VIRUS CLASSIFICATION

Taxonomy is a method to organize and process information. Early taxonomic systems organized viruses based on clearly observable characteristics: the disease or pathology associated with the virus, for example, hepatitis, and/or its epidemiological or ecological characteristics, for example, arboviruses. Later, virus structure (size and shape), reactivity (pH, temperature, etc.), and antigenicity (serological cross-reactivity), were incorporated into classification systems.

Currently, viruses are organized by primary and secondary characteristics. Primary characteristics include (1) genome characteristics (RNA vs. DNA, strandedness, segmentation, circular vs. linear, polarity, haploid vs. diploid) and (2) virion structure (morphology, envelope, nucleocapsid symmetry, capsomere number).
using primary characteristics, viruses can be classified as (1) DNA enveloped, (2) DNA nonenveloped, (3) RNA enveloped, and (4) RNA nonenveloped (Figure 23.2).

Knowing which group a virus belongs to can be clinically useful. For example, DNA viruses tend to be more genetically stable than RNA viruses during the process of replication, meaning that DNA viruses are also antigenically more stable. Enveloped viruses are more susceptible to environmental stresses than non-enveloped viruses and lose their infectivity easily when exposed to lipid solvents or detergents. These general concepts can be useful in devising general strategies for the prevention and control of diseases by viruses in each group.

Virus classification and nomenclature follow the rules of the International Committee on Taxonomy of Viruses (ICTV). The universal system of viral taxonomy is set at the levels of order, family, subfamily, genus, and species. Orders are used to link families that exhibit distant phylogenetic relationships, for example, ancient conserved genes, sequences, or domains. Subfamilies are used only where needed to deal with complex interrelationships among the viruses in a family. Below the level of species, viruses may be identified as “strains” or “variants.” This terminology may be useful for the purpose of diagnostics and vaccine development, but there is no formal taxonomy or classification criterion at this level.

VIRUS NOMENCLATURE

Taxonomical nomenclature for viruses consists of order, family, subfamily, genus, and species (Table 23.2). The names of orders end with the suffix “-virales,” families with the suffix “-viridae,” subfamilies with the suffix “-virinae,” and genera with the suffix “-virus.” There are no formal suffixes at the level of species or lower, that is, types, subtypes, genotypes, serotypes, strains, or variants.

In formal nomenclature, the virus family, subfamily, and genus names are italicized and the first letters capitalized. Species designations are not capitalized (unless they are derived from a place name) nor are they italicized. Examples of proper taxonomical terminology for viruses are the following:

1. order Herpesvirales, family Herpesviridae, subfamily Alphaherpesvirinae, genus Varicellovirus, suid herpesvirus 1 (Aujeszky’s disease virus or pseudorabies virus);
2. order Mononegavirales, family Paramyxoviridae, subfamily Paramyxovirinae, genus Henipavirus, Nipah virus;
3. order Nidovirales, family Arteriviridae, genus Arterivirus, porcine reproductive and respiratory syndrome (PRRS) virus;
4. family Circoviridae, genus Circovirus, porcine circovirus 2;
5. family Orthomyxoviridae, genus Influenzavirus A, swine influenza virus H1N1.

Although there is no formal suffix at the species level, a systemic naming convention exists for some viruses, for example, herpesviruses. Suid herpesvirus 1 is the official name for Aujeszky’s disease virus (also known as pseudorabies virus). Following this convention, “porcine reproductive and respiratory syndrome virus” would more appropriately be termed, “porcine arterivirus.”

### Table 23.1. Comparison of basic properties among monocellular microorganisms

<table>
<thead>
<tr>
<th>Property</th>
<th>Bacteria</th>
<th>Mycoplasma</th>
<th>Rickettsia</th>
<th>Chlamydia</th>
<th>Virus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Size &gt;300 nm a</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Grow on artificial media</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Divide by binary fission</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Contain both DNA and RNA</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Contain muramic acid</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Sensitive to antibiotics</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
</tr>
</tbody>
</table>

aSome mycoplasmas and chlamydia are <300 nm in diameter.

Source: Adapted from Murphy et al. (1999).
In formal use, an example of a complete taxonomical description of a virus would be “porcine reproductive and respiratory syndrome (PRRS) virus is a member of genus Arterivirus, family Arteriviridae. . . .” In informal vernacular usage, all terms are written in lowercase except those derived from place or individual’s names; they are not italicized, do not include the formal suffix, and the name of the taxon follows the name, for example, “the arterivirus family” and “the teschovirus genus.”

LABORATORY METHODS FOR VIRUS CLASSIFICATION

Characterization of Virion Structure
Electron microscopy (EM) is most commonly used to visualize a virus in a clinical specimen and characterize virion morphology (shape and size). The morphology of most viruses is sufficiently distinct to be able to use EM to assign an unknown virus to the correct family. The presence of an envelope and, in some cases, the nucleocapsid symmetry, can be determined by EM. This method is particularly useful when dealing with a nonculturable virus. The method can be used on both fluid specimens (vesicle fluid, urine, feces) and tissues. Low diagnostic sensitivity is the biggest limitation of EM, but the sensitivity of the process and accuracy of virus identification can be improved by including virus-specific antibody in the procedure (immunoelectron microscopy).

Ultrafiltration can be used to estimate the virion’s size. In this procedure, a concentrated, purified virus suspension is passed through a series of membrane filters with different pore sizes (10–300 nm). Filterable viruses (presence and quantity) are detected in each filtrate using other laboratory methods. The virion size is based on the pore sizes of two filters—one allowing virus to pass and the other not allowing virus to pass.
or substantially reducing the amount of viruses passed. While EM estimates the size of the dehydrated virion, ultrafiltration gives an estimate of the hydrated virion. The presence of a viral envelope can usually be determined by exposing the virus to a lipid solvent (ether, chloroform) or detergent (sodium deoxycholate; Triton® X-100, Dow Chemical Company, Midland, MI). The infectivity of enveloped virions is easily destroyed by these reagents, whereas nonenveloped viruses retain their infectivity.

The viral molecular weight \( M \) can be estimated from hydrodynamic data based on the Svedberg equation:

\[
M = \frac{15000 \times \sigma}{S^2}
\]

Table 23.2. Viral taxonomy for orders, families, and genera containing viruses infecting pigs

<table>
<thead>
<tr>
<th>Order</th>
<th>Family</th>
<th>Subfamily</th>
<th>Genus</th>
<th>Species</th>
</tr>
</thead>
<tbody>
<tr>
<td>Herpesvirales</td>
<td>Herpesviridae</td>
<td>Alphaherpesvirinae</td>
<td>Varicellovirus</td>
<td>Pseudorabies virus (suid herpesvirus 1)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Betaherpesvirinae</td>
<td>Unassigned</td>
<td>Porcine cytomegalovirus (suid herpesvirus 2)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Gammaherpesvirinae</td>
<td>Macavirus</td>
<td>Porcine lymphotropic herpesvirus (suid herpesviruses 3–5), malignant</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>catarrhal fever virus (ovine herpesvirus 2)</td>
</tr>
<tr>
<td>Mononegavirales</td>
<td>Filoviridae</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Paranyxoviridae</td>
<td>Paramyxovirinae</td>
<td>Ebolavirus</td>
<td>Reston ebolavirus, Zaire ebolavirus</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Henipavirus</td>
<td>Nipah virus</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Rubulavirus</td>
<td>Porcine rubulavirus, Menangle virus</td>
</tr>
<tr>
<td></td>
<td>Rhabdoviridae</td>
<td>Visculovirus</td>
<td>Betaherpesvirus</td>
<td>Viscular stomatitis virus</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lyssavirus</td>
<td></td>
<td>Rabies virus</td>
</tr>
<tr>
<td>Nidovirales</td>
<td>Arterviridae</td>
<td></td>
<td></td>
<td>Porcine reproductive and respiratory syndrome virus</td>
</tr>
<tr>
<td></td>
<td>Coronaviridae</td>
<td></td>
<td>Alpha coronavirus</td>
<td>Hemagglutinating encephalomyelitis virus</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Torovirinae</td>
<td>Betacoronavirus</td>
<td>Porcine torovirus</td>
</tr>
<tr>
<td></td>
<td>Picornaviridae</td>
<td>Picornaviridae</td>
<td>Enterovirus</td>
<td>Porcine enterovirus B, swine vesicular disease virus</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Cardio virus</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Aphthovirus</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Teshovirus</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Kobuvirus</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Sapelovirus</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Senacaviruses</td>
</tr>
<tr>
<td></td>
<td>Adenoviridae</td>
<td>Metaadenovirus</td>
<td></td>
<td>Porcine adenovirus (A, B, C)</td>
</tr>
<tr>
<td></td>
<td>Anellovirdae</td>
<td>Iotatorquevirius</td>
<td></td>
<td>Torque teno suid virus (1, 2)</td>
</tr>
<tr>
<td></td>
<td>Asfaviridae</td>
<td>Asfivirus</td>
<td></td>
<td>African swine fever virus</td>
</tr>
<tr>
<td></td>
<td>Astroviridae</td>
<td>Mamastrovirus</td>
<td></td>
<td>Porcine astrovirus</td>
</tr>
<tr>
<td></td>
<td>Bunyaviridae</td>
<td>Orthobunyavirus</td>
<td></td>
<td>Akabane virus, Oya virus, Lumbo virus, Tahyna virus</td>
</tr>
<tr>
<td></td>
<td>Caliciviridae</td>
<td>Vesivirus</td>
<td></td>
<td>Vesicular exanthema of swine virus</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sapovirus</td>
<td></td>
<td>Swine sapovirus</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Norovirus</td>
<td></td>
<td>Swine norovirus</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Valovirus</td>
<td></td>
<td>St-Valérien virus</td>
</tr>
<tr>
<td></td>
<td>Circoviridae</td>
<td>Circovirus</td>
<td></td>
<td>Porcine circovirus (1, 2)</td>
</tr>
<tr>
<td></td>
<td>Flaviviridae</td>
<td>Flavivirus</td>
<td></td>
<td>Japanese encephalitis B virus, West Nile virus, Murray Valley encephalitis</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pestivirus</td>
<td></td>
<td>Classical swine fever virus, bovine viral diarrhea virus, border disease</td>
</tr>
<tr>
<td></td>
<td>Hepeviridae</td>
<td>Hepevirus</td>
<td></td>
<td>Swine hepatitis E virus</td>
</tr>
<tr>
<td></td>
<td>Orthomyxovirida</td>
<td>Influenza virus A</td>
<td></td>
<td>Swine influenza virus</td>
</tr>
<tr>
<td></td>
<td>Parvoviridae</td>
<td>Parvovirus</td>
<td></td>
<td>Porcine parvovirus</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Hokovirus</td>
<td></td>
<td>Porcine hokovirus (porcine parvovirus 3')</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Canivirus</td>
<td></td>
<td>Porcine parvovirus 2 (H-1 parvovirus)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Unassigned</td>
<td></td>
<td>Porcine parvovirus 4</td>
</tr>
<tr>
<td></td>
<td>Poxviridae</td>
<td>Saipoxivirus</td>
<td></td>
<td>Swine poxvirus</td>
</tr>
<tr>
<td></td>
<td>Reoviridae</td>
<td>Sedoreovirinae</td>
<td>Rotavirus</td>
<td>Porcine rotavirus</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Spinareovirinae</td>
<td>Orthoreovirus</td>
<td>Porcine reovirus</td>
</tr>
<tr>
<td></td>
<td>Retroviridae</td>
<td>Orthoretrovirinae</td>
<td>Gammaaretrovirus</td>
<td>Porcine endogenous retrovirus</td>
</tr>
<tr>
<td></td>
<td>Togaviridae</td>
<td>Alphavirus</td>
<td></td>
<td>Eastern equine encephalitis virus, Getah virus, Sagiyama virus</td>
</tr>
</tbody>
</table>

*Proposed names.
RNase or DNase and then subjected to gel electrophoresis to determine which enzyme digested the genetic material. Chemical determination uses the diphenylamine reaction for DNA or the orcinol reaction for RNA to characterize the makeup of the genome.

Feulgen staining is a specific cytochemical technique used in histology to indicate the presence of DNA or chromosomal material in cell specimens. It is based on converting DNA, but not RNA, to a purpuric acid by acid hydrolysis with release of the aldehyde functions. The aldehyde functions then give a magenta color (pink or red) when the specimen is treated with Schiff reagent. Optionally, the sample can be counterstained for green background. The Feulgen reaction is a semiquantitative method, unless aldehydes remaining in the cell are those produced only by the hydrolysis of DNA. The reaction is not suitable for living cells.

Acridine orange staining technique can be used to differentiate double-stranded DNA or RNA viruses from single-stranded DNA or RNA viruses in infected cells. Double-stranded DNA or RNA is visualized as a yellow-green fluorescence, whereas single-stranded RNA or DNA is orange-red. In combination with the location of fluorescence in cells (nucleus vs. cytoplasm), both the nucleic acid composition (DNA vs. RNA) and the strandedness of the virus can also be determined.

Electrophoretic techniques can be used to determine if a virus has more than one molecule of double-stranded RNA or DNA. For certain viruses, for example, rotavirus, electrophoretic typing based on migration pattern of RNA segments has been used to determine groups. The polarity (sense) of viral nucleic acid can be determined through transfection or in vitro translation studies with extracted viral genome. Appropriate cells transfected with positive-sense viral genome produce virions since such a genomic material serves as mRNAs translated into proteins. Under specific conditions, an in vitro translation study can demonstrate the same effect.

Genetic techniques are powerful taxonomical tools and increasingly used to identify viruses. Public databases that contain genomic sequences for all viral taxa, such as GenBank®, provide a reference for the taxonomical identification of unknown viruses. Furthermore, polymerase chain reaction (PCR)- or microarray-based assays targeting genetically conserved genes can quickly identify a virus to the level of family or genus.

**Characterization of Specific Viral Phenotypes**

Hemagglutination (HA), the ability to agglutinate red blood cells (RBCs), is an informative virus characteristic. Infectious viruses are not required to conduct the HA assay, as long as the proteins with hemagglutinating activity remain intact and functional. Orthomyxoviruses and paramyxoviruses agglutinate the RBCs of several species at low temperatures (4–22°C), whereas some other viruses can agglutinate RBCs of specific species at a higher temperature (37°C). The specificity of HA can be confirmed by a HA-inhibition assay after virus-specific antiserum is obtained.

Members of several virus families produce hemadsorption. That is, cells infected with these viruses bind RBCs on their cytoplasmic membranes at the sites of virion budding. Hemadsorption can be evaluated once cells permissive to virus infection are identified.

Microscopic examination of formalin-fixed, infected cells stained with hematoxylin and eosin is a useful laboratory technique to demonstrate virus-induced inclusion bodies (intranuclear vs. intracytoplasmic) or syncytial formation in cell culture. These characteristics are useful for the identification of some viruses.

**CHARACTERISTICS OF VIRAL FAMILIES**

Virus families have unique physiochemical and biological characteristics and properties that separate one family from the others (Table 23.3). Some of the unique structural, replicative, or biological features of the families containing viruses infectious for swine are described below. For details, refer to specific chapters.

**DNA Viruses**

**Family Adenoviridae (Chapter 24).** The adenovirus virion is distinct hexagonal shape with lengthy fibers projecting from nucleocapsid. These viruses replicate in the nucleus and are known to modulate the host immune response for their replication. Many adenoviruses cause persistent infection and may be reactivated by immunosuppression. Some of the adenoviruses of humans, cattle, and chickens show oncogenic capacity in laboratory animals, but none cause tumors in its natural host. Porcine adenoviruses tend to have low to moderate pathogenicity and generally do not cause serious disease in swine.

**Family Anelloviridae (Chapter 27).** This is a newly established family with nine genera and includes torque teno viruses (TTVs) of humans and animals. Virions are
nonenveloped and contain a molecule of single-stranded, negative-sense, circular DNA. The viruses share similarity with circoviruses in genomic organization and gene expression. No in vitro cell culture system has been identified and a causal role in disease has not been clearly established in any species.

**Family Asfarviridae (Chapter 25).** African swine fever virus (ASFV) is an important pathogen of domestic swine and some wild suid species and the only member of this family. The virion is enveloped and contains a complex icosahedral capsid composed of ≥1892 capsomers. The viral DNA has covalently closed ends and encodes up to 200 proteins. Replication occurs primarily in the cytoplasm, although the nucleus is needed for viral DNA synthesis. Virions are released by budding or cell lysis. The virus is transmitted by contact and by soft ticks (genus Ornithodoros).

**Family Circoviridae (Chapter 26).** Although the virion contains positive-sense DNA, circoviruses utilize an ambisense transcription strategy (encoding some genes in the viral sense DNA strand and other genes in the complementary sense strand). Virions are extremely stable in the environment. Replication occurs in the nucleus of cells in the S phase of the cell cycle. Identified in the late 1990s, porcine circovirus type 2 (PCV2) is a pathogen with global distribution.

### Table 23.3. Viral families containing animal and human pathogens and their distinguishable physicochemical properties

<table>
<thead>
<tr>
<th>Family</th>
<th>Virion Diameter (nm)</th>
<th>Envelope</th>
<th>Symmetry</th>
<th>Type</th>
<th>Structure</th>
<th>Size (kb)</th>
<th>Virion Polymerase</th>
<th>Virion Assembly</th>
</tr>
</thead>
<tbody>
<tr>
<td>Filoviridae</td>
<td>800–950 × 80</td>
<td>+</td>
<td>H</td>
<td>RNA</td>
<td>ss(−) linear</td>
<td>18.9–19.1</td>
<td>+</td>
<td>Cy</td>
</tr>
<tr>
<td>Filoviridae</td>
<td>250–300 × 200</td>
<td>+</td>
<td>C</td>
<td>DNA</td>
<td>ds linear</td>
<td>130–375</td>
<td>+</td>
<td>Cy</td>
</tr>
<tr>
<td>Filoviridae</td>
<td>175–215</td>
<td>+</td>
<td>I</td>
<td>DNA</td>
<td>ds linear</td>
<td>170–190</td>
<td>−</td>
<td>Cy</td>
</tr>
<tr>
<td>Filoviridae</td>
<td>160–350</td>
<td>±</td>
<td>I</td>
<td>DNA</td>
<td>ss linear</td>
<td>15–18</td>
<td>+</td>
<td>Cy</td>
</tr>
<tr>
<td>Filoviridae</td>
<td>150–600</td>
<td>+</td>
<td>H</td>
<td>DNA</td>
<td>ds linear</td>
<td>120–235</td>
<td>−</td>
<td>Nu</td>
</tr>
<tr>
<td>Filoviridae</td>
<td>150–200</td>
<td>+</td>
<td>H</td>
<td>RNA</td>
<td>ss(+) linear</td>
<td>26.2</td>
<td>−</td>
<td>Cy</td>
</tr>
<tr>
<td>Filoviridae</td>
<td>100–300</td>
<td>+</td>
<td>H</td>
<td>RNA</td>
<td>ss(−) circular</td>
<td>10–14</td>
<td>+</td>
<td>Cy</td>
</tr>
<tr>
<td>Filoviridae</td>
<td>180 × 75</td>
<td>+</td>
<td>H</td>
<td>RNA</td>
<td>ss(−) linear</td>
<td>13–16</td>
<td>+</td>
<td>Cy</td>
</tr>
<tr>
<td>Filoviridae</td>
<td>80–160</td>
<td>+</td>
<td>H</td>
<td>RNA</td>
<td>ss(+) linear</td>
<td>20–33</td>
<td>−</td>
<td>Cy</td>
</tr>
<tr>
<td>Filoviridae</td>
<td>80–120</td>
<td>+</td>
<td>H</td>
<td>RNA</td>
<td>ss(−) linear</td>
<td>10–14.6</td>
<td>+</td>
<td>Cy</td>
</tr>
<tr>
<td>Filoviridae</td>
<td>80–120</td>
<td>+</td>
<td>H</td>
<td>RNA</td>
<td>ss(−) linear</td>
<td>11–22.7</td>
<td>+</td>
<td>Cy</td>
</tr>
<tr>
<td>Filoviridae</td>
<td>80–110</td>
<td>−</td>
<td>I</td>
<td>DNA</td>
<td>ds linear</td>
<td>32–40</td>
<td>−</td>
<td>Nu</td>
</tr>
<tr>
<td>Filoviridae</td>
<td>80–100</td>
<td>+</td>
<td>I</td>
<td>RNA</td>
<td>ss(+) linear</td>
<td>7–11</td>
<td>−</td>
<td>Cy</td>
</tr>
<tr>
<td>Filoviridae</td>
<td>60–80</td>
<td>−</td>
<td>I</td>
<td>RNA</td>
<td>ds linear</td>
<td>16–27</td>
<td>+</td>
<td>Cy</td>
</tr>
<tr>
<td>Filoviridae</td>
<td>65–70</td>
<td>+</td>
<td>I</td>
<td>RNA</td>
<td>ss(+) linear</td>
<td>9.7–11.8</td>
<td>−</td>
<td>Cy</td>
</tr>
<tr>
<td>Filoviridae</td>
<td>50–70</td>
<td>+</td>
<td>I</td>
<td>RNA</td>
<td>ss(+) linear</td>
<td>13–15</td>
<td>−</td>
<td>Cy</td>
</tr>
<tr>
<td>Filoviridae</td>
<td>60</td>
<td>−</td>
<td>I</td>
<td>RNA</td>
<td>ds linear</td>
<td>7</td>
<td>−</td>
<td>Cy</td>
</tr>
<tr>
<td>Filoviridae</td>
<td>50–60</td>
<td>+</td>
<td>I</td>
<td>RNA</td>
<td>ss(−) linear</td>
<td>8.9</td>
<td>+</td>
<td>Nu</td>
</tr>
<tr>
<td>Filoviridae</td>
<td>40–60</td>
<td>+</td>
<td>I</td>
<td>RNA</td>
<td>ss(+) linear</td>
<td>9.5–12.5</td>
<td>−</td>
<td>Cy</td>
</tr>
<tr>
<td>Filoviridae</td>
<td>40–85</td>
<td>−</td>
<td>I</td>
<td>DNA</td>
<td>ds linear</td>
<td>5.3–8</td>
<td>−</td>
<td>Nu</td>
</tr>
<tr>
<td>Filoviridae</td>
<td>40–55</td>
<td>−</td>
<td>I</td>
<td>DNA</td>
<td>ds circular</td>
<td>5.3–8</td>
<td>−</td>
<td>Nu</td>
</tr>
<tr>
<td>Filoviridae</td>
<td>40–48</td>
<td>+</td>
<td>I</td>
<td>DNA</td>
<td>ds circular</td>
<td>3–3.3</td>
<td>+</td>
<td>Nu</td>
</tr>
<tr>
<td>Filoviridae</td>
<td>35–39</td>
<td>−</td>
<td>I</td>
<td>RNA</td>
<td>ss(+) linear</td>
<td>7.4–8.3</td>
<td>−</td>
<td>Cy</td>
</tr>
<tr>
<td>Filoviridae</td>
<td>30–32</td>
<td>−</td>
<td>I</td>
<td>DNA</td>
<td>ss(−) circular</td>
<td>2–4</td>
<td>+</td>
<td>?d</td>
</tr>
<tr>
<td>Filoviridae</td>
<td>27–34</td>
<td>−</td>
<td>I</td>
<td>RNA</td>
<td>ss(+) linear</td>
<td>7.1–7.2</td>
<td>−</td>
<td>?</td>
</tr>
<tr>
<td>Filoviridae</td>
<td>27–30</td>
<td>−</td>
<td>I</td>
<td>RNA</td>
<td>ss(+) linear</td>
<td>6.8–7.9</td>
<td>−</td>
<td>Cy</td>
</tr>
<tr>
<td>Filoviridae</td>
<td>22–30</td>
<td>−</td>
<td>I</td>
<td>RNA</td>
<td>ss(+) linear</td>
<td>7–8.5</td>
<td>−</td>
<td>Cy</td>
</tr>
<tr>
<td>Filoviridae</td>
<td>18–26</td>
<td>−</td>
<td>I</td>
<td>DNA</td>
<td>ss(−) linear</td>
<td>5</td>
<td>+</td>
<td>Nu</td>
</tr>
<tr>
<td>Filoviridae</td>
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<td>−</td>
<td>I</td>
<td>DNA</td>
<td>ss(+) circular</td>
<td>1.7–2.3</td>
<td>−</td>
<td>Nu</td>
</tr>
</tbody>
</table>

Note: Families are listed in order of virion size (large to small).  
*Nuclocapsid symmetry abbreviation: complex (C), icosahedral (I), and helical (H).  
Location where virion assembly takes place: cytoplasm (Cy) and nucleus (Nu).  
†Intracellular virion is not enveloped.  
Information is not available as in vitro cultivation method is not available.  
Source: Adapted from Murphy et al. (1999).
Family Herpesviridae (Chapter 28). Herpesvirus virions are enveloped, possess distinct projections, and contain a unique protein known as tegument between envelope and capsid. Viruses replicate in the nucleus and acquire an envelope by budding through the nuclear membrane. An important common feature of all herpesvirus infections is lifelong, persistent infection (latency) with occasional recrudescence of disease. Gamma herpesviruses are oncogenic. Herpesviruses have been identified in most, if not all, of animal species. Pseudorabies virus (suid herpesvirus 1) and porcine cytomegaloviruses (suid herpesvirus 2) are well-known herpesvirus pathogens of swine. Porcine lymphotropic herpesviruses and ovine herpesvirus 2, the cause of porcine malignant catarrhal fever, also belong in this family.

Family Paroviridae (Chapter 29). Virions are known to be very stable in the environment. Virus replication and virion assembly take place in the nucleus. Typically, replication of paroviruses requires host cells in the S phase of the cell cycle so that viruses can utilize host DNA replication machinery for their DNA replication. Formation of hairpin structure in viral DNA at the beginning of replication is a unique feature of paroviral replication. That structure provides a template to form double-stranded DNA intermediate for further replication. Porcine parovirus (type 1) is an economically important, globally distributed pathogen of pigs.

Family Poxviridae (Chapter 30). Typical poxvirus is an enveloped virion in a “brick” or “dumbbell” shape with very distinctive surface membrane although parapoxvirus virions are ovoid. The virion has a very distinct outer coat layer and a complex inner structure. Viral replication and virion assembly occur in discrete sites within the cytoplasm (called viroplasm), and enveloped virions are released by budding. Poxviruses have been identified in most of animal species. Yet, the viruses tend to have narrow host ranges with exception of vaccinia virus and are transmitted by direct contact (e.g., wounds, abrasions), by fomites, and by aerosol. Mechanical transmission by arthropods has been reported. Swine poxvirus is the only swine pathogen in this family.

RNA Viruses

Family Arteriviridae (Chapter 31). PRRS virus is the only virus of swine in this family. A unique feature of arterivirus replication is gene expression through the synthesis of a nested set of subgenomic mRNAs. Arteriviruses primarily target macrophages and establish chronic, persistent infections, but not latency.

Family Astroviridae (Chapter 32). The name of virion comes from the distinctive five- or six-pointed star shape visible under EM. Virions are nonenveloped, with polyhedral symmetry. Viral replication and assembly take place in the cytoplasm, and the virus is released via cell lysis. Porcine astrovirus has been found in feces from diarrheic pigs, but its role in enteric disease is uncertain.

Family Bunyaviridae (Chapter 33). Bunyavirus virions are spherical and enveloped with fine peplomers, containing three segments of circular helical nucleocapsid formed by base-paired terminal nucleotides. The genome consists of three molecules (L, M, S) of “circular,” negative (or ambisense in some viruses), single-stranded RNA (11–21 kb). Viruses replicate in the cytoplasm and bud from Golgi membranes. Bunyaviruses (Akabane, Oya, Tahyna, and Lumbo viruses) have occasionally been isolated from pigs.

Family Caliciviridae (Chapter 34). On EM, calicivirus virions often show symmetrically arranged “cup”-shaped depressions on their surface. Viral replication and virion assembly take place in the cytoplasm, and the virus is released via cell lysis. Caliciviruses are primarily transmitted via the fecal–oral route. Vesicular exanthema of swine virus is in this family.

Family Coronaviridae (Chapter 35). Coronavirus virions are enveloped and either spherical (coronavirus) or rod/kidney (torovirus) shaped. The display of large “club”-shaped peplomers on the surface of the viral envelope is unique to this family. Like arteriviruses, gene expression is through the synthesis of a nested set of subgenomic mRNAs. Virions are assembled and mature in the cytoplasm by budding through the endoplasmic reticulum and Golgi membranes. Swine viruses in this family include transmissible gastroenteritis virus (TGEV), porcine respiratory coronavirus (PRCV), porcine epidemic diarrhea virus (PEDV), porcine hemagglutinating encephalomyelitis virus (HEV), and porcine torovirus.

Family Filoviridae (Chapter 36). Filovirus virions appear as long filamentous forms that appear pleomorphic (“U,” “6,” circular, or spiral shaped) on EM. Replication takes place in the cytoplasm and assembly involves envelopment via budding of preformed nucleocapsids. Nucleocapsids accumulate in the cytoplasm, forming prominent inclusion bodies. These viruses are considered extremely hazardous, and Biosafety Level 4 (BSL4) facilities are used for laboratory work. Both Reston and Zaire ebolaviruses are infectious for pigs.

Family Flaviviridae (Chapter 37). Flavivirus virions are spherical and have fine peplomers that do not show symmetrical placement. The structure of the viral core is not well understood, although it is believed to possess icosahedral symmetry. Viral replication takes place in
the cytoplasm, but the source of the envelope is uncertain.

The members of genus Flavivirus infectious for swine (Japanese encephalitis virus, West Nile virus, and Murray Valley encephalitis virus) are mosquito-borne. Members of genus Pestivirus infectious for swine (classical swine fever virus, bovine viral diarrhea viruses 1 and 2, and border disease virus) are transmitted primarily by direct and indirect contact.

**Family Hepeviridae (Chapter 39).** Hepevirus virions are spherical and not enveloped. Hepevirus is similar to members of the family Caliciviridae in morphology and genomic organization, but its replicative enzymes are closer to those of rubella virus and alphaviruses of the family Togaviridae. An in vitro cell culture system has not been established. Hepatitis E virus of humans and animals is the only member in this family.

**Family Orthomyxoviridae (Chapter 40).** Orthomyxovirus virions are mostly spherical but occasionally seen in filamentous forms. The viral envelope contains large peplomers with unique function and antigenicity (hemagglutinin and neuraminidase). Viral replication takes place in the nucleus and cytoplasm, and virion assembly occurs via budding from plasma membranes. The most notable pathogen in this family is influenza A virus. Besides cross-species infection, genetic drift (point mutation) and shift (reassortment) drive the genetic, antigenic, and biological evolution of influenza viruses. Transmission of influenza viruses is waterborne among waterfowl and primary by contact among vertebrates.

**Family Paramyxoviridae (Chapter 41).** Paramyxovirus virions are pleomorphic in shape (spherical and filamentous forms occur). The virion is enveloped and contains a herringbone-shaped nucleocapsid. The envelope is covered with large peplomers and contains two major glycoproteins with distinct functions (hemagglutinin and fusion protein). Viral replication takes place in the cytoplasm, and virion assembly occurs via budding on plasma membranes. Paramyxoviruses in swine (blue eye paramyxovirus, Nipah virus, and Menangle virus) are transmitted primarily by close contact.

**Family Picornaviridae (Chapter 42).** Picornavirus is a small nonenveloped virion with icosahedral symmetry. Virions are assembled in the cytoplasm and released via cell lysis. Transmission is horizontal, mainly by contact, fecal–oral, or airborne routes. Significant swine pathogens in this family are foot-and-mouth disease virus, swine vesicular disease virus, porcine teschovirus, and encephalomyocarditis virus.

**Family Reoviridae (Chapter 43).** Virions have a unique look due to the presence of multiple capsid shells (each with icosahedral symmetry) influencing the morphology of different genera. The genome is linear double-stranded RNA, but divided into 10–12 segments among different genera. Replication and assembly take place in the cytoplasm, often in association with granular or fibrillar inclusion bodies. Rotaviruses are a major cause of diarrhea in young pigs, whereas the role of reoviruses in diseases of pigs is not certain.

**Family Retroviridae (Chapter 44).** Retrovirus virions contain a diploid genome (two molecules of linear positive-sense single-stranded RNA arranged as an inverted dimer). Retrovirus replication has two unique features: (1) reverse transcription of viral RNA to double-stranded DNA by viral reverse transcriptase and (2) integration of viral double-stranded DNA into host genome from which transcription and production of full-length of viral genomic RNA occurs. Virion assembly occurs via budding from plasma membranes. The retroviruses are widely distributed in vertebrates and associated with many different diseases, including leukemia, lymphoma, sarcoma, carcinoma, immunodeficiency, autoimmune diseases, and lower motor neuron diseases. In contrast, porcine endogenous retroviruses (PERVs) have not been associated with disease and their significance is related to concerns regarding the safety of pig-derived xenografts for human use.

**Family Rhabdoviridae (Chapter 45).** Rhabdovirus virions are “bullet” shaped. Viral replication takes place in the cytoplasm, and assembly occurs via budding through different cellular membranes depending on the genera. Rabies virus produces a prominent cytoplasmic inclusion body (Negri body) in infected cells. Many of rhabdoviruses are transmitted by arthropods, but transmission of rabies virus is by bite. Rhabdoviruses infectious for swine include vesicular stomatitis virus and rabies virus.

**Family Togaviridae (Chapter 46).** Togavirus virions are spherical, and viral replication involves the synthesis of a subgenomic mRNA corresponding to 3’ end of the viral genome from which the structural proteins are synthesized. Replication takes place in the cytoplasm and assembly involves budding through host cell membranes. Alphaviruses are transmitted between vertebrates by hematophagous arthropods, for example, mosquitoes. Togaviruses reported in swine include eastern equine encephalitis virus, Getah virus, Sagiyama virus, and Ross River virus.

**FURTHER READING**

Baron S, ed. 1996. Medical Microbiology, 4th ed. Galveston, TX: University of Texas Medical Branch.
RELEVANCE

Adenovirus was first isolated from a rectal swab of a pig with diarrhea (Haig et al. 1964). Since the initial report, adenoviruses have been isolated from pigs with diarrhea (Coussement et al. 1981; McAdaragh et al. 1980), encephalitis (Kasza 1966), nephritis (Nietfeld and Leslie-Steen 1993), respiratory disease (Hirahara et al. 1990), and aborted fetuses (Dee 1995), and pigs with no clinical signs (Clarke et al. 1967; Sharpe and Jessett 1967). In general, porcine adenoviruses (PAVs) tend to have low to moderate pathogenicity and do not cause serious disease with significant economic consequences in swine herds.

ETIOLOGY

PAVs are classified within the family Adenoviridae, genus Mastadenovirus. Currently, there are three species and five serotypes, the latter identified by virus neutralization assays (Clarke et al. 1967; Haig et al. 1964; Hirahara et al. 1990; Kadoi et al. 1995; Kasza 1966). Species A includes serotypes 1, 2, and 3; species B includes serotype 4; and species C includes serotype 4 (Büchen-Osmond 2003). Serotype 1 was isolated from the rectal swab of a pig with diarrhea (Haig et al. 1964), types 2 and 3 were isolated from pigs with normal feces (Sharpe and Jessett 1967), type 4 was isolated from the brain of a pig with neurological signs and enteritis (Kasza 1966), and type 5 was isolated from the nasal secretions of pigs with respiratory disease (Hirahara et al. 1990) and the brain of a newborn piglet (Kadoi et al. 1995).

The morphology, structure, and physical properties of PAV are similar to other adenoviruses. Virions are nonenveloped icosahedra 80–90 nm in diameter. The capsid is composed of 252 capsomers, 242 hexamers, and 12 pentamers that occupy the 12 corners of the virion. From each pentamer projects a fiber protein 20–50 nm in length with a terminal knob. The adenovirus genome is a single, double-stranded linear DNA approximately 32–34 kilobases in length (Kleiboeker et al. 1993; Nagy et al. 2001; Reddy et al. 1998). The genome contains sufficient genetic information to code for 10 proteins, but actually encodes up to 40 proteins due to a complex RNA splicing mechanism (Kleiboeker 2006). The ends of the DNA contain inverted terminal repeats, and the replication strategy of PAV is similar to other adenoviruses with early (replication) and late (structural) protein expression.

PAVs can be isolated and amplified in vitro in primary porcine kidney, continuous porcine kidney cells (PK-15), primary thyroid (Dea and Elazhary 1984), and primary testicular cell cultures (Hirahara et al. 1990). Replication in vitro produces a cytopathic effect (CPE) 2–5 days after inoculation. The CPE is characterized by rounding and swelling of infected cells and aggregation into “grapelike” clusters, with eventual detachment of cells from the substrate (Derbyshire et al. 1968). The most notable morphological feature of adenovirus replication is the production of viral intranuclear inclusions. These Cowdry type A intranuclear inclusions have been observed in cell cultures (Derbyshire et al. 1968) and in several tissues, most notably kidneys (Figure 24.1) and intestinal epithelium in the distal jejunum and ileum of naturally and experimentally infected pigs (Coussement et al. 1981; Ducatelle et al. 1982; Sanford and Hoover 1983). Inclusion bodies contain crystalline arrays of virus proteins in the cell nucleus (Koestner et al. 1968).

ROLE IN PUBLIC HEALTH

Swine are the only species known to be susceptible to PAV, although swine can be experimentally infected with human adenovirus (Betts et al. 1962).
CHAPTER 24  PORCINE ADENOVIRUSES

EPIDEMIOLOGY

In general, the host range of adenoviruses is restricted, and there is no known zoonotic transmission of PAV from swine to humans. Adenoviruses are relatively stable, resisting heat inactivation at room temperature for up to 10 days, but readily inactivated by bleach, formaldehyde, alcohol, and phenolic compounds (Derbyshire and Arkell 1971).

Serological surveys indicated that most adult animals have antibodies to adenoviruses, but the incidence of clinical disease is low, suggesting that infections are frequently subclinical. Most of the serological surveys were done in the 1960s to 1970s, and data on the current situation are lacking. Studies indicated 26–53% of the swine population in southwest England had adenovirus group-specific antibodies (Darbyshire 1967; Darbyshire and Pereira 1964). Other reports from England indicated that 50–60% of adult swine had antibodies to adenovirus, as determined by virus neutralization and immunodiffusion tests (Darbyshire 1967; Kasza et al. 1969). The prevalence of adenovirus type 4 in Quebec was found to be considerably lower, that is, 83/540 (15.2%) of swine in one study (Dea and Elazhary 1984) and 64/350 (18.3%) in another (Elazhary et al. 1985).

Most naturally acquired adenovirus infections in swine are horizontally transmitted by fecal–oral or possibly aerosol exposures (Benfield 1990). Vectors are not known to be involved. Since the virus is relatively stable, transmission on inanimate objects such as boots, clothing, bedding, transport vehicles, and feed utensils, is possible.

Most epidemics of adenoviral diarrhea occur in pigs 1–4 weeks of age (Abid et al. 1984; Coussement et al. 1981; Sanford and Hoover 1983). Adenoviruses are most frequently isolated from rectal swabs of weaned pigs and rarely from adult animals (Darbyshire et al. 1966). The presence of high antibody titers in adults may prevent active replication of virus. Shedding of PAV in feces has been reported up to 14 weeks postweaning (Derbyshire et al. 1966), and PAV has been isolated from the brain, nasal tissue, pharynx, lungs, and intestines up to 48 days after experimental inoculation (Kasza 1966). Viral antigen has also been observed in enterocytes by fluorescent antibody (FA) up to 45 days after infection, suggesting that long-term shedding may occur (Kleiboeker 2006).

PATHOGENESIS

PAVs are most commonly associated with gastrointestinal disease in swine (Abid et al. 1984; Coussement et al. 1981; Derbyshire et al. 1966, 1975; Ducatelle et al. 1982; Haig et al. 1964; Sanford and Hoover 1983). Respiratory (Hirahara et al. 1990), reproductive (Dee 1995), and neurological (Edington et al. 1972; Kasza 1966; Shadduck et al. 1967) signs have been associated with PAV infection, but PAV is rarely reported as the principle pathogen in pigs with these clinical presentations.

Adenovirus infection of swine occurs via ingestion and/or inhalation. Primary replication occurs in the tonsil and villous enterocytes and lymphoid tissue in the distal small intestine (Coussement et al. 1981; Ducatelle et al. 1982; Sanford and Hoover 1983; Shadduck et al. 1967; Sharpe and Jessett 1967). In all experimental studies, independent of the route of inoculation, virus replication is always demonstrated in the short, blunt villi overlying lymphoid aggregates or Peyer’s patches in the ileum.

Piglets inoculated orally with PAV-4 developed a watery diarrhea 3–4 days postinoculation (DPI) that lasted for 3–6 DPI. Adenoviral particles were demonstrated in intestinal contents up to 9 DPI. Antigen was detected in villous epithelial cells of the distal jejunum and the ileum as early as 24 hours after inoculation and for at least 15 DPI and, in one pig, up to 45 DPI (Ducatelle et al. 1982). In another study, diarrhea was induced in 8-day-old gnotobiotic piglets within 48 hours postinoculation, and adenovirus inclusions and antigens were demonstrated by light microscopy and immunofluorescence in the short, blunted villi overlying lymphoid aggregates or Peyer’s patches in the ileum (McAdaragh et al. 1980).

Infection of colostrum-deprived pigs can also induce pneumonia and lesions in the kidneys, thyroid, and lymph nodes (Shadduck et al. 1967). Inoculation of pregnant swine can result in abortion, with virus replication demonstrated in fetal tissues (Dee 1995). Adenoviruses may also play a role in coinfections. Serotype 4 adenovirus in combination with Mycoplasma
**h ypoviriae** produced a more severe pneumonia (Kasza et al. 1969), and adenovirus inclusion bodies are more commonly observed in the kidneys of pigs with porcine circovirus-associated disease (PCVAD) (J. Nietfeld, personal communication).

**CLINICAL SIGNS**

Gastrointestinal disease characterized by watery to pasty diarrhea is the most consistent clinical sign observed in pigs naturally or experimentally inoculated with PAV. Diarrhea is typically observed 3–4 days after oronasal inoculation of cesarean-derived, colostrum-deprived piglets and persists for 3–6 days. Pigs may present with mild dehydration and reduced weight gain, but mortalities are rare (Coussement et al. 1981; Derbyshire et al. 1969, 1975; Ducatelle et al. 1982; Sanford and Hoover 1983). Respiratory signs and abortion are also rare clinical presentations.

**LESIONS**

There are no pathognomonic gross lesions of PAV infection in swine. Common histological lesions include villous blunting and the presence of intranuclear basophilic inclusion bodies in enterocytes of the distal jejunum and ileum, sites of primary viral replication (Coussement et al. 1981; Ducatelle et al. 1982). In field cases, affected enterocytes are usually present along the sides and tips of the villi or on the apical border of the short blunt villi over Peyer’s patches. These enterocytes appear to be desquamating, and the nuclei contain large eosinophilic-to-amphophilic inclusion bodies (Sanford and Hoover 1983). Intranuclear inclusion bodies are also commonly observed in the lungs, kidneys (Figure 24.1), and occasionally brain following experimental inoculation (Shadduck et al. 1967).

Shadduck et al. 1967 induced encephalitis only when pigs were inoculated via the intracerebral route; intranasal/oral inoculation produced a nonsuppurative pneumonitis. Intranuclear inclusions were observed in a variety of organs including the lungs, kidneys, and brain. Virus was isolated from these tissues for several weeks after inoculation.

Interstitial nephritis has also been described in a pig naturally infected with adenovirus. Kidney lesions included inflammation and intranuclear inclusion bodies in cells lining the tubules of the medulla. These cells were confirmed to be adenovirus-infected by direct FA staining, electron microscopy, and virus isolation from the kidneys (Nietfeld and Leslie-Steen 1993). An unusual disseminated adenovirus infection in a nursing pig with cutaneous and visceral hemorrhages has also been described (Tang et al. 1995).

**DIAGNOSIS**

Adenovirus infection should be considered in the differential diagnosis of gastrointestinal and possibly respiratory diseases of pigs. Diagnostic techniques used for the identification of PAV include negative stain electron microscopy of feces or intestinal contents, detection of viral antigens by FA, or immunohistochemical staining of infected cells. FA staining using frozen sections is rapid, and results may be available the day of sample submission. Immunohistochemistry (IHC) also offers rapid and specific identification of adenoviruses but usually requires 1–2 days of processing time. Although intranuclear inclusion bodies may be detected in necropsy samples, the presence of PAV should be confirmed by FA, IHC, or virus isolation.

Adenoviruses are routinely isolated in cell culture from fecal samples or homogenates of the lungs or kidneys. PAVs replicate and induce CPE in primary swine kidney cells, PK-15 cells, primary pig thyroid, and pig testicular cells. Interpretation of results can be complicated by the ubiquitous presence of adenoviruses in swine herds. That is, PAV is frequently isolated from the kidneys, spleen, or testes harvested for the production of primary cell cultures (Hirahara et al. 1990). This can be an adventitious agent “problem” when primary pig kidney cells are used as vaccine substrate or in xenotransplantation procedures where pig tissues are used in humans. Once isolated, the serotype of adenoviruses can be determined by virus neutralization assays with type-specific antisera, but this is rarely done because such reagents are not readily available.

Adenovirus infection can be serologically diagnosed by showing a rising antibody titer in the presence of clinical disease. Serological diagnosis is done by virus neutralization assays or an indirect FA test where adenovirus-infected cells are used as substrate to detect antibodies (Dea and Elazhary 1984).

Polymerase chain reaction (PCR) and quantitative polymerase chain reaction (qPCR) techniques have been developed for the detection of PAV serotype 3 in sources of fecal contamination in the environment (Hundesa et al. 2006; Maluquer de Motes 2004). To date, PCR has not been evaluated as a diagnostic tool for the detection of PAV in clinical samples.

**IMMUNITY**

Since most adenovirus infections are asymptomatic or cause moderate disease, there is little information on immunity to PAV. Most adult swine are seropositive to adenovirus. Colostrum-deprived and conventional newborn piglets are free of adenovirus antibodies prior to nursing. After nursing, antibody titers peak within a few days and persist for 8–9 weeks after weaning. Presumably, antibodies are protective, or at least moderate adenovirus infection, because the
frequency of adenovirus shedding increases after weaning as colostral antibody titers decrease (Derbyshire et al. 1966).

PREVENTION AND CONTROL

PAVs generally produce mild disease with few economic consequences, so there is no incentive to produce commercial PAV vaccines. Control and prevention is best maintained by practicing good sanitation procedures that reduce environmental fecal contamination. PAVs have been used as vectors for gene transfer in other species (Bangari and Mittal 2004) and as a vector of choice for delivery and expression of foreign proteins for vaccination (Ferreira et al. 2005).

REFERENCES

African Swine Fever Virus
José Manuel Sánchez-Vizcaíno and Marisa Arias Neira

RELEVANCE
African swine fever (ASF) was first described in 1921 in Kenya when the virus was transmitted from wild African suids to domestic pigs (Sus scrofa), causing a disease with 100% mortality (Montgomery 1921). It was confined to Africa until 1957 when it spread from Angola to Lisbon, appearing in peracute form with nearly 100% mortality (Manso Ribeiro et al. 1958). The disease reached Spain in 1960, and then France (1964), Italy (1967, 1969, 1993), Malta (1978), Belgium (1985), and The Netherlands (1986). ASF remained endemic in Portugal and Spain until 1995 when both countries successfully concluded an intensive eradication program. ASF reached the western hemisphere in the 1970s, including Cuba (1971, 1980), Brazil (1978), the Dominican Republic (1978), and Haiti (1979), but it was eradicated. At present, ASF is endemic in many sub-Saharan countries of Africa. In Europe, ASF is endemic in Sardinia (Italy), and in 2007, it entered the Caucasian region and the Russian Federation where it currently presents a threat to neighboring countries in Europe and Asia.

African swine fever virus (ASFV) is a large, complex, deoxivirus producing a highly contagious hemorrhagic disease in pigs of all breeds and ages. ASF is a notifiable disease, with the potential for rapid dissemination and significant socioeconomic consequences. Such was the case in Spain, where the ASF eradication program (1985–1995) cost an estimated $100 million dollars (Arias and Sánchez-Vizcaíno 2002b). Control of ASF is based on rapid laboratory diagnosis and the enforcement of strict sanitary measures. There is no treatment or effective vaccine available, and for these reasons, ASF is considered a global animal health priority.

ETIOLOGY
ASFV is a large icosahedral DNA virus and the only member of the family Asfarviridae, genus Asfivirus (Dixon et al. 2005). The virion is composed of several concentric layers and an external hexagonal membrane (Figure 25.1) acquired by budding through the cell plasma membrane (Carrascosa et al. 1984). By electronic microscopy, the average diameter of ASFV particles is around 200 nm.

The genomic organization of ASFV is similar to that of poxviruses. The viral genome consists of a single molecule of linear double-stranded DNA approximately 170–190 kilobase pairs in size, depending on the isolate (Blasco et al. 1989). It exhibits covalently close-ended and terminal inverted repeats 2.1–2.5 kilobases in length. The DNA molecule encodes between 151 and 167 open reading frames (ORFs) located on both strands.

The DNA structure is based on two variable ends and a conserved central region of about 125 kilobase pairs (Blasco et al. 1989). The left (38–47 kilobases) and right (13–16 kilobases) variable ends of the genome contain five multigene families (MGFs) called MGF 100, 110, 300, 360, and 505/530 (Yañez et al. 1995). Deletions and insertions of copies (3–20 kilobases) within MGF genes account for the variability in the size of the genome. The large differences in MGF between ASFV isolates suggest that these regions might be associated with the generation of antigenic variability and may provide a mechanism for evading the immune system. Smaller variations in size (<1 kilobase pair) are seen in the central variable region (CVR) of the 125 kilobase pairs conserved central region.
The complete genome sequences of 11 African and European ASFV isolates from different regions and hosts (domestic pigs, warthogs, and ticks), and with different levels of virulence revealed significant genetic diversity at the genomic level (De Villier et al. 2010). The analysis also revealed differences between avirulent and virulent isolates from the same lineage. Virulent isolates contained regions composed of members of the 360 and 505 multicopy gene families not present in avirulent isolates. These regions had previously been identified by functional analysis as important determinants of virulence in macrophages and determinants of tick host range (Burrage et al. 2004; Zsak et al. 2001).

At least 28 structural proteins have been identified in intracellular viral particles (Tabarés et al. 1980), and more than 100 virus-induced proteins have been identified in infected porcine macrophages (Estevez et al. 1986). Attachment proteins p12 and p24 are found in the external membrane of the extracellular particles, while p150, p37, p34, and p14 proteins are localized in the virus core. The external envelope also contains CD2v (hemadsorption protein), the only glycoprotein of the viral particle. Proteins such as the major structural component of the viral capsid, p72, and membrane proteins p54, p30, and p12 are highly antigenic. In total, more than 50 specific proteins are immunogenic and react with sera from infected or recovered pigs. Although their role in inducing protective immunity is unresolved (Neilan et al. 2004), they are very useful as antigens in serological diagnosis.

ASFV does not induce neutralizing antibodies and, for that reason, no serotypic classification scheme has been devised. However, 22 ASFV genotypes have been identified using a genotyping strategy based on partial nucleotide sequencing of the p72 gene (Boshoff et al. 2007). Subtyping is based on full-length sequencing of the p54 gene. Further discrimination among isolates can be done by analysis of the CVR within the B602L gene (Gallardo et al. 2009).

All of the 22 known genotypes are present in Africa. Genotype I isolates are primarily present in West Africa, with the remaining 21 genotypes in the eastern and southern regions of the continent. All ASFV isolates previously present in Europe and the western hemisphere belonged to West Africa genotype I. However, a genotype II isolate similar to those circulating in southeastern Africa was introduced into the Caucasian region in 2007 (EFSA 2010).

ASFV is very stable at pH 4–10 but is inactivated in minutes at pH < 4 or >11.5 in serum-free media (EFSA 2010). In serum, the virus can remain infectious for 6 years at 5°C (41°F) and for days at pH 13.4 in 25% serum. ASFV is inactivated by heating at 60°C (140°F) for 30 minutes (Plowright and Parker 1967) or 56°C (133°F) for 70 minutes (Mebus 1988). Many solvents are able to inactivate the virus by disrupting the lipid envelope, but ASFV is resistant to proteases and nucleases (Plowright and Parker 1967).

ASFV field isolates are recovered in porcine monocytes and macrophages because they do not replicate in conventional cell cultures. For experimental purposes, several ASFV isolates have been adapted to grow in stable cell lines, for example, African green monkey kidney epithelial cells, VERO, MS, and CV. More recently, the COS-1 cell line has been used for the detection, growth, and titration of field isolates, as well as in the generation of laboratory-engineered ASFV (Hurtado et al. 2010).

**ROLE IN PUBLIC HEALTH**

ASFV is not infectious for humans and does not directly affect public health (EFSA 2009). However, ASFV has a serious social and economic impact on the trade of swine, pig by-products, and food security, particularly in countries in which pigs are an important source of protein.

**EPIDEMIOLOGY**

ASF is endemic in more than 20 countries in sub-Saharan Africa, where a number of epidemiological patterns and scenarios have been described. In Europe, ASF is endemic in Sardinia (Italy). Molecular epidemiology studies proved that this resulted from a single introduction of a genotype I isolate in the 1970s (1978). In June 2007, ASF was recognized in the Caucasus region following an outbreak in Georgia. Thereafter, the outbreak spread to Armenia, Azerbaijan, and the Russian Federation, and reached the border with Ukraine and northwestern Russia near the Baltic Sea and also the Barent Sea. These outbreaks were caused by an ASFV
isolate related to the genotype II ASFV circulating in southeastern Africa. It has been shown that the outbreaks resulted from a single introduction of ASFV in Europe in 2007 and that no molecular changes have occurred up to the present (URL (European Union Reference Laboratory for ASF) CISA-INIA Centro de investigación en Sanidad Animal 2010).

Suids, both wild and domestic, are the natural hosts of ASFV. European wild boars are susceptible to ASFV infection and present clinical signs and mortality rates similar to those observed in domestic pigs (McVicar et al. 1981; Sánchez Botija 1982). In contrast, ASFV usually produces inapparent infection in the three African wild suid species that serve as reservoirs: warthogs (Phacochoerus aethiopicus), the giant forest hog (Hylochoerus meinertzhageni), and bushpigs (Potamochoerus porcus) (De Tray 1957).

In Africa, ASFV is maintained by a complex transmission cycle involving African wild suid species, soft ticks, and domestic pigs. In eastern and southern regions, the virus follows an ancient sylvatic cycle involving soft ticks and asymptomatically infected warthogs and bushpigs. Two additional cycles have been described in endemic areas: a domestic pig/tick cycle without warthog involvement and a domestic pig/pig cycle (Jori and Bastos 2009).

In Europe, direct transmission by contact between sick and healthy animals, including domestic pigs and wild boar, is the most common route of transmission. ASFV spreads among domestic pigs via oral or nasal routes of dissemination and exposure (Colgrove et al. 1969). Pigs can also be infected by other routes, including tick bite (Plowright et al. 1969); cutaneous scarring; and intramuscular, subcutaneous, intraperitoneal, or intravenous injection (McVicar 1984).

Several species of soft ticks are ASFV reservoirs and vectors, including Ornithodoros moubata in Africa (Plowright et al. 1969) and Ornithodoros erraticus in the Iberian Peninsula (Sánchez Botija 1963). Indirect transmission by biological vectors like O. erraticus occurred in the Iberian Peninsula, especially in outdoor pig production. The role of these vectors in eastern Europe is unknown. There is an important difference in the epidemiology of the disease in Africa versus Europe related to ASFV replication in soft ticks. Transovarial and transstadial transmission of ASFV has been described for O. moubata (Plowright et al. 1970), but in Europe, only transstadial transmission has been observed in O. erraticus. Ornithodoros savignyi, also present in Africa, can experimentally transmit ASFV to domestic pigs (Mellor and Wilkinson 1985). A number of other tick species are capable of harboring and transmitting ASFV (Groocock et al. 1980). All the Ornithodoros spp. tested to date are susceptible to ASFV infection (EFSA 2010).

The incubation period varies widely (4–19 days), depending on the ASFV isolate and the route of exposure. Domestic pigs infected with virulent isolates begin shedding virus during the incubation period, that is, prior to the observation of clinical signs. After the onset of clinical signs, ASFV is shed at high levels in all secretions and excretions, including nasal secretions, saliva, feces, urine, conjunctival exudate, genital discharges, and bleeding wounds. In parallel with high antibody titers, surviving pigs demonstrate a long-term viremia and virus may be recovered from tissues for weeks or months. Consequently, once ASFV is established in domestic pigs, carrier pigs become an important source of virus and a major consideration in ASF eradication strategies.

Infection in wild African suids typically results in low virus titers in tissues and low or undetectable viremia (Plowright 1981). These levels of virus are sufficient for transmission to domestic pigs through tick vectors but usually not by direct contact between animals. This transmission cycle makes eradication of ASFV in Africa very difficult.

ASFV is very stable in the environment and can remain infectious in contaminated pens for more than 3 days and up to several weeks in pig feces. ASFV can be isolated from the sera or blood stored at room temperature for 18 months and for up to 15 weeks in putrefied blood (EFSA 2009). ASFV persists for weeks to months in frozen or uncooked meat. In cured or processed products, such as Parma ham, infectious virus was not found after 300 days of processing and curing (McKercher et al. 1987). Spanish cured pig meat products, such as Serrano hams and shoulders, were free of viable ASFV by day 140 and Iberian loins by day 112 (Mebus et al. 1993). No infectious ASFV has been found in cooked or canned hams heated to 70°C (158°F). Infectivity of ASFV is lost by 110 days in chilled deboned meat, bone-in meat, or ground pork, and after 30 days in smoked deboned meat (Adkin et al. 2004).

ASFV is very sensitive to inactivation by lipid solvents, detergents, oxidizing agents such as hypochlorite and phenol, and commercial disinfectants in a time- and temperature-dependent relationship. For example, ASFV can be inactivated in 30 minutes by exposure to 2.3% chlorine and 3% ortho-phenylphenol, as well as iodine-containing compounds. Other effective virucidal treatments may use formalin, sodium hydroxide, beta-propiolactone, glyceraldehydes, or acetyl-ethyleneimine (EFSA 2010).

In general, soaps, detergents, and alkalis are effective for disinfecting animal housing, machinery, clothing, vehicles, human habitations, and so on. Disinfectants such as Virkon® are recommended for use in aircraft. Virus-contaminated feed, effluents, and manure may be buried or burned. ASFV-contaminated pig slurry can be treated with 1% sodium or calcium hydroxide at 4°C (39°F) for 3 minutes or for 30 minutes if using 0.5% sodium or calcium hydroxide (Turner and Williams 1981).
PATHOGENESIS

The sites of primary ASFV replication are the monocytes and macrophages of the lymph nodes nearest the point of virus entrance. In the case of oral exposure, the monocytes and macrophages of the tonsils and mandibular lymph nodes are the first involved. Thereafter, the virus spreads through the blood and/or lymphatic system to the sites of secondary replication, that is, lymph nodes, bone marrow, spleen, lungs, liver, and kidneys. Viremia usually begins 4–8 days postinfection (DPI) and, due to the absence of neutralizing antibodies, persists for weeks or months.

ASFV primarily replicates in monocytes and macrophages (Malmquist and Hay 1960; Minguez et al. 1988), but also replicates in endothelial cells (Wilkinson and Wardley 1978), hepatocytes, renal tubular epithelial cells (Gómez-Villamandos et al. 1995), and neutrophils (Carrasco et al. 1996). No infection has been observed in T or B lymphocytes (Minguez et al. 1988). The virus particle enters susceptible cells by receptor-mediated endocytosis (Alcami et al. 1989) and replicates in distinct areas of the cytoplasm close to the nucleus.

ASFV is associated with red blood cell membranes (Quintero et al. 1986) and platelets (Gómez-Villamandos et al. 1996) and causes hemadsorption in affected pigs (Sierra et al. 1991).

The pathogenesis of the hemorrhage observed in the acute form is believed to be phagocytic activation of endothelial cells aggravated by virus replication in the same cells in the final stages of the disease. In the subacute form, hemorrhages are mainly due to an increase in vascular permeability (Gómez-Villamandos et al. 1995). The pathogenesis of the lymphopenia in the acute form has been related to apoptosis of lymphocytes, mainly in the T area of lymphoid organs (Carrasco et al. 1996). However, there is no evidence of virus replication in T or B cells, suggesting that other mechanisms are involved, for example, cytokines or apoptotic mediators released by ASFV-infected macrophages (Oura et al. 1998).

The subacute form is characterized by a transitory thrombocytopenia (Gómez-Villamandos et al. 1996). The alveolar edema observed in the last stages of the acute and subacute forms of ASF (and the primary cause of death) is a consequence of the activation of pulmonary intravascular macrophages (Sierra et al. 1990).

Host genetic factors and/or immunological responses that account for the low levels of virus in tissues or undetectable levels of viremia in wild African suids (warthogs and bushpigs) are unknown (Plowright 1981). Similarly, the reasons for the apparent higher resistance of European wild boars to infection compared with domestic pigs are not known.

CLINICAL SIGNS

Wild African pigs are very resistant to disease and do not generally present any lesions. Domestic pigs and European wild boars exhibit a wide range of clinical signs from acute to chronic. ASF may resemble several other diseases of pigs, especially classical swine fever (hog cholera) and erysipelas.

The incubation period in natural infections ranges from 4 to 19 days. In experimental infections, the incubation period is 2–5 days, depending on the virus dose and the route of inoculation (Mebus et al. 1983). Morbidity rates depend on whether the virus isolate causes acute or subacute disease, the route of exposure, and the presence or absence of bleeding (epistaxis or hemorrhagic diarrhea). Most commonly, morbidity ranges from 40% to 85%. Likewise, mortality depends on the virulence of the ASFV isolate. Highly virulent viruses may cause 90–100% mortality; moderately virulent isolates may cause 20–40% mortality in adult animals and 70–80% in young animals; and low virulent isolates produce mortality rates of 10–30%.

The clinical forms of ASF range from peracute (sudden death with few or no clinical signs) to subclinical or inapparent infection, depending on virus virulence, exposure dose, and exposure route. African ASF isolates generally induce peracute or acute disease, but in endemic areas, subacute to chronic infection may occur.

The acute form of ASF is characterized by loss of appetite, elevated body temperature (40–42°C; 104–108°F), leukopenia, pulmonary edema, extensive necrosis and hemorrhage of lymphoid tissue, hemorrhages in skin (especially the skin of the ears and flanks), and high mortality (Gómez-Villamandos et al. 1995; Mebus 1988; Mebus et al. 1983; Moulton and Coggins 1968). In the final stages, rapid, labored breathing and serous or seromucous nasal secretions caused by pulmonary edema may be observed. In some cases, there may be nasal hemorrhaging, constipation, vomiting, and, to a lesser extent, diarrhea. Hemorrhagic discharge from the anus (melena) is sometimes observed. Exanthemas are very evident (pinkish almost purple skin due to intense hyperemia) and/or cyanotic foci, which appear as irregular purple-colored marks on the skin of the extremities, ears, chest, abdomen, and perineum. Hematomas and necrotic areas may be observed, although these lesions are more intense in pigs infected with moderately virulent isolates. Abortion frequently occurs in gestating females.

In endemic situations, subacute or chronic forms may be present. The subacute form is characterized by transitory thrombocytopenia, leukopenia, and numerous hemorrhagic lesions (Gómez-Villamandos et al. 1997).

Highly virulent ASFV isolates are mainly involved in the peracute and acute forms of the disease. Moderately
virulent isolates can generate a wide range of clinical forms: acute, subacute, or chronic. Isolates of low virulence induce chronic forms of the disease.

**LESIONS**

A wide variety of lesions are seen in ASF, depending on the virulence of the viral strain. The acute and subacute forms are characterized by extensive hemorrhages and lymphoid tissue destruction. Conversely, lesions may be minimal or absent in the subclinical and chronic forms (Gómez-Villamandos et al. 1995; Mebus et al. 1983).

The principal gross lesions are observed in the spleen, lymph nodes, kidneys, and heart (Sánchez Botija 1982). The spleen may be darkened, enlarged, infarcted, and friable (Figure 25.2). Sometimes lesions are large infarcts with subcapsular hemorrhages. Lymph nodes are hemorrhagic, edematous, and friable and often look like dark red hematomas (Figure 25.3). Because of the congestion and subcapsular hemorrhage, cut sections of affected lymph nodes sometimes have a marbled appearance. Kidneys usually have petechial hemorrhages on the cortical (Figure 25.4) and cut surfaces, as well as in the renal pelvis. An intense hydropericardium with serohemorrhagic fluid is present in some cases. Petechial and ecchymotic hemorrhages

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**25.2.** Enlarged and darkened spleen from acute ASF.

**25.3.** Lymph nodes from a normal pig (left), a pig with subacute ASF (center), and a pig with acute ASF (right).

**25.4.** Kidney from a pig with acute ASF showing numerous petechiae on the cortical surface.
may be observed in the epicardium and endocardium. Other lesions can also be observed in acute ASF, such as serohemorrhagic fluid in the abdominal cavity, with edema and hemorrhages throughout the alimentary tract. Congestion of the liver and the gall bladder may be observed, as well as petechial hemorrhages in the mucosa of the urinary bladder. Hydrothorax and petechial hemorrhages of the pleura are frequently found in the thoracic cavity, and the lungs are usually edematous. Intense congestion is observed in the meninges, choroid plexus, and encephalon (Mebus et al. 1983).

Subacute form is similar to the acute form except for milder lesions. The subacute form is characterized by large hemorrhages in lymph nodes and kidney. The spleen is enlarged and hemorrhagic. Congestion and edema can be observed in the lungs, and in some cases, an interstitial pneumonia may be found.

In the acute form of ASF, histopathological lesions are present in blood vessels and in lymphoid organs. These lesions are characterized by hemorrhages, microthrombosis, and damage to endothelial cells with accumulations of dead cells in the subendothelium (Gómez-Villamandos et al. 1995). Hemorrhagic splenomegaly, characteristic of the acute and subacute forms, is a consequence of the loss of the splenic architecture caused by viral replication and the resulting necrosis of the splenic fixed macrophages. The lymphoid tissue destruction in the acute form is mainly observed on the T area of the lymphoid organs, but no evidence of virus replication in lymphocytes has been observed (Carrasco et al. 1996; Mínguez et al. 1988).

The chronic form of ASF is characterized by alterations in the respiratory tract, but lesions in the chronic form may be minimal or absent (Gómez-Villamandos et al. 1995; Mebus et al. 1983). Lesions include fibrinous pleuritis, pleural adhesions, caseous pneumonia, and hyperplasia of the lymphoreticular tissues. Fibrinous pericarditis and necrotic skin lesions are also common (Moulton and Coggins 1968).

DIAGNOSIS

Laboratory studies are essential to establish a definitive diagnosis of ASF because of the similarity of ASF clinical signs and lesions to other hemorrhagic diseases of pigs, for example, classical swine fever (hog cholera), erysipelas, and septicemic salmonellosis. ASF cannot be diagnosed on the basis of clinical signs or gross lesions.

At necropsy, severe pulmonary edema and splenomegaly is observed in animals that die from infection with highly virulent isolates (peracute and acute disease). The spleen becomes purplish black in color and crosses the entire abdominal cavity from one side to the other (Figure 25.2). This lesion, very characteristic of ASF, has been termed hyperemic splenomegaly, hemorrhagic infarction, and hemorrhagic splenitis. Such animals also show hemorrhages in the lymphatic ganglia, particularly in the gastrohepatic and renal ganglia, which almost always involve the cortical and medullar layers, and petechial hemorrhaging in the kidneys, bladder mucosa, pharynx and larynx, pleura and heart, endocardium and pericardium, hydropericardium, ascites, hydrothorax, and hepatic congestion.

A variety of laboratory tests are available for the diagnosis of ASF (Arias and Sánchez-Vizcaíno 2002a; Oura and Arias 2008). Samples that are recommended for laboratory analyses include lymph nodes, kidneys, spleen, lungs, blood, and serum. Tissues are collected for virus isolation or detection. Tissue exudates and serum samples are primarily used for antibody detection but are also useful for virus detection.

The most convenient, safe, and frequently used techniques for the detection and identification of ASFV are direct immunofluorescence (DIF) (Bool et al. 1969), the hemadsorption test (HA) (Malmquist and Hay 1960), and polymerase chain reaction (PCR) (Agüero et al. 2003, 2004; King et al. 2003).

DIF relies on the demonstration of viral antigen in impression smears or frozen tissues sections from spleen, lungs, lymph nodes, or kidneys using a conjugated immunoglobulin against ASFV. DIF is a fast, economical test with high diagnostic sensitivity for the acute form of ASF. However, for subacute or chronic forms, DIF has a diagnostic sensitivity of only 40%. This decrease in sensitivity seems to be the result of the formation of antigen–antibody complexes in the tissues of infected pigs blocking the reaction between the ASFV antigen and ASF conjugate when such tissues are tested in the laboratory (Sánchez-Vizcaíno 1986).

HA, because of its diagnostic sensitivity and specificity, is useful under the widest range of circumstances and should be used to evaluate suspected outbreaks, especially when other tests are negative. HA is based on the attachment of erythrocytes to the external (cytoplasmic) membrane of ASFV-infected porcine macrophages cultured in vitro. Typically, the erythrocytes form a rosette around the infected macrophages before the appearance of ASFV-induced cytopathic effects (Figure 25.5) (Malmquist and Hay 1960). However, a few ASFV field strains have been isolated that induce cytopathic effect in macrophages but do not induce HA (Sánchez Botija 1982). These strains can be identified by PCR or by using DIF on sediments of these cell cultures.

Several PCR-based methods have been validated as consistent, specific, and highly sensitive for the detection of currently circulating ASFV genotypes, as well as both nonhemadsorbing and low virulent virus isolates (URL (European Union Reference Laboratory for ASF), CISA-INIA Centro de investigación en Sanidad Animal 2010). Primer sets and probes are designed to a highly conserved region of the viral genome within the VP72
The presence of anti-ASFV antibodies is indicative of infection because vaccine is not available. Acute forms of the disease cause high mortality, and pigs frequently die before the development of a detectable antibody response. However, in subacute disease, a percentage of pigs recover from infection and produce high levels of ASFV-specific antibodies: immunoglobulin M (IgM) can be detected by 4 DPI and IgG by 6–8 DPI. Following infection, antibody circulates concurrently with virus for up to 6 months (Arias and Sánchez-Vizcaíno 2002a; Wilkinson 1984) and may be detectable for years after the initial exposure. The early appearance and subsequent persistence of antibodies makes them useful for detecting subacute and chronic forms of the disease. For the same reason, they play an important role in testing strategies implemented as a part of eradication programs (Arias and Sánchez-Vizcaíno 2002b). Several techniques have been adapted to ASF antibody detection, but enzyme-linked immunosorbent assay (ELISA) (Sánchez-Vizcaíno 1986; Sánchez-Vizcaíno et al. 1979) and immunoblotting (IB) (Pastor et al. 1987) tests are the most frequently used.

ELISA is the most useful method for large-scale ASF serological studies and well suited for control and eradication programs (Arias and Sánchez-Vizcaíno 2002b). A World Organization for Animal Health (OIE) indirect ELISA and a commercial competitive ELISA are both validated for use under different epidemiological situations. The sensitivity of the ELISA may be adversely affected if samples are poorly preserved (Arias et al. 1993), but ELISAs based on new recombinant proteins do not have this limitation (Gallardo et al. 2006).

The IB is a highly specific, sensitive, and easy-to-interpret technique. It has been used as a confirmatory assay following ELISA testing (Arias and Sánchez-Vizcaíno 2002b), and its use is recommended when sera are suspected to be poorly preserved (Arias et al. 1993).

**IMMUNITY**

The immune responses involved in protection against ASF are poorly understood, and all attempts at developing an effective vaccine have been unsuccessful. Pigs that survive ASFV develop protective immunity against homologous viruses. Likewise, pigs inoculated with attenuated ASFV isolates survive challenge with the parental virulent virus. However, protection is rarely observed upon challenge with heterologous isolates (Ruiz Gonzalvo et al. 1981). Seemingly, the main difficulty in establishing protective immunity is the lack of neutralizing antibodies and the great variability among virus isolates.

Early experiments demonstrated the absence of neutralizing antibodies against ASFV in sera from naturally or experimentally infected pigs. Indeed, ASFV-specific antibodies have never been demonstrated to neutralize virus in the classical sense of neutralization. However, recovered pigs produced normal levels of neutralizing antibodies in response to foot-and-mouth virus vaccine, suggesting that humoral responses were not adversely affected by ASFV infection per se (De Boer 1967).

Passive transfer of humoral immunity by administration of immune serum from ASFV-infected pigs or from other infected animals did not protect against ASFV infection (De Boer 1967; De Boer et al. 1969), and recent work showed that antibodies against the highly antigenic proteins p30, p54, and p72 were not protective (Neilan et al. 2004). Nevertheless, pigs that receive antibodies show partial protection against ASFV, for example, a delay in the onset of clinical signs, amelioration of clinical signs, reduced levels of viremia, and higher survival rates (Onisk et al. 1994; Schlafer et al. 1984a,b; Wardley et al. 1985).

In vivo and in vitro studies have suggested a possible role for antibodies in complement-mediated cell lysis and antibody-dependent cell-mediated cytotoxicity (ADCC). These studies have also suggested an important role for natural killer (NK) cells (Leitao et al. 2001). Cytotoxic T lymphocytes from recovered pigs destroyed infected macrophages (Martins and Leitao 1994), suggesting that cell-mediated immunity could be an important component of the protective response. Thus, some form of protection related to antibody- and cell-mediated immunity occurs in response to ASFV infection.

**PREVENTION AND CONTROL**

When ASF is suspected for any reason, pig movement should be restricted and diagnostic studies performed immediately. It is important to remember that low
Several key actions were basic for the success of the support of the European community. Arias and an coordinated program to eradicate ASF with the In the period 1985–1995, Spain carried out an extensive epidemiological situation and scenarios, economical resources, and the situation in neighboring regions. In endemic areas where mild or inapparent infections have been recognized, such as Sardinia, prevention relies on the control of pig movements and pig products in combination with extensive serological surveys to detect carriers. In areas of Africa where ASF is endemic, the most important aspect of prevention is the control of natural reservoirs, that is, soft ticks (O. moubata) and wild African suids, with the objective of preventing their contact with domestic pigs. In eastern Europe, it will be necessary to understand the role of biological vectors in the cycle and to control the movements of domestic pigs, wild boars, and pig by-products.

Methods to control ASF and eliminate ASFV from herds may differ depending on the region and continent, epidemiological situation and scenarios, economical resources, and the situation in neighboring regions. In the period 1985–1995, Spain carried out an extensive coordinated program to eradicate ASF with the support of the European community (Arias and Sánchez-Vizcaíno 2002b; Bech-Nielsen et al. 1995). Several key actions were basic for the success of the program:

- Elimination of ASF outbreaks, identification and slaughter of ASFV-carrier animals, and depopulation of infected herds. Adequate compensation to the pig producers of the infected holdings is necessary.
- Improvements in animal holding facilities to prevent the spread of the disease, for example, footbaths, sanitary enclosures, and residue and slurry disposal. The biosafety and sanitary measures developed to avoid transmission of the virus between herds played an important role in the eradication of the disease.
- A network of mobile veterinary field teams in charge of the sanitary control of holdings; animal identification; epidemiological surveys; and sample collection for the serological surveillance of breeders, serological control at slaughterhouses, and diagnosis. The mobile veterinary teams also promoted and encouraged pig producers to create sanitary associations.
- Strict control of animal movement and identification of animals moved for fattening or breeding purposes. Vehicles were also required to be properly washed and disinfected.
- Direct involvement and active participation of the farmers were of major importance for the success of the eradication program.

Every country should have an ASF contingency plan prepared and ready to put into place in case of an ASF emergency.

REFERENCES


26 Porcine Circoviruses
Joaquim Segalés, Gordon M. Allan, and Mariano Domingo

RELEVANCE
In the late 1990s, a previously unrecognized porcine circovirus (PCV) was detected in North America and Europe (Allan et al. 1998; Ellis et al. 1998). This virus was distinct from a known PCV contaminant of PK-15 cell cultures (Tischer et al. 1982, 1974). The original cell culture contaminant, nonpathogenic for swine (Allan et al. 1995; Tischer et al. 1986), was designated porcine circovirus type 1 (PCV1), and the new virus associated with clinical disease was named porcine circovirus type 2 (PCV2) (Allan et al. 1999b).

PCV2 infection is associated with postweaning multisystemic wasting syndrome (PMWS) (Clark 1996; Harding 1996), porcine dermatitis and nephropathy syndrome (PDNS) (Rosell et al. 2000b), porcine respiratory disease complex (Kim et al. 2003), and reproductive disease (West et al. 1999). The term “porcine circovirus diseases” (PCVDs) was proposed to group diseases or conditions linked to PCV2 (Allan et al. 2002b). In North America, the term “porcine circovirus-associated diseases” (PCVADs) is commonly used (Opriessnig et al. 2007). Among PCVD, only PMWS has a worldwide impact on swine production. Since 2007, a number of PCV2 commercial vaccines have been licensed, and economic losses attributed to PMWS and subclinical PCV2 infection have been markedly reduced (Kekarainen et al. 2010).

ETIOLOGY
PCVs belong to the genus Circovirus in the family Circoviridae (Segalés et al. 2005a). PCVs are nonenveloped and 12–23 nm in diameter (Figure 26.1) (Rodríguez-Cariño and Segalés 2009; Tischer et al. 1982). The PCV2 nucleocapsid exhibits icosahedral symmetry. Three-dimensional studies showed a polygonal outline containing 60 capsid (Cap) protein elements arranged in 12 slightly protruding pentameric units, giving an overall diameter of about 20.5 nm (Crowther et al. 2003). The circular, covalently closed, single-stranded DNA (ssDNA) genome contains 1759 (PCV1) and 1767–1768 (PCV2) nucleotides (Hamel et al. 1998; Meehan et al. 1998). PCV1 and PCV2 may have a common evolutionary origin, but a common ancestor has not been identified (Olvera et al. 2007).

After PCV infects a cell, the ssDNA is converted to a double-stranded DNA (dsDNA) intermediate known as the replicative form (RF) (Mankertz et al. 2004). The RF is ambisense, with genes encoded by both the viral (positive) and the complementary (negative) strand. PCV2 genes are arranged in 11 putative open reading frames (ORFs) (Hamel et al. 1998), but protein expression has been described for only three. ORF1 (Rep gene) is located on the positive strand and in clockwise orientation. It codes for the nonstructural replicase proteins Rep and Rep’, 314 and 178 amino acids (AAs) in length, respectively (Cheung 2003; Mankertz et al. 1998). ORF2 (Cap gene) is on the complementary strand and oriented counterclockwise. It encodes for the capsid (Cap), the only structural protein (233–234 AA) (Mankertz et al. 2004; Nawagitgul et al. 2000). ORF3 is located on the complementary strand, oriented counterclockwise, and completely overlaps the ORF1 gene. ORF3 codes for a nonstructural protein 105 AA in length. In vitro, the ORF3 protein induces apoptosis in PK-15 cells (Liu et al. 2005). An ORF3-deficient PCV2 mutant was shown to be less virulent in pigs compared with wild-type PCV2 (Karuppannan et al. 2009).

Analysis of PCV2 viruses from around the world showed close phylogenetic relationships and nucleotide sequence identity greater than 93% (Larochelle et al. 2002; Mankertz et al. 2000). Most PCV2 genomic sequences fit into two major groups: PCV2 genotypes...
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a and b (Gagnon et al. 2007; Grau-Roma et al. 2008; Olvera et al. 2007; Segalés et al. 2008; Timmusk et al. 2008). PCV2a is more genetically variable than PCV2b, which suggests that PCV2a is older than PCV2b (Grau-Roma et al. 2008). However, both genotypes are likely to have emerged from a common ancestor approximately 100 years ago and then commenced independent evolutionary trajectories while co-circulating in the same host species and geographical regions (Firth et al. 2009). PCV2a was the most prevalent genotype in clinically affected pigs from 1996 to the early 2000s, whereas PCV2b currently predominates. The emergence of PCV2b in North America and Europe was associated with the appearance of a more severe clinical disease (Carman et al. 2006; Cortey et al. 2011; Timmusk et al. 2008; Wiederkehr et al. 2009). A third genotype (PCV2c) was detected in archival material in Denmark (Dupont et al. 2008; Segalés et al. 2008).

PCV2 strains of the same or different genotype may circulate concurrently in the same pig (Cheung et al. 2007; de Boisseson et al. 2004; Gagnon et al. 2007; Grau-Roma et al. 2008; Hesse et al. 2008). In vivo and in vitro studies provided evidence of viral recombination (Cheung 2009; Hesse et al. 2008; Lefebvre et al. 2009; Olvera et al. 2007), and the emergence of new genotypes could have been the result of recombination between strains coexisting in the same animal. A recombinant virus containing PCV1 ORF1 and PCV2a ORF2 was identified in Quebec (Canada), although its exact origin is under debate due to its resemblance to a chimeric killed vaccine strain (Gagnon et al. 2010).

Initially, similarities in reactivity to monoclonal and polyclonal antibodies led to the conclusion that no major antigenic differences existed among PCV2 strains (Allan et al. 1999b; McNeilly et al. 2001). However, subsequent work revealed antigenic variability among PCV2 genotypes (Lefebvre et al. 2008a; Shang et al. 2009). Despite antigenic differences, immunity induced by one genotype conferred protection against challenge with other genotypes (Opriessnig et al. 2008c). The efficacy of current PCV2 vaccines, all of which are based on PCV2a isolates, against infection with PCV2b is further evidence of antigenic cross-protection (Fort et al. 2008).

Physicochemical and Biological Properties
PCV1 has a buoyant density of 1.37 g/mL in CsCl, does not hemagglutinate erythrocytes from a wide range of species, is resistant to inactivation at pH 3 and by chloroform, and is stable at 70°C (158°F) for 15 minutes (Allan et al. 1994b). The biological and physicochemical properties of PCV2 are not well characterized. Infectivity decreases somewhat in an acid buffer, but PCV2 remains viable even at pH <2. Infectivity is markedly decreased at pH 11–12 (Kim et al. 2009). PCV2 resists inactivation at high temperatures (56°C [133°F] for 1 hour and 75°C [167°F] for 15 minutes), which suggests that the virus is able to remain infectious in the environment at high ambient temperatures, for example, summer (Kim et al. 2009; O'Dea et al. 2008). Exposure of PCV2 (10 minutes to 24 hours) at room temperature to a number of commercial disinfectants (chlorhexidine, formaldehyde, iodine, oxidizing agents, and alcohols) led to a significant reduction in virus titers (Kim et al. 2009; Martin et al. 2008; Royer et al. 2001).

Laboratory Cultivation
Replication of PCVs in vitro is best achieved by inoculation of semiconfluent monolayers of PCV-free PK-15 cells. Since PCV does not produce cytopathic effect, viral replication is monitored by immunofluorescence or immunoperoxidase staining (Allan and Ellis 2000). PCV1 has been shown to replicate in other porcine-derived cell lines and Vero cells (Allan et al. 1994a; Tischer et al. 1982). PCV DNA replication seems to be dependent on cellular enzymes expressed during the S phase of growth (Tischer et al. 1987) or perhaps cell repair (Sánchez et al. 2003). Viral yields may be enhanced by cell treatment with glucosamine, but care must be taken because glucosamine is cytotoxic (Allan and Ellis 2000).

ROLE IN PUBLIC HEALTH
PCVs are not of public health concern, but the discovery of PCV DNA in two rotavirus vaccines intended for use in children caused considerable concern (Kuehn 2010; Victoria et al. 2010). PCV1 DNA was previously detected in swine vaccines (Quintana et al. 2006). These data reflect quality control issues in vaccine production.
Epidemiology

PCV1 is ubiquitous in pig populations, although its prevalence is much lower than PCV2 (Calsamiglia et al. 2002). PCV1 has been detected in wild boar and its nucleotide sequence grouped with PCV1 genomes from domestic swine (Csagola et al. 2008). Likewise, PCV2 is ubiquitous and both domestic and feral swine are infected with PCV2a (Ellis et al. 2003; Pensaert et al. 2004; Shibata et al. 2006). Isolates of PCV2a and PCV2b genotypes (Ellis et al. 2003; Schulze et al. 2004; Sofia et al. 2008).

Nonporcine species, including humans, are not susceptible to PCV2 infection (Allan et al. 2000b; Ellis et al. 2001, 2000; Rodriguez-Arrioja et al. 2003). Mice are the exception, that is, PCV2 can replicate in and transmit between mice to a limited degree (Küpel et al. 2001; Opriessnig et al. 2009a). Suggestive of a possible role as alternate hosts or mechanical vectors, PCV2 has been found in mice and rats from pig farms, but not in rodents collected outside swine herds (Lorincz et al. 2010).

Oronasal exposure is considered the primary route of transmission, but PCV2 has been found in nasal, tonsillar, bronchial, and ocular secretions, feces, saliva, urine, colostrum, milk, and semen (Krakowska et al. 2000; Larochelle et al. 2000; Park et al. 2009; Segalés et al. 2005b; Shibata et al. 2003, 2006). Pigs can also become infected by eating raw tissues from viremic animals (Opriessnig et al. 2009c). Transplacental infection occurred in pregnant sows intrasinally exposed to PCV2 3 weeks prior to farrowing (Ha et al. 2008, 2009; Park et al. 2005). Naïve sows inseminated with semen contaminated with PCV2 exhibited reproductive failure and their fetuses became infected (Madson et al. 2009a). However, it is not known if the quantity of PCV2 naturally shed in semen is sufficient to infect sows or their fetuses.

Transmission of PCV2 among pigs can occur by mixing naïve with infected animals (Albina et al. 2001; Bolin et al. 2001). Direct contact is more efficient than transmission between animals in separate pens (Andraud et al. 2008). Longitudinal studies quantifying PCV2 in serum, nasal excretions, and feces found that most pigs became infected at 4–11 weeks of age, depending on the farm (Carasova et al. 2007; Grau-Roma et al. 2009). A low percentage of sows and piglets may be viremic during lactation (Calsamiglia et al. 2007; Grau-Roma et al. 2009), suggesting the possibility of transmission from sows to nursing piglets (Larochelle et al. 2003; Pensàert et al. 2004; Shibata et al. 2006; Sibila et al. 2004).

PCV2 persistence in individual pigs or groups of pigs has not been studied extensively, but Bolin et al. (2001) isolated virus or detected viral DNA in tissues from experimentally inoculated pigs for up to 125 days postinoculation (DPI). In the field, viral DNA has been detected in serum from pigs for a period up to 22 weeks (Rodríguez-Arrioja et al. 2002). PCV2 DNA was repeatedly found in the sera from pigs 7–70 days of age (Grau-Roma et al. 2009), further evidence that some animals remain persistently infected despite the presence of high levels of PCV2-specific antibodies (McIntosh et al. 2006; Rodriguez-Arrioja et al. 2002).

Risk Factors for Postweaning Multisystemic Wasting Syndrome

Horizontal transmission of PMWS by comingling affected and healthy pigs and between affected and naïve pigs in adjacent pens has been reported (Kristensen et al. 2009), but both infectious and noninfectious factors are believed to play a role in PMWS. Infectious agents considered potential PMWS triggers include infection with porcine parvovirus (PPV) and/or porcine reproductive and respiratory syndrome virus (PRRSV) (Rose et al. 2003; Segalés et al. 2002). Noninfectious risk factors for PMWS are summarized in Table 26.1.

Pathogenesis

Both PCV2a and PCV2b are pathogenic, that is, able to cause PMWS (Allan et al. 1999a; Lager et al. 2007). Following the PMWS pandemic in the early 2000s, an event that appeared to coincide with a genotype shift from PCV2a to PCV2b (Carman et al. 2008; Cortey et al. 2011; Dupont et al. 2008; Timmusk et al. 2008; Wiederkraft et al. 2009), it was suggested that PCV2b strains were more virulent than PCV2a strains. However, the question of a difference in virulence between these genotypes has not been resolved (Harding et al. 2010; Opriessnig et al. 2009b).

There is little information regarding the target cells for initial viral replication, the early events of PCV2 infection, or the cell type(s) that support PCV2 replication in vivo. PCV2 does not code for its own DNA polymerases, and cells in the S phase are presumed necessary for the virus to complete its infectious cycle (Tischer et al. 1987). If so, cells with a high mitotic rate should be the most efficient for viral replication. Initial work suggested that macrophages and lymphocytes did not play a significant role in PCV2 replication (Gilpin et al. 2003; Vincent et al. 2003), but later studies found that they (mainly macrophages) may support replication as well as endothelial and epithelial cells (Hamberg et al. 2007; Pérez-Martín et al. 2007; Rodríguez-Cariño et al. 2010; Yu et al. 2007). Characterization of PCV2-infected leukocyte subpopulations from peripheral blood mononuclear cells (PBMCs) showed that circulating T lymphocytes (CD4+ and CD8+) and, to a lesser extent, B lymphocytes supported PCV2 replication, but not PBMC-derived monocytes (Lefebvre et al. 2008b; Lin et al. 2008; Yu et al. 2007). In PCV2-infected
pigs, the highest concentration of virus was found in the cytoplasm of monocyte/macrophage lineage cells (Rosell et al. 1999; Sánchez et al. 2004). In vitro studies have shown that PCV2 infects these cells after which they become persistently infected with little or no virus replication (Gilpin et al. 2003; Vincent et al. 2003). For this reason, it has been suggested that monocyctic cells serve as a mechanism to disseminate PCV2 within the host, rather than as a primary target for PCV2 replication (Vincent et al. 2003). Ultrastructural studies in cell culture and lymph nodes from PMWS-affected pigs suggested that mitochondria may play a role in PCV2 replication (Rodríguez-Cariño et al. 2010, 2011).

PCV2 viremia is first detected around 7 DPI and peaks at 14–21 DPI (Allan et al. 1999a; Opriessnig et al. 2008b; Rovira et al. 2002). Under field conditions, seroconversion usually occurs at 7–12 weeks of age and antibodies last until at least 28 weeks of age (Rodríguez-Arrioja et al. 2002). Lymphoid tissues contain the highest concentration of PCV2 (Quintana et al. 2003; Rosell et al. 1999), but virus may also be detected in epithelial cells from the kidney and respiratory tracts, endothelial cells, lymphocytes, enterocytes, hepatocytes, smooth muscle cells, and pancreatic acinar and ductal cells (McNeilly et al. 1999; Rosell et al. 1999; Sánchez et al. 2004; Shibahara et al. 2000).

### Pathogenesis of Postweaning Multisystemic Wasting Syndrome

PMWS is a multifactorial disease in which PCV2 infection is a required component. Although PMWS has been reproduced under experimental conditions, a definitive, consistently repeatable disease model is lacking. The most repeatable PMWS models are based on PCV2 inoculation in combination with other infectious and/or noninfectious cofactors (Allan et al. 2004). Reasons for inconsistency in the experimental reproduction of PMWS is not known, but may be related to the origin of pigs, age of the animals at inoculation, immunological status, genetic predisposition, PCV2 strain, type of inoculum, infectious dose, and route of administration. Meta-analysis revealed that the experimental reproduction of PMWS was mostly probable in colostrum-deprived pigs <3 weeks of age inoculated with high doses (>1 × 10^5 tissue culture infectious dose 50% [TCID_{50}] per pig) of PCV2b in combination with another pathogen (Tomás et al. 2008). Harding et al. (2010) reproduced PMWS by inoculating PCV2a and PCV2b genotypes 7 days apart. Dual heterologous PCV2 genotype infection induced severe disease in germ-free pigs, but failed to do so when both inoculated viruses were from the same genotype.

Field observations suggested that certain genetic lines of pigs might be more susceptible to PMWS. This observation was supported by experimental studies in which Landrace pigs were shown to be more likely to develop PMWS than Duroc, Large White, and Pietrain pigs (Opriessnig et al. 2006a, 2009b). However, field studies have reported contradictory results, with the Pietrain boar line showing no effect in one study (Rose et al. 2005) and lower general postweaning and PMWS-associated mortalities in another (López-Soria et al. 2004).

### Table 26.1. Noninfectious risk factors for PMWS

<table>
<thead>
<tr>
<th>Factors Increasing the Risk of PMWS</th>
<th>Factors Decreasing the Risk of PMWS</th>
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<tbody>
<tr>
<td>Animals</td>
<td>Facilities</td>
</tr>
<tr>
<td>• Gender (male)</td>
<td>• Gender (female)</td>
</tr>
<tr>
<td>• Litter of origin</td>
<td>• Separate pit for adjacent fattening rooms</td>
</tr>
<tr>
<td>• Low birth weight</td>
<td>• Shower facilities</td>
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<tr>
<td>• Low weaning weight</td>
<td>• Sorting pigs by sex at nursery stage</td>
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<tr>
<td>• Low weight at the beginning of fattening period</td>
<td>• Greater minimum weight at weaning</td>
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<tr>
<td>Facilities</td>
<td>Management practices</td>
</tr>
<tr>
<td>• Large number of sows</td>
<td>• High level of cross-fostering</td>
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<tr>
<td>• Large pens at nursery and growing ages</td>
<td>• Short empty periods at weaning and fattening</td>
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<tr>
<td>• Proximity to other pig farms</td>
<td>• Large range in age and weight entering the nursery</td>
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<tr>
<td>Vaccination/treatment/ nutrition</td>
<td>• Purchase of replacement gilts</td>
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<tr>
<td>• Vaccination of gilts against PRRSV</td>
<td>• Sows with neck injuries due to poor injection technique</td>
</tr>
<tr>
<td>• Vaccination of sows against <em>Escherichia coli</em></td>
<td>• Early weaning (&lt;21 days of age)</td>
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<tr>
<td>• Use of separate vaccines against <em>Escherichia coli</em> and porcine parvovirus on gilts</td>
<td>• Vaccination of sows against atrophic rhinitis</td>
</tr>
<tr>
<td></td>
<td>• Use of semen from an insemination center</td>
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PRRSV, porcine reproductive and respiratory syndrome virus.

Table modified from Grau-Roma et al. (2011) by permission of the publisher.
Stimulation and/or activation of the immune system of PCV2-infected pigs by other viral infections or non-infectious factors upregulates PCV2 replication and increases viral loads in tissues and serum (Allan et al. 1999a, 2000a; Harms et al. 2001; Kennedy et al. 2000; Krakowka et al. 2001; Rovira et al. 2002). This suggests that PCV2 infection and immunostimulation may be pivotal events in the development of PMWS, although the precise mechanism by which coinfection or immunostimulation triggers the development of PMWS in PCV2-infected pigs is unknown (Kekarainen et al. 2010).

Conversely, regular features of PMWS in severely affected pigs are suggestive of immunosuppression (Darwich et al. 2004; Segalés and Mateu 2006), for example, microscopic lymphoid lesions (Clark 1997; Rosell et al. 1999), the association of PMWS with opportunistic pathogens (Carrasco et al. 2000; Clark 1997; Núñez et al. 2003; Segalés et al. 2003; Zlotowski et al. 2006), and changes in immune cell subpopulations in lymphoid tissues and PBMCs (Chianini et al. 2003; Darwich et al. 2002; Nielsen et al. 2003; Segalés et al. 2001).

The most striking evidence of immunosuppression is the extensive lesions in lymphoid tissues of PMWS-affected pigs, that is, depletion of B and T lymphocytes, an increase in the number of macrophages, and loss or redistribution of interfollicular dendritic cells (Chianini et al. 2003). In lymphoid tissues, depletion of T lymphocytes primarily involved CD4⁺ cells and, to a lesser extent, CD8⁺ cells (Sarli et al. 2001).

Another feature suggestive of immunosuppression in PMWS-affected pigs is the alteration of PBMC subsets, mainly lymphopenia (Darwich et al. 2002; Nielsen et al. 2003). Depletion of B and T lymphocytes was experimentally observed only in PCV2-inoculated pigs that later developed PMWS. Changes in T-cell subsets involved mainly CD4⁺CD8⁻ memory T cells. In PCV2-inoculated pigs that did not show clinical signs, the number of cytotoxic (CD4⁺CD8⁻) and γδ (CD4⁺CD8⁻) T lymphocytes was increased in comparison to that of control pigs, thereby suggesting an active response to PCV2 infection.

Lymphoid depletion and lymphopenia are consistent features of PMWS-affected pigs, but it is not known whether the loss of lymphocytes is a direct or an indirect effect of PCV2 infection. PCV2-infected PBMC undergo morphological changes typical of cellular degeneration (Lefebvre et al. 2008b) and B and T lymphocytes, albeit a low proportion, support PCV2 replication (Pérez-Martín et al. 2007; Yu et al. 2007). Alternatively, lymphoid depletion might be the result of virus-induced apoptosis, but this is controversial (Küupel et al. 2005; Krakowka et al. 2004; Mandrioli et al. 2004; Resendes et al. 2004b; Shibahara et al. 2000).

In vitro studies also suggest that PCV2 is immunosuppressive. For example, the addition of PCV2 to cultured alveolar macrophages altered the production of certain cytokines and/or chemokines (Chang et al. 2006). The altered functionality of PCV2-infected alveolar macrophages may favor the spread of PCV2, as well as render pigs more susceptible to opportunistic and secondary pulmonary infections. PCV2 can impair dendritic cell function in vitro. PCV2 infection of myeloid dendritic cells did not interfere with dendritic cell maturation or their ability to process and present antigen to T lymphocytes (Vincent et al. 2005, 2003), but PCV2 interaction with plasmacytoid dendritic cells (aka natural interferon-producing cells) impaired their responsiveness to danger signals (Vincent et al. 2007). PCV2-induced impairment of dendritic cell function did not require viral replication, but was mediated by viral DNA in a dose-dependent manner; that is, a minimum concentration of dsDNA (RF) was necessary to mediate inhibition (Vincent et al. 2007).

The addition of PCV2 to PBMCs from either healthy or clinically affected pigs altered their cytokine profiles (Darwich et al. 2003a). PCV2 seemingly modulated the specific immune responses to other pathogens as well; that is, PCV2 downregulated the cytokine recall antigen response (Kekarainen et al. 2008b). The PCV2-induced suppression of cytokine expression to recall antigen was associated with the whole virus and certain DNA sequences derived from its genome. In contrast, PCV2 virus-like particles (VLPs) did not show any suppressive effect or modulate interferon-α (IFN-α) responses (Kekarainen et al. 2008a).

Inhibition of some of cytokine responses was mediated by the release of PCV2-induced interleukin-10 (IL-10) by monocytic cells. Ex vivo, increased serum levels of this cytokine in PCV2-infected pigs were associated with the development of PMWS (Darwich et al. 2003b; Stevenson et al. 2006). Recent data suggest that IL-10-producing cells are of myeloid and lymphoid origin, but they are rarely, if ever, infected with PCV2 (Crisci et al. 2010; Doster et al. 2010).

Pathogenesis of Reproductive Disease
PCV2 replicates in in vivo produced zona pellucida-free morulae and blastocysts (Mateusen et al. 2004, 2007), but the relevance of this finding to naturally occurring reproductive disease is unknown. Porcine embryos are susceptible to PCV2 infections and their susceptibility increases with developmental stage (Mateusen et al. 2004). Direct intrafetal inoculation at 57, 75, and 92 days of gestation showed that PCV2 replicated in all inoculated fetuses, with significantly higher replication in fetuses inoculated at 57 days of gestation (Sánchez et al. 2001). PCV2-susceptible cells in fetuses include cardiomyocytes, hepatocytes, and cells of the monocyt/macrophage lineage (Sánchez et al. 2003). Myocarditis-like lesions were observed in the heart, and cardiac tissues contained the highest virus titer and the highest proportion of infected cells.
Pensaert et al. (2004) reported mummification in fetuses inoculated at 57 days of gestation, with transmission of PCV2 to neighboring, noninoculated fetuses. At farrowing, fetuses inoculated at 75 days of gestation were stillborn, whereas fetuses inoculated at 92 days of gestation showed no lesions. In both cases, cardiac tissues were virus positive and pigs were PCV2 antibody positive at birth.

Intranasal inoculation of pregnant sows or artificial insemination with PCV2-contaminated semen produced infection in fetuses/newborn piglets and reproductive failure (Madson et al. 2009a; Park et al. 2005). Park et al. (2005) intranasally inoculated six sows with PCV2 3 weeks before the expected farrowing date. Subsequently, three sows aborted and three farrowed prematurely. PCV2 antigen and nucleic acid were detected in lymphoid and nonlymphoid tissues from stillborn and live-born piglets, but no PMWS-like or cardiac lesions were observed. Madson et al. (2009a) artificially inseminated three sows with PCV2a-contaminated semen and three with PCV2b-contaminated semen. Sows that received semen with PCV2b became pregnant and maintained pregnancy to term, but most of the fetuses were mummified with myocardial lesions associated with PCV2 antigen. Sows inseminated with PCV2a-contaminated semen failed to become pregnant. Whether this was due to early embryonic death caused by PCV2a or another factor was undetermined.

**Clinical Signs and Lesions**

PCV2 is ubiquitous and most PCV2 infections are subclinical. In the field, the proportion of PCV2-infected pigs and their viral load increases gradually after lactation and coincident with the decline in maternal immunity. Most pigs in a PMWS-affected population become infected with PCV2, with some proportion of the population developing PMWS. Experimental infections comparing PCV2-inoculated groups with negative controls rarely detected significant differences in production parameters (Fernandes et al. 2007), but the evaluation of production records on farms without clinical disease showed that the use of PCV vaccine reduced mortality and increased average daily gain (ADG) (Reindl et al. 2010).

**Postweaning Multisystemic Wasting Syndrome**

PMWS most commonly affects pigs at 2–4 months of age. The highest infectious pressure occurs at the time PMWS appears in the population (Grau-Roma et al. 2009). At that time, clinically affected pigs have a higher concentration of virus in the serum, shed higher levels of virus, and demonstrate a weaker antibody response compared with subclinically infected pigs (Fort et al. 2007; Grau-Roma et al. 2009). Morbidity in affected farms is commonly 4–30% (occasionally 50–60%), and mortality ranges from 4% to 20% (Segalés and Domingo 2002).

PMWS is characterized clinically by wasting, pallor of the skin, respiratory distress, diarrhea, and occasionally, icterus (Figure 26.2) (Harding and Clark 1997). Enlarged subcutaneous lymph nodes are a common finding in the early clinical phases of PMWS.

PMWS lesions are primarily found in lymphoid tissues, and enlargement of lymph nodes is the most prominent feature of the early clinical phase of PMWS (Clark 1997; Rosell et al. 1999). Normal sized, or even atrophied, lymph nodes are usually seen in more advanced phases of PMWS (Segalés et al. 2004), and the thymus is frequently atrophied in diseased pigs (Darwich et al. 2003a). Characteristic histopathological lymphoid lesions in PMWS-affected pigs include lymphocyte depletion with infiltration by large histiocytic cells and giant multinucleate cells (Figure 26.3) (Clark 1997; Rosell et al. 1999). In thymus, cortical atrophy is a prominent finding (Darwich et al. 2003a). Cytoplasmic viral inclusions may be found in histiocytes or dendritic cells (Figure 26.4).

Lungs may be enlarged, noncollapsed, and rubbery in consistency, in a diffuse or patchy distribution. These findings correspond microscopically to interstitial pneumonia. Peribronchial fibrosis and fibrinous bronchiolitis occurs in advanced cases (Clark 1997; Segalés et al. 2004).

In a few cases of PMWS, the liver is enlarged or atrophied, pale, and firm, with a fine granular surface that corresponds microscopically to widespread cytopathic changes and inflammation (Clark 1997; Segalés et al. 2004). Pigs may show generalized icterus at this latter stage. Microscopic lesions in the liver may vary
from mild lymphohistiocytic hepatitis to massive inflammation, with apoptotic bodies, disorganization of hepatic plates, and perilobular fibrosis (Rosell et al. 2000a).

Some pigs show white spots in the kidney cortex (nonpurulent interstitial nephritis). Foci of lymphohistiocytic inflammatory infiltrates may be seen in many tissues of pigs affected by PMWS (Segalés et al. 2004). Brain lesions consisting primarily of vasculitis have occasionally been described (Correa et al. 2007; Seeliger et al. 2007).

**Porcine Dermatitis and Nephropathy Syndrome**

PDNS affects nursery, growing, and adult pigs (Drolet et al. 1999). The prevalence of PDNS is usually <1% (Segalés et al. 1998), although higher frequency has been described (Gresham et al. 2000). Mortality approaches 100% in pigs older than 3 months versus approximately 50% in younger pigs. Severe, acutely affected pigs die within a few days after the onset of clinical signs. Surviving pigs tend to recover and gain weight 7–10 days after the beginning of the syndrome (Segalés et al. 1998).

PDNS-affected pigs are anorexic and depressed, with little or no pyrexia (Drolet et al. 1999). They may be prostrate, reluctant to move, and/or stiff-gaited. The most obvious sign of PDNS is the presence of irregular, red-to-purple macules and papules in the skin, primarily on the hind limbs and perineal area (Figure 26.5), but sometimes more generally distributed. With time, the lesions become covered by dark crusts. The lesions gradually fade, sometimes leaving scars (Drolet et al. 1999). Macules and papules are seen microscopically as necrotic and hemorrhagic skin associated with necrotizing vasculitis (Segalés et al. 1998). Necrotizing vasculitis is a systemic feature. Studies on tissue sections from pigs with PDNS have failed to consistently demonstrate PCV2 antigen or nucleic acid associated with PDNS vascular lesions.

Pigs that die acutely with PDNS have bilaterally enlarged kidneys with a fine granular cortical surface, small reddish pinpoint cortical lesions, and edema of the renal pelvis (Segalés et al. 2004). These lesions correspond to a fibrinonecrotizing glomerulitis with nonpurulent interstitial nephritis. Pigs with prolonged disease may show chronic glomerulonephritis (Segalés et al. 1998). Normally, both skin and renal lesions are present in PDNS, but in few occasions, skin or renal lesions may occur alone. Lymph nodes may be enlarged...
and red in color. Splenic infarcts may also be present (Segalés et al. 1998). Microscopically, lymphoid lesions similar to PMWS are frequently observed in PDNS-affected pigs (Rosell et al. 2000b).

Reproductive Disease

PCV2 has been linked to late-term abortions and stillbirths (West et al. 1999), but the contribution of PCV2 infection to reproductive failure in the field is unclear. That is, some reports suggest that its occurrence is rare (Ladekjaer-Mikkelsen et al. 2001; Maldonado et al. 2005; Pensaert et al. 2004; Sharma and Saikumar 2010), while others suggest that 13–46% of aborted fetuses and/or stillborns are infected by PCV2 (Kim et al. 2004; Lyoo et al. 2001).

In PCV2-associated reproductive disease, stillborn and nonviable neonatal piglets show chronic, passive, hepatic congestion and cardiac hypertrophy with multifocal areas of myocardial discoloration (West et al. 1999). The primary microscopic lesion corresponds to a nonsuppurative, fibrotic, and/or necrotic myocarditis (Mikami et al. 2005; West et al. 1999).

DIAGNOSIS

A farm with an evidence of PCV2 infection (by polymerase chain reaction [PCR] or serology) and no clinical signs compatible with PCVD is, by definition, a subclinically infected farm. The respiratory form of PRRSV and all diseases and conditions that cause wasting must be differentiated from PMWS (Harding and Clark 1997). Differential diagnoses for PDNS include any condition that causes red-to-dark discoloration of the skin, as well as conditions that cause petechial hemorrhages in the kidneys (Segalés 2002). Special note should be made of the similarity of gross lesions between PDNS, classical swine fever, and African swine fever. The reproductive form of PCV2 is clinically indistinguishable from other swine diseases that cause late-term abortions and stillbirths.

Postweaning Multisystemic Wasting Syndrome

A diagnosis of PMWS in a pig or group of pigs is warranted if the following criteria are fulfilled (Sorden 2000):

1. growth retardation and wasting, frequently with dyspnea and enlargement of inguinal lymph nodes and occasionally with jaundice;
2. moderate-to-severe characteristic histopathological lesions in lymphoid tissues;
3. moderate-to-high amounts of PCV2 within the lesions in lymphoid and other tissues of affected pigs.

This case definition does not exclude the concomitant presence of other diseases together with PMWS. Neither clinical signs nor gross lesions observed in suspected PMWS-affected pigs are sufficient to diagnose the disease. A herd diagnosis of PMWS is based on the occurrence of a clinical process characterized mainly by wasting and mortality in excess of the expected and/or historical level for the farm and the individual diagnosis of PMWS, as described above, in a number of pigs. This case definition is very useful in an epidemic situation, but the evolution of PMWS to a more chronic, milder form with lower mortality has added to the difficulty of establishing a diagnosis in farms suspected of PMWS that subsequently responded to PCV2 vaccination.

Porcine Dermatitis and Nephropathy Syndrome

Detection of PCV2 is not included in the diagnostic criteria for PDNS. Rather, the diagnosis of PDNS is based on two main criteria (Segalés 2002):

1. the presence of hemorrhagic and necrotizing skin lesions, primarily on the hind limbs and perineal area, and/or swollen and pale kidneys with generalized cortical petechiae;
2. presence of systemic necrotizing vasculitis and necrotizing, fibrinous glomerulonephritis.

Reproductive Disease

The diagnosis of PCV2-associated reproductive disease includes three criteria (Segalés et al. 2005a):

1. late-term abortions and stillbirths, sometimes with evident hypertrophy of the fetal heart;
2. the presence of heart lesions characterized by extensive fibrosing and/or necrotizing myocarditis;
3. the presence of high amounts of PCV2 in myocardial lesions and other fetal tissues.

Recent studies suggested that this case definition is useful for the diagnosis of acute stages of reproductive failure, whereas quantitative PCR can be used as a sensitive diagnostic method within a wider time span (Hansen et al. 2010). Serology is not useful for the detection of intrauterine infection with PCV2 (Hansen et al. 2010).

Laboratory Confirmation

Several methods have been developed to detect PCV2 in tissues. In situ hybridization (ISH) and immunohistochemistry (IHC) are the most widely used tests for the diagnosis of PCVD (McNeilly et al. 1999; Rosell et al. 1999). PCV2 nucleic acid or antigen in PMWS- and PDNS-affected pigs is usually found in the cytoplasm of histiocytes, multinucleate giant cells, and other monocyte/macrophage lineage cells, as well as in other cell types (Segalés et al. 2004). In aborted and mummi-
fied fetuses, PCV2 is mainly found in the myocar-dio-
cyte (Madson et al. 2009a; West et al. 1999).

A strong correlation has been observed between the
quantity of PCV2 seen in tissues and the severity of
microscopic lymphoid lesions in PMWS (Figure 26.6)
(Rosell et al. 1999). Since the amount of PCV2 in
damaged tissues is the primary difference between
PMWS-affected pigs and PCV2 subclinically infected
pigs, techniques that allow PCV2 quantification in
tissues and/or serum could potentially be used to diag-
nose PMWS (Brunborg et al. 2004; McNeilly et al. 2002;
Olvera et al. 2004). However, while quantitative PCR
shows potential for the diagnosis of PMWS on a popu-
lation basis, histopathology in combination with the
detection of PCV2 in tissues is required for the diagno-
sis of PMWS in individual pigs (Grau-Roma et al. 2009).
Qualitative PCR techniques should not be used to diag-
nose PCVD because the virus is ubiquitous, and posi-
tive results in the absence of clinical disease are
common.

Serological assays for the detection of antibodies to
PCV2 have been developed (Segalés and Domingo
2002), but diagnosis of PCVD using serological tech-
niques is problematic because PCV2 is ubiquitous and
seroconversion patterns are relatively similar in PMWS-
affected and nonaffected farms. PCV2 antibody dynam-
ics are of interest because of their potential role in
monitoring PCV2 vaccination (Fachinger et al. 2008;
Meerts et al. 2005; Pogranichniy et al. 2000; Resendes et al. 2004a;
Steiner et al. 2009). In the field, seroconversion occurs
in both subclinically infected and PMWS-affected pigs
(Grau-Roma et al. 2009; Larochelle et al. 2003;
Rodriguez-Arrioja et al. 2002). Some field studies have
found no difference in total anti-PCV antibody titer
levels between non-PMWS and PMWS-affected pigs
(Larochelle et al. 2003; Sibila et al. 2004), but others
have reported weaker humoral immune responses in
PMWS pigs (Grau-Roma et al. 2009; Meerts et al. 2006).
Under experimental conditions, delayed responses or
low total antibody titers have been associated with
PMWS (Bolin et al. 2001; Ladekjaer-Mikkelsen et al.
2002; Meerts et al. 2006; Okuda et al. 2003; Rovira et al.
2002). Most of these studies have shown that PCV2
persists in tissues and blood of subclinically infected
and PMWS-affected pigs in the presence of high total
antibody titers, but did not discriminate between neu-
tralizing versus non-neutralizing antibodies.

PCV2-infected pigs develop PCV2-specific neutral-
zizing antibodies at 10–28 DPI (Fort et al. 2007; Meerts
et al. 2005; Pogranichniy et al. 2000). Under experi-
mental conditions, low neutralizing antibody titers
have been associated with increased PCV2 replication
and the development of PMWS. Meerts et al. (2006)
investigated the dynamics of neutralizing antibodies in
naturally infected pigs and found that maternally
derived neutralizing antibodies were passively trans-
ferred to all piglets, and none of the pigs that developed
PMWS seroconverted for neutralizing antibodies. Fort
et al. (2007) showed that the levels of neutralizing
antibodies were correlated with the clinicopathological
status of naturally infected pigs.

Thus, an insufficient humoral response (Bolin et al.
2001; Okuda et al. 2003; Rovira et al. 2002) and, in
particular, a poor neutralizing antibody response (Fort
et al. 2007; Meerts et al. 2006), is associated with
increased viral replication, severe lymphoid lesions,
and the eventual development of PMWS. However, it
has also been suggested that excessive PCV2 serum
antibody titers may trigger PDNS (Wellenberg et al.
2004).

Data regarding cell-mediated immune responses in
PCV2-infected pigs is relatively sparse (Fort et al. 2009a;
Meerts et al. 2005; Steiner et al. 2009). Pigs with sub-
clinical PCV2 infections develop specific humoral and

**IMMUNITY**

Under field conditions, colostral antibodies decline
during the lactating and nursery periods, followed by

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**Figure 26.6.** Mesenteric lymph node. Marked presence of PCV2 nucleic acid in the cytoplasm of multinucleate giant cells and macrophages (dark stained cells) in a PMWS-affected pig (in situ hybridization to detect PCV2; fast green counterstain; original magnification: 100×).
T-cell responses, although with relatively slow kinetics. The kinetics of the helper and cytotoxic T-cell responses, as measured by the number of IFN-γ-secreting cells (SCs), are dependent on the individual animal and the time postinoculation at which the cells are assayed. Overall, current data on the adaptive immune response against PCV2 infection suggest that the cell-mediated response, as measured by IFN-γ-SCs, together with a significant neutralizing antibody response, is primarily responsible for viral clearance in infected animals (Fort et al. 2009a). It is hypothesized that a failure in one or the other or both responses might result in PMWS development.

PREVENTION AND CONTROL

PMWS is a multifactorial disease that can be controlled by use of PCV2 vaccines. All current vaccines are based on PCV2a strains (Kekarainen et al. 2010).


In sows, vaccination reportedly increased fertility and reduced returns to service (Kekarainen et al. 2010). However, one study using a PCV2 commercial subunit vaccine (Madson et al. 2009b) found that vertical transmission of PCV2 could occur in PCV2-vaccinated dams exposed at 56 days of gestation, although clinical protection was achieved. PCV2 infection and/or low serological titers to PCV2 in sows at farrowing increased the overall mortality of their offspring to PMWS (Allan et al. 2002a; Calsamiglia et al. 2007). Therefore, measures that increase maternal immunity and decrease sow viremia at farrowing should diminish piglet PMWS mortality in problem herds (Pejsak et al. 2009).

PCV2 vaccine efficacy in pigs may rely on humoral immunity, either maternally acquired (sow vaccination) or actively induced (pig vaccination) (Fort et al. 2008, 2009b; Opriessnig et al. 2010). However, low antibody responses, as well as lack of antibody development after vaccination, do not necessarily correlate with lack of protection. Cell-mediated immunity is also assumed to be important for protection (Fenaux et al. 2004; Pérez-Martín et al. 2010). Development of cell-mediated immunity in response to a PCV2 piglet subunit vaccine was demonstrated by the production of PCV2-specific IFN-γ-SC induced in vaccinated animals (Fort et al. 2009b). It is noteworthy that high levels of maternal-derived antibodies can interfere with active seroconversion following vaccination (Fort et al. 2009b), although the effect on vaccine efficacy under field conditions has not been sufficiently evaluated.

Prior to the advent of vaccines, prevention and control focused on eliminating environmental and infectious cofactors and triggers of PMWS, for example, “Madec’s 20-point plan” (Ellis et al. 2004; Madec et al. 2000; Segalés et al. 2005a). These factors still have relevance in the control of PMWS.

In addition, changes in the diet reportedly led to partial control of PMWS on some farms, although results are contradictory (Segalés et al. 2005a). One study found that conjugated linoleic acid (CLA) ameliorated the effect of PCV2 experimental infection on the immune system (Bassaganya-Riera et al. 2003), but these data have not been corroborated.

Some experimental and field studies suggested that immune activation from infection or vaccination might be an important trigger for PMWS (Krakowka et al. 2001, 2007; Kyriakis et al. 2002). From a practical point of view, eliminating efficacious vaccines from health programs may present a greater risk to the population than the threat of inducing PMWS in a small percentage of pigs. However, producers with PMWS-affected herds should consider rescheduling vaccinations so as not to coincide with PCV2 infections (Opriessnig et al. 2004).

REFERENCES

RELEVANCE

In 1997, a previously unrecognized virus was detected in a Japanese patient with posttransfusion non-A-G hepatitis (Nishizawa et al. 1997). The virus was initially denoted TT virus (TTV), referring to the initials of the patient. It was later renamed torque teno virus from Latin “torques” (necklace) and “tenuis” (thin) in reference to the organization of the TTV DNA genome (Todd et al. 2005). Human TTVs have been associated with liver diseases, respiratory disorders, hematological disorders, and cancer. However, a causal role for TTV has not been established in any species, and therefore, these viruses are considered nonpathogenic commensal inhabitants of vertebrates (Simmonds et al. 1999; Zein 2000).

Two species of TTV have been described in pigs: torque teno sus virus type 1 (TTSuV1) and 2 (TTSuV2). Research on swine TTSuV is very recent, with the first epidemiological studies published in 2004 (McKeown et al. 2004) and 2005 (Bigarré et al. 2005). TTSuVs have been linked to porcine circovirus type 2 (PCV2)-associated diseases (PCVAD), such as postweaning multisystemic wasting syndrome (PMWS) (Ellis et al. 2008; Kekarainen et al. 2006) and a porcine dermatitis and nephropathy syndrome (PDNS)-like pathological condition (Krakowka et al. 2008).

ETIOLOGY

Increasing numbers of TTVs have been placed into the newly created family *Anelloviridae*. All members of the family share similar genomic organization, although they are genetically very distinct. Currently, the family consists of nine genera based on host species. Genus *Iotatorquevirus* contains the two species known to be infectious for swine. TTSuV classification is based on several tentative pairwise identity (PI) thresholds: variants (>95% PI), subtypes (85–95% PI), types (67–85% PI), species (55–67%), and genus (36–55%) (Huang et al. 2010).

TTV virions are icosahedral, and nonenveloped, with a diameter of 30–32 nm. The buoyant density in cesium chloride is 1.31–1.33 g/cm$^3$ for TTV in serum and 1.33–1.35 g/cm$^3$ for TTV in feces (Okamoto et al. 1998).

The TTV genome has negative polarity and ranges from 2.1 to 3.8 kilobases in size, depending on the host species (2.8 kilobases for TTSuVs) (Okamoto et al. 2002). Complete genomic sequencing of species-specific TTVs infecting humans, nonhuman primates, tupaia (shrew), domestic swine, feline, and canines showed that the genomic organization is preserved among species (Inami et al. 2000; Niel et al. 2005; Okamoto et al. 2001, 2002). The untranslated region (UTR) of swine TTSuV occupies 24% of the viral genome (Okamoto et al. 2002). This region contains highly conserved sequences preserved among all TTVs. As shown in other circular single-stranded DNA (ssDNA) viruses, the UTR contains sequences regulating viral replication and gene transcription (Mankertz et al. 2004). Promoter and enhancer elements within the UTR differentially regulate gene transcription, depending on the cell line (Kamada et al. 2004; Suzuki et al. 2004).

The genomic organization and predicted transcriptional profile of TTSuVs resemble those of TTVs of humans, although the sequence similarity is less than 50% (Niel et al. 2005; Okamoto et al. 2002). Three mRNAs of different sizes are transcribed from the TTSuV genome (Okamoto et al. 2002). By analogy with...
circoviruses, open reading frame (ORF)1 (635 amino acids) encodes the putative coat protein and presents sequence motifs that are characteristic for replication-associated proteins of circular ssDNA viruses. ORF2 (73 amino acids) contains sequences characteristic of tyrosine phosphatases. ORF3 (224 amino acids) function cannot yet be predicted. Recent studies showed the generation of up to six different proteins by human TTVs using a concerted strategy of alternative splicing (Qiu et al. 2005).

The wide distribution of TTSuV in tissues (Aramouni et al. 2010; Bigarré et al. 2005) suggests that the virus can replicate in distinct cells, but no in vitro cell culture system has been identified. Mitogen-stimulated peripheral blood mononuclear cell (PBMC) cultures infected with human TTV reportedly released infectious virus (Maggi et al. 2001; Mariscal et al. 2002). On the other hand, replicative forms of the viral genome were detected in the liver, bone marrow cells, and PBMCs, suggesting that these cells would replicate the virus (Okamoto et al. 2000; Zhong et al. 2002).

ROLE IN PUBLIC HEALTH

There is no documented transmission of swine anelloviruses to humans. Swine TTSuV DNA has been found in human drugs containing components of swine origin, but the biological significance is undetermined (Kekarainen et al. 2009).

EPIDEMIOLOGY

A retrospective study showed that TTSuVs have been widespread, but undetected, in pig populations since at least 1985 (Segalés et al. 2009). No comprehensive study on geographical distribution has been done, but TTSuVs appear to be ubiquitous in pigs in Canada, China, France, Germany, Italy, Korea, Spain, Thailand, and the United States (Bigarré et al. 2005; Kekarainen et al. 2006; Martelli et al. 2006; McKeown et al. 2004). Therefore, it is assumed that the virus is present worldwide.

TTVs have been found in several domestic animal species, including swine, cattle, sheep, cats, dogs, and chickens (Leary et al. 1999; Okamoto et al. 2002), as well as nondomestic animals such as wild boar (Martínez et al. 2006) and tupaia (shrew) (Okamoto et al. 2001). Each host has its own, genetically distinct TTV, which is considered species specific. However, human TTV can infect chimpanzees under experimental conditions (Luo et al. 2000), and genetically similar TTSuVs circulate between wild boar and domestic pigs.

TTSuVs are apparently transmitted both vertically and horizontally. TTSuVs can be detected in fetal tissues and blood, semen, and colostrum (Kekarainen et al. 2007; Martínez-Guinó et al. 2009, 2010), indicating the potential for vertical transmission. In fact, vertical transmission could be very important in maintaining the virus in swine populations. Animals tested during the first weeks of life may be negative for TTSuV DNA if only serum is analyzed (Sibila et al. 2009b), but most and perhaps all animals (including fetuses) are polymerase chain reaction (PCR) positive when tissues are tested (Aramouni et al. 2010). The route of fetal infection is uncertain, but may reflect virus-contaminated semen or transplacental transmission.

TTSuV is detected in nasal and fecal samples from a small percentage of piglets from their first week of age (Sibila et al. 2009a). Fecal excretion seems to be low during the first 15 weeks of age, but nasal detection of TTSuVs seems to increase with age (30% for TTSuV1 and 55% for TTSuV2). Increasing prevalence and increasing viral load in tissues with age suggests that transmission is efficient, probably by the oral–nasal exposure.

The current inability to propagate virus in vitro precludes studies on the virus’ persistence in the environment or susceptibility to disinfectants. However, the physicochemical properties of TTSuVs are thought to be similar to those of the circoviruses.

PATHOGENESIS

The primary site of virus replication is unknown. In fetuses, the highest concentration of TTSuV is found in the lungs, heart, spleen, and kidneys, which suggest they contain a significant number of cells supporting the initial viral replication (Aramouni et al. 2010). In older animals, high concentrations of virus are found in all tissues. Whether this reflects actively replicating virus within these tissues or virus accumulated over time is uncertain.

TTSuVs may be found in a high proportion of apparently healthy animals (Kekarainen and Segalés 2009), which suggests that infection by itself does not produce clinical signs. However, TTSuVs have been associated with economically important diseases like PMWS (Ellis et al. 2008; Kekarainen et al. 2006) and a PDNS-like condition (Kراكowka et al. 2008). Potentially, TTSuVs could differ genetically and in clinical manifestation.

CLINICAL SIGNS AND LESIONS

At present, no clinical signs are associated with TTSuV infection and no clinicopathological experimental studies involving conventional pigs have been done. Inoculation of gnotobiotic pigs with TTSuV1-containing tissue homogenate caused mild interstitial pneumonia, transient thymic atrophy, membranous glomerulonephropathy, and modest lymphocytic-to-histiocytic liver infiltrates (Karakowka and Ellis 2008). One-half of gnotobiotic pigs inoculated intraperitoneally with TTSuV1 and 7 days later oronasally with PCV2 developed acute fatal PMWS (Ellis et al. 2008). No PMWS
was observed if TTSuV or PCV2 was the only inoculum or if PCV2 preceded the inoculation with TTSuV inoculation. Thus, TTSuV infection could be a cofactor in the development of PMWS.

**DIAGNOSIS**

No assays for the detection of antibodies or protocols for virus isolation have been described. Several PCR-based assays have been developed (Segalés et al. 2009), including a quantitative PCR (qPCR) for TTSuV1 and TTSuV2 in the serum (Gallei et al. 2009; Lee et al. 2010) and a nonspecific qPCR that detects both TTSuV species and bovine TTV (Brassard et al. 2010).

**IMMUNITY**

There are no studies on immune responses against TTSuV. Based on the recent work of Aramouni et al. (2010), it can be postulated that high viral loads in older animals reflect an inefficient anti-TTSuV immune response. Furthermore, in utero infection prior to the gestational age at which the fetus becomes immunologically responsive and evidence of lifelong infection in pigs raises the possibility of immunotolerance. Further studies are needed to understand these observations.

**PREVENTION AND CONTROL**

The impact of TTSuV infection and the consequences for herd health have not yet been established.

**REFERENCES**

Herpesviruses infect a wide variety of animals from mollusks to mammals. More than 200 distinct herpesvirus species have so far been recognized. Herpesviruses belong to the most complex and most widespread viruses. They are grouped in the order *Herpesvirales* based on the morphology of the virion and biological characteristics of their replication cycle (Davison 2010; Davison et al. 2009).

Herpesviruses infecting reptiles, birds, and mammals, which show a significant homology in their genomic sequence and gene arrangement, constitute the family *Herpesviridae*, whereas the phylogenetically more distant herpesviruses infecting amphibia and fish make up the family *Alloherpesviridae*. The hitherto single identified herpesvirus from mollusks (ostreid herpesvirus 1 isolated from oysters) represents the type and only member of the *Malacoherpesviridae*.

In addition to virion morphology, all herpesviruses share the capacity to establish a state of latency resulting in lifelong association with the infected host. During latency, viral gene expression is restricted and only serves the maintenance of the latent state without production of infectious virus. After reactivation, infectious virus is again produced and spreads to infect other susceptible individuals. This striking biological feature is a hallmark of herpesvirus infections and explains their evolutionary success.

Within the *Herpesviridae*, three subfamilies have been established based on distinct biology and supported by genetic analyses: *Alphaherpesvirinae*, *Betaherpesvirinae*, and *Gammaherpesvirinae*. Herpesviruses relevant for swine are present in all three subfamilies of the *Herpesviridae* (Table 28.1).

*Alphaherpesvirinae* have a rapid lytic replication cycle and establish latency (primarily) in neurons of sensory ganglia. They contain four genera: *Simplexvirus* with human herpes simplex virus 1 (HSV-1; taxonomically correctly designated as human herpesvirus 1 [HHV-1]) as type member also contains HSV-2 (HHV-2) as well as bovine herpesvirus 2 (BoHV-2; bovine mammillitis virus); *Varicellovirus* with the human varicella-zoster virus (VZV or HHV-3) and bovine herpesviruses 1 and 5 (BoHV-1, infectious bovine rhinotracheitis virus; BoHV-5, bovine encephalitis herpesvirus), and equine herpesviruses 1 and 4 (EHV-1, EHV-4); *Iltovirus* with infectious laryngotraechitis virus (gallid herpesvirus 1); and *Mardivirus* with Marek’s disease virus (gallid herpesvirus 2) as type member.

Members of the *Betaherpesvirinae* generally specify larger genomes than either the alpha- or the gammaherpesviruses, and are characterized by extended replication cycles and strict host specificity. Genera *Cytomegalovirus* (type member human cytomegalovirus or HHV-5), *Muromegalovirus* (type member murid herpesvirus 1 or murine cytomegalovirus), *Roseolovirus* (type member human herpesvirus 6), and *Proboscivirus* (type member elephantid herpesvirus 1) have been recognized.

The *Gammaherpesvirinae* comprise herpesviruses with transforming potential and preferential association with lymphocytes grouped into genera *Lymphocryptovirus* (type member Epstein–Barr virus [EBV] or HHV-4), *Rhadinovirus* (type member Kaposis’s sarcoma herpesvirus [KSHV] or HHV-8), *Macavirus* (type member malignant catarrhal fever [MCF] virus or alcelaphine herpesvirus 1 [AIHV-1]), and *Percavirus* (derived from perissodactyl and carnivore) containing EHV-2 and -5 and mustelid herpesvirus 1.
Table 28.1. Herpesviruses of swine

<table>
<thead>
<tr>
<th>Taxonomic Name</th>
<th>Trivial Name</th>
<th>Genus</th>
<th>Subfamily</th>
</tr>
</thead>
<tbody>
<tr>
<td>Suid herpesvirus 1</td>
<td>Pseudorabies virus (Aujeszky's disease virus)</td>
<td>Varicellovirus</td>
<td>Alphaherpesvirinae</td>
</tr>
<tr>
<td>Suid herpesvirus 2</td>
<td>Porcine cytomegalovirus</td>
<td>Unassigned</td>
<td>Betaherpesvirinae</td>
</tr>
<tr>
<td>Suid herpesvirus 3</td>
<td>Porcine lymphotrophic herpesvirus 1</td>
<td>Macavirus</td>
<td>Gammaherpesvirinae</td>
</tr>
<tr>
<td>Suid herpesvirus 4</td>
<td>Porcine lymphotrophic herpesvirus 2</td>
<td>Macavirus</td>
<td>Gammaherpesvirinae</td>
</tr>
<tr>
<td>Suid herpesvirus 5</td>
<td>Porcine lymphotrophic herpesvirus 3</td>
<td>Macavirus</td>
<td>Gammaherpesvirinae</td>
</tr>
<tr>
<td>Ovine herpesvirus 2</td>
<td>Sheep-associated malignant catarrhal fever virus</td>
<td>Macavirus</td>
<td>Gammaherpesvirinae</td>
</tr>
</tbody>
</table>

*Whereas pigs are the main/sole hosts for SuHV1–5, OvHV-2 is primarily found in sheep but causes spillover infections in pigs.


Herpesvirus Morphology

Herpesvirus particles consist of four structural elements: the core, which contains the linear, double-stranded DNA genome; the icosahedral nucleocapsid; a lipid envelope derived from the host cell into which viral proteins, mostly glycoproteins, are embedded; and the tegument, a proteinaceous structure linking the envelope and nucleocapsid similar to the matrix in RNA viruses (Figure 28.1). Whereas basic morphology of the herpes virion appears identical in all members of the Herpesvirales, the diameter of the virus particle varies around ~200 nm depending on the amount of tegument. The ~100-nm-diameter capsid shell exhibits icosahedral symmetry with a triangulation number of 16, thus consisting of 162 capsomers with 150 hexons and 12 pentons.

Receptor-binding proteins as well as major immunogens are located in the viral envelope. Most of them are modified by the addition of carbohydrates and, thus, represent glycoproteins. Within the Herpesviridae glycoproteins B (gB), gH, gL, gM, and gN are conserved. Glycoprotein gB and a heterodimer of gH and gL constitute the core machinery mediating fusion between virion envelope and the host cell membrane, whereas the gM–gN heterodimeric complex functions during virion assembly.

Herpesvirus Genomic Organization and Gene Expression

The genomes of herpesviruses consist of linear, double-stranded DNA molecules varying in size between ~123,000 base pairs (simian varicellovirus) and ~300,000 base pairs (koi herpesvirus) encoding between 70 (VZV) and 252 (human cytomegalovirus [HCMV; HHV-5]) predicted proteins. Many herpesvirus genomes contain, besides singular “unique” sequences, extensive repetitive sequences that can either occur as tandem repeats at one site or in different locations in the viral genome in parallel or antiparallel orientation. Approximately 40 genes and gene products are conserved in all members of the Herpesviridae (Mettenleiter et al. 2008). They are mostly arranged in gene blocks and encompass proteins required for capsid structure and maturation, several tegument and envelope proteins, the replication, cleavage, and packaging machinery for the viral genome, and proteins with enzymatic function in nucleotide metabolism. Approximately half of the genes/proteins of each herpesvirus are not essential for viral replication in cell culture, including those relevant for transmission and spread in vivo and for immune modulation and evasion.

Herpesvirus Replication

Herpesvirus replication is a complex process (Figure 28.2). Adsorption of free virions to target cells is mediated by virally encoded glycoproteins embedded in the virion envelope. This interaction triggers fusion between the virion envelope and the host cell membrane. Fusion primarily occurs at the cell surface, but may also be executed after endocytosis. After the release of the nucleocapsid into the cytosol, it is transported by cellular motor proteins along microtubules to the
nuclear pore, where it docks with one vertex oriented toward the pore and viral genomic DNA is released into the nucleus through the nuclear pore.

In the nucleus, the linear viral genome circularizes, and viral gene expression ensues in a cascade-like fashion in the order immediate-early (α-), early (β-), and late (γ-) gene expression. Capsid assembly occurs in the nucleus, and the resulting nucleocapsids leave the nucleus for final maturation in the cytosol by budding at the inner nuclear membrane, thereby acquiring a primary envelope that fuses with the outer nuclear membrane to release nucleocapsids into the cytosol. Viral tegument proteins now attach to the nucleocapsid prior to final (secondary) envelopment by a second budding process into vesicles of the trans-Golgi network, resulting in the formation of a complete virion within a cellular secretory vesicle. Release of mature virus particles then occurs by fusion of the vesicle with the plasma membrane. This prototypic replication cycle appears to be valid for all herpesviruses, although more or less subtle deviations may apply (Mettenleiter et al. 2009).

Spread of herpesvirus infections occurs not only via free virions but also by direct cell-to-cell spread. It is unclear whether complete virions or subviral particles (e.g., nucleocapsids) mediate this direct spread.

The success of herpesviruses is mainly due to their ability to establish lifelong latency in the infected host.
Thus, the viral genome persists indefinitely even after clinical signs disappear. Spontaneous reactivation results in the formation and shedding of infectious virions, which can infect susceptible animals. Alphaherpesviruses establish latency primarily in sensory neurons after replication in peripheral epithelial cells, whereas latency of betaherpesviruses is established in extraneural sites, for example, secretory glands. Latent gammaherpesviruses are mostly present in the cells of the lymphoreticular system, primarily B and T cells. Establishment and maintenance of the latent state are achieved by different mechanisms in the different herpesvirus subfamilies. However, they have in common a restricted expression of specific latency-related genes with a concomitant absence of lytic viral gene expression.

**SUID HERPESVIRUS 1 (AUJESZKY’S DISEASE VIRUS; PSEUDORABIES VIRUS)**

**Relevance**

Although the taxonomic name suid herpesvirus 1 (SuHV-1) indicates that the natural host of pseudorabies virus (PRV) is pigs, clinical PRV was first described in 1813 in cattle. This was because PRV infection in swine, particularly in older animals, may produce only innocuous respiratory signs or may be clinically inapparent, whereas productive infection in other susceptible species is invariably fatal and characterized by severe central nervous signs. Thus, the rabies-like clinical picture in cattle prompted the use of the term “pseudorabies” in Switzerland in 1849. Likewise, “mad itch” was used to describe the disease in cattle in the United States in the first half of the 19th century because PRV causes excessive pruritus.

In 1902, the Hungarian physician Aladár Aujeszky reported the isolation of the infectious agent from a diseased ox, a dog, and a cat, and differentiated it from rabies (Aujeszky 1902). It could be passaged in rabbits reproducing the typical clinical signs. Guinea pigs and mice were also found to be susceptible, whereas chicken and doves were resistant. Thus, the disease became widely known as Aujeszky’s disease (AD). It was not until 1931 that Richard Shope established that the agent of “mad itch” was also present in domestic pig holdings in the United States. Erich Traub in Germany was the first to cultivate PRV *in vitro* in organ explants in 1933. One year later, Sabin and Wright reported a serological relationship between PRV and herpes simplex virus, resulting in the inclusion of PRV into the herpesvirus group.

**Role in Swine Health**

Whereas PRV exhibits a wide host range capable of infecting basically all mammals except higher primates, only pigs are able to survive a productive infection and are thus considered the natural host. PRV infections in swine soared after the Second World War, particularly in Europe, when intensive pig breeding and farrowing were established. In the 1970s, PRV became a major scourge of pigs worldwide, distributed primarily by global movement of animals and animal products.

Although field isolates and strains differ in virulence, they can cause devastating losses by fatal infection of piglets and abortions in pregnant animals. Pigs exhibit a pronounced age resistance against PRV, with younger animals more susceptible to fatal infections characterized by neuronal signs, such as ataxia, convulsions, and sudden death. In contrast, older animals (>1 year) primarily present with respiratory distress or even subclinical infection. In pregnant animals, infection of fetuses results in resorption, mumification, or abortion.

Early eradication programs focused on elimination (culling) of infected herds met with some success, for example, in Great Britain or Scandinavian countries. However, advances in molecular biology produced the first so-called marker vaccines that allowed serological differentiation between vaccinated and infected animals. These vaccines provided the basis for cost-effective control and, eventually, PRV eradication (van Oirschot 1999).

**Etiology**

PRV belongs to the genus *Varicellovirus* in the subfamily *Alphaherpesvirinae* of the family *Herpesviridae* (Davison 2010). Only a single serotype is known. Isolates were initially differentiated by the restriction pattern of viral genomes after cleavage with sequence-specific endonucleases, such as Bam HI or Kpn I (Herrmann et al. 1984).

In 2004, the complete genome sequence of PRV assembled from various strains was described (Klupp et al. 2004). The PRV genome encompasses 143,461 nucleotides and contains at least 72 open reading frames (ORFs) encoding 70 different proteins (Figure 28.3). The viral genome consists of two unique portions, the long (UL) and short (US) unique regions, and two large inverted repeats (terminal repeat [TR] and internal repeat [IR]), which bracket the US. This results in inversion of the US relative to the UL and the existence of two isomeric forms of the viral genome, that is, a class D herpesvirus genome.

**Laboratory Cultivation.** Due to its broad host range, many cell lines and primary cell cultures from different species are permissive for PRV. Cell lines generally used for PRV replication and experimental analysis include PK-15, Madin–Darby bovine kidney (MDBK) cells, or primate Vero cells. PRV undergoes lytic replication resulting in cytopathic effect (CPE) manifested either by immediate cell destruction (plaque formation) or by formation of syncytia due to the fusion activity of viral glycoproteins exposed at the cell surface. The appear-
28.3. Predicted PRV transcript and gene organization. The linear form of the PRV genome is shown with UL being followed by IR, US, and TR. The predicted locations of open reading frames, 5' and 3' untranslated regions, DNA repeats, splice sites, and origins of replication (Ori) are depicted (from Klupp et al. 2004, reprinted with permission of the American Society of Microbiology). LLT, large latency transcript; UTR, untranslated region.
ance of CPE depends on the infectious dose but is routinely seen in 24–72 hours. Infected cells can be unambiguously identified by immunostaining using PRV-specific monoclonal antibodies (mAbs). Genetically engineered viral variants expressing reporter proteins such as β-galactosidase or fluorescent proteins can be used to track virus infection in cell culture and in experimental animal infections. These viruses are also used for neuronal circuit tracing based on the capacity of PRV to spread transsynaptically in neuronal networks (Curanovic and Enquist 2009).

Role in Public Health
Although isolated reports describe putative infections of humans with PRV, they are not conclusive, and it is generally accepted, and has been demonstrated even by self-inoculation, that humans are resistant against natural PRV infection (Jentzsch and Apostoloff 1970).

Epidemiology
AD has an almost worldwide distribution, except for Norway, Australia, and most of the Southeast Asian islands. It occurs particularly in regions with dense pig populations. The disease had not existed widely outside eastern Europe before the 1960s, but had spread nearly globally by the end of the 1980s assisted by the emergence of more virulent PRV strains and changes in swine management, notably total confinement of large numbers of pigs and continuous farrowing. As a result, PRV became one of the most important infectious diseases of domestic pigs.

Due to increased control efforts and the strict implementation of national eradication programs, AD virtually disappeared from domestic pigs in several parts of the world in recent decades. In Europe, PRV has been eliminated from domestic pig populations in Austria, Cyprus, the Czech Republic, Denmark, Finland, France (except single departments), Germany, Hungary, Luxembourg, The Netherlands, Sweden, Switzerland, Slovakia, and Great Britain (England, Scotland, and Wales). Canada, New Zealand, and the United States are also free of AD (Hahn et al. 2010; MacDiarmid 2000; Müller et al. 2003). In PRV-free countries, vaccination is prohibited. AD is still endemic in eastern and southeastern Europe, Latin America, Africa, and Asia. However, more countries are implementing national eradication programs to eliminate PRV.

Despite successful elimination of PRV from domestic pigs, the disease is widespread in populations of non-domestic swine, including feral pigs, wild boar, and hybrids, around the world (Müller et al. 2000, 2011). Although AD in wild boar generally has not impacted the AD-free status of domestic pigs, infected wild boars represent a constant danger for reintroduction of PRV into free herds and regions.

In Europe, PRV is present in wild boar in many countries, for example, France, Germany, Spain, Italy, Slovenia, Croatia, and the Czech Republic. Seroprevalences in these populations range from 4% to 66% at the national level (Müller et al. 2010, 2011). PRV is also endemic in feral swine populations in the United States (Hahn et al. 2010). Although the prevalence of PRV infection in wild boar in other parts of the world is not known, isolations of PRV strains from wild boar have been reported across Europe, as well as from feral pigs in the United States (Hahn et al. 2010; Müller et al. 2010).

Molecular characterization of a large number of PRV isolates using Bam HI restriction fragment length polymorphism revealed four major genome types. Type I is found predominantly in the United States and central Europe, whereas types II and III circulate in central Europe and northern Europe, respectively. Type IV is restricted to Asia. Within the major genome types, several subtypes can be distinguished (Christensen 1995; Herrmann et al. 1984) aided by phylogenetic analyses of PRV field strains using partial sequence analysis of the gC gene (Fonseca et al. 2010; Hahn et al. 2010).

Susceptible Species. Pigs are the only natural host for PRV, but the virus can naturally infect cattle, sheep, cats, dogs, and rats causing fatal disease (Pensaert and Kluge 1989). Infections have also been reported in brown bear, black bear, Florida panther, raccoon, coyote, deer, and farm fur animal species (mink and foxes) (Banks et al. 1999; Bitsch and Munch 1971; Glass et al. 1994). Only swine (Suidae) are able to survive a productive PRV infection (Enquist et al. 1998). Whether other members of the artiodactyl suborder Suina, for example, Tayassuidae (peccaries) and possibly hippopotamuses, are susceptible to PRV is not known. Therefore, reports of low PRV seroprevalence (<1%) in free-ranging peccaries in the southwestern United States have to be interpreted with caution (Corn et al. 1987). Of the laboratory species, the rabbit is the most susceptible and develops intense local pruritus at the inoculation site. Guinea pigs are less susceptible and may resist subcutaneous inoculation, but succumb to intracerebral or intraperitoneal inoculation (Ashworth et al. 1980).

Transmission. In general, rather high quantities of virus, for example, >1 × 10^4.5 median tissue culture infectious dose (TCID50), are necessary to infect animals, except piglets (1 × 10^2 TCID50). Thus, PRV is not very contagious (Wittmann 1991). Larger quantities of virus are necessary for oral than for intranasal infection (Jakubik 1977).

The virus is spread primarily by direct contact between swine or by contact with PRV-contaminated fomites, for example, contaminated bedding and water, meat products and carcasses of rats, raccoons, swine, and other infected animals. The mucosae of the nasal
Infectious PRV is shed in saliva, nasal and oropharyngeal secretions, and aerosols. Virus titers in nasal and oropharyngeal swabs for 18–25 days with virus titers of up to $10^6$ TCID$_{50}$. At the peak of virus excretion, one pig may excrete $1 \times 10^6$ TCID$_{50}$ into the air during a 24-hour period (Müller et al. 2001; Wittmann 1991). Virus shedding starts 1–2 days after infection, prior to the onset of viremia and clinical signs, reaching a peak at 2–5 days and lasting up to 17 days. Transplacental transmission leads to considerable virus shedding during abortion and birth (Beran 1991; Blaha 1989). Virus can also be found in vaginal and preputial secretions, in ejaculate for up to 12 days, and for 2–3 days in milk. It is occasionally shed in urine and has been detected in rectal swabs, but not in feces, for up to 10 days (Wittmann 1991).

**Persistence in the Environment.** Infectious PRV is resistant to environmental conditions, depending on pH, humidity, and temperature (Pejsak and Truszczynski 2006; Wittmann 1991). On average, about 50% infectivity is destroyed within 24 hours (Schoenbaum et al. 1990). Ultraviolet light and dry or arid conditions inactivate the virus. However, it is stable at pH 4–12, and even at extreme pH values of 2.0 and 13.5, complete inactivation can take 2–4 hours (Benn dorf and Hantschel 1963). It is relatively heat resistant and stable at normal or low temperatures, remaining infectious at 25°C (77°F), 15°C (59°F), and 4°C (39°F) for about 6, 9, and 20 weeks, respectively. Inactivation may occur within weeks at certain subzero temperatures, for example, −18°C to −25°C (0°F to −13°F), and at −40°C (−40°F), PRV remains stable for years. At higher temperatures, it is inactivated rapidly between 60 and 1 minute at 60°C (140°F) and 100°C (212°F), respectively.

In slurry, PRV remains infectious for 1–2 months, depending on the season (Kretzschmar 1970). At high virus doses ($10^{6.5}$ TCID$_{50}$/mL), however, infectious virus could still be detected after 27 weeks at 4°C (39°F), and 15 weeks at 23°C (73°F). In aerated slurry at pH 9.6 and 44°C (111°F), infectivity disappeared in 8–21 days. Infectious PRV persisted in soil for 5–6 weeks, on hay and straw for 15 and 40 days, on sacks and wood for 10 and 15 days in summer and in winter, respectively (Wittmann 1991). In waste food fermented by *Lactobacillus acidophilus*, the virus was inactivated at 20°C (68°F) and 30°C (86°F) within 24 hours, but remained infectious at 10°C (50°F) for 48 hours and at 5°C (41°F) for 96 hours. Heating of waste food to 70°C (158°F) or 80°C (176°F) destroyed the virus within 10 or 5 minutes, respectively. Maturation of pig meat at 4°C (39°F) does not inactivate the virus. However, in meat, the virus is believed to be inactivated at −18°C (0°F) within 40 days and after heat treatment of meat and meat products at 80°C (176°F) (Donaldson 1983).

**Susceptibility to Disinfectants.** Orthophenolphenate compounds, peracetic acid, formalin, 2% sodium hydroxide, trisodium phosphate iodide disinfectants, 1–2% quaternary ammonium compounds, hypochlorites, and chlorine (chlorhexidine) solutions are suitable disinfectants (Beran 1991), with a slightly reduced effectiveness in the presence of organic matter. For large-scale disinfection, calcium chloride preparations dissolved in water, crude chloramines, and preparations containing at least 1% active formaldehyde may be used. Lime ($20$ kg Ca(OH)$_2$/m$^3$) is recommended for disinfecting slurry. PRV is relatively resistant to changes
in pH between 4 and 12 (Benndorf and Hantschel 1963); hence, pure acid and lye preparations can only be used as disinfectants to a limited extent. The same applies to phenol- and alcohol-based disinfectants (Blaha 1989).

**Pathogenesis**

After oronasal infection of the natural host and primary replication in epithelial cells of the upper respiratory tract, the virus gains access to neurons innervating the facial and oropharyngeal area, in particular, the olfactory, trigeminal, and glossopharyngeal nerves. By fast axonal retrograde transport, it spreads centripetally and reaches the cell bodies of infected neurons, where either lytic or latent infection ensues. PRV is also able to cross synapses to infect neurons of higher order (Pomeranz et al. 2006). Viremia disseminates it to many organs, where the virus replicates in epithelia, vascular endothelium, lymphocytes, and macrophages (Kritas et al. 1999; Mettenleiter 2000).

Replication of PRV in the CNS is characterized by nonsuppurative meningoencephalitis causing severe central nervous disorders (Enquist 1994; Pensaert and Kluge 1989). Trigeminal ganglia, sacral ganglia, and tonsils are considered prime sites of latency in pigs. The demonstration of the sacral ganglia as the most common sites of PRV latency in feral swine supported the hypothesis that these viruses are primarily transmitted venereally and not by the respiratory route, as is common in domestic swine, in which the trigeminal ganglia are the predominant sites of virus latency (Romero et al. 2001). In nonporcine species, PRV is rather strictly neuroinvasive (Mettenleiter 2000).

**Virulence Factors.** PRV strains differ widely in virulence, and the isolate involved affects the severity of disease in pigs and the quantity and duration of virus shedding (Maes et al. 1983). Virulence also affects the tissue tropism of infecting PRV strains. Whereas highly virulent PRV strains are predominantly neuroinvasive, strains of moderate or low virulence exhibit weak neuroinvasiveness, but distinct pneumotropism. Highly adapted or attenuated PRV strains have acquired a tropism for the reproductive system (Romero et al. 2001).

PRV virulence is controlled by multiple genes (Lomniczi and Kaplan 1987; Lomniczi et al. 1984). Proteins determining virulence are found among viral membrane glycoproteins, virus-encoded enzymes, and nonessential capsid-associated proteins (Mettenleiter 2000). According to their role in viral replication in cell culture, glycoproteins (g) are either nonessential (gC, gE, gG, gl, gM, gN) or essential (gB, gD, gH, gK, gl, gL). In terms of virulence, glycoproteins that mediate attachment of PRV to target cells, gC and gD, are of special interest because they may directly determine viral tropism. Virus-encoded enzymes, for example, thymidine kinase or dUTPase, involved in nucleic acid metabolism are major determinants of virulence, and their inactivation leads to strong attenuation of the virus. Neuroinvasiveness and virulence are also determined by viral envelope glycoproteins (Card et al. 1992; Karger and Mettenleiter 1993). Glycoprotein gE is one of the key proteins in neuroinvasion both in the trigeminal and olfactory pathways. Deletion of the gene encoding gE significantly decreases virulence and results in restricted neuronal infection (reviewed in Enquist et al. 1998). Glycoprotein gC, a major viral membrane protein required for efficient adsorption of virus in cell culture, has no obvious role in determining the pattern of neuronal infectivity but appears to function with gE to influence neurovirulence. Besides gE and thymidine kinase, inactivation of several other PRV genes has been shown to result in the attenuation of the virus. In fact, inactivation of many genes, whose products are nonessential for viral replication in cell culture, decreases PRV virulence to a variable extent (for details, see Mettenleiter 2000).

**Latency.** A hallmark of herpesviruses is their capacity to persist in a latent state for the lifetime of the host (Wittmann and Rziha 1989). In PRV infection, latency is established primarily in the neurons of the trigeminal and sacral ganglia, but also in the tonsils (Romero et al. 2003). No infectious virus is produced during latency, but viral genomic DNA persists extrachromosomally (Brown et al. 1995; Cheung 1995; Gutekunst 1979; Rziha et al. 1986). Under control of a latency-active promoter (LAP; Jin et al. 2000), only a small portion of the viral genome is transcribed from part of the internal repeat and adjoining U, region (Cheung 1989; Priola et al. 1990) into an 8.4-kb RNA, which overlaps in antiparallel orientation the mRNA coding for the major immediate-early protein, IE 180. Thus, it has been hypothesized that hybrid formation of the two transcripts may modulate the establishment and/or maintenance of latency and reactivation (Mettenleiter et al. 2008). However, the exact molecular mechanism of the establishment of and reactivation from latency remains unclear.

Because of the potential to reactivate and shed infectious PRV, latently infected animals are a major threat to disease control. Reactivation can occur by stress (transport, handling, temperature) or hormonal imbalance (gestation, farrowing). Latent virus can be detected by demonstration of viral DNA or latency-associated transcripts using polymerase chain reaction (PCR) or nucleic acid hybridization techniques. Since no latent antigen is produced in PRV, specific serological detection is not possible. Reactivation can be induced experimentally, for example, administration of high doses of corticosteroids produces virus shedding and transmission (Mengeling et al. 1992). Interestingly, precolonization of sites of latency appears to interfere with
subsequent colonization after challenge (Schang et al. 1994). Although live-attenuated vaccine strains are limited in their replication in the animal, they may also induce latency, although with reduced efficiency.

**Clinical Signs**

The incubation period in pigs is affected by the infectious dose, the route of infection, and the host species. The incubation period normally ranges from 1 to 8 days, but may take up to 3 weeks. In other susceptible animal species, the course of disease is peracute with incubation periods of 2–3 days.

Infection of pigs with PRV produces a high fever, followed by anorexia, listlessness, dyspnea, excessive salivation, vomiting, trembling, and eventually marked incoordination, especially of the hind legs. Involvement of the respiratory tract with coughing, sneezing, dyspnea, and aspiration pneumonia may occur. In adult swine, high morbidity is predominantly due to respiratory involvement.

The presence and severity of clinical signs, as well as morbidity and mortality, depend on the age and immunological status of the pig (Nauwynck 1997). Furthermore, the route of infection and the virulence of the PRV strain are important factors (Schmidt et al. 2001).

In general, PRV infections in fully susceptible swine result in high morbidity and mortality, especially in juvenile animals in which meningoencephalitis and viremia-associated signs predominate. In neonatal pigs less than 7 days of age, the disease may be characterized by sudden death with few, if any, clinical signs. In 2- to 3-week-old piglets, severe signs of central nervous system (CNS) involvement, for example, trembling, incoordination, convulsion, tremor, ataxia, and paralysis, are seen (Figure 28.4) with mortality up to 100%. Older animals (3–6 weeks of age) may show neurological signs, but usually develop age-dependent resistance. Mortality may decrease to 50% by the fourth week of age and to less than 5% in 5-month-old pigs and even lower as the age of the infected pigs increases.

Clinical signs can be present for 6–10 days. Animals may recover within a few days, but lose weight over the course of the disease. In finishing and fattening pigs, because of the population density, clinical signs can amplify and animals often die from secondary bacterial pneumonia. Signs in gilts and sows depend on the phase of gestation and include embryonic death, resorption of fetuses, mummified fetuses, abortion, or stillbirth, in addition to respiratory signs and fever. Pigs surviving a PRV infection become latently infected (Nauwynck 1997).

In the case of coinfections with other swine viruses, for example, porcine reproductive and respiratory syndrome virus (PRRSV), porcine circovirus type 2 (PCV2), and swine influenza virus (SIV), a severe and often fatal proliferative and necrotizing pneumonia (PNP) may develop in weaning and postweaning pigs (Morandi et al. 2010).

Natural resistance to PRV in particular pig breeds associated with specific quantitative trait loci has been described (Reiner et al. 2002), but the mechanism is unclear.

In PRV-infected nondomestic swine, clinical signs are rare, indicating that circulating PRV variants are highly adapted to the host population (Müller et al. 2001). In Spain and Germany, rare cases of spontaneous clinical AD in juvenile wild boar have been reported, indicating that these field viruses can induce disease in wild pigs that is clinically and pathologically identical to AD in domestic pigs (Gortazar et al. 2002; Schulze et al. 2010).

For other susceptible animal species, a peracute fatal course of infection is characteristic. Often, extreme pruritus resulting in severe self-mutilation is the only clinical sign observed. Dead mice, rats, dogs, or cats on farms are telltale signs for the presence of PRV prior to

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**Figure 28.4.** PRV infection in piglets. Central nervous signs observed after intranasal infection of 4- to 6-week-old piglets with virulent PRV include ataxia and convulsions.
the onset of clinical signs in infected pigs. This also applies to hunting dogs.

Lesions

Gross Lesions. There are no AD-specific gross lesions in pigs, and changes are often absent or minimal. Gross lesions may occur in non-neural tissues, including lymphoid organs, and respiratory, digestive, and reproductive tracts. Particularly in young suckling pigs lacking passive immunity, multifocal necrosis is observed in these tissues, as well as in the liver, spleen, and adrenal glands. Typically, exudative keratoconjunctivitis, serous to fibrinonecrotic rhinitis, laryngitis, tracheitis, and necrotizing tonsillitis may be present.

The CNS is free of gross lesions except for leptomeningeal hyperemia.

Gross lesions in the upper respiratory tract are most common, including rhinitis with patchy epithelial necrosis and necrotizing laryngotracheitis, often in conjunction with multifocal tonsillar necrosis. Lesions in the lower respiratory tract may be pulmonary edema and scattered small foci of necrosis, hemorrhage, or bronchointerstitial pneumonia (Becker 1964). However, the pulmonary lesions are less consistent and are composed of areas of reddening and consolidation scattered throughout the lungs, especially focused on the cranioventral lung lobes.

Multiple, small foci (1–3 mm in diameter) of acute hemorrhagic necrosis characteristic of alphaherpesviral infections may be seen in the liver, spleen (Figure 28.5), lungs, intestines, adrenals, and placenta.

In sows, necrotizing placentitis and endometritis with thickened, edematous wall of the uterus are observed after abortion (Kluge and Maré 1978). Aborted fetuses may be macerated or, occasionally, mummified (stillbirth, mummified fetuses, embryonic death, infertility [SMEDI]). In fetuses or neonatal pigs, necrotic foci in the liver and spleen, lungs, and tonsils are common (Kluge and Maré 1976). PRV infection may also cause edema of the scrotal region.

Microscopic Lesions. Microscopic lesions in pigs reflect the neuroinvasive and epitheliotropic properties of PRV. CNS lesions are characterized by a nonsuppurative meningoencephalomyelitis in the gray and white matter, and ganglioneuritis of the trigeminal and paravertebral ganglia (Figure 28.6: Baskerville et al. 1973). Death may occur before neuronal degeneration or non-

28.5. Multifocal acute coagulative necroses (two of them marked by arrows) of the spleen parenchyma after infection with PRV (courtesy of Dr. W. Thiel, Detmold Germany).

28.6. (A) Diffuse lymphoplasmacytic ganglioneuritis in the trigeminal ganglion with degeneration and necrosis of perikarya, and (B) multifocal to coalescing nonsuppurative encephalitis in the medulla with neuronal degeneration and perivascular cuffing, 8 days after PRV experimental PRV infection.
suppurative inflammatory reactions in the brain become visible. If animals survive long enough, CNS injury can be marked, leading to focal neuronal degeneration and necrosis, with neuronophagia, satellitosis, and gliosis. Especially, young piglets tend to develop panencephalitis with the most severe lesions in the cerebral cortex, brain stem, spinal ganglia, and basal ganglia of the brain.

Perivascular cuffing consists predominantly of pyknotic and karyorrhectic mononuclear cells. Similar lesions exist in the spinal cord, especially in the cervical and thoracic segments. Meninges covering affected areas of the brain and cord may be thickened because of mononuclear cell infiltration.

Intranuclear eosinophilic inclusion bodies are not commonly detected in pigs, but can be present in the neurons, astrocytes, oligodendroglia, and endothelial cells. Lymphoplasmacytic inflammation with neuronal degeneration of the gastric myenteric plexus is also described.

Epithelial lesions consist of multifocal areas of coagulative or lytic, partially hemorrhagic, necrosis in the liver, tonsils, lungs, spleen, placenta, and adrenals with the presence of the characteristic intranuclear inclusions. Viral intranuclear inclusion bodies are much more common in lesions outside the nervous system (Kluge et al. 1999). They are present in tonsil crypt epithelial cells adjacent to necrotic foci and are frequently found in the airway epithelium, within the connective tissue, and cells sloughed into alveolar spaces. However, the specificity of the lesions must be confirmed by immunostaining.

Mucosal epithelial necrosis and submucosal infiltrations of mononuclear cells occur in the upper respiratory tract (Baskerville 1971, Baskerville et al. 1973). In the lungs, necrosis of the bronchi, bronchioles, and pneumocytes is found. Also, peribronchial mucous gland epithelium may be involved in the inflammatory process. Alveolar edema and cellular infiltration may be multifocal or diffuse. Lymphocytes, macrophages, and, less frequent, plasma cells and neutrophils are the characteristic inflammatory cells.

In the uterus, multifocal-to-diffuse lymphohistocytic endometritis and vaginitis and necrotic placentitis with coagulative necrosis of chorionic fossae develop (Bolin et al. 1985; Kluge et al. 1999). Intranuclear inclusion bodies are present in degenerate trophoblasts associated with necrotic lesions (Kluge et al. 1999; Kluge and Maré 1978).

In the male reproductive tract, degeneration of seminiferous tubules and necrotic foci in the tunica albuginea of the testicles may be observed (Hall et al. 1984). Boars with exudative periorchitis have necrotic and inflammatory lesions in the serosa covering the genital organs. Spermatozoa abnormalities occur.

Aborted or stillborn piglets usually exhibit no evidence of encephalitis, but foci of necrosis may be found in the liver and other parenchymatous organs, together with focal bronchial necrosis and interstitial pneumonia. Focal necrosis of the mucosal epithelium involving the muscularis mucosa and tunica muscularis develops in the intestines (Narita et al. 1984b). Intranuclear inclusion bodies may be present in degenerative crypt epithelial cells.

The occurrence of hemorrhage and fibrin exudation results from the involvement of connective tissue and endothelium. Necrotizing vasculitis of the arterioles, venules, and lymphatic vessels around the tonsils and submaxillary lymph nodes is observed in piglets (Narita et al. 1984a). Endothelial nuclei are pyknotic and karyorrhectic, and the vessel walls are infiltrated by neutrophils. Intranuclear inclusion bodies are often present in affected endothelial cells (Kluge et al. 1999).

**Diagnosis**

**Differential Diagnosis.** Several infectious and noninfectious diseases may produce clinical signs in pigs similar to pseudorabies, including rabies, porcine polioencephalomyelitis (teschovirus infection), classical and African swine fever, Nipah virus infection, Japanese encephalitis, hemagglutinating encephalomyelitis, bacterial meningoencephalitis such as *Streptococcus suis* infection, swine influenza, salt poisoning, hypoglycemia, organic arsenic or mercury poisoning, congenital tremors, encephalomyocarditis (EMC), other diseases causing abortion, infections with highly virulent strains of PRRSV, and PCV2 infection.

In species other than the pig, PRV is rather strictly neuroinvasive (Mettenleiter 2000). In those animals, diseases of the CNS, such as rabies, scrapie (sheep), and bovine spongiform encephalopathy (BSE), and diseases or conditions causing persistent itching need to be excluded.

**Pathological Examination.** In pigs, the trigeminal ganglia, olfactory ganglia, and tonsils are the preferred tissues for isolation or detection of PRV. The virus can also be recovered from other organs, for example, lungs, spleen, liver, kidneys, lymph nodes, and pharyngeal mucosa. In latently infected pigs, virus isolation is most successful from the trigeminal ganglia in domestic pigs and the sacral ganglia in feral pigs.

PRV antigen can be detected either in cryosections by immunofluorescence or in formalin-fixed paraffin-embedded tissues by immunohistochemistry. PRV-DNA can be visualized by in situ hybridization. In nonporcine species, the segment of the spinal cord that innervates the pruritic area of the skin should be collected. The affected area of the skin, together with the subcutaneous tissues, should also be submitted.

Samples for virus isolation should be sent to the laboratory under cold conditions. *Postmortem* serum can also be collected for serology. Serological tests can also be performed on meat juice.
**Laboratory Confirmation.** Rapid detection of viral infection is essential for the effective control of PRV. Clinical observations are only sufficient to lead to a suspicion of AD because the infection produces no pathognomonic clinical signs or gross postmortem lesions in swine. Therefore, laboratory confirmation is required.

**Virus Detection.** Viral antigen can be detected using immunoperoxidase and/or immunofluorescence staining with polyclonal or mAbs on impression smears and cryosections of tissues, for example, brain, lungs, and tonsils (Allan et al. 1984; Onno et al. 1988). Diagnosis is confirmed by virus isolation in conventional cell cultures requiring ~2–5 days, depending on the time of development of virus-specific CPE.

A variety of cells can be used for the recovery of PRV, including rabbit lung (ZP), rabbit kidney (RK-13), hamster kidney (BHK-21), porcine kidney (PK-15, SK6), African green monkey kidney (VERO), mink lung (ML), ferret kidney (FK), ovine fetal lung (OFL), bovine turbinate (BT), and turkey embryo kidney (TEK) cells (Onyekaba et al. 1987). In general, a porcine kidney cell line is employed under routine laboratory conditions.

PRV can be isolated from secretions and excretions and from tissues, for example, brain, tonsils, lungs, and spleen, of infected animals. In latently infected pigs, the trigeminal ganglia and tonsils are the most consistent sites for virus isolation. As there is no CPE characteristic of PRV, and CPE may vary with the prevailing PRV strain and the cell line used, virus identity is confirmed by immunofluorescence, immunoperoxidase, or neutralization assays using specific antisera or mAbs. In the absence of any obvious CPE, blind passages should be performed. Rapid detection of PRV by shell vial technique has been described (Tahir and Goyal 1995).

Viral RNA can be detected by direct filter hybridization (Belak and Linne 1988), DNA hybridization dot-blot assay (McFarlane et al. 1986), and PCR (Jestin et al. 1990). Each of these molecular techniques has advantages and disadvantages in terms of sensitivity, specificity, ease of performance, cost, and speed (Pen-saert and Kluge 1989). Detection of PRV in secretions or organ samples using PCR is the method of choice. In general, primers should target regions of the genome conserved among PRV strains. Several conventional PCRs targeting genes encoding gB, gC, gD, or gE have been established (Müller et al. 2010; Schang and Orsorio 1993), but there is as yet no internationally agreed-upon standard. PRV-specific nested and real-time quantitative reverse transcription-polymerase chain reaction (RT-PCR) assays have been described (Tombácz et al. 2009; van Rijn et al. 2004) with the capacity to differentiate between wild-type and gene-deleted vaccine viruses (Ma et al. 2008).

**Antibody Detection.** At one time, the virus neutralization (VN) test was considered the reference standard serum antibody assay (Bitsch and Eklildsen 1982), but it has been widely replaced by enzyme-linked immunosorbent antibody assays (ELISAs). Robust and sensitive indirect or competitive ELISAs detect antibodies against the complete PRV or against distinct viral antigens (Toma 1982). The latex agglutination test (LAT; Rodgers et al. 1996) and immunoblotting are alternatives. VN and LAT are highly reliable but cannot differentiate between antibodies resulting from natural infection or vaccination. The development of ELISAs able to detect serum antibodies against gE (or gC or G) (van Oirschot et al. 1986) allowed for the differentiation of infection from vaccination and led to the “marker” or differentiating infected from vaccinated animal (DIVA) concept. These ELISAs became a key part of AD eradication programs.

**Immunity**

The major immunogens of PRV that elicit antibody-dependent and cell-mediated immunity have been identified (Mettenleiter 1996). Most are envelope glycoproteins, although the major immediate-early protein also induces an antibody response. The attachment glycoprotein gC is a major target of complement-independent neutralizing antibodies (Hampl et al. 1984; Lukacs et al. 1985) and T-cell-mediated immune responses (Ober et al. 2000; Zuckermann et al. 1990). Complement-independent neutralizing antibodies directed against gB and gD have also been identified (Hampl et al. 1984; Lukacs et al. 1985). These antibodies function by inhibiting attachment (anti-gC, anti-gD) or penetration (anti-gB) of virions. Correlated with these findings, subunit vaccines containing gB, gC, or gD, as well as DNA vaccines expressing these proteins, were able to confer at least some level of immunity against challenge virus infection (Gerds et al. 1997; Mettenleiter 1996).

Like other herpesviruses, PRV also tries to avoid cellular immune responses by immune evasion. Binding of antibodies to viral glycoproteins exposed at the surface of infected cells results in their internalization, which may hide them from the immune system (van de Walle et al. 2003). This effect is mediated by endocytosis motifs in the C-terminal intracellular domains of gD and gB (Ficinska et al. 2005). The gE–gI complex exhibits Fc receptor activity (Favoreel et al. 1997), which may also contribute to immune evasion as does binding of complement factor 3 by PRV gC (Huemer et al. 1992). Immune evasion is also effected by blocking major histocompatibility complex (MHC) class I-dependent antigen presentation by gN-mediated inhibition of transport of peptides from the cytosol to the endoplasmic reticulum by TAP (Koppers-Lalic et al. 2005).
Despite these immune evasion mechanisms, solid immunity against PRV infection can be induced by vaccination with inactivated viruses, live modified viruses, protein antigens, or DNA. In particular, modified live viruses attenuated either by passaging in vitro in cell culture or in embryonated poultry eggs have proven highly effective in decreasing clinical signs of AD (Bartha 1961), although they do not prevent infection by and subsequent latency of field virus. However, precolonization of ganglia by latent vaccine virus reduces latency of superinfecting wild-type PRV (Schang et al. 1994).

**Immune Responses.** Onset of the immune response to PRV in infected and vaccinated pigs is rapid. However, slight variations in the development of the immune response may depend on the PRV strain, the route of infection, and individual pig immune competency.

PRV-specific serum antibodies are already present when the animals show clinical signs of disease (Kretzschmar 1970). Using highly sensitive serological assays, antibodies can be detected as early as 5–7 days postinoculation, but not until 12 days postinoculation by VN. In infected animals, antibodies are almost exclusively immunoglobulin M (IgM) up to 7 days postinoculation, which persist until day 18, while in vaccinated animals, they have almost disappeared by this time (Müller et al. 2001; Rodák et al. 1987). IgG antibodies appear sooner in infected (day 7 postinoculation) than in vaccinated (day 10 postinoculation) pigs reaching higher mean titers. In contrast, IgA antibodies seem to be present only in infected pigs from day 10 postinoculation.

Similar antibody dynamics and distribution can be detected in oropharyngeal swabs, except that IgG and IgM titers are considerably lower than in the serum, whereas IgA titers in oropharyngeal swabs are higher than in the serum (Rodák et al. 1987). About 3 weeks postinoculation, IgG antibodies reach maximum levels and, in general, persist for the life of the pig. Only in exceptional cases, virus-neutralizing antibodies were reported to have disappeared after 2–3 months (Blaha 1989).

**Protective Immunity.** Immunity after infection is durable and very stable and protects against viremia and clinical disease. Even massive intracerebral inoculation can be resisted. However, sterilizing immunity is not achieved. Although a significantly higher infectious dose is required to provoke exogenous infection, those virus doses are easily shed within an infected herd (Blaha 1989). In latently infected pigs, reactivation of latent PRV due to immunosuppression or new exposure to virulent virus may result in increasing virus-neutralizing antibody titers. However, there is no evidence that reactivation of latent virus is associated with any unique immunological response (Mengeling 1991).

**Maternal Immunity.** In both domestic and nondomestic pigs, PRV-specific antibodies are transferred by immune sows to their offspring even years after infection. In general, maternally derived virus-neutralizing antibodies, mostly IgG, can be detected 14–15 weeks postpartum. Their duration is dependent on the original concentration (Iglesias and Trujano 1989; Müller et al. 2005). With 11 and 21 days in domestic pigs and wild boar, respectively, the half-life of maternal antibodies is considerably longer than known for other infections. However, anti-PRV antibodies can be detected by ELISA as long as 27 weeks postpartum, that is, twice as long as in VN assays (Müller et al. 2005; Tenhagen et al. 1995).

Maternally derived immunity prevents the transmission of PRV in newborn piglets and is able to protect against clinical disease after infection by limiting virus replication in the CNS. However, the correlation between level of maternal immunity and protection against neuroinvasion in maternally immune neonatal pigs is dependent on the PRV strain. High virus-neutralizing antibody titers protect neonatal pigs almost completely against neural invasion, whereas low titers do not (Kritas et al. 1999). Maternally derived antibodies inhibit the ability of piglets to respond to vaccination (Tielen et al. 1981; Weigel et al. 1995), but recombinant vaccinia virus vaccines expressing PRV glycoproteins are able to circumvent maternally derived antibodies and stimulate active immunity (Brockmeier et al. 1997).

**Prevention and Control**

Due to the rapid increase in AD in the 1970s, test and slaughter programs were initiated in several countries, including England and Denmark in the early 1980s. Although costly, they succeeded in eliminating AD from national pig herds, although new outbreaks did occur due to introduction of virus by trade or air. In other countries, control of disease, but not infection, was achieved by blanket vaccination with inactivated (particularly in breeding animals) and modified live virus vaccines (in finishers). Whereas vaccines were efficacious in reducing disease, they did not lead to the elimination of virus, since none of them prevented latent infection and subsequent reactivation and shedding of virulent field virus.

The first major improvement occurred in 1986 when the first recombinant DNA-derived modified live virus vaccine was licensed in the United States (Kit and Kit 1991). It carried a genetically engineered deletion of the thymidine kinase gene, a gene that is relevant for virulence. At about the same time, it was discovered that several classical AD vaccine strains, for example, the Bartha strain (Bartha 1961), carried deletions of the
gene encoding immunogenic glycoprotein gE (Mettenleiter et al. 1985) that did not impair their potency as vaccines. After the development of an ELISA to measure anti-gE antibodies in the animal (van Oirschot et al. 1986), the combination of marker vaccine and differential ELISA made it possible to discriminate between vaccinated, PRV-uninfected animals (PRV-positive but gE-negative) from wild-type PRV-infected (gE-positive) animals. Subsequently, additional nonessential glycoproteins, for example, gC or gG, were also deleted by genetic engineering and used as markers with appropriate serological assay systems. Thus, marker vaccines against AD were the first genetically modified live vaccines used on a wide scale (Quint et al. 1987). The combination of highly efficacious DIVA vaccines and accurate differential ELISAs has made eradication of AD from large areas of the world practical and feasible.

**PORCINE CYTOMEGALOVIRUS**

**Relevance**

Porcine cytomegalovirus (PCMV) infection was originally designated “inclusion body rhinitis” (sometimes abbreviated as “IBR” but not to be confused with “infectious bovine rhinotracheitis”) based on the histopathological observation of basophilic intranuclear inclusion bodies in cytomegalic cells of the nasal mucosa of pigs with rhinitis (Done 1955). Ultrastructural investigations demonstrated herpes virions in epithelial cells of the turbinate mucous glands, lachrymal and salivary glands, and the renal tubuli. The virus grew slowly in cell culture and produced cytomegaly with large intranuclear inclusions (reviewed in Yoon and Edington 2006).

Infections with PCMV are ubiquitous. PCMV exists in nearly all pig populations, but clinical disease is rare with the exception of young piglets, in which a fatal, systemic disease develops. In immunologically susceptible herds, the virus causes fetal and piglet mortality, running, rhinitis, pneumonia, and sometimes neurological signs (Yoon and Edington 2006).

**Etiology**

PCMV (SuHV-2) belongs to the subfamily Betaherpesvirinae of the family Herpesviridae but is not assigned to any genus (Table 28.1; Davison 2010). Recent studies of the polymerase (gB) and major capsid protein genes indicate that PCMV is genetically closer to human herpesviruses 6 and 7 than to cytomegaloviruses (Rupasinghe et al. 2001; Widen et al. 2001).

PCMV particles exhibit typical herpes virion morphology (Duncan et al. 1965; Valicek and Smid 1979). No distinct PCMV serotypes or genotypes have been identified, although some genetic variation was noted in polymerase and gB genes among PCMV isolates of different geographical origins (Widen et al. 2001). Possible antigenic variability has also been reported (Tajima and Kawamura 1998). The virus is sensitive to chloroform and ether. Virus infectivity is preserved at subzero temperatures (Booth et al. 1967). PCMV can be isolated in porcine pulmonary macrophages and propagated up to maximum titers of $1 \times 10^5–6$ TICD<sub>50</sub>/mL in vitro, for example, in primary pig lung (PL) cells, primary swine testicle (ST), PK-15 cell line, and porcine turbinate (PT) (Yoon and Edington 2006).

Basophilic intranuclear and, occasionally, small aci- dophilic intracytoplasmic inclusions (Figure 28.7) are present in infected cytomegalic cells (Watt et al. 1973). Since CPEs are lacking in cell culture, confirmatory immunostaining is necessary (Figure 28.8).

**Role in Public Health**

PCMV is ubiquitous in pig populations, induces latent infections in pigs, and is similar to human and primate cytomegaloviruses (Garkavenko et al. 2004; Tucker et al. 1999). Although no human infections have been reported, the potential use of live porcine cells, tissues, and organs for xenotransplantation has led to concerns regarding exposure of immunocompromised humans
pod vectors have not been reported. Natural infection with PCMV is limited to pigs. The virus does not replicate in mice, rabbits, dogs, cattle, or chicken embryos. However, Mueller et al. (2002) detected virus replication in tissues of baboons that received xenografts of porcine origin.

PCMV is transmitted horizontally via the oronasal route, but congenital transmission is well documented (Yoon and Edington 2006). Infection most commonly occurs perinatally in commercial pig holdings (Watt 1978).

PCMV can be recovered from nasal and ocular discharge, urine, and cervical fluid (Yoon and Edington 2006). The majority of pigs shed PCMV in nasal secretions between 3 and 8 weeks of age (Plowright et al. 1976). This suggests that infection is usually acquired by contact within the infected cohort. Virus recrudescence from latent infections is possible (Edington et al. 1976c; Narita et al. 1985).

The stability of PCMV in the environment is unknown. No specific disinfectants are recommended.

**Pathogenesis**

The primary replication site of PCMV is the nasal mucosa and/or the lachrymal or Harderian glands. Cell-associated viremia follows primary replication 14–21 days postinfection in animals older than 3 weeks (Edington et al. 1976c, 1977). Shedding in nasal secretions lasts from 10 to over 30 days. Congenitally infected pigs excrete virus until death (Edington et al. 1977). Excretion in cervical fluids from pregnant sows is found concomitant with fetal deaths.

The site of secondary viral replication varies with age. In nursery or growing pigs, PCMV spreads to the nasal mucosal glands, Harderian and lachrymal glands, kidney tubules, and more rarely, the epididymis and mucous glands of the esophagus. Hepatocytes and duodenal epithelium are rarely infected.

In the fetus or neonate, there is predominantly infection of capillary endothelium and sinusoids of lymphoid tissues, thus resulting in systemic spread of PCMV and generalized lesions (Edington et al. 1977, 1988). This observation is important in terms of PCMV eradication from pigs bred for xenotransplantation. That is, spleens from donor animals should be examined as part of quality control procedures (Clark et al. 2003).

**Clinical Signs**

Incubation period for PCMV may be 10–20 days (Edington et al. 1977). During viremia, animals become depressed and anorexic. Neonates may die without any clinical signs, whereas others exhibit shivering, sneezing, respiratory distress, poor weight gain, and rhinitis. Sometimes a black discoloration can be observed around the eyes due to conjunctival discharge. The disease is generally self-limiting. In pigs older than 3...
weeks, the course of disease is usually subclinical to mild, but can lead to death of the fetus or newborn pig. Affected pigs show respiratory signs, for example, sneezing, catarrhal nasal exudate and discharge, and coughing with dyspnea, and develop rhinitis or neurological disease (Yoon and Edington 2006). There is no link between PCMV infection and atrophic rhinitis (Edington et al. 1976b). Embryonic death and infertility may occur (Edington et al. 1977, 1988; L’Ecuyer et al. 1972; Yoon et al. 1996).

PCMV has been associated with porcine respiratory disease complex (PRDC) in pigs of different age (Orr et al. 1988). A significant correlation between PCMV and PCV2 infection was found, and it is speculated that the presence of PCMV causes exacerbation of PRDC (Hansen et al. 2010).

Although infection is usually subclinical in older pigs if uncomplicated, morbidity after congenital or neonatal infection is 100%. The mortality in a naive herd can be 10%, but it may increase to 50% in the presence of secondary bacterial or viral infections (Yoon and Edington 2006).

**Lesions**

Whereas epithelia are the target tissue in older pigs, generalized infection affects reticuloendothelial tissues in the fetus or neonate (Edington et al. 1976a).

Macroscopic changes are usually only seen in piglets less than 3 weeks of age with systemic PCMV infection, in which case catarrhal rhinitis, hydrothorax and hydropericardium, pulmonary and subcutaneous edema, and renal petechiation can be present. In fetal infections, stillbirths, mummification, embryonic death, and infertility are seen.

Microscopically, 8- to 12-µm basophilic intranuclear inclusion bodies, cytomegaly, and karyomegaly are seen in the nasal mucous glands (Figure 28.9), acinar and duct epithelium of Harderian and lachrymal glands, and renal tubular epithelium. The major sites of replication develop focal lymphoid hyperplasia (Figure 28.10).

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**28.9.** The basophilic intranuclear inclusion, translucent halo, and defined nuclear membrane are prominent in the enlarged superficial mucous gland epithelium of an animal 18 days after experimental intranasal inoculation (hematoxylin and eosin [H&E]; ×480).

**28.10.** The lamina propria is heavily infiltrated with lymphocytes and plasma cells 24 days after inoculation with PCMV. Many of the acini of the mucous glands still show cytomegaly and prominent inclusion bodies (hematoxylin and eosin [H&E]; ×120).
Interstitial nephritis and random focal gliosis in the CNS with inclusion bodies can be additional findings, with a predilection for the choroid plexus, cerebellum, and olfactory lobes (Yoon and Edington 2006). In the acute fatal syndrome, most basophilic inclusions are seen in the capillary endothelium and sinusoidal cells of the lymphoid tissues. Multifocal edema or hemorrhage results from vascular damage. Mononuclear cells and macrophages with inclusions are found in the blood vessels, alveoli, and spleen. Focal hepatocellular necrosis and inclusions in glomerular capillary endothelium are further lesions.

**Diagnosis**

PCMV-associated diseases must be differentiated from infection with classical swine fever virus, enterovirus, parvovirus, PRRSV, PCV2, and PRV. Virus isolation or PCR-based assays for detection of viral DNA can be used for positive identification (Fryer et al. 2001; Hamel et al. 1999; Widen et al. 1999). Antemortem samples of choice are swabs with nasal secretion or scrapings and whole blood (Edington et al. 1976a; Watt et al. 1973).

Preferred postmortem samples are turbinate mucosa, lungs, pulmonary macrophages obtained by lung lavage, and kidneys. In cases of early reproductive failure, PCMV can occasionally be demonstrated in the brain, liver, and bone marrow of fetuses (Yoon and Edington 2006).

Virus isolation is possible on primary or immortalized cells. Viral antigen can be detected by immunostaining on frozen tissue sections. The combination of inclusion bodies, cytomegaly, and karyomegaly is pathognomonic (Yoon and Edington 2006). PCMV infection in a herd is confirmed by serology using serum samples from grower–finishers. ELISAs have been described and adapted to differentiate IgG and IgM responses (Tajima et al. 1994). It should be noted that no PCMV antibodies are induced by in utero infection. Therefore, antibody is not expected in colostrum-deprived neonatal sera.

**Immunity**

After experimental infection, antibodies are detected by indirect immunofluorescent antibody (IFA) tests 2–3 weeks after inoculation. IFA-detectable antibodies peak at ~6 weeks and remain at high levels for at least 10–11 weeks (Edington et al. 1976c, 1977). Development of serum antibody levels detectable by IFA coincides with the disappearance of viremia, but nasal excretion of virus continues for another 2–3 weeks. Piglets with congenital and neonatal infections do not seroconvert but excrete virus and develop fatal, systemic infections (Edington et al. 1977). Piglets acquire maternal antibodies and these provide some protection, but virus is shed even in the presence of circulating maternal antibody in PCMV-endemic farms (Plowright et al. 1976). Maternal antibodies persist for approximately 2 months (Tajima et al. 1994).

**Prevention and Control**

No vaccine or specific treatment for PCMV is available. Introduction of new stock into herds poses a significant risk due to reactivation of latent infections or primary infection of susceptible herds.

**PORCINE LYMPHOTROPIC HERPESVIRUS**

**Relevance**

The detection by PCR of the genetic material of two porcine herpesviruses in leukocytes and lymphoid organs of healthy pigs (Sus scrofa) led to the discovery of porcine lymphotropic herpesvirus 1 (PLHV-1) and porcine lymphotropic herpesvirus 2 (PLHV-2). Sequence analysis revealed that they were the first porcine herpesviruses belonging to the subfamily Gammaherpesvirinae (Ehlers et al. 1999a). In 2003, a third porcine gammaherpesvirus, porcine lymphotropic herpesvirus 3 (PLHV-3), was identified (Chmielewicz et al. 2003a).

Little is known about the pathogenic potential of the PLHVs. However, the presence of these viruses in apparently healthy pigs (Chmielewicz et al. 2003a; Ehlers et al. 1999a; Ulrich et al. 1999) raised concerns about the safety of pig-to-human xenotransplantation. These concerns were reinforced by the high worldwide prevalence of the PLHVs, the difficulties in eliminating them from pigs bred for use as tissue and organ donors (Tucker et al. 2003), and the association of PLHV-1 with a porcine lymphoproliferative disease of high mortality (Goltz et al. 2002; Huang et al. 2001).

**Etiology**

Approaches to characterize the PLHV genomes were hampered by the fact that propagation of these viruses in cell culture was not successful. Therefore, the genomes were amplified directly from PLHV-positive pig samples by a PCR-based genome walking technique. About 101 kilobases, 68 kilobases, and 98 kilobases of PLHV-1, -2 and -3, respectively, were characterized and found to harbor more than 60 genes with counterparts in other herpesviruses (Chmielewicz et al. 2003a; Goltz et al. 2002; Lindner et al. 2007). Upon comparison of genes conserved among the Herpesviridae, the PLHVs were then classified by the International Committee on Taxonomy of Viruses (ICTV) as members of the subfamily Gammaherpesvirinae, and named suid herpesvirus 3 (PLHV-1), suid herpesvirus 4 (PLHV-2), and suid herpesvirus 5 (PLHV-3) (Davison et al. 2009). Close relatives of the PLHVs were also found in other suid species, that is, *Phacochoerus africanus* and *Sus barbatus* (Ehlers and Lowden 2004).

Phylogenetic analysis revealed that the PLHVs are most closely related to a group of ruminant gammaherpesviruses (Figure 28.11). Members of this group cause...
B-cell line L23, was found to carry PLHV-3 genomes (Chmielewicz et al. 2003a). However, attempts to induce efficient lytic replication failed (B. Ehlers, unpublished data). Attempts to isolate PLHV from primary samples were not reported. Therefore, a lytic cell culture system is not available.

Role in Public Health

The PLHVs have the potential to cause a lymphoproliferative disease of high mortality in experimentally immunosuppressed pigs that resembles the post-transplantation lymphoproliferative disease (PTLD) in humans. The PLHVs are closely related to AlHV-1 and OvHV-2, which are innocuous in their natural hosts, but cause MCF in other animals. Porcine cells, tissues, and organs offer a potential solution to the shortage of organs available for human-to-human allotransplantation (Yang and Sykes 2007), but the pathogenic potential and worldwide prevalence of the PLHVs in pigs has raised concerns regarding the consequences of pig-to-human xenotransplants in immunosuppressed humans (Ehlers et al. 1999a; Mueller and Fishman 2004).

Epidemiology

Knowledge on the epidemiology of the PLHVs is limited, but there is growing evidence that they are
present in pigs and their relatives in the family Suidae worldwide. PLHVs were detected in domestic pigs (S. scrofa domestica) from Germany, Italy, France, Belgium, Denmark, Ireland, the United Kingdom, the United States, Australia, and Vietnam, and in wild boar (S. scrofa) from Germany, the United States, and Australia (Chmieliewicz et al. 2003a, b; Ehlers et al. 1999a; Garkavenko et al. 2004; Goltz et al. 2002; McMahon et al. 2006; Tucker et al. 2003; Ulrich et al. 1999; B. Ehlers, unpublished data). PLHV-1 sequences were also amplified from S. barbatus, S. barbatus, S. celebensis, and Phacochoerus africanus. PLHV-3 sequences were identified in Babyrousa babyrussa and a sequence with 98% identity to the PLHV-3 DPOL gene in S. barbatus (Ehlers and Lowden 2004).

The prevalence of the PLHVs was determined with real-time PCR in German domestic pigs. Depending on the sample type (blood, spleen, and lungs), prevalences of 48–62% (PLHV-1), 16–41% (PLHV-2), and 54–78% (PLHV-3) were estimated. In German wild boar, PLHV-1 was detected in 1 of 19 and PLHV-2 in 18 of 19 bone marrow samples (Ulrich et al. 1999). Spleens of domestic pigs from Ireland were 74%, 21%, and 45% positive for PLHV-1, PLHV-2, or PLHV-3, respectively (McMahon et al. 2006). In New Zealand, 95% of domestic piglets (20 weeks) and pigs (>6 months) were PCR-positive for PLHV-2. PLHV-1 was not detected and PLHV-3 was not tested (Garkavenko et al. 2004). In the United Kingdom, PLHV sequences were amplified in 80% and 67% of adult Large White pigs and adult miniature swine, respectively (Tucker et al. 2003). These data indicated a high PLHV prevalence in commercial and experimental pig herds. They further revealed a remarkably low level of intraspecies sequence variation (well below 1%) in coding regions. This indicated that the PLHVs are genetically stable and well adapted to their natural host.

Very few data allow conclusions about the mode of PLHV transmission. Cesarean-derived pigs were found to be far less frequently infected with PLHVs than conventionally reared pigs. This reduced rate may indicate that PLHVs are rarely transmitted in utero, but frequently postpartum (Tucker et al. 2003). Further evidence for horizontal transmission as the major route emerged by repeated analysis of piglets after birth using ELISA and PCR. These data indicated de novo PLHV infection by contact to the infected dam and subsequent seroconversion (Figure 28.12; Brema et al. 2008).

Pathogenesis, Clinical Signs, and Lesions
A clinical disease associated with PLHV infection under field conditions is not known. The closely related

![Figure 28.12](https://example.com/image.png)

**28.12. Analysis of PLHV antibody titers in piglets of different ages.** Sera were obtained from newborn pigs up to 5 months of age and split into nine age groups (days 0–7, 8–14, 15–21, 22–30, 31–60, 61–90, 91–120, 121–150, and 151–156). All sera were examined for anti-PLHV antibodies by ELISA. The number of samples (n) in each group is shown below each box plot. Outliers (circles) and extreme values (stars) are also shown. ELISA was performed using 1.25 µg antigen (N-terminal part of PLHV-I glycoprotein B) per well and 1:50 diluted swine sera. In addition, peripheral blood leukocyte samples were analyzed for PLHV-1 DNA by PCR. The percentages of PCR-positive samples are listed in the box at the bottom of the figure (from Brema et al. 2008, reprinted by permission of the publisher).
gammaherpesviruses naturally infecting wildebeest (AlHV-1) and sheep (OvHV-2) can be horizontally transmitted to cattle or pigs and cause MCF, a lymphoproliferative disease of high mortality (Russell et al. 2009). Therefore, the PLHVs might have a similar pathogenic potential, either in foreign hosts or in their natural host. The latter may be difficult to observe since most commercial pigs are slaughtered at approximately 6 months of age.

Evidence for the pathogenicity of the PLHVs under experimental conditions came from immunosuppressed miniature swine subjected to allogeneic hematopoietic stem cell transplantation. A high incidence of PTLD was observed. B-cell proliferation occurred, and the majority of the animals died (Huang et al. 2001). In the PTLD-affected individuals, high PLHV-1 genome copy numbers were detected. In addition, transcripts of several PLHV-1 genes were demonstrated in PTLD pigs, but not in healthy pigs, strongly suggesting a causative role of PLHV-1 in PTLD (Goltz et al. 2002; Huang et al. 2001). Similar findings were reported after allogeneic spleen transplantation in miniature swine (Dor et al. 2004).

The clinical signs of experimental porcine PTLD resemble those of human PTLD associated with EBV, a human gammaherpesvirus. Lethargy, fever, anorexia, enlarged lymph nodes, and elevated numbers of leukocytes were observed. Pigs suffering from PTLD showed enlarged lymphoreticular organs, airway obstruction, and respiratory failure. Microscopically, a mixture of immunoblasts, plasmacytoid cells, and plasma cells were typical of porcine PTLD (Huang et al. 2001).

Pigs have the potential to be used as cell, tissue, and organ donors in pig-to-human xenotransplantation. The theoretical concern is that PLHVs might recombine with human herpesviruses in the human xenotransplant recipients, giving rise to recombinants with novel pathogenic properties. In addition, activation of human herpesviruses through PLHVs might occur in xenotransplant patients leading to lytic infection and rejection of the transplanted organ (Santoni et al. 2006).

Diagnosis

Nucleic acid-based and antibody-based diagnostic assays are available for PLHV diagnosis. Three independent real-time PCR assays have been developed to quantify PLHV-1, PLHV-2, or PLHV-3 genome copy numbers in porcine samples (Chmielewicz et al. 2003a). Single-round conventional PCR assays for the specific detection of a certain PLHV or the simultaneous detection of PLHV-1 and -2 have also been published (Chmielewicz et al. 2003a; Ehlers et al. 1999a). The very low level of PLHV intraspecies variation (<1% in coding regions) ensures the conservation of the primer binding sites used in the PCR and real-time PCR assays.

In addition, a panherpes consensus PCR has been designed for the universal detection of all mammalian and avian herpesviruses with degenerate primers (Ehlers et al. 1999b). This assay readily amplifies PLHV DNA polymerase sequences (Chmielewicz et al. 2003a; Ehlers et al. 1999a). However, many porcine specimens are infected with more than one PLHV. Due to the degenerate nature and universal binding properties of the consensus primers, sequences of only one virus are preferentially amplified from a mixture. Therefore, the simultaneous diagnosis of multiple PLHV infections in a single specimen is usually not successful using the panherpes consensus PCR.

With the PCR assays described above, the PLHVs were frequently detected in blood leukocytes, in lymphoreticular organs (spleen, lymph nodes, tonsils, bone marrow), and in the lungs (Chmielewicz et al. 2003a; Ehlers et al. 1999a; Ulrich et al. 1999). Since organ samples are not readily available from live pigs, routine PLHV diagnosis relies on testing white blood cells. This has to be taken with caution since PLHV are more frequently detected in the spleen and lungs than in the blood (Chmielewicz et al. 2003a).

Only one serological assay has been published, an ELISA for the detection of anti-PLHV antibodies. The assay was used for the analysis of sera from groups of pigs, differing by age and origin. Seropositivity ranged from 38% (piglets) to 90% (gilts) and 100% (breeding sows, miniature pigs, and pigs for slaughter). Compared with the percentages of PCR-positive samples in the same groups of sera (20%, 80%, and 0–75%, respectively), this ELISA is suitable for PLHV diagnosis (Brema et al. 2008).

Immunity

The type and efficacy of immune responses against the PLHVs are largely unknown. Test systems relying on viral cell culture are not available, and infection studies with cultured viruses are not possible at present. An ELISA for the detection of anti-PLHV antibodies in porcine sera has been developed, using recombinant gB of PLHV-1 as antigen. A group of 12 piglets was tested repeatedly after birth until the age of 156 days for the presence of anti-PLHV antibodies. At birth, antibodies of probable maternal origin were detected, which declined to background levels during the first 3 weeks of life. Thereafter, seroconversion due to de novo PLHV infection by contact with the infected dam (or already infected piglets) was observed (Brema et al. 2008) (Figure 28.12).

In the blood, the PLHVs infect predominantly B cells. This was assessed with real-time PCR analysis of microbead-sorted B cells, T cells, and macrophages (Chmielewicz et al. 2003b). In immunosuppressed miniature swine developing PTLD, a rise of PLHV-1 genome copies and a concomitant expansion of the B cells were observed (Huang et al. 2001). In addition, transcripts of an ORF were found in the PTLD-affected pigs, which encodes a protein for B-cell entry (Goltz et al. 2002).
Prevention and Control
Concerns regarding the safety of pig-to-human xenotransplantation make PLHV-free donor pigs a desirable goal. Early weaning of piglets failed to exclude PLHV (Mueller et al. 2005), but a markedly reduced prevalence of PLHVs was achieved using cesarean-derived, barrier-reared breeding conditions, (Tucker et al. 2003). These results indicated that the derivation of PLHV-free animals is a long-term, but realistic objective.

Ovine Herpesvirus 2 Causing Porcine Malignant Catarrhal Fever
Relevance. MCF is a sporadic, systemic gammaherpesvirus infection of ungulates. It was first described as a fatal disease, primarily of cattle, characterized by high fever, copious nasal discharge, corneal opacity, generalized lymphadenopathy with lymphopenia, inflammation, necrosis of mucosal surfaces, and vasculitis. In Europe, an association with sheep was recognized as a prerequisite (Götze and Liess 1930), whereas in Africa, the source of infection was wildebeests (Connochaetes taurinus), which are inapparent carriers (Plowright et al. 1960). Later, the sheep-associated (SA) form has been reported worldwide in a wide variety of species belonging to the subfamily Bovinae and the family Cervidae (Hüssy et al. 2000; Müller-Doblies 1998; Plowright 1990).

A naturally occurring disease similar to MCF has been described in pigs, particularly in Scandinavian countries (Løken et al. 1998). However, the first cases of MCF in pigs were reported from Italy and Germany, where single sows developed clinical signs resembling the disease in cattle (Kurtze 1950; Morcelli 1901). For decades, overt disease in swine seemed to be limited to Norway but has since occurred in Finland, Sweden, and North America (Alcaraz et al. 2009; Bratberg 1980; Gauger et al. 2010; Gyrting 1974; Holmgren et al. 1983; Løken et al. 2009; Okkenhaug and Kjelvik 1995; Syrjälä et al. 2006). Other cases of porcine MCF were also reported from Switzerland (Pohlzen et al. 1974). Although the etiology had not been clarified, in most reports, contact with sheep was mentioned before the disease was noted (Albini et al. 2003a).

Porcine MCF is a very rare, poorly documented disease of swine (Alcaraz et al. 2009). The small number of reported cases of MCF is mainly based on the fact that the clinical signs can be subtle and nonspecific and diagnostic tools are not generally available (Albini et al. 2003a). Compared with other herpesviral diseases of pigs, porcine MCF does not pose a significant threat to the porcine population, even if undiagnosed in the field.

Etiology
There are two etiologically distinct forms of MCF: (1) a wildebeest-associated form (WA-MCF), caused by AlHV-1, and (2) a sheep-associated form (SA-MCF), occurring worldwide and caused by OvHV-2 (Baxter et al. 1993; Meier-Trummer et al. 2010). Based on their molecular biology, both belong to the genus Macavirus within the subfamily Gammaherpesvirinae and are closely related to PLHV1–3 (Figure 28.11). In contrast to AIHV-1, there is no permissive cell culture system for OvHV-2.

Public Health
OvHV-2 infection in humans has not been reported.

Epidemiology
Knowledge about porcine MCF caused by OvHV-2 is sparse, although a few cases with proven etiology were recently reported from European countries and North America (Albini et al. 2003a,b; Alcaraz et al. 2009). This may reflect the limited availability of molecular diagnostic assays and the possibility that a considerable number of cases may not be recognized. However, it cannot be excluded that the low frequency of MCF cases in pigs is due to the low susceptibility of pigs.

In general, gammaherpesviruses have a narrow host range (Ackermann 2005, 2006; Meier-Trummer et al. 2010). However, OvHV-2 affects a broader range of natural hosts such as sheep, goats, cattle, bison, swine, mule deer, and, at least experimentally, rabbits and hamsters (Ackermann 2006; Albini et al. 2003b; Jacobsen et al. 2007; Li et al. 2003; Løken et al. 1998; O’Toole et al. 2007). Sheep and goats remain healthy upon infection, whereas the other susceptible hosts develop MCF (Meier-Trummer et al. 2010).

The exact mode of transmission of OvHV-2 is uncertain, but there is convincing evidence that the predominant mode is via nasal secretions (Li et al. 2004; Løken et al. 2009) by contact or aerosol, mainly from lambs under 1 year old (Russell et al. 2009). However, descriptions of outbreaks of SA-MCF in cattle on farms with no contact with sheep have also been documented (Kersting 1985). Pigs and sheep usually do not share close environments, which might also explain the infrequent presentation of MCF in pigs (Alcaraz et al. 2009).

MCF-susceptible species are thought to be dead-end hosts that do not transmit virus, thus limiting the spread of disease during outbreaks (Russell et al. 2009). OvHV-2 transmission by the respiratory route of sheep had strongly been postulated on the basis of the detection of infectivity in nasal secretions (Taus et al. 2005).

Pathogenesis
OvHV-2 DNA can be detected in tissues of pigs with MCF, but not in healthy pigs. Simultaneous infections with OvHV-2 and other porcine gammaherpesviruses have never been observed (Albini et al. 2003a,b).

MCF in ruminants has an autoimmune-like pathology, mainly caused by the cytotoxic action of noninfected lymphocytes under the regulatory influence of a
small number of infected cells. Cerebral vasculitis and perivasculitis in MCF-affected cattle and bison contain CD8⁺ OvHV-2-infected lymphocytes in large numbers, as shown by in situ PCR. Therefore, the pathogenesis of MCF may be due to the direct action of virus-infected, dysregulated cytotoxic T cells at sites of lesions (Russell et al. 2009). Whether this model also applies for pigs is not yet known.

Clinical Signs

The clinical signs and lesions in pigs are similar to those described for MCF in cattle. Usually, pigs of any age over 3 months can be affected (Løken et al. 1998).

No reliable data are available concerning the incubation period in pigs. Cattle can show clinical signs as soon as 9 days after exposure to sheep, but some cases occur 70 days or longer after contact. In bison exposed to sheep, the incubation period is often a month or more.

Clinically, porcine MCF is associated with a high and persistent fever (40.5–42°C; 105–107.6°F), anorexia, depression, weight loss, recumbency, a high pulse rate, and expiration dyspnea with loud snoring stenotic sounds. Strained respiration is caused by foul-smelling nasal discharge and partially dried crusts in and around the nostrils, obstructing the upper airways. Ocular discharge, bilateral corneal edema, and keratoconjunctivitis often develop. Signs of CNS disease may appear, mostly as ataxia (central vestibular), hyperesthesia, tremors, and balance loss with convulsions (Alcaraz et al. 2009). In the cases reported from Norway, diarrhea has been occasionally seen, but all four Swiss cases had diarrhea (Løken et al. 1998). Multiple to coalescent small, slightly elevated red foci are seen in the skin. Pregnant sows may abort before death occurs (Albini et al. 2003b).

Hematological changes can include lymphopenia and a moderate neutrophilia. There can be biochemical abnormalities such as increases in urea, total bilirubin, and creatinine (Løken et al. 1998).

Incubation period and severity of clinical signs vary with the virus, the host, and other factors that are not completely understood. Evidence suggests that subclinical or clinical courses are possible, depending on whether the animals were stressed by handling. The clinical course of the disease is usually short, with death generally occurring after 2–4 days (Løken et al. 1998).

Lesions

Macroscopic lesions can be scant and not typical. Usually, pigs with MCF are in good body condition. They have cyanotic areas or petechiation in their skin. The hyperemic skin can be covered by fine crusts. The lymph nodes are moderately enlarged, hyperemic, and moist on cut surfaces. The mucosa of the respiratory tract is characterized by hyperemia and covered by mucopurulent exudate. The lungs are congested and edematous; catarrhal or suppurative bronchitis and bronchopneumonia may develop. The spleen and liver can be engorged. No consistent gross lesions are reported for the alimentary tract. Kidneys are occasionally swollen and pale, and multiple cortical grayish-red foci up to 5 mm in diameter can be present. The CNS appears normal upon gross examination, although hyperemic meninges may be seen. Corneal opacity, conjunctivitis, and acral cyanosis are observed at necropsy (Albini et al. 2003b; Løken et al. 1998).

The most consistent histological finding, and the hallmark of MCF in pigs, is acute vasculitis in the CNS and other organs. This is characterized by the presence of numerous adventitial and transmural mononuclear cells and focal and segmental fibrinoid necrosis of the vessel walls in many of the tissues, including myocardium, spleen, CNS, skin, and kidneys. The lymphoproliferative vasculitis and associated degeneration and necrosis are most prominent in the media and adventitia of medium-sized and small arteries (panarteritis).

In the skin, perivascular and intravascular accumulations of mononuclear inflammatory cells occur in the dermis associated with subepithelial edema and focal epidermal necrosis. Lymph nodes show hyperplasia of lymphoid cells within the paracortex with scattered areas of necrosis. The alveolar septa of the lungs are thickened with increased numbers of lymphocytes and plasma cells. Perilobular and periportal accumulations of lymphoid cells are found in the liver. Multifocal, lymphoplasmacytic interstitial nephritis, with necrotizing arteritis and periarteritis involving blastic lymphoid infiltrates, occurs in the kidney (Figure 28.13).

Cerebrum, cerebellum, and meninges show mild-to-moderate perivascular cuffs and transmural infiltra-

28.13. Kidney; large blood vessel with vasculitis and transmural fibrinoid necrosis (hematoxylin and eosin [H&E]; with permission from Alcaraz et al. 2009).
tions of mononuclear cells, including some histiocytes and very few neutrophils with minimal karyorrhectic debris and plasma exudation into Virchow–Robin spaces. Ocular lesions consist of corneal edema and lymphoplasmacytic conjunctivitis. The uvea and retina are heavily infiltrated by lymphocytes, especially prominent around the blood vessels and in vessel walls (Løken et al. 1998). Lymphocytic optic neuritis can also be present. Similar moderate to abundant lymphoid infiltrates might be present in the zona glomerulosa of the adrenal cortex, and the intestinal submucosa (Alcaraz et al. 2009). Rarely, intranuclear inclusion bodies can be found.

Diagnosis
Differential diagnoses include AD, classical swine fever, African swine fever, porcine enterovirus infection, PCV2 infection, and rabies. Since lesions found at necropsy are not sufficiently specific for an etiological diagnosis, histopathology including molecular biological tests is necessary to confirm MCF. As in cattle, panarteritis is the most significant histological lesion. Consistent changes are seen in the brain. In particular, it is beneficial to microscopically investigate the epidural carotid rete mirabile around the pituitary gland for characteristic vasculitis, as in cattle. A combination of arteritis, lymphoid hyperplasia, and multifocal intraepithelial lymphocytic infiltrates is very characteristic for MCF and is essentially consistent with the classical head-and-eye form of MCF seen in ruminants (Løken et al. 1998).

Diagnosis of MCF depends on a combination of clinical signs, histopathology, and detection of virus-specific antibodies in blood or DNA in peripheral blood leukocytes or lymphoid tissue samples (Baxter et al. 1993). The OIE recognizes histopathology as the definitive diagnostic test for cattle, but laboratories have adopted other approaches, such as PCR assays that detect OvHV-2-DNA sequences. Using a mAb (15A) specific for a conserved antigen, a competitive inhibition (CI)-ELISA test has been developed and refined (Li et al. 1994, 2001). A direct ELISA has been developed recently that offers a simple and inexpensive alternative. Conventional and real-time (quantitative) PCR assays allow sensitive confirmation of the presence of OvHV-2 in infected pigs and may also be useful for phylogenetic and epidemiological studies in natural and MCF-susceptible hosts (Albini et al. 2003a; Baxter et al. 1993; Hüssy et al. 2001; Russell et al. 2009).

Immunity
Prophylactic immunization is not available.

Prevention and Control
Despite the low rate of porcine MCF in Europe and North America, it is important to discourage contact between pigs and sheep to minimize any infections.

ACKNOWLEDGMENT
We thank Viola Damrau for helping in the preparation of this chapter.

REFERENCES
RELEVANCE

Porcine parvovirus (PPV) was first isolated in 1965 by Anton Mayr and coworkers in Munich, Germany, as a contaminant of a porcine primary cell line used for the propagation of classical swine fever virus (Mahnel 1965, cited after Mayr et al. 1968; Siegl 1976). It took several years to link this virus to endemic reproductive disorders and to show that PPVs were distributed worldwide (Cartwright and Huck 1967; Joo et al. 1976a; Mengeling and Cutlip 1976).

Infection with PPV causes reproductive losses in swine characterized by stillbirths, mummification, embryonic death, and infertility (SMEDI). The affected sow does not typically show clinical signs, and virus transmission to the fetuses only occurs if she is seronegative. PPV is probably the most important cause of reproductive failure in pigs worldwide.

Novel parvoviruses recently identified in swine include porcine parvovirus 2, porcine hokovirus, and porcine parvovirus 4. DNA analyses confirmed their membership in the family Paroviridae, but they are either phylogenetically distinct within the genus Parovirus or are members of the genus Bocavirus (Blomström et al. 2009; Cheung et al. 2010; Hijiikata et al. 2001; Lau et al. 2008). The role of these viruses in swine health has not been determined.

ETIOLOGY

PPV is a member of the genus Parovirus within the subfamily Parovirinae of the family Paroviridae. It is a true autonomous parvovirus, as it does not need helper viruses for replication. Like the closely related parvoviruses of carnivores, canine parvovirus and feline panleukopenia virus, the PPV virion is about 28 nm in diameter and consists of 60 copies of the structural protein VP1/VP2, about 90% of which are VP2 and 10% VP1 molecules. The capsid structure is characterized by a simple T = 1 icosahedral symmetry (Simpson et al. 2002).

The PPV genome is a single-stranded DNA molecule of about 5000 bases. Like all parvoviruses, complex palindromic hairpin structures located at each terminus are required for DNA replication. The genome encodes four proteins transcribed from two promoters. “Alternative splicing” extends the coding capacity of the small genome. Two nonstructural proteins, NS1 and NS2, function in the replication of the virus, particularly for DNA replication. Two structural proteins (VP1 and VP2) are transcribed and translated from the parovirus genome. The smaller protein (VP2) is produced by splicing from the same RNA template as the larger protein (VP1). Thus, the entire VP2 sequence is present in the VP1 sequence, but the latter has a unique amino terminus of about 120 amino acids (see Cotmore and Tattersall 2006 for a review of parvovirus genome organization and gene expression). Some molecules are posttranslationally trimmed by proteases to create the minor protein VP3.

Sequence analyses of recent isolates suggested active evolution of PPV. Specifically, sequence alignments and phylogenetic studies of the capsid protein gene (VP1) revealed a new cluster of viruses characterized by specific nucleotide and amino acid changes (Zimmermann et al. 2006) (Figure 29.1). Preliminary data indicated that these “new” viruses were spreading through European pig populations and perhaps worldwide. The appearance of “new” viruses could be important because changes in the capsid protein influence the
antigenic properties of the virus. There is only one PPV serotype and all isolates show a high degree of cross-reactivity in various serological tests, for example, virus neutralization or hemagglutination inhibition (HI) assays. However, differences in cross neutralization in the “new” viruses have been demonstrated using sera raised against “classic” PPVs (Zeeuw et al. 2007). Whether the “new” virus type is a new antigenic type or a new genotype will require further study.

PPV agglutinates erythrocytes of a variety of animal species, including rat, monkey, chicken, guinea pig, and human (blood group 0) (Siegl 1976). The virus can be cultured in several porcine cell lines (PK-15, SPEV, swine testicle cells, and others), causing a marked cytopathic effect.

ROLE IN PUBLIC HEALTH
There is no evidence that PPV is infectious for humans or plays any role in public health.

EPIDEMIOLOGY
PPV is endemic in most of the world. The virus readily replicates in susceptible pigs, although clinical signs (reproductive losses) only occur in pregnant females. The virus is shed in feces and other secretions from acutely infected pigs, and the epidemiology of PPV is shaped by the capacity of the virus to resist inactivation in the environment. That is, PPV can remain infectious for months in the environment and contaminated pens or facilities and may therefore be a constant source of new infections.

The virus can be transported between herds via fomites, for example, the clothes, boots, and equipment. Likewise, rodents functioning as mechanical vectors have reportedly introduced the virus into herds. The virus can also be introduced into populations by infected boars. Whether PPV is shed in the semen of infected boars or whether PPV in semen represents environmental contamination is unresolved. Regardless, there are numerous reports on PPV in the semen of naturally infected boars (Cartwright and Huck 1967; Ruckerbauer et al. 1978).

PPV is resistant to inactivation by ethanol (70%) and quaternary ammonium (0.05%), as well as low concentrations of sodium hypochlorite (2500ppm) and peracetic acid (0.2%), but is readily inactivated by aldehyde-based disinfectants and higher concentrations of sodium hypochlorite (25,000ppm) and hydro-
gen peroxide (7.5%). The virus is relatively heat stable and may resist dry (but not moist) heat at 90°C (194°F) (Eterpi et al. 2009).

The introduction of PPV into a herd does not cause immediate problems if a sufficient proportion of the sows are immune through vaccination or natural exposure. However, the virus is able to replicate even in vaccinated pigs (Jóźwik et al. 2009). This was demonstrated by a sharp rise in antibody titers in vaccinated sows. Thus, virus circulation within a population cannot be completely prevented by vaccination.

### PATHOGENESIS

The pathogenesis of PPV reflects the ability of the virus to infect the fetus (Mengeling et al. 2000). However, it is not clear how PPV actually crosses the porcine transplacental barrier. As in other viruses, PPV could reach the fetus in one of three ways: in body fluids, such as blood or lymph; by progressive replication through continuous placental cell layers; or in cells, such as macrophages or lymphocytes (Mengeling et al. 2000).

After primary replication in lymphoid tissues, PPV is distributed systemically via cell-free viremia (Brown et al. 1980; Paul et al. 1980). However, the swine epitheliochorial placenta is composed of six tissue layers that completely separate maternal from fetal blood circulation, and placental cells are so closely connected that they do not allow the passage of even small molecules, for example, antibodies. Placental cells are not susceptible to PPV infection, and PPV has not been demonstrated in placental tissues, so it is not likely that the virus can cross the barrier through progressive replication (Mengeling et al. 1978). Therefore, it is most probable that the virus reaches the fetus via immune cells. Studies have reported both the presence of the virus in lymphoid tissues from pigs (Lucas et al. 1974; Mengeling et al. 2000) and fetal lymphocytes in the circulatory system of pregnant sows (Rudek and Kwiatkowska 1983). Virus replication in macrophages has not been observed, but phagocytized PPV remains infectious for an extended time (Paul et al. 1979).

Once in the fetus, PPV encounters an environment conducive to virus replication because of the high mitotic index of most tissues of the developing fetus. The virus can be detected in many tissues and organs, suggesting that there is no specific tissue tropism (Wilhelm et al. 2005).

PPV enters cells through a series of interactions that culminate in the release of viral genetic material into a cell compartment in which replication can occur (Harbison et al. 2008). The entry mechanisms of PPV are unclear, but include clathrin-mediated endocytosis, or macroendocytosis, followed by transportation through the endosomal pathway (Boisvert et al. 2010). Endosomal trafficking and acidification are essential for PPV to enter in the nucleus (Boisvert et al. 2010). Endosome acidification results in reversible modifications of the virus capsid that allow the virus to escape from the endosome (Farr et al. 2005; Vihinen-Ranta et al. 2002). The phospholipase A2 motif (PLA2) must be externalized from the capsid. This motif activity is essential for breaking the vesicular membranes, resulting in the formation of pores (Girod et al. 2002). After the virus arrives in the nucleus, PPV replicates using the cell’s own replication mechanism. The virus replicates in cells in replication phase (S) using the cellular DNA polymerase for DNA replication. This explains the requirement for cells with a higher replication index (Rhode 1973).

### Factors Affecting the Severity of Disease

Several PPV biotypes with marked differences in pathogenicity are recognized (Choi et al. 1987; Kresse et al. 1985; Mengeling and Cutlip 1975; Mengeling et al. 1984). Some PPVs are completely nonpathogenic and do not cause disease even if experimentally inoculated into a fetus; others can cause disease even in immunocompetent fetuses, that is, after day 70 of gestation (see Table 29.1).

The genetic basis of pathogenicity has not been resolved, but the structural protein VP1 appears to play a major role. Presumably, pathogenicity or virulence is determined (at least in part) by tissue tropism (Bergeron et al. 1996). In vitro studies using recombinant viruses derived from pathogenic (Kresse) and nonpathogenic (NADL-2) PPVs identified “allotropic determinants” and showed that single amino acids in the capsid protein affected an isolate’s capacity to replicate in certain cell lines. Comparisons between Kresse and NADL-2 genomes showed that the noncoding regions

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<th>Virus Isolate</th>
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<th>Fetal Death After Intrauterine Inoculation</th>
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been described (Brown et al. 1980; Dea et al. 1985; Duhamel et al. 1991). These reports represent rare findings in which the etiological role of the virus remains to be fully established.

Even under experimental conditions, gilts and boars infected with PPV remain clinically healthy, except for reproductive losses in seronegative gilts or sows (Mengeling and Cutlip 1976; Mengeling and Paul 1981; Thacker et al. 1987; Zeeuw et al. 2007). A moderate and transient lymphopenia may be observed 5–10 days postinoculation, regardless of gender or age (Joo et al. 1976a; Mengeling and Cutlip 1976; Zeeuw et al. 2007). The early phases of infection and the incubation period are not well defined. Apparently, the virus first replicates in the tonsils and oral/nasal cavities. After 1–3 days, the virus reaches the lymphatic system and causes a cell-free viremia. Transplacental transmission and subsequent embryo/fetal infection occur around 15 days after inoculation of susceptible gestating females with PPV (Brown et al. 1980; Mengeling et al. 1978; Paul et al. 1980).

Reproductive clinical signs correlate to the stage of gestation at which infection occurs (Figure 29.2). At the beginning of gestation, the conceptus is protected by the zona pellucida and is not susceptible to infection. Thereafter and until approximately day 35 of gestation, PPV infection results in embryonic death and maternal resorption of fetal tissues. About gestation day 35, fetal organogenesis is essentially complete and ossification of the fetal skeleton begins. PPV infection after this time typically results in fetal death followed by mummification. At or about day 70 of gestation, the fetus is able to mount an effective immune response and eliminate the virus. After day 70, fetal infection is subclinical, and the piglet is born with anti-PPV antibodies (Bachmann et al. 1975; Joo et al. 1977; Lenghaus et al. 1978; Mengeling et al. 2000).

Reproductive clinical signs correlate to the stage of gestation at which infection occurs (Figure 29.2). CLINICAL SIGNS

Maternal reproductive failure is the major and only well-established clinical sign of PPV infection. Reproductive losses are typically low in vaccinated herds, but PPV can cause devastating abortion storms in unvaccinated herds or in situations in which the vaccine was administered incorrectly. Diarrhea and skin lesions, with PPV and PPV-like structures in diarrheic feces and isolation of PPV from “vesicle-like” skin lesions, have been described (Brown et al. 1980; Dea et al. 1985; Duhamel et al. 1991). These reports represent rare findings in which the etiological role of the virus remains to be fully established.

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The pathogenicity of PPV is also influenced by the presence of other viruses. In particular, the recognition of low-level PPV contamination in the inoculum used in experiments that reproduced postweaning multisystemic wasting syndrome (PMWS) in gnotobiotic pigs (Ellis et al. 1999) led to the recognition that PPV in combination with porcine circovirus type 2 (PCV2) can increase the severity of the PMWS lesions (Kennedy et al. 2000). However, PPV coinfection is not a necessary requirement for the development of PMWS (Ellis et al. 2004).

CLINICAL SIGNS

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PPV is widespread among swine, but the frequency of reproductive losses is difficult to estimate because evidence of infection may appear weeks after infection, that is, an increase in the return to estrus index or the observation of affected litters. Likewise, diagnostic tests on fetal tissues often produce false-negative results,
possibly due to the autolyzed state of fetal tissues and the high antibody titers in the sows.

The use of serum antibody titers to detect PPV infection is complicated by the common use of inactivated vaccines. However, serological surveys in various regions of the world showed that PPV antibodies were present in 70–100% of the herds (Foni and Gualandi 1989; Nash 1990; Oravainen et al. 2005; Robinson et al. 1985). PPV titers ≥512 (a typical postvaccination titer) were demonstrated in approximately 40% of animals from vaccinated herds (Oravainen et al. 2005).

**LESIONS**

Experimental PPV inoculation in boars, gilts, and sows does not produce gross lesions (Bachmann et al. 1975; Lenghaus et al. 1978; Mengeling and Cutlip 1976; Thacker et al. 1987). Embryonic death followed by resorption of fluids and soft tissues is the most common sequel to PPV infection. Gross lesions in fetuses include a variable degree of stunting before other external changes are evident. Occasionally, blood vessels on the body surface become prominent due to congestion and leakage of blood into connective tissues. Congestion, edema, hemorrhage with accumulation of serosanguinous fluids in body cavities, and hemorrhagic discoloration, which becomes progressively darker after death and dehydration (mummification), are typical of PPV infection (Figure 29.3). The placenta can be dehydrated and brown to gray in color and the extra-fetal fluid volume reduced (Joo et al. 1977; Lenghaus et al. 1978).

After fetuses become immunocompetent, no macroscopic changes are observed postinfection (Bachmann et al. 1975).

PPV has also been associated with cutaneous lesions in piglets. Kresse et al. (1985) associated PPV with an epidemic disease in piglets characterized by slit-like erosions and vesicle-like lesions involving the oral cavity and snout. Whitaker et al. (1990) associated PPV with a necrotic and exudative dermatitis in piglets. However, experimental cutaneous inoculation of this virus into piglets produced no lesions, which led to the conclusion that PPV may only predispose piglets to secondary skin disease (Lager and Mengeling 1994).

Microscopic lesions have been observed in tissues of gilts necropsied after their fetuses were infected by transuterine inoculation of the virus. Seronegative gilts infected at 70 days of gestation and necropsied at 12 and 21 days postinoculation had focal accumulation of mononuclear cells adjacent to the endometrium and in deeper layers of the lamina propria. There was also marked perivascular cuffing of plasma cells and lymphocytes in the brains, spinal cord, and choroid of the eye (Hogg et al. 1977). When fetuses were inoculated at time points earlier in gestation (35, 50, and 60 days) and the dams necropsied 7 and 11 days later, the lesions were similar. However, at that time uterine lesions were more severe and included extensive cuffing of mononuclear cells around myometrial and endometrial vessels (Lenghaus et al. 1978). Only focal accumulations of lymphocytes were detected in the uteri of gilts that were seropositive when their fetuses were inoculated (Cutlip and Mengeling 1975).

**Figure 29.3.** Litters of inoculated pregnant sows on day 90 of gestation displaying distinct levels of lesions. The sows were exposed to different PPV isolates (27a and NADL-2) on day 40 of gestation. The fetuses of each litter are placed according to their position in the uterus with the most cervical-positioned fetuses at the top (Zeeuw et al. 2007).
Consider PPV when reproductive consequences compatible with PPV infection are observed, for example, an increase in the return to estrus index or delays in parturition with increased numbers of mummified fetuses and smaller litters, especially in first or second parity females. A mix of normal pigs and mummified fetuses that died at different development stages in the same litter is a strong indication of PPV infection. PPV infection does not normally cause abortions and does not cause clinical signs in adults (Mengeling 1978; Mengeling and Cutlip 1975). Given this clinical picture, the differential diagnosis should also include pseudo-rabies (Aujeszky’s disease), brucellosis, leptospirosis, porcine reproductive and respiratory syndrome (PRRS), toxoplasmosis, nonspecific bacterial uterine infection, and others.

Laboratory submissions for the confirmation of PPV infection should include mummified fetuses and fetal remains. Detection of viral antigen in fetal tissues by immunofluorescence (IF) is a reliable procedure for the diagnosis of PPV (Mengeling 1978; Mengeling and Cutlip 1975). Alternatively, paired serum samples from gilts and sows can be used to document PPV infection. However, serum should be collected at the time of reproductive failure and a second sample 2–4 weeks later. Serum or fluids from fetuses, stillborn...
situ hybridization (Waldvogel et al. 1995), are sensitive techniques for the detection of virus in clinical samples. For routine diagnostics, polymerase chain reaction (PCR) is the most useful technique for the detection of PPV in fetal tissues, semen, and other samples. Numerous PCR protocols have been described (Chen et al. 2009; Gradil et al. 1994; Miao et al. 2009; Molitor et al. 1991; Prikhod’ko et al. 2003; Soares et al. 1999; Wilhelm et al. 2006), including multiplex PCRs (Cao et al. 2005; Huang et al. 2004; Kim and Chae 2003), especially for the concurrent detection of PPV and PCV2. These methods are considered to possess higher diagnostic sensitivity and specificity than hemagglutination and are better suited for the detection of PPV in autolyzed tissues.

Serology may be useful for the diagnosis of PPV when fetal tissues are not available; however, the normally high prevalence of PPV in populations and the time lag between infection and the observation of reproductive losses often present challenges to the interpretation of results. For these reasons, paired serum samples should be evaluated in the context of changes in antibody titers between the two samples. Since the virus cannot cross the placental barrier, antibody-positive fluids or sera from fetuses, and pigs prior to consumption of colostrum, is indicative of an intrauterine infection.

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The HI assay is commonly used for detection and quantification of PPV-specific serum antibodies. Importantly, HI results may be affected by incubation temperature and the source of erythrocytes. Serum to be assayed in the HI test is usually pretreated by heat inactivation (56°C [133°F], for 30 minutes) followed by adsorption with erythrocytes (to remove nonspecific hemagglutinins) and kaolin (to remove or reduce nonspecific inhibitors of hemagglutination) (Mengeling 1972; Morimoto et al. 1972).

The enzyme-linked immunosorbent assay (ELISA) format is a preferable alternative to HI because it can be standardized and automated for high-throughput testing. Furthermore, it does not require pretreatment of serum before testing (Hohdatsu et al. 1988; Westenbrink et al. 1989). Differential ELISAs can distinguish vaccinated animals from animals infected with PPV (Madsen et al. 1997; Qing et al. 2006). Inactivated vaccines only elicit antibodies against the structural proteins (VP), whereas differential ELISAs detect antibodies produced against the nonstructural (NS1) proteins that are expressed during virus replication in infected pigs.

**IMMUNITY**

Piglets from seropositive dams are protected by colostral antibodies consumed in the first day of life. Antibodies against PPV are 10 times more concentrated in the colostrum relative to serum. Antibody is produced by the piglet starting in the second week of life. In most piglets, or umbilical cord serum before ingestion of colostrum ingestion can also be tested for PPV-specific antibodies.

The virus grows readily in renal or testicular cells. Primary cell cultures present a higher risk of contamination with adventitious agents and are composed of cells with a lower index of division. Therefore, continuous cell lines (ESK, PK-15, SK6, ST, STE, and SPEV) are typically used for virus propagation and titration (Mengeling 1972; Zimmermann et al. 2006). Cytopathic effects of PPV in cell cultures include intranuclear inclusions, pyknotic nucleus, granulations, irregular shape, slow replication, and subsequent cell death (Cartwright et al. 1969; Mengeling 1972). IF microscopy may be used to confirm PPV infection of cell cultures and to titrate virus (Johnson 1973; Mengeling 1978) (Figure 29.5). Alternatively, since PPV produces a viral hemagglutinin, the virus may also be titrated based on the hemagglutinating activity of PPV for erythrocytes of certain species (Joo et al. 1976b; Siegl 1976). Infectious PPV is slowly, but progressively, lost after fetal death (Mengeling and Cutlip 1975). The likelihood of successful recovery of virus will depend on the condition of fetal tissues at the time of collection, but attempted virus isolation from autolyzed tissues is unproductive.

Hybridization techniques, like nonradioactive probes after DNA extraction (Oraveerakul et al. 1990) and in situ hybridization (Waldvogel et al. 1995), are sensitive techniques for the detection of virus in clinical samples. For routine diagnostics, polymerase chain reaction (PCR) is the most useful technique for the detection of PPV in fetal tissues, semen, and other samples. Numerous PCR protocols have been described (Chen et al. 2009; Gradil et al. 1994; Miao et al. 2009; Molitor et al. 1991; Prikhod’ko et al. 2003; Soares et al. 1999; Wilhelm et al. 2006), including multiplex PCRs (Cao et al. 2005; Huang et al. 2004; Kim and Chae 2003), especially for the concurrent detection of PPV and PCV2. These methods are considered to possess higher diagnostic sensitivity and specificity than hemagglutination and are better suited for the detection of PPV in autolyzed tissues.

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pigs, maternal antibody levels decrease steadily and reach nondetectable titers after around 20 weeks of age. In some cases however, they may persist for up to 9 months and may interfere with the ability of young gilts to respond to vaccination. Antibodies and PPV DNA can be demonstrated in infected animals, once maternal antibodies have waned (Streck et al. 2011).

Active immunity after field infection or vaccination develops within a few days. Antibodies are detected by HI or virus neutralization tests as early as 6 days postinfection. There is a marked difference between antibody titers induced by commercial inactivated vaccines versus infection with field virus. By HI, vaccine antibody titers are typically ≤1:500 or lower, whereas the infection titers regularly exceed 1:2000. The persistence of antibodies has been described for 4 months to 4 years (Johnson et al. 1976; Joo and Johnson 1977). Antibodies may prevent clinical disease, but infection and subsequent shedding of field virus occurs (Jóźwik et al. 2009). Cellular immunity has also been described, and the proliferation of some virus-specific CD4+CD8+ T cells proliferating after PPV antigen contact have been demonstrated (Ladekjaer-Mikkelsen and Nielsen 2002).

PREVENTION AND CONTROL

PPV is prevalent in the pig population and highly stable in the environment. These factors make it difficult to establish and maintain breeding populations free of the virus. A more practical goal in commercial herds is to maintain herd immunity against PPV. Historically, swine producers used various approaches to infect gilts with PPV before the first breeding, for example, intentional infection of gilts to PPV by exposure to virus-contaminated tissues from affected litters. Approaches of this type are both unreliable and dangerous because they can result in the dissemination of other pathogens in the population, for example, classical swine fever virus. More preferable and reliable is regular vaccination of breeding females.

Most commercial vaccines are based on chemical inactivation (formalin, beta-propiolactone, or binary ethyleneimine) of tissue culture-derived virus adjuvanted with oil or aluminum hydroxide. These vaccines induce antibody titers sufficient to prevent disease, but not infection (Jóźwik et al. 2009). In controlled studies, antibody titers stimulated by inactivated vaccines were detected for 4–13 months after vaccination (Joo and Johnson 1977; Vannier et al. 2006). Therefore, regular revaccination of breeding sows at 4–6 month intervals may be necessary to maintain protective immunity in sows.

Modified live virus (MLV) vaccines have also been developed. Vaccination with MLVs induces a long-lasting immune response, with viremia and shedding of the vaccine virus occurring for a short time postvaccination. There are few reports on MLVs and most are based on NADL-2 virus as the vaccine virus (Paul and Mengeling 1980, 1984). Parenteral transmission was more effective than oral administration and the quantity of virus administered was related to subsequent virus shedding and antibody titers. In all cases, transplacental transmission was prevented. Limited experimental infections of pregnant sows with the reference strain Impfstoffwerke Dessau-Tornau (IDT), strain Stendal, strain NADL-2, and the field isolate PPV-143 revealed no transmission of these viruses to fetuses and the induction of a very strong humoral immune response (Jóźwik et al. 2009; Zeeuw et al. 2007).

Several subunit vaccines have been described, with most based on expression of the viral VP2 protein in a baculovirus system. They provided protection comparable with inactivated, full-virus vaccines (Antonis et al. 2006).

Review of the current vaccination strategy against PPV infection is warranted. The use of inactivated full-virus vaccines for protection against the closely related canine and feline parvoviruses is no longer common, having been replaced by MLVs. The few licensed inactivated vaccines remaining are used for special purposes, for example, vaccination of exotic zoo felids. MLVs in carnivores induce a long-lasting immune response that provides protection for several years. In swine, the appearance of “new” genotypes or antigenic types of PPV needs to be closely watched. New PPV vaccines that induce longer-lasting immunity and protect against all the prevalent virus strains circulating in pig populations are needed.

REFERENCES

RELEVANCE

Swinepox (SWP) is a mild, acute disease of swine characterized by typical poxviral lesions of the skin. SWP is distributed worldwide and is usually associated with poor sanitation. Morbidity may be high in individual herds where young pigs are most severely affected, but mortality is usually negligible. Clinical signs and epidemiology are usually sufficient for SWP diagnosis.

ETIOLOGY

SWP virus (SWPV) is the sole member of the genus Suipoxvirus in the family Poxviridae (Moyer et al. 2000). The SWPV virion is morphologically similar to vaccinia virus, exhibiting a brick-like structure approximately 320 × 240 nm in horizontal section (Figure 30.1) (Blake-more and Abdussalam 1956; Cheville 1966a; Teppema and De Boer 1975). The virion is composed of a central biconcave core or nucleoid bordered by two ellipsoid lateral bodies and at least two lipid membranes (Blakemore and Abdussalam 1956; Cheville 1966a; Conroy and Meyer 1971; Kim and Luong 1975; Smid et al. 1973; Teppema and De Boer 1975). SWPV is ether-sensitive.

The SWPV genome is a double-stranded DNA molecule of 146 kilobase pairs predicted to contain 150 genes. It shares features common with other members of the Poxviridae, including a conserved central genomic region containing genes necessary for intracytoplasmic replication and virion structure (Afonso et al. 2002). Although genetically related to viruses of other poxviral genera (Capripoxvirus, Leporipoxvirus, Yatapoxvirus), SWPV represents a distinct poxvirus genus and contains in terminal genomic regions a unique comple-

iment of genes predicted to affect virus/host interaction and aspects of SWPV virulence and host range (Afonso et al. 2002; Massung et al. 1993).

Despite reports of limited antigenic cross-reactivity, SWPV is antigenically distinct, as evidenced by the failure of SWPV antibodies to cross-protect, cross-neutralize, or efficiently immunoprecipitate viral proteins of other poxviruses (De Boer 1975; Massung and Moyer 1991; Meyer and Conroy 1972; Ouchi et al. 1992; Shope 1940).

Although some isolates have been reported to induce first-passage cytopathic effect (CPE) on swine cells (Afonso et al. 2002; Paton et al. 1990), SWPV generally replicates poorly on initial isolation in swine cell cultures. It requires multiple passages before inducing CPE, but maintains pathogenicity for swine (Garg and Meyer 1972; Kasza and Griesemer 1962; Kasza et al. 1960; Meyer and Conroy 1972). CPE, characterized by cytoplasmic vacuoles and inclusion bodies, nuclear "vacuoles," and cell rounding and clumping (Borst et al. 1990; De Boer 1975; Kasza et al. 1960; Meyer and Conroy 1972), is seen 3–5 days postinoculation (PI), and isolated plaques remain relatively small in size (<1.5 mm) (Kasza et al. 1960; Massung and Moyer 1991; Meyer and Conroy 1972). Infection of cell cultures results in detectable expression of early and late SWPV mRNA by 4 and 8 hours PI, respectively, and viral protein synthesis by 4 hours PI (Massung and Moyer 1991).

Most attempts to grow or adapt SWPV for growth in nonswine cell cultures or on chicken chorioallantoic membranes have failed (Garg and Meyer 1972; Kasza et al. 1960; Meyer and Conroy 1972), indicating a host-range restriction for replication at the cellular level. Culture-adapted SWPV has been shown to replicate,
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mal inoculation of rabbits (Datt 1964). This restricted host range suggests that swine represent the reservoir of SWPV in nature.

SWP is present worldwide. Limited serological survey data from Europe indicated that 8–19% of swine serum samples contained anti-SWPV antibodies (De Boer 1975; Paton et al. 1990). Young swine are most often affected, as adult swine rarely present with clinical disease (Kasza et al. 1960; McNutt et al. 1929). Morbidity rates can be high (up to 100%), but mortality is generally negligible (less than 5%) (De Boer 1975). Overall, SWP is of little economic consequence.

Natural transmission of SWP is not well understood, but is often associated with poor sanitation. SWP has been associated with louse (Haematopinus suis) infestation. Lice are able to mechanically transmit SWPV and are thought to affect the extent and distribution of cutaneous lesions, which often occur in less keratinized abdominal and inguinal regions (Kasza et al. 1960; Manninger et al. 1940; Shope 1940). However, SWP without evidence of louse involvement has been described, suggesting a role for other insect vectors or the possibility of horizontal transmission (De Boer 1975; Jubb et al. 1992; Paton et al. 1990; Schwarte and Biester 1941). Vertical SWPV transmission is indicated by sporadic cases of congenital infection resulting in stillborn fetuses with generalized lesions (Afonso et al. 2002; Borst et al. 1990; Paton et al. 1990).

**PATHOGENESIS**

SWPV may enter the host through preexisting skin abrasions and preferentially replicates in epidermal keratinocytes of the stratum spinosum (Meyer and Conroy 1972). Although mature viral particles have been observed in epidermal basal cells (Teppema and De Boer 1975) and viral antigen has been detected in dermal macrophages (Cheville 1966b), there is no indication that these cell types support virus replication. With the exception of moderate changes in superficial lymph nodes, tissues other than the skin are rarely affected. Infectious virus can be readily isolated from the skin of infected animals as early as 3 days post-intradermal inoculation (Kasza and Griesemer 1962) and from regional lymph nodes only if skin lesions are severe (Kasza and Griesemer 1962). A viremic stage has been suggested to account for virus spread from the primary to secondary sites of replication in the skin and for congenital infection, however, virus has not been isolated from the blood of infected animals (Borst et al. 1990; Kasza and Griesemer 1962; Paton et al. 1990; Shope 1940).

Functional studies on SWPV pathogenesis are lacking. The complement of virus genes with putative roles in virulence and host range suggests that modulation of host immune responses and inhibition of apoptosis may play a role in the course of the disease (Afonso...
et al. 2002; Kawagishi-Kobayashi et al. 2000; Massung et al. 1993). SWPV contains genes with sequence similarity to cellular and viral genes encoding CD47, and proteins that bind interferon (IFN)-α/β, IFN-γ, tumor necrosis factor (TNF)-α, interleukin-18 (IL-18), and CC chemokines. Products of these genes potentially modulate host immune responses, including natural killer (NK) and T-cell responses, and may facilitate SWPV replication and dissemination. SWPV TNF-binding protein has been shown to interact with high affinity with porcine TNF but not with TNF from other species (Rahman et al. 2006). SWPV encodes proteins similar to host major histocompatibility complex (MHC)-I, NFκB activators, Bcl-2 family proteins, serine proteases, and PKR, some of which in other poxviruses are known to interfere with or delay inflammatory responses at the site of virus replication.

CLINICAL SIGNS

SWPV causes eruptive dermatitis in pigs. Animals up to 3 months of age are the most susceptible, with adults usually developing a mild, self-limiting form of the disease. Multiple cutaneous lesions are commonly found on the flanks, belly, inner side of the legs, ears, and less frequently, on the face of affected animals (De Boer 1975; Jubb et al. 1992; Kim and Luong 1975; McNutt et al. 1929; Olufemi et al. 1981; Schwarte and Biester 1941). Lesions can also be found in the teats of sows and on the face, lips, and tongue of suckling pigs (Olufemi et al. 1981). In congenital infections, lesions are observed over the entire body and in the oral cavity (Borst et al. 1990; Paton et al. 1990). When virus transmission is associated with mechanical vectors, the distribution of lesions tends to reflect the vector’s preferred feeding areas.

The incubation period is thought to be 4–14 days under field conditions (De Boer 1975; McNutt et al. 1929) and 3–5 days after intradermal or intravenous virus inoculation (Kasza and Griesemer 1962; Schwarte and Biester 1941), although longer periods have been described (Shope 1940). Initial lesions are flat, pale, rounded areas 3–5 mm in diameter (maculae). Over the course of 2 days, these progress to papulae 1–2 mm in height, 1–2 cm in diameter, and occasionally confluent (Figure 30.2). The appearance of papulae may be accompanied by a slight and transient increase in body temperature and loss of appetite (Kasza and Griesemer 1962; Kasza et al. 1960). A true vesicle stage is absent or transient (Borst et al. 1990; Datt 1964; Kasza and Griesemer 1962; Meyer and Conroy 1972). The lesions usually become umbilicated and shrink about a week after appearing. They are replaced by crusts, which are eventually shed, leaving discolored spots (Kasza and Griesemer 1962). Complete recovery is observed 15–30 days postexposure, but it may be delayed if secondary bacterial infection occurs (De Boer 1975; McNutt et al. 1929; Miller and Olson 1980; Schwarte and Biester 1941).

LESIONS

The most conspicuous histological change caused by SWPV infection is hydropic degeneration of stratum spinosum keratinocytes (Figure 30.3A,B) (Borst et al. 1990; Cheville 1966a; Kasza and Griesemer 1962; McNutt et al. 1929; Meyer and Conroy 1972; Olufemi et al. 1981; Paton et al. 1990; Schwarte and Biester 1941; Teppema and De Boer 1975). Hyperplasia of epidermal cells is not as marked as in poxviral infections of other mammals, an observation that might be related to the lack of an SWPV-encoded homolog of the poxviral epidermal growth factor-like gene (Afonso et al. 2002; McNutt et al. 1929; Schwarte and Biester 1941). The cytoplasm of affected cells is brightened and enlarged, contains eosinophilic inclusion bodies resembling poxviral type B inclusion bodies (Teppema and De Boer 1975) (Figure 30.3B), and reacts strongly with antibodies against viral antigens (Cheville 1966b). Hydropic degeneration and inclusion bodies are also observed in the outer root sheaths of the hair follicles (Kasza and Griesemer 1962; Meyer and Conroy 1972). The nucleus of affected cells exhibits margination of chromatin and a large, central “vacuole” resembling the nuclear clearing observed in sheeppox virus-infected keratinocytes (Figure 30.3B) (Cheville 1966a; Kasza and Griesemer 1962; McNutt et al. 1929; Meyer and Conroy 1972; Plowright and Ferris 1958; Teppema and De Boer 1975). No significant fluid accumulation is observed between keratinocytes. Apical keratinocytes undergo necrosis at later stages of infection. Leukocyte
infiltration is observed in the underlying dermis and, to a lesser degree, the affected epidermis (Figure 30.3A), with few viral antigen-containing dermal macrophages (Cheville 1966b). When involved, the inguinal lymph nodes present edema, hyperemia, hyperplasia, and few virus antigen-containing cells (Cheville 1966a; Kasza and Griesemer 1962).

Ultrastructurally, infected cells exhibit a marked decrease in keratin precursors (tonofilaments) and loss of intercellular interdigitations characteristic of the stratum spinosum (Cheville 1966a; Teppema and De Boer 1975). Individual inclusion bodies consist of electrondense central cores surrounded by lamellar bodies and maturing viral particles (viroplasm) (Cheville 1966a; Conroy and Meyer 1971; Kim and Luong 1975; Smid et al. 1973; Teppema and De Boer 1975). The large, well-defined nuclear “vacuole” can be more accurately described as a region of low electron density, which lacks a surrounding membrane and contains cross-striated fibrils similar to those observed in the cytoplasm.

**DIAGNOSIS**

Presumptive SWP diagnosis is based on the observation of pox lesions on the skin of affected animals. Differential diagnosis includes vesicular diseases, allergic skin reactions, sunburn, bacterial dermatitis, ringworm, and skin parasitosis (Acarus (Tyroglyphus) spp., sarcoptic mange) (Blood and Radostits 1989; Yager and Scott 1985). SWPV involvement can be confirmed by electron microscopy and histopathology and includes pathognomonic epidermal changes: ballooned stratum spinosum keratinocytes containing cytoplasmic eosinophilic inclusion bodies and a “vacuolated” nucleus. SWPV involvement may be definitively confirmed using SWPV-specific antibodies to neutralize virus isolated on swine cell cultures (primary swine kidney cells, PK-15 cell line) (Borst et al. 1990; Meyer and Conroy 1972; Paton et al. 1990) or by performing immunocytochemistry on tissue samples or infected cell cultures (Garg and Meyer 1973; Mohanty et al. 1989; Paton et al. 1990). Papular/pustular exudate or crusted materials are clinical samples of choice for virus isolation. At least seven blind passages should be attempted before considering the sample negative (De Boer 1975).

Virus neutralization and precipitating antibody tests to detect SWPV-specific antibodies in convalescent serum have been described (De Boer 1975; Shope 1940). Since swine do not reliably develop high levels of neutralizing antibodies (Kasza et al. 1960; Shope 1940), negative test results should be interpreted with caution.

The availability of the SWPV genome sequence and the identification of unique SWPV gene sequences will permit the development of rapid, sensitive, and specific polymerase chain reaction-based detection and diagnostic assays (Afonso et al. 2002).

Although differential diagnosis of SWP from a vaccinia virus infection in pigs was important when human smallpox vaccination was practiced, this no longer represents a diagnostic concern (Shope 1940).

**IMMUNITY**

Convalescent swine are resistant to SWPV challenge, indicating that infection induces protective immunity (De Boer 1975; Garg and Meyer 1972; Kasza et al. 1960; Schwarte and Biester 1941; Shope 1940). However, the
immune mechanisms associated with protection are not known. SWPV neutralizing activity is present in swine sera as early as 7 days PI. However, low neutralizing titers, delayed kinetics of antibody response, and lack of neutralizing antibodies at 50 days PI have been reported (Kasza et al. 1960; Meyer and Conroy 1972; Shope 1940; Williams et al. 1989). Suckling pigs may also be protected by maternal antibody (Manninger et al. 1940), although high neonatal mortality rates have been observed (Olufemi et al. 1981). Decreased mitogen and SWPV-induced proliferative responses have been observed in peripheral blood mononuclear cells from experimentally infected swine (Williams et al. 1989).

**PREVENTION AND CONTROL**

No specific treatment for SWPV exists. Antibiotic regimens are recommended for the treatment of secondary bacterial infection. Given that SWP is of relatively low economic impact, no vaccine has been developed. Good animal husbandry, including ectoparasite control, should be practiced.

**REFERENCES**


RELEVANCE

In the late 1980s, severe outbreaks characterized by severe reproductive losses, respiratory disease, reduction in growth rate, and increased mortality were reported in the United States (Hill 1990; Keffaber 1989; Loula 1991). Initial efforts to identify the etiology of the outbreaks were unsuccessful. Clinically similar outbreaks occurred in Germany in November 1990 (OIE 1992). No common link was found between the outbreaks in Germany and the United States (Anonymous 1991). Spreading rapidly, over 3000 outbreaks were documented in Germany in May 1991 and across Europe in the following 4 years (Bøtner et al. 1994; Edwards et al. 1992; OIE 1992; Pejsak and Markowska-Daniel 1996; Plana Duran et al. 1992a; Valiček et al. 1997). In Asia, outbreaks occurred in Japan in 1988 (Hirose et al. 1995) and in Taiwan in 1991 (Chang et al. 1993).

The etiology of the disease was established in 1991 when Koch’s postulates were fulfilled with a previously unrecognized RNA virus (Terpstra et al. 1991a; Wensvoort et al. 1991). Shortly thereafter, the virus was isolated in the United States (Collins 1991; Collins et al. 1992) and Canada (Dea et al. 1992a,b). The first virus isolates in The Netherlands and the United States were designated Lelystad virus and swine infertility and respiratory syndrome (SIRS) virus (BIAH-001), respectively. European researchers introduced the term “porcine reproductive and respiratory syndrome” (PRRS) in 1991 (Terpstra et al. 1991b).

The origin of porcine reproductive and respiratory syndrome virus (PRRSV) remains unknown. Regardless, the virus has become endemic in most swine-producing regions of the world and control remains problematic. Neumann et al. (2005) estimated annual PRRS losses to U.S. pig producers at $560.32 million annually. A more recent study estimated losses in the United States at $668.58 million annually, exclusive of costs related to PRRSV vaccination, treatment, diagnostics, and biosecurity (Holtkamp et al. 2011). For this reason, elimination of PRRSV from herds, regions, and countries is considered the best solution.

ETIOLOGY

Taxonomy and Classification

PRRSV is classified with equine arteritis virus (EAV), lactate dehydrogenase-elevating virus (LDV), and simian hemorrhagic fever virus in the family Arteriviridae. The families Arteriviridae, Coronaviridae, and Roniviridae comprise the order Nidovirales. The PRRSV species forms two major genetic lineages represented by type 1 and type 2 genotypes. The two prototype genomes, Lelystad virus (type 1) and VR-2332 (type 2), vary by –44% in nucleotide sequence. Both types were discovered around 1990: type 1 viruses in Europe and type 2 viruses in North America. Today, both types share worldwide distributions, with type 1 predominant in Europe and type 2 predominant in North America and Asia.

Phylogenetic analyses, based largely on open reading frame (ORF) 5, which encodes the major envelope glycoprotein (GP5), have showed that both type 1 and type 2 PRRSVs are remarkably diverse. Intratype pairwise nucleotide sequence variation up to 30% is present in type 1 viruses, and it exceeds 21% in type 2 viruses. Although the origin of PRRSV is not known, the extensive differences between type 1 and type 2 PRRSVs...
suggests that their ancestor(s) evolved independently in ecologically or geographically distinct environments for an extended period, possibly in a nonswine species reservoir.

Multiple genetic lineages are described in type 1 PRRSVs. The lineages present in Russia, Belarus, Ukraine, Lithuania, and Latvia, have significantly larger diversity and likely earlier divergence times than other type 1 lineages. These data suggest that type 1 PRRSV was established in the former Soviet Union countries long before the first reported epidemic outbreak in western Europe, suggesting that this region was the primordial reservoir in which type 1 PRRSV evolved (Stadejek et al. 2006). The apparent segregation of independent genetic viral lineages in western and eastern Europe is consistent with the historical fact that swine populations in the two regions were unconnected until political changes in Europe in the 1990s facilitated animal movement between the two.

Nine diverse lineages have been defined in the phylogeny of type 2 PRRSV ORF 5 (Shi et al. 2010). Seven lineages are predominated by North American isolates and two are exclusively constituted by East Asian isolates. Asian lineages, as well as some North American post-pandemic lineages, may have diverged prior to the original pandemic lineage, indicating that, like type 1 PRRSV, transmission and evolution of type 2 PRRSV was extensive prior to the recognition of PRRS as a new swine disease. The presence of type 2 PRRSV in Asia appears to be due primarily to introductions of North American lineages, followed by local diversification leading to new disease outbreaks and increased virulence (An et al. 2007, 2010; Hu et al. 2009; Shi et al. 2010).

The taxonomic emphasis on ORF5 may obscure important genetic variation in other regions of the PRRSV genome. Thus, a fuller picture of PRRSV genetic relationships and evolutionary origins may be facilitated by whole genome analyses and comparisons of multiple protein-coding regions, including the polymerase gene, which is widely used in RNA viral evolutionary analyses.

**Other Methods of Describing Porcine Reproductive and Respiratory Syndrome Virus Isolates.** Restriction fragment length polymorphism (RFLP) typing is based on identifying restriction endonuclease cleavage patterns in ORF5 (Wesley et al. 1998). Restriction endonucleases cleave a nucleic acid chain at a predetermined sequence of nucleotides; thus, viruses with the same RFLP pattern contain the same restriction cleavage sites and were initially assumed to have similar sequences that reflected phylogenetic relationships. However, RFLP typing has several major weaknesses: (1) It was never adapted to type 1 PRRSVs (with one exception), (2) RFLP types do not correlate with phylogenetic relatedness of field isolates, (3) the RFLP pattern is not a stable characteristic and can change as a virus is passed in pigs (Cha et al. 2004; Yoon et al. 2001), and (4) the RFLP identification terminology has become too complex. By mid-2011, at least 4 MluI digestion patterns, 72 HindII digestion patterns, and 9 SacII digestion patterns were recognized (K. Rossow, personal communication).

Serotyping has also been explored as a method of grouping PRRSV isolates. Immune serum from pigs infected with, and monoclonal antibodies produced against, type 1 or type 2 PRRSVs react preferentially with PRRSVs of the same type, but it has been difficult to identify distinct serological relationships within a type. This problem is exacerbated by differences in the strain of the antibody response among individual pigs to PRRSV infection (Johnson et al. 2007). Serotyping based on virus neutralization (VN) is a possibility, but neutralizing antibodies may not be detected in all pigs (Nelson et al. 1994). Then, too, VN has not been shown to reproducibly identify related clusters of viruses, and cross-neutralization has not been related to efficacious cross-protection against challenge with virulent PRRSV. On the contrary, immune cross-protection appears to be broad between viruses in which there is no evidence of serological cross-neutralization (Opriessnig et al. 2005).

Glycotype clustering of PRRSV isolates by predicted N-linked glycosylation patterns in GP5 may provide information on immunological cross-reactivity. However, it has not been shown to provide generally applicable insights to the biological behavior or properties of PRRSV, and it has not been applied to type 1 PRRSV. A recent typing scheme applicable to both type 1 and type 2 PRRSVs has been described (Kim 2008). Its limitation is the absence of published data describing either the basis of the method or its validation.

The issue of quasispecies confounds attempts at grouping PRRSVs within a type. RNA viruses with high mutation rates are proposed to exist in an infected host as a cloud of mutationally distinct genomes. Theoretically, genetic diversity increases reproductive fitness and may be necessary for evolutionary success. Quasispecies might help explain the exceptional genetic diversity of PRRSV and facilitate emergence of mutants resistant to existing immunity. However, sequential pig-to-pig passages of PRRSV did not increase the genetic variation within individual pigs, and no evidence for immunological selection of escape mutants has been described in experimental or field settings (Chang et al. 2002, 2009; Goldberg et al. 2003). Thus, the role or significance of quasispecies in PRRSV is unclear.

**Physicochemical and Biological Properties**

PRRSV is a small, enveloped, positive-sense, single-stranded RNA virus. The virion includes an infectious RNA genome of approximately 15 kilobases in a
proteinaceous nucleocapsid, surrounded by a lipid-containing envelope with five or six structural proteins. Virions are small, pleomorphic spheroids ~50–70 nm in diameter, with small surface projections that cover the entire virion surface (Benfield et al. 1992; Spilman et al. 2009). The buoyant density of infectious viral particles is ~1.18–1.22 g/cm³ in cesium chloride (Benfield et al. 1992). PRRSV is stable in media, serum, and tissue homogenates at ~70°C (~94°F), but its half-life decreases with increasing temperature. It is inactivated by lipid solvents and is highly unstable in solutions containing low concentrations of ionic or nonionic detergents due to loss of infectivity through disruption of the viral envelope.

**Genomic Organization and Gene Expression**

The genomic organization of PRRSV is similar to that of other arteriviruses, consisting of ~15,000 nucleotides organized into nine ORFs that are expressed from genomic and subgenomic (sg) mRNAs. ORFs 1a and 1b comprise 80% of the genome and encode the protein cleavage, homologous recombination, and RNA replication machinery required for viral transcription, replication, and immunomodulation. ORF 1a and ORF 1ab are translated as large polypeptides, which are then proteolytically processed into approximately 12 nonstructural proteins (NSPs).

Seven ORFs (2, 2b, and 3–7) located downstream of ORF 1ab at the 3′ end of the genome encode the viral structural proteins. Expression is accomplished by formation of subgenomic (sgm) messages containing the viral 5′ leader sequence ligated at a conserved leader-body junction site upstream of each ORF. Protein expression occurs by translation of the first ORF downstream of the leader sequence in each sgmRNA, except in sgmRNA2 and sgmRNA5, each of which encodes two proteins in different reading frames (Johnson et al. 2011; Wu et al. 2005).

The formation of sgmRNA is nonrandom, since sgmRNA7 is most abundant, followed by sgmRNA6 and sgmRNA5. The abundance of structural proteins follows the same trend, with nucleocapsid (N) being most abundant, followed by the M and GP5 products of ORF6 and ORF5. The noncoding untranslated 5′ leader sequence and 3′ untranslated sequence downstream of ORF7 are conserved within type 1 and type 2 PRRSVs. These sequences are essential for viral replication.

A third RNA species, heteroclite RNA, consists of genomic RNA ligated at noncanonical junction sites downstream of the 5′ leader and within structural protein-coding ORFs in the 3′ end (Yuan et al. 2000). Heteroclite RNAs are produced under all conditions of viral growth, vary in size, but all lack nsp9, encoding the polymerase. They appear to be incorporated into virions and can be translated (Yuan et al. 2004). However, their contribution to infection and disease remains to be determined.

**Nonstructural Proteins.** PRRSV NSPs are encoded in one of two large polyproteins translated from the full-length genomic RNA molecule. ORF 1a is translated into a large polyprotein of ~260–277 kDa that is proteolytically cleaved into smaller active proteins including four proteases (nsp1-alpha, nsp1-beta, nsp2, and nsp4) that carry out the cleavage events in ORF 1a and ORF 1b. PRRSV has an unusual RNA loop structure at the terminus of ORF 1a that can bypass the stop codon and continue translation in a second ORF, resulting in an ORF 1ab polyprotein containing an additional ~160–170 kDa of protein. The proteases in ORF 1a cleave the additional polyprotein from ORF 1b into approximately six proteins that contain the replicase activity, including a helicase, an RNA-dependent RNA polymerase, and an endoribonuclease. The replication complex is located intracellularly in a perinuclear region consistent with the Golgi complex.

PRRSV envelope proteins display a similar localization, suggesting that key viral biosynthetic and assembly events occur in the Golgi complex. In addition to endoproteolytic activity, nsp1-alpha and nsp1-beta may contribute to viral pathogenicity by blocking type 1 interferon (IFN) synthesis directly or via inhibition of signaling pathways (Beura et al. 2010; Chen et al. 2010). The nsp2 shows extensive size polymorphisms due to variable, in-frame deletions that are frequently observed in highly virulent variants. However, the nsp2 region does not appear to determine virulence (Zhou et al. 2009a).

**Structural Proteins.** The most abundant structural protein is N, a small (15 kDa), highly basic, polypeptide that interacts with the viral RNA in the assembly of infectious particles. It is expressed at high levels in infected cells and represents 20–40% of the total protein content of the virion. It is active in nuclear shuttling and localization to the nucleolus and may influence nuclear processes during replication, possibly through rRNA precursor processing and ribosome biogenesis (Yoo et al. 2003). Its abundant expression and antigenicity make it a good target for immunodiagnostic assays, but a role for these antibodies in immune protection has not been demonstrated.

The two major envelope proteins are (1) a nonglycosylated matrix (M) envelope protein that lacks a signal sequence and accumulates in the endoplasmic reticulum, and (2) GP5 with which M forms disulfide-linked heterodimers that are incorporated into the virion envelope. GP5, after cleavage of the signal peptide, contains an ectodomain of about 30 amino acids in length and is predicted to contain 2–5 N-linked glycans. Due to variable glycosylation, it migrates at 25–35 kDa on sodium dodecyl sulfate (SDS)-polyacrylamide gels. The GP5-M heterodimer is essential for virion formation, but GP5-M alone is not sufficient for viral infectivity (Das et al. 2010; Wissink et al. 2005).
The 29- to 30-kDa GP2, 45- to 50-kDa GP3, and 31- to 35-kDa GP4 glycoproteins are present in low amounts and form a trimeric envelope protein complex. The presence of all three proteins is required for their assembly and incorporation into the virion and for viral infectivity (Wissink et al. 2005). The trimeric structure mediates infection alone, or through an interaction with GP5 (Das et al. 2010; Wissink et al. 2005). These observations explain why replacement of EAV GP5 with PRRSV GP5 did not change the tropism of EAV to porcine macrophages (Dobbe et al. 2001). The minor envelope glycoprotein complex mediates infection of permissive porcine cells through an interaction with CD163 (Calvert et al. 2007; Das et al. 2010).

**Laboratory Cultivation**

Type 1 and type 2 PRRSVs grow primarily in porcine macrophages of the lungs and lymphoid tissues and in dendritic cells, but not in monocytes. In the laboratory, PRRSV is cultivated on alveolar macrophages isolated from young pigs and on simian kidney cells, especially African green monkey MA-104 cells and its derivative, the MARC-145 cell line. Type 1 PRRSV isolates grow readily on porcine macrophages but are difficult to adapt to simian cell culture. Type 2 PRRSV isolates grow preferentially on macrophages but are frequently adaptable to simian cell culture with several blind passages, whereas cell culture-adapted vaccine viruses are preferentially isolated on MARC-145 cells (de Abin et al. 2009). Variation in the ability of type 1 and type 2 PRRSV to grow in vitro suggests that both porcine macrophages and simian cells should be used for virus isolation (VI) whenever possible.

Characterization of host cell surface molecules involved in PRRSV infection, especially CD163 and sialoadhesin, has resulted in the development of transgenic PK-15 and CHO cell lines that permit infection by vaccine viruses and field isolates (Delrue et al. 2010; Van Gorp et al. 2008). Established cell lines that are readily permissive to type 1 and type 2 PRRSV field isolates will facilitate VI, especially for type 1 isolates.

**PUBLIC HEALTH**

PRRSV is not infectious for humans or cells of human origin and has no public health significance.

**EPIDEMIOLOGY**

**Geographical Distribution**

PRRSV is present in most swine-producing regions of the world, with a few significant exceptions. In Europe, countries free of PRRSV include Sweden (Carlsson et al. 2009; Elvander et al. 1997), Norway (OIE 1997), Finland (Bøtner 2003), and Switzerland (Corbellini et al. 2006). In Oceania, New Caledonia (OIE 1996), New Zealand (Motha et al. 1997), and Australia (Garner et al. 1997) are PRRSV-free. In South America, Argentina (Perfumo and Sanguinetti 2003), Brazil (Ciacci-Zanella et al. 2004), Cuba (Alfonso and Frías-Leporeau 2003), and some areas of the Caribbean are thought to be free of PRRSV.

Accurate estimates of the prevalence of infection with field virus in specific countries or regions are not readily available, but within pig-dense infected regions, 60–80% of herds are typically infected. Seroprevalence estimates are complicated by the presence of vaccine virus-derived antibodies produced by vaccination or infection. Virus from modified live virus (MLV) vaccines is shed and transmitted in the field (Bøtner et al. 1997; Christopher-Hennings et al. 1996, 1997; Menge ling et al. 1998).

**Susceptible Species**

A number of species have been determined not to be susceptible to PRRSV, including mice, rats (Hooper et al. 1994; Rosenfeld et al. 2009), and guinea pigs (J. Zimmerman, unpublished data). Wills et al. (2000b) found no evidence of PRRSV replication in cats, dogs, mice, opossums, raccoons, rats, skunks, house sparrows, or starlings. Zimmerman et al. (1997) reported that mallard ducks (Anas platyrhynchos) were susceptible to PRRSV, but subsequent workers have not replicated these results. Feral swine are susceptible to PRRSV, but serosurveys indicate that infection in free-ranging feral swine animals is relatively rare (Albina et al. 2000; Lutz and Wurm 1996; Oslage et al. 1994; Saliki et al. 1998; Wyckoff et al. 2009). The susceptibility of other species within superfamily Suoidae (Sus spp., peccaries, warthogs, and babirusa) for PRRSV is unknown.

**Routes of Shedding**

Infected animals shed virus in saliva (Wills et al. 1997a), nasal secretions (Christianson et al. 1993; Rossow et al. 1994a), urine (Wills et al. 1997a), semen (Swenson et al. 1994a), and occasionally in feces (Christianson et al. 1993). Pregnant susceptible females inoculated in late gestation shed virus in mammary secretions (Wagstrom et al. 2001). Virus variants vary significantly in the level of shedding by pigs (Cho et al. 2006).

Shedding of virus in semen is a concern because of the extensive use of artificial insemination in pig production. The duration of semen shedding varies widely among boars (Christopher-Hennings et al. 1996). Christopher-Hennings et al. (1995a) detected viral RNA in the semen of experimentally infected boars for up to 92 days postinoculation (DPI) and isolated PRRSV from the bulbourethral gland of a boar euthanized 101 DPI. Semen shedding of MLV vaccine virus occurred for up to 39 days in one study, but prior vaccination eliminated or reduced shedding upon challenge (Christopher-Hennings et al. 1997).
**Persistent Infection**

PRRSV produces a chronic, persistent infection in pigs. This is the single most significant epidemiological feature of PRRSV infection. Persistent PRRSV infection has been documented through transmission experiments and by detection of virus in animals. A number of studies have reported detection of infectious virus for 100–165 DPI, particularly from tonsil or lymphoid tissues (Allende et al. 2000; Benfield et al. 2000b; Fangman et al. 2007; Horter et al. 2002; Wills et al. 1997b). Persistent infection is not a function of pig age at the time of infection. Persistence occurs regardless of whether the pig is exposed in utero (Benfield et al. 1997, 2000b; Rowland et al. 1999), as a young animal, or as an adult (Bierk et al. 2001; Christopher-Hennings et al. 1995a; Fairbanks et al. 2002; Zimmerman et al. 1992). The mechanisms by which the virus is able to persevere in the face of an active immune response have not been identified, but apparently do not involve evasion of immunity through continual in vivo viral mutation. Chang et al. (2002, 2009) found relatively low rates of mutation in persistently infected animals.

**Transmission**

Swine are susceptible to PRRSV by several routes of exposure, including intranasal, intramuscular, oral, intraterine, and vaginal. The probability that a given dose will infect an animal differs by the route of exposure. Yoon et al. (1999) reported that exposure to 20 or fewer PRRSV particles by intramuscular exposure resulted in infection. Hermann et al. (2005) estimated the dose required to infect one-half of the exposed animals, the infectious dose 50 (ID$_{50}$), for oral and intranasal routes of exposure to be $1 \times 10^{3.3}$ TCID$_{50}$ and $1 \times 10^{4.0}$ TCID$_{50}$, respectively. Based on the data from Benfield et al. (2000a), the ID$_{50}$ for exposure via artificial insemination is approximately $1 \times 10^{4.5}$ TCID$_{50}$.

Infectivity may vary among PRRSVs. Cutler et al. (2011) estimated the ID$_{50}$ for aerosol exposure to isolate MN-184 at $1 \times 10^{0.26}$ TCID$_{50}$. In contrast, Hermann et al. (2009) reported an ID$_{50}$ of $1 \times 10^{1.1}$ TCID$_{50}$ by aerosol exposure using isolate VR-2332. Both estimates were produced by the same laboratory under similar conditions.

Pigs are extremely susceptible to infection via parenteral exposure (breaks in the skin barrier) and generally less susceptible to other routes. In the field, parenteral exposure may occur by standard husbandry practices such as ear notching, tail docking, teeth clipping, tattooing, and inoculations with medications and biologics. Likewise, because PRRSV is present in oral fluid for weeks following infection, parenteral exposure may occur via bites, cuts, scrapes, and/or abrasions that occur during aggressive interactions among pigs. Bierk et al. (2001) associated transmission with aggressive behavior between carrier sows and susceptible contacts. Other behaviors that result in exchange of blood and oral fluid, such as tail biting and ear biting, may also function in transmission.

Indirect transmission of PRRSV to susceptible swine can occur by a number of routes (reviewed by Cho and Dee 2006), including contact with contaminated fomites, PRRSV-contaminated needles, personnel contaminated with oral fluid and blood from infected pigs, and exposure to insect vectors (houseflies and mosquitoes). Indirect transmission by aerosols is dependent on the viral variant and environmental factors. Risk factors for the airborne spread of PRRSV from a shedding source population to an at-risk population include directional winds of low velocity with sporadic gusts, low temperatures, high relative humidity, and low sunlight levels (Dee et al. 2010).

**Vertical Transmission**

PRRSV is transmitted from viremic dams transplacentally to fetuses, resulting in fetal death or birth of infected pigs that are weak or appear normal (Bøtner et al. 1994; Christianson et al. 1992; Terpstra et al. 1991a). Some pigs in affected litters may escape infection. PRRSV can replicate in fetuses 14 days of gestational age or older, but infection of fetuses during the first two-thirds of gestation is uncommon because most PRRSVs cross the placenta efficiently only in the last trimester of pregnancy (Christianson et al. 1993; Lager and Mengeling 1995; Mengeling et al. 1994; Prieto et al. 1996a,b). Transit is independent of the reproductive virulence of the virus isolate. Park et al. (1996) showed that PRRSV isolates of low and high virulence for fetuses cross the placenta with equal efficiency when sows are inoculated at 90 days of gestation.

**Transmission within Herds**

PRRSV tends to circulate within a herd indefinitely. Endemicity is driven by persistent PRRSV infection in carrier animals and the continual availability of susceptible animals through either birth, purchase, or loss of protective immunity. The virus is perpetuated by a cycle of transmission from dams to pigs either in utero or postpartum, or by commingling susceptible animals with infected animals. Under conditions in which susceptible and infectious pigs are mixed, for example, at weaning, a large proportion of the population may quickly become infected. Dee and Joo (1994a) reported 80–100% of pigs in three swine herds were infected by 8–9 weeks of age, and Maes (1997) found 96% of market hogs sampled from 50 herds to be positive. However, marked differences in infection rates between groups, pens, or rooms of animals may be observed in endemically infected herds. Houben et al. (1995) even found transmission to vary within litters. Some littermates seroconverted as early as 6–8 weeks of age, but other individuals reached 12 weeks of age, the end of the monitoring period, still free of PRRSV infection.
Transmission between Herds
The role of infected pigs, virus-contaminated semen, and aerosols in herd-to-herd transmission is firmly established (Dee 1992; Dee et al. 2010; Mousing et al. 1997; Otake et al. 2010; Weigel et al. 2000). Mortensen et al. (2002) found that PRRSV entered negative herds through the introduction of animals and semen and through area spread from neighboring farms, which they attributed to aerosol transmission. Torremorell et al. (2004) attributed over 80% of new infections in commercial systems to area spread from neighboring units, the movement of pigs in PRRSV infected transports, the lack of compliance of the biosecurity protocols, or possibly, introduction via insects.

Proximity to infected herds is a well-recognized risk factor. The risk of a herd becoming infected increases with the density of PRRSV-positive neighboring herds, but decreases with distance between herds. Le Potier et al. (1997) found that 45% of herds suspected to have become infected through area spread were located within 500 m (0.3 mi) of the postulated source herd and only 2% were 1 km from the initial outbreak.

On the other hand, Goldberg et al. (2000) evaluated the ORF5 gene sequences from 55 field isolates collected in Illinois (USA) and eastern Iowa (USA) and found that the genetic similarity of isolates did not correlate with their geographical distance. On that basis, they concluded that PRRSV was most commonly introduced into herds through animals or semen, as opposed to mechanisms associated with spread from neighboring herds.

Stability in the Environment
Shedding of virus in saliva, urine, and feces results in environmental contamination and creates the potential for transmission via fomites. PRRSV is fragile and quickly inactivated by heat and drying. At 25–27°C, infectious virus was not detected on plastic, stainless steel, rubber, alfalfa, wood shavings, straw, corn, swine starter feed, or denim cloth, beyond day zero (Pirtle and Beran 1996). PRRSV can remain infectious for an extended time under specific conditions of temperature, moisture, and pH. PRRSV is stable for months to years at temperatures of −70 and −20°C. Jacobs et al. (2010) observed no difference in inactivation rate among four type 2 isolates in solution and estimated the virus half-life at 4°C (39°F)—155 hours, 10°C (50°F)—84.5 hours, 20°C (68°F)—27.4 hours, and 30°C (86°F)—1.6 hours.

The thermal stability of PRRSV in serum and tissues is similar to that described for virus stored in media. PRRSV was isolated from 47%, 14%, and 7% of porcine serum samples stored at 25°C for 24, 48, and 72 hours, respectively. When serum was stored at 4°C or −20°C, PRRSV was isolated from 85% of the samples after 72 hours (Van Alstine et al. 1993). PRRSV is stable at pH 6.5–7.5, but infectivity is rapidly lost at pH below 6 and above 7.5 (Benfield et al. 1992; Bloemraad et al. 1994).

Disinfection
PRRSV is inactivated by lipid solvents, for example, chloroform and ether (Benfield et al. 1992). PRRSV is highly unstable in solutions containing low concentrations of detergents, which disrupt the envelope with concomitant release of the noninfectious core particles and loss of infectivity (Snijder and Meulenberg 2001). At “room temperature,” Shirai et al. (2000) reported complete inactivation of PRRSV with chlorine (0.03%) in 10 minutes, iodine (0.0075%) in 1 minute, and a quaternary ammonium compound (0.0063%) in 1 minute. Decontamination protocols involving drying, thermo-assisted drying, and foaming disinfectants containing glutaraldehyde and quaternary ammonium chloride compounds are effective in inactivating PRRSV in farrowing rooms and transport vehicles in cold and warm climates (Dee et al. 2004, 2005a,b).

PATHOGENESIS
PRRS viral infection has been studied in germ-free, cesarean-derived/colostrum-deprived, or conventional pigs (Duan et al. 1997b; Halbur et al. 1995b, 1996a; Rossow et al. 1994a, 1995, 1996a). Following exposure, viral replication initially occurs primarily in local permissive macrophages and then rapidly spreads to lymphoid organs, lungs, and, less consistently, to other tissues. Virulent variants of PRRSV cause viremia as early as 12 hours in some pigs, and in all pigs by 24 hours postinoculation along with viral infection of lymphoid tissues and lungs. Viral loads peak in serum, lymph nodes, and lungs by days 7–14 with virus titers of $1 \times 10^2$–$10^4$ TCID$_{50}$/mL of serum or gram of tissue. The highest viral titers are consistently reported in the lungs.

PRRSV replicates primarily in differentiated subsets of monocyte-derived cells that display a 220-kDa glycoprotein receptor (sialoadhesin) and a transmembrane glycoprotein (CD163). Sialoadhesin binds PRRSV and mediates entry by receptor-mediated endocytosis (Duan et al. 1998; Kreutz and Ackermann 1996; Nauwynck et al. 1999; Vanderheijden et al. 2003; Wissink et al. 2003), while CD163 is essential for release of internalized PRRSV from early endosomes to the cytoplasm, a prerequisite for viral replication (Calvert et al. 2007; Van Gorp et al. 2009). The predominant differentiated cells known to support PRRSV replication include pulmonary alveolar macrophages (PAMs) and pulmonary intravascular macrophages (PIMs) in the lungs (Thanawongnuwech et al. 1997a; Wensvoort et al. 1991) and macrophages in lymphoid tissues (Duan et al. 1997b). Thus, the highest titers of PRRSV and most significant lesions are found in these tissues during acute PRRS.
Maturity and/or activation of PAM, and presumably other macrophages, is required for PRRSV replication (Duan et al. 1997a; Molitor et al. 1996, 1997; Thacker et al. 1998). PRRSV replicates in the subset of differentiated PAM with maximal ability to phagocytose bacteria and produce superoxide anion for bacterial killing in phagolysosomes (Molitor et al. 1996). Both PAM and PIM harvested from younger pigs replicate PRRSV to higher titers compared with older pigs (Mengeling et al. 1995; Thanawongnuwech et al. 1998b). Similarly, when young and older pigs are inoculated with identical PRRSV inocula, younger pigs replicate virus to higher titers and shed more virus than older pigs (Cho et al. 2006; van der Linden et al. 2003). PRRSV will also replicate in microglia (Molitor et al. 1997), but will not replicate in all monocyte-derived cells such as peripheral blood monocytes, peritoneal macrophages, and bone marrow progenitor cells (Duan et al. 1997a,b).

The largest amount of PRRSV antigen and/or nucleic acid is observed in the lungs and lymph nodes. Antigen/nucleic acid is also consistently observed in perivascular and intravascular macrophages in the heart, brain, kidneys, and elsewhere, and is inconsistently observed in alveolar, bronchial, and nasal epithelium; endothelium; fibroblasts; spermatids; and spermatoocytes (Halbur et al. 1995a,b, 1996a; Magar et al. 1993; Pol et al. 1991; Rossow et al. 1996a; Sirinarumitr et al. 1998; Sur et al. 1997; Thanawongnuwech et al. 1997a).

Generally, clinical disease and consistent lesions correspond to the time and sites of highest viral titers, that is, 7–14 DPI in lung and lymph nodes. In contrast, in stillborn and congenitally infected live-born pigs, viral antigen and nucleic acid are in largest amounts in lymphoid organs, but not lung (Cheon and Chae 2001).

After peaking, virus titers in serum decrease rapidly. Most pigs are no longer viremic by 28 DPI, although viral RNA has been detected in serum by reverse transcription-polymerase chain reaction (RT-PCR) up to 251 DPI (Duan et al. 1997b; Wills et al. 2003). The duration of viremia may be slightly longer in congenitally infected pigs and is consistently demonstrated by isolation of virus for up to 48 days after birth and infrequently by RT-PCR for up to 228 days (Rowland et al. 2003).

Following viremia, congenitally and postnatally infected pigs are persistently infected with virus in tonsil (Wills et al. 1997b) and/or lymph nodes, especially inguinal and sternal (Bierk et al. 2001; Xiao et al. 2004), for extended periods of time. Virus has been detected by VI for as long as 132–157 DPI (Rowland et al. 2003; Wills et al. 1997b). Persistent virus is produced in lymphoid tissues by a low level of continuous replication (Allende et al. 2000).

**Mechanisms of Cell Injury**

Replication of PRRSV in macrophages in lung and lymphoid tissues, and to a lesser extent other tissues, induces lesions and clinical disease by a variety of mechanisms. These include apoptosis of infected cells, apoptotic death of proximate noninfected cells (indirect or bystander apoptosis), induction of inflammatory cytokines, induction of polyclonal B-cell activation, and reduction in bacterial phagocytosis and killing by macrophages that results in increased susceptibility to septicemia (also likely due to other forms of immunomodulation by PRRSV).

Direct and indirect (bystander) apoptosis is a major cause of cell death. During acute infection when virus titers are high, only a small portion of macrophages in scattered foci are infected with PRRSV (Duan et al. 1997b; Mengeling et al. 1995), but there are large numbers of apoptotic mononuclear cells distributed diffusely (Sirinarumitr et al. 1998; Sur et al. 1998). Apoptotic cells rarely contain PRRSV and are in greatest numbers at 10–14 DPI. This suggests that PRRSV induces apoptosis in noninfected cells in proximity to infected cells. Most apoptotic cells are morphologically typical of macrophages; fewer are typical of lymphocytes.

Cell death in PRRSV-infected cells is by necrosis as well as by apoptosis involving activation of caspases and a mitochondria-mediated pathway (Costers et al. 2008; Lee and Kleiboeker 2007). The cause of PRRSV-induced indirect apoptosis is unknown, but is likely due to substances released from, or secreted by, infected macrophages, for example, p25, apoptogenic cytokines, reactive oxygen species, or nitric oxide (Choi and Chae 2002; Labarque et al. 2003; Suárez 2000).

Secretion of proinflammatory cytokines from PRRSV-infected macrophages likely results in effects that are both positive (recruitment of leukocytes, initiation of immune response, and reduction in viral replication) and negative (increased vascular permeability resulting in pulmonary edema and bronchial constriction). Studies demonstrated variable elevations in cytokine levels in bronchoalveolar lavage from PRRSV-infected versus uninfected control pigs, including IFN-γ, tumor necrosis factor (TNF)-α, interleukin (IL)-1, IL-6, IL-10, and IL-12 (Choi et al. 2001; Suradhat and Thanawongnuwech 2003; Thanawongnuwech et al. 2003; van Gucht et al. 2003). Production of these cytokines is predominately by macrophages located in alveolar septa in foci of inflammation and by lymphocytes positive for IFN-γ (Choi et al. 2001; Chung and Chae 2003; Thanawongnuwech et al. 2003). Of these cytokines, TNF-α, IL-1, and IL-6 are proinflammatory and known to promote influx and activation of leukocytes, increased microvascular permeability (pulmonary edema), and induction of systemic effects such as pyrexia, anorexia, and lethargy. TNF-α and IL-1 can also cause bronchial hyperreactivity and constriction with asthma-like symptoms.

PRRSV replication in lymphoid organs is also associated with polyclonal B-cell activation. Grossly, this appears as nodular lymphoid hyperplasia and
microscopically as follicular lymphoid hyperplasia (Lamontagne et al. 2001). Inoculation of neonatal gnotobiotic pigs with PRRSV results in lymphoid hyperplasia, extremely elevated levels of all classes of serum immunoglobulins (of which only 1% are specific for PRRSV), circulating immune complexes, immune complex deposition on glomerular basement membranes with associated cellular inflammation, and induction of autoantibodies to Golgi antigens and double-stranded DNA (dsDNA) (Lemke et al. 2004).

CLINICAL SIGNS


Clinical presentation of PRRS varies greatly between herds, ranging from asymptomatic to devastating. Clinical signs of PRRSV are influenced by virus variant, host immune status, host susceptibility, exposure to lipopolysaccharides (LPSs), concurrent infections, and other management factors (Blaha 1992; White 1992a).

Clinical disease in a herd is primarily the consequence of acute viremia in individuals (Collins et al. 1992; Pol et al. 1991; Terpstra et al. 1991a) and transplacental transmission resulting in reproductive failure (Terpstra et al. 1991a). Because PRRSV isolates vary in virulence, low virulence isolates can cause completely subclinical epidemic or endemic infections in herds (Morrison et al. 1992), whereas highly virulent isolates can cause severe clinical infections that appear differently, depending on the herd’s immune status.

Clinical epidemics occur when PRRSV enters an immunologically naive herd or production site and all ages are affected. Endemic PRRS occurs in herds that have homologous immunity to the infecting PRRSV. In endemic PRRS, clinical disease is observed in susceptible subpopulations, usually in nursery–grower pigs when maternal immunity decays, and/or in replacement gilts or sows that have previously escaped infection, as well as their congenitally infected progeny.

Antigenic variation is great enough among variants of PRRSV, that entry of a new, relatively unrelated virus can cause an epidemic in an endemically PRRSV-infected herd or region. This phenomenon was recently described with the emergence of a highly virulent type 2 PRRSV, with deletions in the Nsp2 gene that rapidly spread through eastern China (Li et al. 2007; Tian et al. 2007; Zhou et al. 2008).

Epidemic Infection

The first phase of a PRRS epidemic lasts 2 or more weeks and is characterized by anorexia and lethargy in 5–75% of animals of all ages because of acute viremia. It begins in one or more stages of production and quickly spreads in 3–7 or more days, depending on size and composition of the site, to all stages of production. Individuals are anorexic for 1–5 days. The spread of the disease through a segregated group of pigs usually requires 7–10 or more days, giving rise to the descriptive term “rolling inappetence.” Clinically affected animals may also be lymphopenic, pyretic with rectal temperatures from 102 to 106°F (39–41°C), hyperpneic and dyspneic, or have transient “blotchy” cutaneous hyperemia or cyanosis on extremities.

The second phase may begin before the first phase of acute illness is completed and continues for 1–4 months. This phase is characterized by reproductive failure, primarily in sows that were viremic in their third trimester, and by high preweaning mortality in their live-born progeny. When reproductive performance and preweaning mortality return to near pre-outbreak levels, endemic infection of most herds continues.

Sows. During the phase of acute illness, 1–3% of litters may be lost in sows that are at 21–109 days of gestation. This is recognized as overt abortions or, later, as irregular returns to estrus or nonpregnant sows (Hopper et al. 1992; Keffaber 1989; Loula 1991; White 1992a). Also, observed inconsistently in acutely ill sows are agalactia (Hopper et al. 1992), incoordination (de Jong et al. 1991), and/or a dramatic exacerbation of endemic diseases, such as sarcocystic mange, atrophic rhinitis, or cystitis/pyelonephritis (White 1992a).

Mortality in sows is typically 1–4% during acute illness and is sometimes associated with pulmonary edema and/or cystitis/nephritis (Hopper et al. 1992; Loula 1991). A few cases of severe acute PRRS in sows have been described with 10–50% abortions, up to 10% mortality, and nervous signs such as ataxia, circling, and paresis (Epperson and Holler 1997; Halbur and Bush 1997).

Late-term reproductive failure begins at approximately 1 week and continues for up to 4 months. Not all affected sows are clinically ill during acute PRRS. Typically, 5–80% of sows farrow on days 100–118 of gestation and have litters composed of any combination of normal pigs, weak variably sized pigs, and dead pigs that are fresh stillborn (intrapartum death), autolytic (brown), partially mummified, or completely mummified fetuses. Typically, pigs born dead comprise 0–100% of each affected litter and 7–35% of the total pigs born in a farrowing group. In time, there is a shift from predominantly stillborn pigs and large partially mummified pigs, to smaller more completely mummified pigs, to small weak-born pigs, to pigs of normal
size and vigor (Keffaber 1989; Loula 1991; White 1992a). In some herds, the majority of abnormal pigs are born alive, premature, weak, and small, but few are born dead (Gordon 1992). Periparturient mortality in sows may be 1–2% (de Jong et al. 1991; Keffaber 1989). Surviving sows on the subsequent breeding often have delayed return to estrus and low conception rates.

**Boars.** During acute illness, in addition to anorexia, lethargy, and respiratory clinical signs, boars may lack libido and have variable reduction in semen quality (de Jong et al. 1991; Feitsma et al. 1992; Prieto et al. 1994). Changes in sperm occur 2–10 weeks after infection with virus and include reduced motility and acrosomal defects, but it is unclear whether conception rates are affected (Lager et al. 1996; Prieto et al. 1996a,b; Swenson et al. 1994b; Yaeger et al. 1993). However, of much greater significance is semen shedding of PRRSV that can result in venereal transmission to sows (Swenson et al. 1994b; Yaeger et al. 1993).

**Suckling Pigs.** During the 1- to 4-month phase of late-term reproductive failure, there is high preweaning mortality (up to 60%) in pigs born prematurely or at term associated most consistently with listlessness, emaciation/starvation, splaylegged posture, hyperpnea, dyspnea (“thumping”), and chemosis. Seen less commonly are tremors or paddling (Keffaber 1989; Loula 1991), slight doming of foreheads (Gordon 1992), anemia and thrombocytopenia with consequent hemorrhage from navels and elsewhere, and an increase in bacterial polyarthritis and meningitis (Hopper et al. 1992; White 1992a). Watery diarrhea was commonly reported in the United Kingdom (Gordon 1992; Hopper et al. 1992; White 1992a) and less commonly elsewhere (Keffaber 1989; Leyk 1991).

**Weanling and Grower Pigs.** Acute PRRSV infection in nursery or grower–finisher pigs is characterized most consistently by anorexia, lethargy, cutaneous hyperemia, hyperpnea and/or dyspnea without coughing, rough hair coats, and variable reduction in average daily gain creating uneven groups (Moore 1990; White 1992b). Frequently reported is a higher than usual incidence of endemic diseases and elevated mortality of 12–20% (Blaha 1992; Keffaber et al. 1992; Loula 1991; Moore 1990; Stevenson et al. 1993; White 1992a). Diseases most commonly reported include streptococcal meningitis, septicemic salmonellosis, Glässer’s disease, exudative dermatitis, sarcoptic mange, and bacterial bronchopneumonia.

**Endemic Infection of Herds**

Once introduced into a herd, PRRS becomes endemic in nearly all cases. In endemically infected herds, PRRS is most often seen as regular or occasional outbreaks of typical acute PRRS in susceptible nursery or grower–finisher pigs (Keffaber et al. 1992; Stevenson et al. 1993). Clinical signs are also observed in groups of susceptible gilts or replacement boars exposed to PRRSV after introduction into the herd (Dee and Joo 1994b; Dee et al. 1996; Grosse-Beilage and Grosse-Beilage 1992), but may also be seen in susceptible sows. Acute clinical disease in gilts or boars is as described for epidemics. The reproductive consequences depend on the number of gilts/sows infected and the stage of their reproductive cycle when infected, both of which may vary widely (Torrison et al. 1994). If few gilts are infected on an ongoing basis, then there may be scattered abortions, irregular returns to estrus, nonpregnant gilts, and late-term reproductive failure with abnormal litters typical of PRRS. These may only be recognized if records are evaluated on a parity-specific basis (White 1992b). Alternatively, gilts may escape exposure to PRRSV until there is a significant subpopulation of susceptible gilts in various stages of gestation. In this situation, endemic PRRS in the breeding herd manifests as periodic mini-outbreaks of PRRS in gilts and, less commonly, sows, that are identical to those in an epidemic (Dee and Joo 1994b).

**Factors Affecting the Severity of Disease**

Differences in the expression of clinical disease are incompletely understood and affected by many factors, including virus variant, immune status (discussed elsewhere), host susceptibility, exposure to LPS, and concurrent infections. Management factors, for example, pig flow, building design, and temperature regulation, likely have impact but are poorly defined.

PRRSVs differ genetically (Li et al. 2007; Murtaugh et al. 1995; Tian et al. 2007), antigenically (Nelson et al. 1993; Wensvoort et al. 1992), in severity of induced respiratory disease and lesions (Halbur et al. 1995b, 1996a,b; van der Linden et al. 2003), and in severity of induced reproductive disease (Mengeling et al. 1998; Park et al. 1996). High virulence viruses, as compared with low virulence viruses, are known to result in significantly more viral antigen in lung and lymphoid tissues (Halbur et al. 1996a), viremia of higher peak titer and of longer duration (Grebennikova et al. 2004; van der Linden et al. 2003), and more pulmonary IFN-γ-producing cells (Thanawongnuwech et al. 2003).

A few PRRSV inoculation studies in purebred animals have suggested differences in disease between breeds. Halbur et al. (1998) reported significant differences in pulmonary lesions, number of pulmonary PRRSV antigen-positive cells, incidence of myocarditis, and incidence of encephalitis. Christopher-Hennings et al. (2001) reported differences in duration of PRRSV shedding in semen of infected boars.

Bacterial LPS (endotoxin) is a major component of bacterial cell walls and is known to be in high levels in dust in poorly ventilated swine buildings (Zejda et al. 1994). Intratracheal administration of LPS in
PRRSV-inoculated pigs as compared with pigs given only PRRSV or LPS resulted in consistently more severe clinical respiratory disease that was temporally associated with 10- to 100-fold elevations of IL-1, IL-6, and TNF-α, but not differences in gross and microscopic lung lesions or number of inflammatory cells in bronchoalveolar lavage fluids (Labarque et al. 2002; van Gucht et al. 2003).

Infection with PRRSV renders pigs more susceptible to some bacterial and viral diseases and has an additive or synergistic effect with some other bacteria or viruses to create more severe disease than either agent alone. Postnatal and congenital infection with PRRSV leaves pigs more susceptible to development of septicemia with Streptococcus suis (Feng et al. 2001; Galina et al. 1994). Studies have demonstrated that a likely mechanism is replication in and killing of PIMs and PAMS, as well as reduction in ability of virus-infected PIMs and PAMS to phagocytose and kill bacteria (Thanawongnuwech et al. 1997b, 1998a,b, 2000a,b). This mechanism may be operative in rendering pigs with acute PRRS more susceptible to septicemia by other bacteria, but experimental proof is lacking. Infection of weanling pigs with PRRSV also renders them more susceptible to development of bronchopneumonia due to Bordetella bronchiseptica (Brockmeier et al. 2000). This might be due to PRRSV infection of PAMS and resulting reduction in ability to kill bacteria (Thanawongnuwech et al. 1997b). Acute PRRS increases incidence and severity of clinical disease following inoculation with Salmonella choleraesuis (Wills et al. 2000a). Infection with PRRSV also significantly enhances the replication of porcine circovirus type 2 (PCV2), resulting in more severe PRRS viral pneumonia as well as lesions of PCV2-associated porcine multisystemic wasting syndrome (Allan et al. 2000; Harms et al. 2001). Attempts to experimentally confirm field observations of increased susceptibility in PRRSV-infected pigs to diseases caused by confirm field observations of increased susceptibility in 2000; Harms et al. 2001). Attempts to experimentally

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LESIONS

Postnatal Lesions

Similar lesions are described in all ages of pigs with PRRSV infection. The severity and distribution of lesions varies with the virulence of the infecting virus (Done and Paton 1995; Halbur et al. 1996b). Most inoculation studies in which lesions have been described were in suckling or weaned pigs 1–70 days of age (Collins et al. 1992; Dea et al. 1992c; Halbur et al. 1995b, 1996a;b; Pol et al. 1991; Rossow et al. 1994a, 1995). Gross and microscopic lesions are consistently observed from 4 to ≥ 28 DPI in the lungs and lymph nodes, where most viral replication takes place. Later, only microscopic lesions are inconsistently observed, beginning at approximately 7–14 DPI, in the kidneys, brain, heart, and elsewhere where there is much less virus; mostly in perivascular and intravascular macrophages and endothelial cells. Microscopic lesions are also in the uterus of dams following reproductive failure and in the testicles of boars. Lesions unique to pigs inoculated at ≤ 13 days of age include periocular edema 6–23 DPI, scrotal edema 11–14 DPI, and subcutaneous edema 2–7 DPI (Rossow et al. 1994a, 1995).

Lungs have interstitial pneumonia from 3 to ≥ 28 DPI that is most severe 10–14 DPI. Mild lesions are in cranial lungs or diffuse. Affected parenchyma is resilient, slightly firm, noncollapsing, mottled gray-tan, and moist. Severe lesions are diffusely distributed, and parenchyma is mottled or diffusely red-tan, noncollapsing, firm and rubbery, and very moist. Microscopically, alveolar septa are expanded by macrophages, lymphocytes, and plasma cells and may be lined by hyperplastic type II pneumocytes. Alveoli may contain necrotic macrophages, cell debris, and serous fluid. Lymphocytes and plasma cells form cuffs around the airways and blood vessels. Rarely, PRRSV antigen is described in few bronchial epithelial cells with cell swelling and loss of cilia (Done and Paton 1995; Pol et al. 1991). In field cases of PRRS, especially in nursery and grower–finisher pigs, PRRS pulmonary lesions are often complicated or obscured by lesions of concurrent bacterial and/or viral diseases.

Lesions in lymph nodes are observed 4–28 or more DPI (Dea et al. 1992c; Halbur et al. 1995b; Rossow et al. 1994a,b, 1995). Many lymph nodes in most pigs are enlarged from 2 to 10 times normal. Early postinoculation, enlarged nodes are edematous, tan, and moderately firm. Later, nodes are firm and white or light tan in a nodular or diffuse pattern. Uncommonly, there are multiple fluid-filled 2- to 5-mm-diameter cortical cysts. Microscopic lesions are predominantly in germinal centers. Early in the course of infection, germinal centers are necrotic and depleted. Later, germinal centers are very large and composed of blast-type lymphocytes. The cortices may contain small cystic spaces lined by endothelium and containing proteinaceous fluid, lymphocytes, and multinucleate prokaryocytes (Rossow et al. 1994b, 1995). Microscopically, there may be mild lymphoid necrosis, depletion, and/or hyperplasia in the thymus, in periarteriolar lymphoid sheaths of the spleen, and in lymphoid follicles in tonsil and Peyser’s patches (Halbur et al. 1995b; Pol et al. 1991).
Mild to moderate multifocal lymphohistiocytic vasculitis and perivascular myocarditis may develop in the heart ≥ 9 DPI (Halbur et al. 1995a, 1996b; Rossow et al. 1994a, 1995). Less commonly, mild myocardial fibrillar necrosis and lymphocytic cuffing of Purkinje fibers is described (Rossow et al. 1995).

Mild lymphohistiocytic leukoencephalitis or encephalitis involving cerebellum, cerebrum, and/or brain stem may develop ≥ 7 DPI (Collins et al. 1992; Halbur et al. 1996b; Rossow et al. 1995; Thanawongnuwech et al. 1997a). There is segmental cuffing of blood vessels by lymphocytes and macrophages and multifocal gliosis. Necrotizing vasculitis was also described in one field case of PRRS with neurological clinical disease (Thanawongnuwech et al. 1997a).

Kidneys occasionally have mild periglomerular and peritubular lymphohistiocytic aggregates from 14 to 42 DPI (Cooper et al. 1997; Rossow et al. 1995). Cooper et al. (1997) described mild to severe segmental vasculitis that was most severe in the pelvis and medulla. Affected vessels had swollen endothelium, pooled subendothelial proteinaceous fluid, fibrinoid medial necrosis, and intramural and perivascular aggregates of lymphocytes and macrophages.

By 12 hours postinoculation, the nasal mucosal epithelium may have clumped or absent cilia and epithelial cell swelling, loss, or squamous metaplasia (Collins et al. 1992; Halbur et al. 1996b; Pol et al. 1991; Rossow et al. 1995). By 7 DPI, lymphocytes and macrophages are in the epithelium and propria-submucosa.

Microscopic lesions are frequently in the uterus of pregnant sows with natural or experimental PRRS (Christianson et al. 1992; Lager and Halbur 1996; Stockhofe-Zurwieden et al. 1993). The myometrium and/or endometrium are edematous with lymphohistiocytic perivascular cuffs. Less commonly, there is segmental lymphohistiocytic vasculitis in small vessels and microseparations between endometrial epithelium and placental trophoblasts that contain eosinophilic proteinaceous fluid and cell debris.

Atrophy of seminiferous tubules is seen in 5- to 6-month-old boars 7–25 DPI (Sur et al. 1997). Atrophic tubules have PRRSV antigen and nucleic acid in germinal epithelial cells, giant cells with 2–15 nuclei, and apoptosis and depletion of germ cells.

**Fetal Lesions**

PRRS reproductive failure should be suspected when litters are delivered at ≥100 days of gestation, but before term, and are composed of variable proportions of clinically normal pigs, small- or normal-sized weak pigs, dead variably autolyzed pigs, and mummies. Lesions in fetuses and stillborn pigs are uncommon and rarely contribute to a definitive diagnosis of PRRS. An absence of lesions in fetuses does not rule out PRRS. Segmental edematous and hemorrhagic enlargement of umbilical cords in aborted or stillborn pigs is an inconsistent lesion that is highly suggestive of PRRSV.

PRRSV-infected litters contain variable numbers of normal pigs, small weak pigs, and dead pigs that are either fresh stillborn (intrapartum deaths), autolytic stillborn (prepartum deaths), or partially mummified/ completely mummified fetuses. Dead pigs are commonly coated with a thick brown mixture of meconium and amniotic fluid, a nonspecific finding that suggests fetal stress and/or hypoxia (Lager and Halbur 1996; Stockhofe-Zurwieden et al. 1993). Most lesions in fetuses are nonspecific and due to sterile in utero autolysis.

PRRSV-specific gross and microscopic lesions are few and inconsistent. These are best observed in fetuses with little or no in utero autolysis (Bøtner et al. 1994; Collins et al. 1992; Done and Patton 1995). Lesions are more commonly seen in live-born PRRS-infected littermates that die or are sacrificed within a few days after birth. Gross fetal lesions include perirenal edema, edema of the splenic ligament, mesenteric edema, ascites, hydropneumothorax, and hydropneumonitis (Dea et al. 1992c; Lager and Halbur 1996; Plana Duran et al. 1992b). Microscopic lesions are mild and nonsuppurative and include segmental arteritis and periarteritis in lung, heart, and kidney (Lager and Halbur 1996; Rossow et al. 1996b); multifocal interstitial pneumonia with occasional hyperplasia of type II pneumocytes (Plana Duran et al. 1992b; Sur et al. 1996); mild periportal hepatitis (Lager and Halbur 1996); myocarditis with loss of myocardial fibers (Lager and Halbur 1996; Rossow et al. 1996b); and multifocal leukoencephalitis (Rossow et al. 1996b).

An uncommon, but diagnostically discriminating lesion is segmental hemorrhagic enlargement of the umbilical cord up to three times normal diameter that is caused by segmental necro supplicative and lymphohistiocytic vasculitis (Lager and Halbur 1996).

**Unique Lesions Described in Asia**

An epidemic of PRRS caused by a variant of type 2 PRRSV spread across eastern China in the summer of 2006 affecting over 2 million pigs with an average mortality of 20% (Li et al. 2007; Tian et al. 2007; Zhou et al. 2008). Gross lesions in field cases were as described above for PRRS with the addition of lesions unique to PRRS including petechiae and ecchymoses on renal cortices, hepatic capsule, and visceral pleura. Occasionally, splenic infarcts and hematuria were observed. However, in inoculation studies using virus isolates from infected farms, only lesions more typical of PRRS and none of these unique lesions were produced (Li et al. 2007; Zhou et al. 2008). It is unclear if these “unique” lesions attributed to PRRS were caused by the offending variant of PRRSV or to concurrent bacterial pathogens. Reports described concurrent
infections with streptococci, *Escherichia coli*, *H. parasuis*, and others (Li et al. 2007; Tian et al. 2007).

**DIAGNOSIS**

The possible involvement of PRRSV is suggested in any herd with reproductive diseases in breeding swine and/or respiratory disease in pigs of any age. Production records in herds with clinically active PRRSV show increased abortions, early farrowings, stillbirths, pre-weaning mortality, and nonproductive sow days. However, the absence of overt clinical signs does not mean that a herd is free of PRRSV infection.

Postnatal virulent PRRSV produces consistent gross lesions of interstitial pneumonia and enlarged lymph nodes in all ages of swine. These lesions are suggestive of PRRSV, but are not diagnostic since a variety of other viral and bacterial diseases can cause similar lesions. Typical microscopic lesions of PRRSV sometimes allow a strong presumptive diagnosis. However, a definitive diagnosis always requires demonstration of PRRSV.

Table 31.1 summarizes the various diagnostic assays and their recommended use. Depending on the region, the differential diagnosis may include classical swine fever virus, cytomegalovirus, hemorrhagglutinating encephalomyelitis virus, leptospirosis, parvovirus, PCV2, pseudorabies (*Aujeszky’s disease*) virus, swine influenza virus, and teschovirus (Halbur 2003). Commonly, concurrent viral and/or bacterial infections complicate the clinical picture and diagnosis. Thus, a definitive diagnosis requires diagnostic laboratory confirmation of the presence of virus, viral products, and/or antibodies (Table 31.1).

**Pathological Evaluation**

There are no pathognomonic gross or microscopic lesions for PRRSV and aborted fetuses and stillborn pigs rarely have lesions of diagnostic value. Gross lesions of interstitial pneumonia and enlarged lymph nodes may be observed in infected pigs of all ages (Lager and Halbur 1996; Stevenson et al. 1993). Microscopically, interstitial pneumonia is the primary lesion.

**Virus Isolation**

Although the kinetics of virus replication vary significantly among isolates, the virus is generally detected in higher amounts and for a longer time in younger pigs, reaching peak levels at 4–7 DPI before declining to undetectable levels by 28–35 DPI. Viremia may persist for 28–42 DPI in suckling, weaned, and grower pigs and for 7–21 DPI in sows and boars (Christopher-Hennings et al. 1995a,b; Kittawornrat et al. 2010; Mengeling et al. 1996). Infectious virus and/or viral RNA can be demonstrated in pulmonary lung lavage, oral fluid, tonsil, and lymph nodes for several weeks after cessation of viremia (Horter et al. 2002; Mengeling et al. 1995; Ramirez et al. 2011; Rowland et al. 2003; Wills et al. 2003). Virus persists longer in tonsil and lymph nodes than in serum, lung, and other specimens. For example, virus was isolated from tonsil and oropharyngeal scrapings at 130 and 157 DPI, respectively, under experimental conditions (Rowland et al. 2003; Wills et al. 2003).

Specimens for VI should be refrigerated (4°C) immediately after collection and shipped to the diagnostic laboratory within 24–48 hours. The virus is heat-labile and has a narrow range of pH stability (Benfield et al. 1992; Bloemraad et al. 1994; Jacobs et al. 2010; Van Alstine et al. 1993).

Virus can be isolated on PAMs or sublines (CL-2621, MARC-145) of the African monkey kidney cell line, MA-104 (Benfield et al. 1992; Kim et al. 1993). PAMs are reportedly more sensitive than MARC-145 cells for VI and the presence of Fc receptors on PAMs could improve the success of isolating PRRSV in the presence of antibodies (Yoon et al. 2003).

Isolates of PRRSV vary in their ability to replicate in PAMs and MA-104 cells (Bautista et al. 1993), suggesting that VI should be conducted on both cell types for optimum recovery of virus from clinical specimens (Yoon et al. 2003). The use of MARC-145 cells may bias toward isolation of modified live vaccine viruses because vaccine viruses are adapted to MA-104 cells. The use of PAMs is mandatory for successful isolation of type 1 (European) and European-like viruses (Christopher-Hennings et al. 2002; Wensvoort et al. 1991).

Isolation of PRRSV on cell culture may be confirmed by RT-PCR or by visualizing viral antigens in the cytoplasm of infected cells by fluorescent antibody (FA) or immunohistochemistry (IHC) using PRRSV-specific monoclonal antibodies (Nelson et al. 1993). Negative-stain electron microscopy (EM) can also be used to visualize virus particles in cell culture fluids.

**Detection of Virus**

Lung, tonsil, lymph nodes, heart, brain, thymus, spleen, and kidney may be fixed in 10% neutral buffered formalin for microscopic evaluation and IHC (Halbur et al. 1994; Van Alstine et al. 2002; Yaeger 2002). The combination of histopathology and IHC allows the visualization of viral antigens in the cytoplasm of cells within or contiguous to microscopic lesions (Halbur et al. 1994). Tissues should be processed within 48 hours of fixation to avoid degradation of PRRSV antigens and loss of IHC-positive cells (Van Alstine et al. 2002). Lesions and viral antigens are best observed during acute infection (4–14 DPI) when viral titers and quantities of viral antigen in the cytoplasm of infected cells are highest. Detection of PRRSV antigens in lung by IHC requires the examination of at least five sections of anteroventral lung to identify >90% of PRRSV-infected pigs (Yaeger 2002).

Viral antigen can also be detected in frozen lung sections by FA (Benfield et al. 1992; Halbur et al. 1996a;
Table 31.1. Summary of the use of diagnostic assays for the detection of PRRSV infection

<table>
<thead>
<tr>
<th>Assay</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>In Utero Infection</th>
<th>1–28 DPI</th>
<th>30–90 DPI</th>
<th>≥90 DPI</th>
<th>Optimum Window of Detection</th>
</tr>
</thead>
<tbody>
<tr>
<td>VI</td>
<td>Moderate</td>
<td>High</td>
<td>Samples from live-born pigs preferred; samples from stillborn or mummies of limited diagnostic value; see list of tissues for acute 1–28 DPI; umbilical cords and umbilical cord blood also appropriate</td>
<td>Serum, oral fluid, lungs, tonsils, lymph nodes, lung lavage, heart, kidney, spleen, thymus</td>
<td>Tonsils, oropharyngeal scrapings, serum, lung lavage</td>
<td>Tonsils, oropharyngeal scrapings; poor success rate</td>
<td>Serum and most tissues positive from 1 to 35 DPI and longer in lymphoid tissues</td>
</tr>
<tr>
<td>FA</td>
<td>Moderate</td>
<td>Results depend on PRRSV isolate that infected the pigs</td>
<td>Lung or bronchoalveolar macrophages (lung lavage)</td>
<td>Lung lavage and direct culture of macrophages from lavage sample</td>
<td>Not recommended—lacks diagnostic sensitivity</td>
<td>4–14 DPI</td>
<td></td>
</tr>
<tr>
<td>IHC</td>
<td>Moderate</td>
<td>High</td>
<td>Most tissues from live-born pigs</td>
<td>See list for VI</td>
<td>Limited success with lymphoid tissues from 30–70 DPI</td>
<td>Not recommended—lacks diagnostic sensitivity</td>
<td>4–14 DPI</td>
</tr>
<tr>
<td>RT-PCR</td>
<td>High</td>
<td>High</td>
<td>Virus detected in most tissues from live-born pigs; limited success with thoracic fluid and tissues from stillborn fetuses</td>
<td>Oral fluid, serum, semen, or blood swab from boars, samples listed for VI</td>
<td>Tonsils, oropharyngeal scrapings, and lung lavage; good success rate</td>
<td>Results similar to VI, with occasional reports of prolonged detection by RT-PCR</td>
<td></td>
</tr>
<tr>
<td>ELISA</td>
<td>High</td>
<td>High</td>
<td>Antibodies in umbilical cord blood, but only in VI- or RT-PCR-positive piglets</td>
<td>Oral fluid, muscle transudate (meat juice), serum</td>
<td>Serum</td>
<td>Antibodies detected 9–14 DPI and persist for at least 12 months</td>
<td></td>
</tr>
<tr>
<td>IFA</td>
<td>High</td>
<td>Results depend on PRRSV isolate used in the test</td>
<td>Antibodies in umbilical cord blood, but only in VI- or RT-PCR-positive piglets</td>
<td>Muscle transudate (meat juice)</td>
<td>Serum</td>
<td>Antibodies detected 9–14 DPI and persist up to 5 months</td>
<td></td>
</tr>
<tr>
<td>VN</td>
<td>Low</td>
<td>Results depend on PRRSV isolate used in the test</td>
<td>Not detected in live-born or stillborn piglets</td>
<td>Serum</td>
<td>Serum</td>
<td>Initial detection variable, but usually 28–46 DPI and persist up to 12 months</td>
<td></td>
</tr>
</tbody>
</table>

VI, virus isolation; FA, fluorescent antibody on frozen samples; IHC, immunohistochemistry using formalin-fixed tissues; RT-PCR, reverse transcriptase-polymerase chain reaction; ELISA, commercial HerdChek® PRRS IDEXX; IFA, indirect fluorescent antibody; VN, virus neutralization.
Rossow et al. 1995). Tissues should be collected during peak viral replication. The FA test is faster and less expensive than IHC, but has the disadvantage of requiring fresh tissue.

Both IHC and FA use monoclonal antibodies to detect viral nucleocapsid antigen in the cytoplasm of infected cells. Interpretation of both tests is dependent on the operator's ability and skill to differentiate positive results from nonspecific background staining. Positive IHC or FA results may be confirmed by VI or RT-PCR.

Development of an immunochromatographic test strip for the rapid detection of PRRSV has been reported (Zhou et al. 2009b). To perform the test, sera or tissue homogenate is added to a mixture containing monoclonal antibodies to the nucleocapsid (N) and membrane (M) proteins of PRRSV. Virus binds to the antibody and the antigen–antibody complexes are then captured on chromatographic test strips. Positive reactions are detected using a labeled antibody that binds to the monoclonal antibodies. The assay is not commercially available at this time.

The first PRRSV RT-PCR was developed to detect PRRSV in semen and serum of boars (Christopher-Hennings et al. 1995b, 1996, 2001). This approach made it possible to detect PRRSV in semen without the need to attempt VI or bioassay. Subsequently, numerous PRRSV PCRs have been described, and PCR continues to be an area of rapid development. Two commercial real-time reverse transcription-polymerase chain reaction (qRT-PCR) assays are currently available in North America and many other parts of the world for the detection of both type 1 and type 2 PRRSVs. The primary concerns with PRRSV RT-PCR assays have been false-positive results, inability to detect genetically diverse isolates, ability to detect both type 1 and type 2 genotypes with a single assay, and the lack of uniform assay performance between laboratories. The continued development of PCR technology and the wider availability of standardized commercial assays will continue to lead to improvements in these areas.

In acute infections, serum and tissues appropriate for VI are the preferred specimens for PCR. In boars, serum is the preferred sample for detection of PRRSV during the first 6 DPI (Reicks et al. 2006a,b), whereas virus can be detected in semen up to 92 DPI with a mean of 35 DPI (Christopher-Hennings et al. 1995a,b, 2001). Pooling of serum, semen, or blood swabs is not recommended as this reduces the sensitivity of RT-PCR (Rovira et al. 2007). In persistently infected swine, PRRSV nucleic acid has been detected up to 86 DPI in lymph nodes (Bierk et al. 2001), 105 DPI in oropharyngeal scrapings (Horter et al. 2002), and 251 DPI in serum and tonsil homogenates (Wills et al. 2003) by PCR.

Oral fluid specimens collected from individual or pens of pigs and tested by qRT-PCR may be used for surveillance of PRRSV in populations (Prickett et al. 2008b). Depending on the pattern of circulation in the population, PRRSV may be detected for 2 weeks to several months (Ramirez et al. 2011). Kittawornrat et al. (2010) detected virus in oral fluid specimens collected from individual boars for at least 3 weeks. PCR-detectable virus is stable in oral fluid, but samples should be frozen or refrigerated to preserve target integrity until tested (Prickett et al. 2010). Oral fluid samples should be tested using PCRs verified to function adequately in the oral fluid matrix (Chittick et al. 2011). For surveillance, a sampling interval of 2–4 weeks appeared to be effective for the detection of virus in swine herds with circulating PRRSV (Prickett et al. 2008a; Ramirez et al. 2011).

Depending on the application, the fact that PCR cannot differentiate between infectious and noninfectious virus may be important to the interpretation of the results. Agreement between PCR and detection of live PRRSV by swine bioassay was 94% and 81% for semen and oropharyngeal scrapings, respectively (Christopher-Hennings et al. 1995b; Horter et al. 2002), suggesting that PCR-positive specimens are likely to contain infectious virus. However, virus stability studies found that qRT-PCR results did not reflect the inactivation of infectious PRRSV over time (Hermann et al. 2006; Jacobs et al. 2010). Thus, PCR can detect inactivated PRRSV, and inactivated PRRSV is relatively stable in the environment.

A recent technical development termed loop-mediated isothermal amplification (LAMP) offers the advantage of RT-PCR that does not require the sophisticated equipment used in most laboratories for PCR. The technique is similar to RT-PCR in that a specific fragment of the DNA is amplified, but it can be done at a constant temperature in a water bath or heat block rather than a thermal cycler. The amplified product is visualized by the amount of turbidity (correlates with amount of amplified DNA) in the reaction tube or the addition of SYBR® green into reaction mixtures resulting in visualization of a color change from yellow to green (Li et al; 2009; Rovira et al. 2009). While RT-LAMP tests have lower diagnostic sensitivity than RT-PCR, there is potential for this technique to be adapted in laboratories where RT-PCR instrumentation is too expensive or technical to be implemented (Rovira et al. 2009).

**Characterization of Isolates**

Prior to the ready availability of sequencing, PRRSV isolates were differentiated using ORF5 restriction fragment polymorphism (RFLP) (Umthun and Mengeling 1999; Wesley et al. 1998). Increasingly, it is recognized that RFLP patterns are an insensitive method for characterizing genetic relatedness among viruses. That is, genetically diverse PRRSV can share similar RFLP patterns. Sequencing and phylogenetic analyses provide
more precise and accurate descriptions of results for differentiating isolates of PRRSV. Unfortunately, RFLP designations are still commonly used in the field to characterize PRRSVs.

Sequencing is usually done on ORF5 and ORF6 with direct PCR products from diagnostic samples to avoid the potential bias of selection, mutation, or nucleotide changes by passage in cell culture. The ORF5 sequences are highly variable, and there is an extensive databank of sequences available for comparison. Dendograms (phylogenetic tree) shows phylogenetic relationships based on genetic similarity/dissimilarity. Identity matrices can depict similarity and difference among genomic sequences.

**Detection of Antibody**

Serological diagnosis is favored by practitioners because serum is easily collected in quantities for multiple tests and stored for future reference. Antibodies can also be detected in oral fluid specimens (Prickett et al. 2010). The demonstration of seroconversion (negative to positive) using acute and convalescent serum samples is the definitive method to serologically diagnose PRRSV infection. Increasing titers of PRRSV-specific antibody demonstrated by indirect fluorescent antibody (IFA) or rising enzyme-linked immunosorbent assay (ELISA) S/P ratios in a group of infected animals can also indicate PRRSV infection.

Serology is not a valid approach for diagnosis of PRRSV in previously infected or vaccinated herds, because serological assays cannot differentiate among antibodies resulting from the initial infection, reinfection, or vaccination. Single serum samples are of limited use due to the high prevalence of PRRSV in herds. For that reason, a single positive serological result does not prove a causal role for PRRSV in a clinical diagnosis. Detection of antibodies in nursing and nursery pigs may represent maternal antibodies that persist until pigs reach 3–5 weeks of age.

The three tests most commonly used to detect antibodies to PRRSV are IFA, ELISA, and VN. The IFA detects immunoglobulin M (IgM) and immunoglobulin G (IgG) antibodies as early as 5 and 9–14 DPI, respectively (Joo et al. 1997). IgM antibodies persist for 21–28 DPI, whereas IgG antibodies peak at 30–50 DPI and are undetectable 3–5 months after infection. Test sensitivity is impacted by the technical skill of laboratory personnel and antigenic differences between the PRRSV isolate used in the IFA and the field virus that induced antibodies in the pig. IFA is generally used to confirm suspected false-positive ELISA results, but has also been used to detect PRRSV antibodies in muscle transudate and oral fluid samples in surveillance programs (Molina et al. 2008a; Prickett et al. 2010).

The commercial ELISA (HerdChek® X3 PRRS ELISA, IDEXX Laboratories Inc., Westbrook, ME) is the “reference standard” for the detection of antibodies to PRRSV. The assay is sensitive, specific, standardized, and rapid. A study done by three diagnostic laboratories indicated a 99.9% diagnostic specificity using 1445 negative sera (Iowa State University 2010). The test targets antibodies to the nucleocapsid antigens for both North American and European-like PRRSVs. Antibodies can be detected as early as 9 DPI, peak at 30–50 DPI, and then decline to negative levels 4–12 months after infection. The ELISA antibody response of individual pigs is highly variable, and the assay should not be used to estimate the stage of infection (Roberts 2003).

Interpretation of ELISA negative and positive samples collected at a single point in time is difficult. Negative ELISA samples have several possible interpretations: (1) Pigs were not infected; (2) pigs were recently infected and have not seroconverted; (3) pigs are persistently infected, but have become seronegative; (4) pigs have cleared the infection and reverted to seronegative; or (5) pigs are negative due to low test sensitivity (Yoon et al. 2003). Pigs negative for antibodies on ELISA can be persistently infected, as demonstrated by isolation of infectious virus or detection of viral RNA in tonsil, lymphoid tissues, or oropharyngeal scrapings (Fangman et al. 2007; Horter et al. 2002).

In addition to the commercial ELISA that utilizes the nucleocapsid protein as the antigen, studies have also shown that nsp1, 2, and 7 induce high levels of antibody during PRRSV infections. An ELISA was developed using nsp2 and nsp7 to monitor antibody responses in pigs to both North American and European isolates of PRRSV. Antibodies were detected at 14 DPI and, during the first 126 DPI, the results of the ELISA tests using nsp2 or nsp7 as antigen correlated well with those of the 2X ELISA kit. The nsp7 ELISA also resolved 98% of samples with suspected false-positive 2X ELISA results. Thus, an nsp7-based ELISA has potential as an alternative or confirmatory test for the commercial 2X ELISA kit (Brown et al. 2009).

The VN assay detects antibodies capable of neutralizing a constant amount of PRRSV in cell culture. The test is highly specific, but antibodies do not develop until 1–2 months after infection (Benfield et al. 1992). These antibodies typically peak 60–90 DPI and persist up to 1 year after infection. Like IFA, the magnitude of the VN response is highest when homologous virus is used in the assay. The VN assay has not been standardized between laboratories and is not generally used as a routine diagnostic test.

**IMMUNITY**

Infection with PRRSV induces protective immunity, although it is slow to develop. For example, protective immunity is the mechanism underlying the elimination of PRRSV from populations using “herd closure” (Schaefer and Morrison 2007; Torremorell et al. 2002). In herd closure, pigs sharing a common airspace are
exposed to live virus by inoculation or vaccination with modified live vaccine, and no additional animals are introduced for a period of at least 200 days. Complete elimination of virus, that is, sterilizing immunity, is shown by the absence of new infections when susceptible animals are introduced to the population.

Live virus is necessary for induction of protection. Killed virus, subunit proteins, and other experimental vaccines that do not contain live PRRSV have not induced effective protection against PRRSV. The properties that confer protection have not been identified, despite extensive examination of major envelope proteins and testing of recombinant viruses containing nonstructural and structural coding regions implicated in virulence or protection.

Susceptibility to infection decreases with age, that is, sows are substantially more resistant to infection than are weaned pigs (Klinge et al. 2009). Immune induction may be independent of age, but findings related to infection and immunity in young pigs should be interpreted cautiously when applied to older swine. Immunity to PRRSV appears to be the same for type 1 and type 2 PRRSVs, but the majority of the research has been conducted on type 2 PRRSVs.

**Innate Immunity**

Early studies showed that PRRSV suppressed IFN-α production and innate antiviral responses were largely absent in the lungs following PRRSV infection (Albina et al. 1998; van Reeth et al. 1999). Direct inhibitory mechanisms included PRRSV NSP interference in innate signaling pathways that disrupted both type 1 IFN and inflammatory cytokine expression (Beura et al. 2010; Chen et al. 2010; Luo et al. 2008; Sun et al. 2010). Induction of IL-10 expression in cells and tissues also increased in serum following in vivo infection (Faaberg et al. 2009; Suradhat et al. 2003). However, focused and global responses (Suradhat and Thanawongnuwech 2003; Pirzadeh and Dea 1997; Vanhee et al. 2010; Weiland et al. 1999; Wissink et al. 2003; Yang et al. 2000), consistent with the evidence that envelope glycoproteins GP2, GP3, GP4, GP5, and nonglycosylated M exist in a multimeric complex that mediates permissive cell infection against many viral structural and NSPs (Brown et al. 2009; de Lima et al. 2006; Johnson et al. 2007). IgM switches to IgG at ∼2–4 weeks. IgG peaks at ∼4–5 weeks and persists for a long period (Molina et al. 2008b; Mulupuri et al. 2008). Variation in strength and kinetics of antibody responses among individual proteins is present, especially within the ecto- and endodomains of GP5 and M (Molina et al. 2008b; Mulupuri et al. 2008). The appearance of IgG antibodies and the time to peak antibody concentration to GP5 and M are delayed compared with a coadministered irrelevant protein antigen (Mulupuri et al. 2008).

It appears that humoral immunity to PRRSV, once acquired, is highly durable. Interestingly, anti-N antibodies may decay even though virus is present in lymphoid tissues. Since many serodiagnostics are based on nucleocapsid antigen, it is possible to misdiagnose immune animals as nonimmune (Batista et al. 2004).

Anatomically, actively secreting and memory PRRSV-specific B cells reside in diverse lymphoid tissues, especially lymph nodes draining the lungs and genital areas. Spleen and tonsil are major reservoirs of PRRSV-specific B cells. In sharp contrast to mice, the bone marrow is not a site of immune induction or a memory B-cell reservoir (Mulupuri et al. 2008).

Antibodies specific for GP4 and GP5 neutralize viral infectivity (Jiang et al. 2008; Ostrowski et al. 2002; Pirzadeh and Dea 1997; Vanhee et al. 2010; Weiland et al. 1999; Wissink et al. 2003; Yang et al. 2000), consistent with the evidence that envelope glycoproteins GP2, GP3, GP4, GP5, and nonglycosylated M exist in a multimeric complex that mediates permissive cell infection (Das et al. 2010; Wissink et al. 2005). These disparate interactions might explain the observed diversity of host pathogenic and immunological responses to PRRSV, but leave unanswered the significance of an innate response in anti-PRRSV immunity.

**B-cell Response**

Humoral immunity to PRRSV appears within 1 week of infection against many viral structural and NSPs (Brown et al. 2009; de Lima et al. 2006; Johnson et al. 2007). It is possible to misdiagnose immune animals as nonimmune (Batista et al. 2004).

Anatomically, actively secreting and memory PRRSV-specific B cells reside in diverse lymphoid tissues, especially lymph nodes draining the lungs and genital areas. Spleen and tonsil are major reservoirs of PRRSV-specific B cells. In sharp contrast to mice, the bone marrow is not a site of immune induction or a memory B-cell reservoir (Mulupuri et al. 2008).

Antibodies specific for GP4 and GP5 neutralize viral infectivity (Jiang et al. 2008; Ostrowski et al. 2002; Pirzadeh and Dea 1997; Vanhee et al. 2010; Weiland et al. 1999; Wissink et al. 2003; Yang et al. 2000), consistent with the evidence that envelope glycoproteins GP2, GP3, GP4, GP5, and nonglycosylated M exist in a multimeric complex that mediates permissive cell infection (Das et al. 2010; Wissink et al. 2005). Hence, antibody-mediated neutralization may be due to direct interference or steric hindrance.

The role of neutralizing antibodies in controlling PRRSV infection is controversial. The appearance of
neutralizing antibodies in primary infection mainly occurs after viremia is already resolved, but neutralizing antibody titer is the best predictor of level and duration of viremia (Molina et al. 2008b). Administration of PRRSV-specific antibodies to pigs may enhance or inhibit infection depending on titer (Yoon et al. 1996), or prevent reproductive disease and viral transmission to offspring (Osorio et al. 2002). The key neutralization epitope in GP5 is reportedly highly conserved (Plagemann et al. 2002), but immunogenicity and viral neutralization are sensitive to variation in glycosylation (Ansari et al. 2006). The GP4 neutralization epitope is highly variable, so that neutralization is only homologous (Vanhee et al. 2010).

In contrast to the well-established correlation between neutralizing antibody titer and protection against influenza viruses, a clear link between VN and protection against PRRSV in pigs has not been established. It seems likely that additional factors, including genetic variation in neutralizing epitopes, variation in immunodominance, epitope masking by glycosylation, and variation in host response, may be involved. Since neutralizing antibodies can control viremia, there may be a role for antibody-based strategies as an immunotherapeutic.

**T-cell Response**

T-cell immunity is poorly understood in swine due to an inability to expand antigen-specific T-cell populations in vitro and a lack of tools and reagents to examine antigen-specific responses in vitro or in vivo. In the case of PRRSV, except for one study, it has been difficult to demonstrate protein-specific T-cell proliferation or cytotoxicity in classical cell culture systems (Bautista et al. 1999). New approaches may address this problem (Jeong et al. 2010). IFN-γ enzyme-linked immunosorbent spot (ELISPOT) assay using live PRRSV in leukocyte cultures has shown a consistent T-cell response to PRRSV infection, but its significance is uncertain (Xiao et al. 2004). The source of IFN-γ is difficult to determine since it is produced by Th1-helper T cells, activated cytotoxic T cells, and natural killer cells. IFN-γ-secreting cells often increase with pig age, but there is no correlation with resolution of PRRSV infection (Klinge et al. 2009).

Cytokine expression that might indicate T-cell responses indicative of Th-1 or Th-2 regulation may not be informative in pigs (Murtaugh et al. 2009). Regulatory T cells (Tregs) and the Th3 cytokine, IL-10, are induced by PRRSV or PRRSV-infected dendritic cells (Gomez-Laguna et al. 2009; Silva-Campa et al. 2009, 2010; Wongyanin et al. 2010). Tregs may be important in PRRSV since the delayed induction of immunity and prolonged infection indicate the potential for a T-cell response that is suppressive rather than ameliorative.

**Protective Immunity**

Exposure to PRRSV elicits protection against subsequent challenge. Immunological protection is premised on the induction of memory B and T cells that persist in the body after all evidence of primary infection is gone. Memory B cells against structural and nonstructural viral proteins are present before viremia is extinguished (Mulupuri et al. 2008). They are abundant in lymphoid tissues, and highest in tonsil, which is also the predominant site of viral persistence.

Even though memory B cells appear to be quite abundant, there is no anamnestic response to viral challenge (Foss et al. 2002). Pigs consistently show substantial resistance to infection, but show no significant change in antibody levels, which is a hallmark of memory. In the cases that have been well studied, prevention of infection is profound (Foss et al. 2002). These observations are relevant to vaccine development since they raise the possibility that the mechanism of immunological protection may not be entirely dependent on memory lymphocytes. In the related arterivirus of mice (LDV), resistance to infection is attributed to the depletion of permissive macrophages (Cafruny et al. 2003). Resistance in swine could be based on a similar mechanism.

In the time before sterilizing immunity is established, it is possible that resistance to challenge could be due to ongoing immunity to the first exposure. One flaw in the understanding of protection is that almost all challenge experiments are performed before the first infection has been resolved. In these cases, it is not certain that memory is involved. In commercial pig production, the duration of PRRSV infection (persistent infection) often exceeds the life span of market pigs (Lager et al. 1997).

**Cross-protection**

The tremendous genetic diversity in both type 1 and type 2 PRRSVs has significant implications for immune protection strategies. Vaccines based on specific isolates are dependent on induction of cross-protective immunity. If immunity is largely isolate specific, then effective vaccine-based protection may be impractical, if not unattainable. However, cross-protection studies in the growing pig consistently show significant improvement in clinical indicators of health, lung pathology and histopathology, and growth performance (Johnson et al. 2004; Mengeling et al. 2003; Opriessnig et al. 2005). Studies in pregnant sows are less common, but also show significant improvement in reproductive performance (Lager et al. 1999; Mengeling et al. 1999).

The efficacy of cross-protection in the field is a major concern, particularly for reproductive PRRS. PRRS outbreaks are known to occur in herds with solid immunity due to regular vaccination or inoculation programs, and high but incomplete protection giving rise to a few viremic piglets may lead to outbreaks in downstream
nurseries. By contrast, vaccine intervention in a finisher outbreak that results in partial reduction in disease severity and reduces economic losses at market may be judged a success. While evidence for immune cross-protection is convincing, its uneven translation to the field indicates that biological factors, in addition to viral genetic variation, affect its efficacy. These factors may include physiological variation in pig susceptibility to infection (Klinge et al. 2009) and genetic variation in host tolerance to PRRSV infection (Halbur et al. 1997; Lewis et al. 2009; Petry et al. 2007).

Maternal Immunity
The appearance of PRRSV in weaned pigs has been correlated with loss of maternal antibody, and the duration of maternal protection was correlated with neutralizing antibody titer (Chung et al. 1997). However, piglets nursing on nonimmune sows showed less severe disease and shorter duration of viremia after challenge than did piglets nursing on immune sows (Shibata et al. 1998). The finding may indicate antibody-dependent enhancement of infection, or additional infection in utero or from virus shed in milk (Wagstrom et al. 2001; Yoon et al. 1996).

PREVENTION AND CONTROL

Prevention
The objective of PRRSV prevention programs is to stop the entry of PRRSV into negative herds and the introduction of new viral variants into PRRSV-infected herds (Dee et al. 2001; Pitkin et al. 2009). Current protocols include the use of quarantine facilities and testing protocols for incoming breeding stock, sanitation and drying protocols for transport vehicles and incoming supplies, personnel entry protocols such as shower-in facilities or Danish entry systems, and insect control programs, that is, screens, habitat management, and insecticides.

For herds in swine-dense regions, PRRSV prevention may include the use of air filtration or air treatment systems. Filtration has been shown to effectively reduce the risk of the introduction of PRRSV and other airborne agents, such as *M. hyopneumoniae*, under both laboratory and field conditions (Dee et al. 2010; Pitkin et al. 2009). Under field conditions, Spronk et al. (2010) demonstrated that air filtration prevented PRRSV infection in two large breeding herds located in swine-dense regions, whereas five nonfiltered herds of similar size became infected during the same observation period.

Control
Specific treatments for PRRS are not available. Therefore, the objective of PRRS control is to limit the adverse effects of the virus in the various stages of production. However, achieving consistent PRRS control in endemically infected herds is problematic.

Gilt Acclimatization. In breeding herds, the control of virus circulation is based on the use of replacement animals that have developed immunity to PRRSV prior to their introduction into the herd (Dee 2003; FitzSimmons and Daniels 2003). Gilt acclimatization relies on one of three general strategies to develop immunity: (1) contact with PRRSV-infected animals, (2) intentional exposure to PRRSV, or (3) vaccination (Dee et al. 1994; FitzSimmons and Daniels 2003). Typically, seronegative replacements are exposed to PRRSV or vaccinated with MLV vaccine in an acclimatization or isolation unit (FitzSimmons and Daniels 2003). These animals are introduced into the breeding herd when they are no longer viremic and, therefore, less likely to transmit virus to herd mates. Exposure of replacement gilts at 2–4 months of age provides time for the development of immunity and resolution of infection well before their introduction into the breeding herd. All exposure methods utilize PRRSV-negative gilts as the starting point. Regardless of the method of exposure, achieving consistent PRRSV infection and recovery is a major challenge.

Contact with PRRSV-infected animals may be used in continuous-flow gilt development units to infect incoming PRRSV-negative animals. PRRSV-infected weaned pigs and cull sows can also be used as donor sources. However, as the breeding herd becomes immune over time, virus transmission within the breeding herd stops, replacements fail to become infected, and gilt acclimatization may not be achievable.

Methods of intentional exposure include feedback with PRRSV-contaminated tissues from weak-born piglets and stillbirths during an outbreak, and exposure to serum collected from viremic pigs at the same farm (Batista et al. 2002; Dee 2003; FitzSimmons and Daniels 2003). The use of intentional exposure using serum containing viable field virus has recently increased due to the increased genetic heterogeneity among PRRSVs and the perception that commercial vaccines do not induce sufficient cross-protection against newly identified PRRSV variants. However, this method has inherent risks and requires thoughtful application and high quality control standards.

MLV vaccines may be used to develop protective immunity and bring consistency to gilt exposure. The primary limitation of MLV PRRSV vaccines is limited cross-protection among PRRSV variants. When using MLV vaccine products, the entire population housed in the same airspace should be vaccinated at one time and managed all-in/all-out. Killed vaccine products may be used in gilt acclimatization protocols as a complement to MLV vaccine or following exposure with field virus, but by themselves are considered to be of limited efficacy.

Breeding-herd Control. The consistent application of acclimatization protocols for incoming replacements
results in the resolution of clinical signs in the breeding herd and the production of PRRSV-negative pigs. Additional procedures directed at breeding/gestating animals may not be required.

MLV vaccines have been used to reduce the presence of susceptible animals in the breeding herd (Dee 1996; Gillespie 2003), expedite the production of PRRSV-negative pigs (Gillespie 2003; Rajic et al. 2001), and, in the face of outbreaks, to limit field virus replication (although success of the latter measure is still under discussion). Currently, some vaccines are approved for use in nonpregnant females, whereas others are not. Vaccination of negative pregnant sows during the third trimester of gestation has been reported to result in fetal infection (Mengeling et al. 1996).

Intentional exposure to serum collected from acutely affected pigs, i.e., during the viremic phase, has also been used in the face of acute outbreaks and as a tool to broaden breeding herd immunity to existing PRRSV variants. The procedure should be considered experimental, and veterinarians should exercise caution in its use. If applied during an outbreak, intentional exposure will not stop clinical losses, but the duration of the outbreak and time to the production of PRRSV-negative weaned pigs may be shortened.

A temporal interruption in the introduction of replacement animals (temporal herd closure) may minimize the effects of PRRSV in the face of a recent infection or accelerate production of PRRSV-negative weaned pigs (Dee et al. 1994; Torremorell et al. 2003). That is, herd stability is improved by discontinuing the introduction of recently infected replacement animals. Periods of 2–4 months may be sufficient to minimize the effects of the virus, but not to eliminate it.

**Pig Management.** In the suckling piglet population, strategies to control PRRS are based on management practices intended to limit the spread of virus among litters (Henry 1994; McCaw 2000, 2003). Measures include restricting cross-fostering to the first 24 hours of life, humanely destroying severely affected piglets, and maintaining strict all-in/all-out animal flow of the nursery.

Control of chronic PRRSV in the weaned pig population is challenging. In continuous-flow sites, the cycle of transmission is maintained by transmission of the virus to recently weaned piglets from older, infected pigs.

Partial depopulation is a control strategy that consists of a strategic adjustment in the pig flow to prevent the lateral spread of PRRSV within chronically infected populations. Partial depopulation can produce significant improvements in average daily gain, mortality, and the overall economic performance of the nursery (Dee et al. 1997; Dee et al. 1993). Limitations to this strategy include the logistics involved in depopulating large nurseries and the fact that it may need to be repeated periodically to maintain performance.

Mass MLV vaccination and unidirectional pig flow is a strategy to eliminate the virus from growing pigs without depopulation (Dee and Phillips 1998). This strategy has been used to limit the spread of PRRSV in commercial settings when emptying a site is not a possibility. MLV vaccination has also proven to be useful for limiting the effects of field virus in weaned pigs. Pigs at risk of exposure to field viruses from lateral introduction, that is, in pig-dense areas, can have significant performance advantages if they have been vaccinated previously with MLV (Waddell et al. 2008).

Control measures in the weaned pig population should also include the control of infections aggravated by concurrent PRRSV infection, for example, *H. parasuis*, *S. suis*, and swine influenza virus. Appropriate vaccination and medication protocols need to be determined for the individual infections.

**Eradication.** Elimination of PRRSV from a population is justified by clear improvements in pig health and productivity. Spontaneous elimination of PRRSV from a herd has been described (Freese and Joo 1994) but is a near impossibility in current production systems. The successful elimination of PRRSV from infected farms can be achieved using well-established protocols: total depopulation/repopulation, partial depopulation, segregated early weaning, test and removal, and herd closure (Dee and Molitor 1998; Torremorell et al. 2003). Successful PRRSV elimination in the breeding herd relies on the introduction of negative, nonexposed replacement animals at a time when PRRSV is no longer circulating. Successful control strategies, as described earlier, will prepare the population for eradication since eventually an immune, virus-free population is developed. A successful elimination plan also requires the implementation of strict biosecurity measures in order to prevent the herds from becoming reinfected (Torremorell et al. 2004).

Total herd depopulation and repopulation is a very successful technique, but is costly and may only be justifiable if the elimination of other concurrent diseases is desired. This strategy may be the only feasible alternative for farrow-to-finish herds where ongoing replication of PRRSV in the growing population does not allow for the elimination of the virus through other methods.

Partial depopulation is indicated for the elimination of the virus from growing pigs when shedding from the breeding population has completely stopped (Dee et al. 1997; Dee et al. 1993). This technique may be sufficient to eliminate the virus from small farms. However, when used in large units (>500 sows), it requires the application of additional strategies, for example, herd closure or test and removal, to eliminate the virus from the
breeding herd prior to attempting elimination in the pig flow. Segregated early weaning has also been used to produce PRRSV-negative pigs from infected sows. However, depending on the level of PRRSV activity in the sow herd, production of PRRSV-negative batches may not be consistent (Donadeu et al. 1999; Gramer et al. 1999). All-in/all-out segregated production of batches of weaned pigs is needed to maximize the success of this technique. In addition, the establishment of PRRSV-negative herds from positive sources is possible when negative piglets are produced by the combination of several of these techniques (Torremorell et al. 2002).

PRRSV elimination through herd closure is based on the fact that PRRSV does not persist in an immune population (Molina et al. 2008b; Torremorell et al. 2003). This strategy mimics the principles followed for transmissible gastroenteritis virus (TGEV) eradication, whereby all animals are exposed to the virus and no replacement animals are introduced as long as there is a possibility that they could be exposed to the virus (Harris et al. 1987). For elimination of PRRSV, a herd closure of at least 200 days is recommended, but variations may be needed depending on the status of the farm and pig flow. Introduction of negative replacement animals should be followed by attrition or scheduled culling of the previously infected animals. This strategy will develop a negative population of breeding animals over time. The success rate using this strategy is above 90% for farms with segregated off-site production. Production management practices, such as off-site breeding projects and others, can minimize the economic effects of herd closure.

Elimination of PRRSV by test and removal consists of testing serum samples from all individuals in the breeding herd for anti-PRRSV antibody and PRRSV (RT-PCR) and removing all positive animals from the farm. Candidate herds for test and removal include herds with segregated production and more than 12 months since the last clinical episode of PRRS, as well as herds with an estimated prevalence below 25%.

Complete eradication of PRRSV has been achieved in Chile and Sweden (Carlsson et al. 2009; Torremorell et al. 2008). In North America, regional projects for the elimination of PRRSV have been undertaken and are expanding (Corzo et al. 2010). Regional programs require significant coordination and collaboration by all members involved and are crucial to maintain significant geographical areas virus-free.

**Vaccines.** Several studies have established that vaccination against PRRSV can result in protective immunity, moderate clinical signs, and reduced shedding of field virus (Cano et al. 2007a,b; Gorcyca et al. 1995; Hesse et al. 1996; Mengeling 1996; Plana Duran et al. 1995). A variety of MLV and inactivated products are available, depending on the geographical region. In general, MLV vaccines are considered to induce a more efficacious immune response than inactivated vaccines, although there are concerns regarding cross-protection and the safety of some products. Inactivated vaccines are generally considered to provide poor protection, but when used in combination with MLV vaccines or in previously infected animals, these may stimulate an anamnestic response and induce the production of neutralizing antibodies.

When used in the field, vaccines have met with variable degrees of success. These differences may be due to differences in the commercial products available and how the products are utilized. Likewise, the results may reflect differences in the virus variants circulating in different regions and/or they may relate to the issue of cross-protection. In addition, field reports have raised the possibility of reversion to virulence by attenuated vaccine virus (Nielsen et al. 2001). Vaccine virus behaves very similarly to field viruses in terms of transmission, persistence, transplacental transmission and congenital infection, shedding in semen, and the length of time required to induce protective immunity. Additional research is needed to provide safer and more efficacious products for the control of PRRSV.

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CHAPTER 31 PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS (PORCINE ARTERIVIRUS)
SECTION III VIRAL DISEASES


Porcine Astroviruses

Gábor Reuter and Nick J. Knowles

RELEVANCE

Porcine astroviruses were first recognized when diarrheic feces were examined by electron microscopy (Bridger 1980; Geyer et al. 1994; Shimizu et al. 1990; Shirai et al. 1985). Their causal role in porcine enteric disease remains undetermined. In some species, for example, humans, turkey, and sheep, astroviruses have been linked to enteric disease (Mendez and Arias 2007). In contrast, bovine astroviruses failed to cause clinical disease when inoculated into calves (Woode and Bridger 1978; Woode et al. 1984). There is evidence that astroviruses from different animal species are antigenically distinct (Mendez and Arias 2007). For example, antibodies against porcine astroviruses do not react with bovine astroviruses (Bridger 1980).

ETIOLOGY

Porcine astroviruses are members of the family Astroviridae, which is divided into two genera, Mamastrovirus (astroviruses of mammals) and Avastrovirus (astroviruses of birds). Approximately 30-nm diameter and nonenveloped, astrovirus particles are distinctive in appearance, with some particles showing a five- or six-pointed star surface pattern when viewed by negative stain electron microscopy (Figure 32.1; Bridger 1980; Shimizu et al. 1990). Not all particles show this distinctive appearance, and care is needed so as not to confuse them with the indistinct appearance of noroviruses, particularly in the presence of antibody. The genome is composed of positive sense, single-stranded RNA 6.4–7.9 kilobases in length, excluding the poly(A) tail at the 3′ end, consisting of three open reading frames (Mendez and Arias 2007).

A study of evolutionary genomic relationships showed that human and animal astroviruses belonged to phylogenetically distinct genomic clusters (Figure 32.2; Jonassen et al. 2001; Kapoor et al. 2009; Lukashov and Goudsmit 2002; Reuter et al. 2011). Multiple lineages (and serotypes) of astroviruses have also been identified within the same species, including humans (classical HAstV1-8, HAstV-MLB1, and HMOAstVs), bats, turkeys, and pigs. Thus, astroviruses are genetically highly diverse, with each lineage potentially representing an independent origin. Genetic data supported the hypothesis that cross-species transmission events involving humans and different animal species, possibly through unknown intermediate hosts, may have occurred in the past. Given the diversity of astroviruses, interspecies transmission could be underestimated because no common molecular probes, antibodies, or oligonucleotide primers are available to identify astrovirus strains across different species (Mendez and Arias 2007).

A few porcine astrovirus genomes from Japan (Jonassen et al. 2001; Lukashov and Goudsmit 2002; Wang et al. 2001), Czech Republic (Indik et al. 2006), and Hungary (Reuter et al. 2011) have been partially characterized. Phylogenetic analysis of the capsid region suggested that genetically different lineages of porcine astroviruses exist in piglets, probably representing more than one species (PAstV-1 and PAstV-2) (Figure 32.2). PAstV-2 was detected in Hungary (Reuter et al. 2011).

The physicochemical and biological properties of porcine astroviruses have not been widely studied. In Japan and the Czech Republic, cytopathic astroviruses from diarrheic pigs were successfully isolated on porcine kidney cell lines by incorporating trypsin into the medium (Indik et al. 2006; Shimizu et al. 1990).
Immunofluorescent cells and astrovirus particles were also detected. A virus with a buoyant density of 1.35 g/mL was cloned, and a serum-virus neutralization test was developed. The isolate was stable to treatment with lipid solvents and resisted heating at 56°C (133°F) for 30 minutes, but showed some susceptibility to acid treatment at pH 3.0. Five structural proteins with molecular masses from 13–39 kDa were identified. At present, the number of astrovirus structural proteins is uncertain and has varied in studies with astroviruses from different animal species (Mendez and Arias 2007).

ROLE IN PUBLIC HEALTH

There is no evidence that porcine astroviruses are zoonotic. In general, astroviruses are considered to be species specific, and interspecies infection with porcine astroviruses has not been confirmed.

EPIDEMIOLOGY

Porcine astroviruses have been identified in feces of pigs in the United Kingdom (Bridger 1980), Japan (Shimizu et al. 1990), South Africa (Geyer et al. 1994), Czech Republic (Indik et al. 2006), and Hungary (Reuter et al. 2011), indicating a worldwide distribution. In a serological survey in Japan, 39% of 128 pigs in eight herds had serum neutralizing antibodies to porcine astrovirus PAstV-1 (Shimizu et al. 1990). All but one herd had antibody, and the in-herd prevalence ranged from 7% to 83%. Transmission is presumed to be fecal-oral.

32.1. The 30-nm diameter, nonenveloped astrovirus particles are distinctive in appearance with some particles showing a five- or six-pointed star pattern on their surface when viewed by negative stain electron microscopy (from Bridger 1980 by permission of the publisher).

32.2. Unrooted neighbor-joining tree showing the relationships between the capsid proteins of porcine astroviruses and other representative members of the family. Porcine astroviruses (PAstV-1 and PAstV-2) are boxed. Members of the two accepted genera (Mamastrovirus and Avastrovirus) are within the shaded areas.
PATHOGENESIS, CLINICAL SIGNS, LESIONS, AND IMMUNITY

In most mammalian species, astrovirus infections are associated with gastroenteritis. In humans, astroviruses were found to be the third to fourth most common cause of viral diarrhea in young children worldwide. Porcine astroviruses have been associated with severe diarrhea in natural infections, but only in the presence of other known enteric pathogens (Bridger 1980; Shimizu et al. 1990). Mild diarrhea developed in 4-day-old pigs after oral inoculation with porcine astrovirus, PASTV-1, grown in cell culture (Shimizu et al. 1990). Diarrhea commenced 1 day after inoculation and continued for 5–6 days. Cytopathic astrovirus was recovered from the feces and pigs were seroconverted. Similarly, porcine astrovirus-induced mild diarrhea was detected in 7-day-old gnotobiotic piglets in a per os experimental inoculation study in the Czech Republic (Indik et al. 2006). Tissues were not evaluated, but it is reasonable to hypothesize that porcine astroviruses might cause an intestinal pathology (especially small intestine) that leads to the clinical signs commonly observed in pre- and postweaning diarrhea. Nothing is known about antigenic differences between porcine astroviruses or the immunity induced by infection with these viruses.

DIAGNOSIS

Assays have not been developed for routine diagnosis, but methods such as electron microscopy, isolation on cell culture, identification with immunofluorescence, and detection of nucleic acids by reverse transcription-polymerase chain reaction (RT-PCR) can be used to diagnose natural porcine astrovirus infection. The virus neutralization and immunofluorescent antibody assays can be used to demonstrate PASTV-1 seroconversion (Shimizu et al. 1990). However, the diagnostic performance of these methods for the laboratory diagnosis of porcine astrovirus infections has not been evaluated.

PREVENTION AND CONTROL

Porcine astroviruses may be just one of several enteric viruses that contribute to pre- and postweaning diarrhea. Elimination of astroviruses from infected farms would be difficult to achieve because they are apparently widely distributed in swine populations and stable in the environment. Efforts to eliminate astroviruses would be difficult to justify on the basis of their clinical effects. Assuming that lesions caused by infection with astroviruses are confined to the intestinal tract, oral rehydration would be expected to be effective in affected pigs. There are no commercially available vaccines.

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Bunyaviruses

Chih-Cheng Chang

RELEVANCE

Although bunyaviruses are important viral pathogens of humans and ruminants, serological surveys have also detected bunyavirus infections (Akabane, Oya, Tahyna, and Lumbo viruses) in pig populations in several parts of the world (Arunagiri et al. 1991; Huang et al. 2003; Hubálek et al. 1993; Kono et al. 2002; Lim et al. 2007). Akabane virus has been isolated from pigs in Taiwan and Korea (Huang et al. 2003; Lim et al. 2007), and Oya virus was isolated from a pig suspected of Nipah virus infection in Malaysia (Kono et al. 2002).

ETIOLOGY

Bunyaviruses are single-stranded, negative-sense, enveloped viruses 80–100 nm in diameter. The genome is composed of three segments of negative-sense RNA that differ in size: large (L), medium (M), and small (S) (Elliott 1985, 1990). The L segment encodes a large polypeptide (L protein) with replicase and transcriptase activities (Jin and Elliott 1991, 1992). The M segment encodes two structural glycoproteins (G1 and G2) and a nonstructural protein (NSm) (Elliott 1985; Fazakerley et al. 1988; Fuller and Bishop 1982; Gentsch and Bishop 1979; Gerbaud et al. 1992). These glycoproteins, inserted in the membrane of the virion and projected onto the surface, are critical in viral virulence, attachment, cell fusion, and hemagglutination activity (Schmaljohn 1996). The nucleocapsid (N) and a nonstructural protein (NSs) are encoded by overlapping reading frames in the S segment (Elliott 1990).

The family Bunyaviridae consists of more than 300 viruses in five genera, including four genera of viruses that infect vertebrates (Bunyavirus, Hantavirus, Nairovirus, and Phlebovirus). Except for genus Hantavirus, bunyaviruses are arthropod-borne viruses (arboviruses) categorized based on common antigenic relationships and biochemical analyses (Bishop 1985; Elliott 1990, 1997). Genus Bunyavirus contains at least 170 viruses in serogroups Bunyamera, Bwamba, California, Guama, and Simbu (Calisher 1996). Bunyaviruses detected in swine include viruses in the Simbu serogroup (Akabane and Oya viruses) and California serogroup (Lumbo and Tahyna viruses) (Calisher 1996; Elliott 1997).

ROLE IN PUBLIC HEALTH

Four bunyaviruses (Akabane, Oya, Tahyna, and Lumbo viruses) are known to be infectious for pigs. Of these, humans are not susceptible to either Akabane or Oya viruses (Bryant et al. 2005). Tahyna virus and the closely related Lumbo virus are widespread in some human populations, occasionally with clinical consequences (Gould et al. 2006; Vapalahti et al. 1996). The role of pigs in the complex ecology of bunyaviruses is undefined, but likely to be minor.

EPIDEMIOLOGY

The geographical distribution of specific bunyaviruses reflects the distribution of its vector(s) and host(s). Viruses in the Simbu serogroup are found worldwide, except Europe, and generally transmitted to vertebrates by culicoid midges and mosquitoes (Calisher 1996). Likewise, the California serogroup is composed of mosquito-borne viruses (Elliott 1997).
Akabane virus (Simbu serogroup) was first isolated from mosquitoes in Japan in 1968. Midges and mosquitoes are vectors of Akabane virus (Jennings and Mellor 1989; Kurogi et al. 1987; Oya et al. 1961) and herbivores, including cattle, sheep, giraffe, horses, and goats, the natural vertebrate hosts (Al-Busaidy et al. 1988; Cybinski et al. 1978). However, recent work has shown that Akabane virus may be prevalent in pig populations in Asia (Huang et al. 2003; Lim et al. 2007) with biting midges (Culicoides spp.) serving as the primary vectors (Hsu et al. 1997; Huang et al. 2003). In pigs, Akabane virus transmission may not be completely arthropod dependent; that is, infection occurred following experimental oronasal exposure (Huang et al. 2003).

In Korea, a survey found that 37% of pigs were seropositive for Akabane virus, with positive pigs on all of the 15 farms surveyed (Lim et al. 2007). In Taiwan, a national survey (Huang et al. 2003) found that approximately 75% of pigs were serum antibody positive for Akabane virus, with seropositivity rates in sow breeding herds reaching 99%. A high percentage of positive results in young pigs (98%) suggested the presence of maternal antibodies, whereas seropositivity declined to 17% for 20-week-old pigs, then returned to high levels (71%) in late finishers. Seropositivity rates for Akabane virus in cattle in Taiwan were also high (96%).

Sequential analysis of Taiwanese Akabane virus isolates from swine (NT-14) and cattle (PT-17) showed a high degree of homology (99.6%) for the small (S) RNA segment. This suggested that pigs were involved in the Akabane virus–host–vector transmission cycle in Taiwan and that NT-14 and PT-17 may have the same origin.

Serosurveys for other bunyaviruses have also been done in swine populations. A study of Oya virus (Simbu serogroup) in Malaysia found that 93% of 360 pigs sampled on 24 farms in six states were serum antibody positive (Kono et al. 2002). Hubálek et al. (1993) reported that 38% of wild boars (Sus scrofa) in the Czech Republic were seropositive for Tahyna virus (California serogroup). Seroconversion of pigs to Lumbo virus, another member of the California serogroup, was reported in Sri Lanka (Arunagiri et al. 1991).

PATHOGENESIS

Little research on the pathogenesis of bunyaviruses in pigs has been reported. Huang et al. (2003) infected 4-week-old pigs with Akabane virus (isolate NT-14) under experimental conditions by oronasal exposure. Viremia occurred 1–6 days postinoculation. During this period, the virus was isolated from a variety of tissues (spleen, lungs, brain, small intestine, lymph node, thymus, and salivary gland) and persisted in the tonsil for 14 days after inoculation. The virus was also isolated from oronasal discharge, but not feces. Naive pigs in direct contact with inoculated pigs did not become infected.

CLINICAL SIGNS AND LESIONS

Akabane virus infection is inapparent in adult swine (Huang et al. 2003), although whether Akabane virus induces lesions in pregnant sows or their fetuses is not known. Under experimental conditions, Akabane virus (isolate NT-14) did not produce gross lesions, but mild lymphocytic encephalitis and cerebral vasculitis were observed (Huang et al. 2003). Clinical signs and lesions have not been described for Tahyna or and Lumbo viruses. Lesions caused by Oya virus in pigs have not been described, but Kono et al. (2002) suggested that Oya virus could cause clinical signs similar to Nipah virus infection.

DIAGNOSIS

Bunyaviruses may be recovered in suckling mice or on susceptible cell culture systems, including hamster lung (HmLu-1), baby hamster kidney (BHK-21) cells, monkey kidney cells (Vero, MA-104, MARC-145), and mosquito cell lines (Bryant et al. 2005; Gerdes 2008; Huang et al. 2003; Kono et al. 2002). Techniques for bunyavirus detection and identification include transmission electron microscopy, serology (Huang et al. 2003), immunohistochemistry, reverse transcriptase-polymerase chain reaction (Bryant et al. 2005; Huang et al. 2003), and sequencing (Saeed et al. 2001).

PREVENTION AND CONTROL

No specific treatments or vaccines are available for bunyavirus infection in pigs. Bunyaviruses are susceptible to most common disinfectants, for example, hypochlorite, detergents, chlorhexidine, and phenols.

REFERENCES

OVERVIEW

The family Caliciviridae consists of five genera: Lagovirus, Vesivirus, Norovirus, Sapovirus, and Nebovirus (Clarke et al. 2011). The Lagovirus genus is comprised of two species, rabbit hemorrhagic disease virus and European brown hare syndrome virus, while the Vesivirus genus is comprised of two species, vesicular exanthema of swine virus (VESV) and feline calicivirus, plus a number of unassigned viruses. The Norovirus, Sapovirus, and Nebovirus genera each consist of a single species, Norwalk virus, Sapporo virus, and Newbury I virus, respectively. Three recently discovered caliciviruses are phylogenetically distinct from the existing genera and are proposed to belong to three novel genera: Tulane virus (isolated from Rhesus macaques) (Farkas et al. 2008), St-Valérien virus (isolated from pigs) (L’Homme et al. 2009b), and chicken calicivirus (Wolf et al. 2011). Caliciviruses infecting pigs belong to the species VESV, Norwalk virus, and Sapporo virus and the unclassified St-Valérien virus.

Calicivirus virions are nonenveloped with icosahedral symmetry. They are 27–40 nm in diameter by negative stain electron microscopy (Wawrzkiewicz et al. 1968) and 35–40 nm by cryo-electron microscopy and X-ray crystallography (Prasad et al. 1999). The capsid is composed of 90 dimers of the major structural protein VP1 arranged on a T = 3 icosahedral lattice. In noroviruses, the VP1 forms a subunit comprised of a shell and two protruding domains. A characteristic feature of calicivirus capsid architecture is the 32 cup-shaped depressions at each of the icosahedral five-fold and threefold axes. In negative stain virus preparations, some cup-shaped depressions appear distinct and well defined, while in others, these depressions are less prominent.

Caliciviruses have single-stranded, positive-sense genomic RNA (positive-strand RNA) organized into either two or three major open reading frames (ORFs). The nonstructural proteins are encoded in the 5′ part of the genome and the structural proteins in the 3′ part. Replication occurs in the cytoplasm, and two major positive-strand RNA species are found in infected cells: (1) The genome-sized, positive-strand RNA serves as the template for the translation of a large polyprotein that undergoes cleavage by a virus-encoded protease to form the mature nonstructural proteins; and (2) a subgenomic-sized, positive-strand RNA, coterminous with the 3′ terminus of the genome is the template for the translation of the VP1 as well as the 3′ terminal ORF product VP2. A double-stranded RNA (dsRNA) corresponding in size to full-length genomic RNA has been identified in cells infected with feline calicivirus and San Miguel sea lion virus (SMSV), indicating that replication occurs via a negative-strand intermediate.

Virion molecular mass (Mr) is about 15 × 10^6. Virion buoyant density is 1.33–1.41 g/cm^3 in CsCl and 1.29 g/cm^3 in glycerol-potassium tartrate gradients. Virion S20w is 160–187S (Wawrzkiewicz et al. 1968). Physicochemical properties have been established for some members of the family. Generally, caliciviruses are stable in the environment, and many strains are resistant to inactivation by heat and certain chemicals (ether, chloroform, and mild detergents) (Wawrzkiewicz et al. 1968). Enteric caliciviruses are acid stable, while vesiviruses are labile below pH 4.5–5.0 (Wawrzkiewicz et al. 1968).

VESICULAR EXANTHEMA OF SWINE VIRUS

Vesicular exanthema of swine (VES) is an acute, highly infectious disease characterized by fever and formation of vesicles on the mucosal and cutaneous surfaces.
of blisters on the snout, oral mucosa, soles of the feet, the coronary band, and between the toes. In pigs, the clinical disease is indistinguishable from foot-and-mouth disease, vesicular stomatitis, and swine vesicular disease.

Originally confined to California, VES became widespread in the United States during the 1950s, but a vigorous campaign to eradicate the disease was successful. In 1959, the United States was declared free of VES, and the disease was designated a foreign animal disease; it has never been reported as a natural infection of pigs in any other parts of the world.

Since 1972, a virus indistinguishable from VESV, designated as SMSV, has been isolated from throat and rectal swabs from premature and 4-month-old California sea lion pups, dead and weanling northern fur seal pups, and nursing northern elephant seal pups. It has also been isolated from vesicular lesions on marine mammals, commercial seal meat produced in Alaska, and perch-like fish collected from tidal pools off the southern California coast. SMSV isolated from both fish and marine mammals is capable of producing VES in pigs. In addition, caliciviruses isolated from throat and rectal swabs from dairy calves cause clinical vesicular exanthema in exposed pigs.

**Etiology**

Genus Vesivirus is comprised of two species: Vesicular exanthema of swine virus and Feline calicivirus. A number of other vesiviruses, isolated from dogs and mink, and as cell culture contaminants, are distinct from the two species and remain unclassified. The species VESV is classified into approximately 40 serotypes (although not all have been serologically compared) (Neill et al. 1995). Thirteen are known as VESV (VESV-B34, -A48, -BS1, -CS2, -DS3, -ES4, -F55, -G55, -HS4, -IS5, -JS6, -K54), 17 as SMSV (SMSV-1, -2, -4 to -7, SMSV-9 to -11, SMSV-13 to -17, SMSV-FADDL 7005, SMSV-693M, SMSV-3709), and the remainder named after the hosts from which they were first isolated, that is, California sea lion virus (strain McAll), Steller sea lion virus (SSLV) V810, SSLV-V1415, bovine calicivirus (BCV) Bos-1, BCV Bos-2, cetacean calicivirus Tur-1, primate calicivirus Pan-1, reptile calicivirus Cro-1, walrus calicivirus, skunk calicivirus, rabbit calicivirus, and human calicivirus.

Seven VESV serotypes isolated during the 1930s and 1940s have since been lost or destroyed and have not been compared with later serotypes. SMSV-3 was found to be a mixture of SMSV-1 and SMSV-2. Partial genome sequencing of SMSV-8 and SMSV-12 shows them to be distinct from other members of the species VESV (N. J. Knowles and S. M. Reid, unpublished data). Members of the species VESV all replicate in mammalian cell cultures (e.g., monkey kidney or porcine kidney) usually causing a rapid and destructive CPE.

**Role in Public Health**

These viruses are not thought to be of public health significance; however, human infection by two different marine mammal serotypes has been reported. SMSV-5 has been recovered from vesicular lesions on the palms and soles of a researcher working with the virus (Smith et al. 1998b). Another individual, who handled diseased Steller sea lions (Eumetopias jubatus) in the Bering Sea, developed blistering of the hard palate, upper lip, and facial area. A novel vesivirus (strain MCAII) was isolated from throat washings sampled 30 days post-onset (Smith et al. 1998b).

**Epidemiology**

VESV/SMSV and related viruses have been found along the Pacific Coast of North America as far north as the Bering Sea. Outbreaks of VES occurring between 1932 and 1951 in pigs in the United States were limited to California. However, in 1951, one serotype (BS1) spread to 41 states and the District of Columbia. Two further serotypes appeared in New Jersey in 1954 and 1956.

Natural infections have been found in pigs, pinnipeds, cetaceans, cattle, horses, skunk, primates (including humans), reptiles, and fish (Smith et al. 1998a). SMSV-7 and -17 have been isolated from a sea lion liver fluke (Zalophatrema sp.) and a mussel (Mytilus californianus), respectively (Smith et al. 1998a). Experimentally, at least six of the SMSV serotypes have been shown to cause vesicular disease in pigs indistinguishable from that caused by VESV (Berry et al. 1990; Bresse and Dardiri 1977; Gelberg and Lewis 1982; Smith et al. 1974, 1980; Van Bonn et al. 2000).

The principle form of transmission during outbreaks of vesicular exanthema in the United States was thought to be by feeding untreated contaminated garbage; however, spread by direct contact of infected animals occurs readily (Madin 1975).

**Clinical Signs and Lesions**

Following an elevated temperature, vesicles appear at one or more of the following sites: snout, lips, tongue, mucosa of the oral cavity and on the sole, interdigital space, and coronary band of the foot (Madin 1975). Lesions may also appear on the teats, particularly in nursing sows.

**Diagnosis**

Presumptive diagnosis in pigs is based on fever and the presence of typical vesicles, which normally rupture within 24–48 hours to form erosions. Laboratory differentiation from other vesicular disease-causing viruses, that is, foot-and-mouth disease virus (Aphthovirus, Picornaviridae), swine vesicular disease virus (Enterovirus, Picornaviridae), and vesicular stomatitis virus (Vesiculovirus, Rhabdoviridae), is essential.

Virus may be detected by a variety of serologically based laboratory tests, including complement fixation.
for vesicular disease to appear in pigs remains an ever-present threat.

**Porcine Caliciviruses (Noroviruses and Sapoviruses)**

Porcine enteric caliciviruses (PECs) (noroviruses and sapoviruses) were first recognized when diarrheic feces from postweaning and nursing pigs in the United Kingdom and the United States were examined by electron microscopy (Bridger 1980; Saif et al. 1980). Porcine caliciviruses have not been widely studied and much is unknown concerning their role in naturally occurring swine disease. This is in contrast to the recognized role of caliciviruses in sporadic and epidemic acute gastroenteritis in humans.

Human caliciviruses belong to two genera, *Norovirus* (formerly Norwalk-like viruses) and *Sapovirus* (formerly Sapporo-like viruses), in the family *Caliciviridae* (Mayo 2002). Viruses in both genera (porcine noroviruses and porcine sapoviruses) are now accepted as common infectious agents on pig farms, and it seems possible that caliciviruses may have a significant role in porcine enteric disease. Public health concerns over potential cross-species transmission and animal reservoirs for caliciviruses have been raised (Mattison et al. 2007; Reuter et al. 2010; Wang et al. 2005a). However, the question is still open. Based on limited data, there is no clear evidence until now for the direct threat to human health of known porcine caliciviruses.

**Porcine Noroviruses**

Noroviruses, possessing a 27- to 32-nm nonenveloped capsid and an indistinct morphology, were reported in swine in 1980 (Bridger 1980) (Figure 34.1). The genome is composed of positive-sense, single-stranded RNA...
7.3–7.7 kilobases in length, excluding the poly (A) tail at the 3′ end. It is composed of three ORFs encoding a polyprotein that undergoes protease processing to produce several nonstructural proteins, including an RNA-dependent RNA polymerase (RdRp), a major capsid protein (VP1), and a minor capsid protein (VP2) (Green 2007).

Noroviruses are genetically highly diverse. Phylogenetic analysis of the virus capsid has been used to designate five genogroups (G) named GI to GV (Zheng et al. 2006). Viruses within each genogroup have been grouped into genotypes, 1–8 in GI, 1–19 in GII, 1–2 in GIII, and 1 each in GIV and GV (Wang et al. 2005a; Zheng et al. 2006). Due to the high sequence diversity between porcine norovirus genotypes (Wang et al. 2005a), homologous recombination probably also occurs distinct from human noroviruses (Sugieda et al. 1998). But formed three separate genotypes (11, 18, and 19) (AB074893), grouped with the GII human noroviruses, the prototype porcine norovirus strain Sw918/1997/JP (Figure 34.2). Porcine norovirus sequences, including and other biological properties. In general, noroviruses are characterized by stability in the environment and nothing is known about their physicochemical and other properties. In general, noroviruses are characterized by stability in the environment and relative resistance to inactivation.

**Role in Public Health.** The close genetic and antigenic relationships between human and porcine noroviruses raise public health concerns regarding their potential for cross-species transmission and as reservoirs for noroviruses. But there is no evidence for the direct threat of porcine noroviruses to human health.

**Epidemiology.** Noroviruses have been identified in humans, swine, cattle, sheep, mice, and lion. It is assumed that natural norovirus transmission is fecal–oral.

Norovirus RNA was detected relatively infrequently (0.4–6%) in pigs by RT-PCR in 4 of 1017 normal slaughtered pigs collected in 1997 from 26 Japanese farms (Sugieda et al. 1998), 2 of 100 pooled fecal samples from fattening pigs (3–9 months of age) on 100 farms in The Netherlands (van der Poel et al. 2000), 6 of 275 fecal samples from normal adult swine in the United States, and 1 of 17 fecal samples from normal swine under the age of 2 years in Hungary (Reuter et al. 2007). These figures are likely to be an underestimate of porcine norovirus prevalence as PCR primers designed for human noroviruses were used, and pre- and post-weaning diarrheic piglets were not examined.

The geographical distribution indicates the worldwide occurrence of porcine noroviruses among pigs in farms. Little is known about the association of porcine noroviruses with natural disease. It is not established if porcine noroviruses are species specific, but it has been postulated that the close genetic similarity of porcine noroviruses and human noroviruses may mean that porcine noroviruses are a reservoir of human infection (Sugieda and Nakajima 2002; van der Poel et al. 2000).

**Pathogenesis, Clinical Signs, Lesions, and Immunity.** All porcine noroviruses were detected from pigs without clinical signs. Subclinically infected pigs may be natural reservoirs for noroviruses. The isolate QW101/2003/US (genotype 18) replicated in gnotobiotic pigs with fecal shedding coincident with mild diarrhea (Wang et al. 2005a).

In an experiment using GII.4 human norovirus, 48 (74%) of 65 gnotobiotic piglets developed mild diarrhea (Cheetham et al. 2006). Histopathological examination showed mild lesions in the proximal small intestine of only one of the seven pigs. Evidence was found for replication of the virus in intestinal enterocytes in 18 of 31 cases. Seroconversion after postinoculation day 21 was detected by antibody ELISA in 13 of 22 virus-inoculated pigs, indicating human norovirus replication in pigs.

Seroprevalence of GII norovirus in swine was 97% in the United States and 36% in Japan (Farkas et al. 2005). Immune responses against porcine noroviruses, protective immunity, and/or the role of maternal antibodies have not been assessed. It may be assumed that protective immune mechanisms are similar to those for other enteric virus pathogens. Porcine norovirus infections in pigs may potentially provide useful insights into protective immunity of the equivalent viruses of humans.
However, the sensitivity of molecular tests for laboratory diagnosis of porcine norovirus infections has not been evaluated.

Prevention and Control. Assuming that porcine norovirus epidemiology and immunology are similar to

Diagnosis. No diagnostic tests for porcine noroviruses have been developed for use outside the research laboratory. Porcine noroviruses have been detected using electron microscopy, RT-PCR, and real-time RT-PCR (Reuter et al. 2007; Sugieda and Nakajima 2002; Sugieda et al. 1998; van der Poel et al. 2000; Wang et al. 2005a). However, the sensitivity of molecular tests for laboratory diagnosis of porcine norovirus infections has not been evaluated.

Prevention and Control. Assuming that porcine norovirus epidemiology and immunology are similar to
Porcine rotaviruses, it is likely that these viruses persist in the environment, and it may be impossible to eliminate the infection from pig herds and prevent natural infection of piglets. However, the persistence of porcine enteric viruses in animal waste depends on the waste treatment technology (Costantini et al. 2007). It is also likely that sows pass on maternal antibody in colostrum and milk and thus limit infection and damage in the gut of nursing piglets. Treatment with oral rehydration fluids is likely to be successful.

Porcine Sapoviruses

Sapoviruses, possessing a 30- to 35-nm nonenveloped capsid and typical calicivirus morphology with clear cup-shaped depressions, were reported in swine in the United States in 1980 (Saif et al. 1980) and genetically characterized as sapovirus in 1999 (Guo et al. 1999). The genome is composed of positive-sense, single-stranded RNA 7.3–7.5 kilobases in length, excluding the poly (A) tail at the 3’ end. It is composed of two ORFs encoding a polyprotein that undergoes protease processing to produce several nonstructural proteins, including an RdRp and a major capsid protein (VP1) in ORF1 and minor capsid protein (VP2) in ORF2 (Green 2007).

Sapoviruses are genetically highly diverse. Phylogenetic analysis of the major virus capsid protein has been used to designate five official genogroups (G) named GI to GV (Green 2007). However, further novel sapovirus genogroups (GVI–GX) identified in swine were also proposed (Reuter et al. 2010; Wang et al. 2005b). Within each genogroup, viruses have been grouped into genotypes, 1–3 in GI, 1–3 in GII, and 1 each in GIII–GX (Green 2007; Reuter et al. 2010). Due to the extremely high sequence diversity between these genogroups, it has been suggested that they may represent distinct virus species. Human sapoviruses belong to GI, GII, GIV, and GV. Porcine sapovirus belongs to genogroups GIII, GVI, GVII, GVIII, GIX, and GX (Figure 34.2). GIII strains were detected the most frequently in pigs (8–67%) and pig farms (7–88%) by RT-PCR in several countries, including Belgium (Mauroy et al. 2008), Brazil (Barry et al. 2008), Canada (L’Homme et al. 2009a), China (Shen et al. 2009), Hungary (Nagy et al. 1996; Reuter et al. 2007), Italy (Martella et al. 2008), Japan (Shirai et al. 1985; Sugieda and Nakajima 2002; Sugieda et al. 1998), The Netherlands (van der Poel et al. 2000), South Korea (Kim et al. 2006), United Kingdom (Bridger 1980), the United States (Guo et al. 1999; Saif et al. 1980), Venezuela (Martinez et al. 2006), and in six European countries (Reuter et al. 2010). The geographical distribution indicates the worldwide occurrence and endemic circulation of porcine sapoviruses among pigs and pig farms. Pigs are infected with porcine sapovirus early in life (Reuter et al. 2010). The one study conducted to examine the prevalence of porcine sapoviruses related to PEC/Cowden showed that at least 83% of 30 sow serum samples from Ohio pig herds with PEC-associated postweaning diarrhea had antibodies reactive to PEC/Cowden (Guo et al. 2001b).

It has not been established whether porcine sapoviruses are species specific. At present, sapoviruses have been identified only in humans, swine, and mink. It is assumed that the main natural transmission mode is fecal–oral. In general, sapoviruses are characterized by stability in the environment and relative resistance to inactivation.

Pathogenesis. Experimental infections with the porcine sapovirus PEC/Cowden produced enteric lesions and disease (Flynn et al. 1988; Guo et al. 2001a). Unusual for viral enteric pathogens, disease and small intestinal lesions in the duodenum and jejunum resulted from intravenous inoculation of PEC/Cowden, as well as by oral exposure. Viral replication in enterocytes was demonstrated by immunofluorescence with anti-PEC/Cowden antiserum. Sapovirus particles were demonstrated in intestinal contents and in the blood.
stream, the first time viremia has been associated with an enteric calicivirus. The mechanism by which the virus reached the small intestine and villous enterocytes from the blood stream was not determined. When infected by the oral route, fecal shedding of the sapovirus PEC/Cowden occurred for up to 9 days. When infected by the intravenous route, fecal shedding was observed for at least 8 days.

**Clinical Signs.** With PEC/Cowden, the incubation period ranged from 2 to 4 days after oral inoculation and clinical signs of anorexia and diarrhea persisted for 3–7 days (Flynn et al. 1988; Guo et al. 2001a). All inoculated pigs became infected and developed clinical signs ranging from mild to severe diarrhea. Control pigs and pigs infected with the tissue culture-adapted PEC failed to develop clinical signs, although intestinal lesions were observed in the exposed pigs. Porcine sapoviruses are one of the viral agents that cause diarrhea in swine.

**Lesions.** Infection with PEC/Cowden produced lesions indistinguishable from those produced by other enteric viral pathogens (Flynn et al. 1988; Guo et al. 2001a). Lesions included shortening, blunting, fusion or absence of duodenal and jejunal villi and, by scanning electron microscopy, an irregular microvillus coat on enterocytes. Crypt cell hyperplasia and a reduction of villus/crypt ratios occurred with cytoplasmic vacuolation and infiltration of polymorphonuclear and mononuclear cells into the lamina propria.

**Diagnosis.** No diagnostic tests for porcine sapoviruses have been developed for use outside the research laboratory. Porcine sapoviruses have been detected using electron microscopy, RT-PCR, and real-time RT-PCR (Reuter et al. 2010; Sugieda and Nakajima 2002; Sugieda et al. 1998; van der Poel et al. 2000; Wang et al. 2005a). However, the sensitivity of molecular tests for laboratory diagnosis of porcine sapovirus infections has not been evaluated. An antigen and antibody ELISA was developed and used to study PEC/Cowden (Guo et al. 2001b).

**Immunity.** Immune responses, protective immunity induced by porcine sapoviruses, and the role of maternal antibodies, have not been assessed. It may be assumed that protective immune mechanisms are similar to those for other enteric virus pathogens. However, the finding of an extraintestinal phase to the pathogenesis of a porcine sapovirus may mean that other immune strategies might be used for their control.

**Prevention and Control.** Assuming that porcine sapovirus epidemiology and immunology are similar to porcine rotaviruses, it is likely that these viruses persist in the environment, and it may be impossible to eliminate the infection from pig herds and prevent natural infection of piglets. However, the persistence of porcine enteric viruses in animal waste depends on the waste treatment technology (Costantini et al. 2007). It is also likely that sows will pass on maternal antibody in colostrum and milk and thus limit infection and damage in the gut of nursing piglets. Treatment with oral rehydration fluids is likely to be successful.

**ST-VALÉRIEN VIRUS**

In 2009, a report described a novel calicivirus from pigs in Quebec, Canada (L’Homme et al. 2009b). Genomic analysis revealed a positive-sense RNA genome of 6409 nucleotides encoding two major ORFs. Phylogenetic analysis showed that these viruses form a unique cluster with a common root with the noroviruses and the Tulane virus isolated from captive juvenile Rhesus macaques. The genus name “Valovirus” has been suggested with the St-Valérien virus as the prototype (Di Martino et al. 2011).

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Coronaviruses
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OVERVIEW
Based on recent taxonomic revisions (Carstens 2010), the family Coronaviridae within the order Nidovirales consists of two subfamilies: (1) Coronavirinae comprised of the genera Alphacoronavirus, Betacoronavirus, and Gammacoronavirus; and (2) Torovirinae comprised of the genera Torovirus and Bafinivirus (Gonzalez et al. 2003).

Four swine coronaviruses (CoVs) have been identified: transmissible gastroenteritis virus (TGEV) first described in 1946; porcine respiratory coronavirus (PRCV), a spike gene deletion mutant of TGEV isolated in 1984; porcine epidemic diarrhea virus (PEDV) isolated in 1977; and the porcine hemagglutinating encephalomyelitis virus (HEV) isolated in 1962. In pigs, CoVs and toroviruses (ToVs) affect a variety of organs, including the gastrointestinal and respiratory tracts, the peripheral and central nervous systems (CNS), and the mammary glands. Most ToVs and PRCV induce mainly asymptomatic infections in pigs, whereas TGEV, PEDV, and HEV infections can result in fatal enteric or nervous diseases.

Swine CoVs comprise two distinct genera, Alphacoronavirus and Betacoronavirus, but share replication strategies common to Coronaviridae. TGEV and PRCV belong to the Alphacoronavirus 1 species that also contains closely related CoVs of domestic cats and dogs. PEDV and two human CoVs (229E and NL63) are separate species in the same genus (Alphacoronavirus). HEV is genetically distinct and belongs to the Betacoronavirus genus. Together with bovine, human OC43, equine, and canine respiratory CoVs, HEV is a member of the Betacoronavirus 1 species. For each swine CoV, only a single serotype is recognized.

CoVs are enveloped and pleomorphic, with an overall diameter of 60–160 nm as viewed by negative stain electron microscopy (EM) (Figure 35.1) (Okaniwa et al. 1968). Most have a single layer of club-shaped spikes (S protein) 12–25 nm in length, but HEV and some other betacoronaviruses have a second shorter layer of surface spikes, the hemagglutinin-esterase (HE) protein.

Genomic Organization and Gene Expression
Swine CoVs contain one large, polyadenylated, single-stranded, genomic RNA (~30 kDa) of positive-sense polarity. The genome organization, replication strategy, and expression of viral proteins are similar to those of other human and animal CoVs (Enjuanes and Van der Zeijst 1995; Gonzalez et al. 2003; Laude et al. 1993). CoV genomic RNA is infectious. The complete genomes of the Purdue and Miller strains of TGEV are 28,546–28,580 nucleotides long and share 96% overall identity (Penzes et al. 2001; Zhang et al. 2007). Most CoVs have a buoyant density in sucrose of 1.18–1.20 g/mL. The phospholipids and glycolipids incorporated into the virus envelope are derived from the host cell, and thus, the envelope composition is host cell dependent (Enjuanes and Van der Zeijst 1995).

CoVs contain four structural proteins: a large surface glycoprotein (spike or S protein visible as the corona; Figure 35.1), a small membrane protein (E), an integral membrane glycoprotein (M), and a nucleocapsid protein (N). However, HEV also contains an HE protein (Carstens 2010).

The N protein (47 kDa) interacts with viral RNA to form a helical ribonucleoprotein complex. This structure, in association with M protein, forms an internal icosahedral core in TGEV. The 29–36 kDa M glycoprotein is embedded in the viral envelope by three to four membrane-spanning regions. In TGEV, the hydrophilic
protein mutations in attenuated TGEV strains or the natural TGEV deletion mutant, PRCV include a serine/alanine mutation at amino acid 585 position associated with induction of virus-neutralizing antibody, as well as receptor (aminopeptidase N) binding (Zhang et al. 2007).

Aminopeptidase N has been identified as the TGEV and PEDV cell receptor (Delmas et al. 1992; Li et al. 2007). The receptor-binding and major neutralizing site (site A) on the S protein of TGEV is located within the same domain (Godet et al. 1994). TGEV binding to sialic acid residues on glycoproteins of target cells was proposed to initiate infection of intestinal enterocytes (Schwegmann-Wessels et al. 2002). Treatment of TGEV with sialidase enhanced hemagglutinin (HE) activity (Noda et al. 1987; Schultze et al. 1996). The HE activity resides in the N-terminal region of the TGEV S protein, a region that is missing from the PRCV S protein; thus, the determination of HE activity (Schultze et al. 1996) could potentially differentiate PRCV and TGEV strains.

Contrasts and Comparisons
Seven CoVs are related antigenically or by their genomic sequence (Enjuanes and Van der Zeijst 1995): TGEV, PRCV, canine coronavirus (CCoV), feline infectious peritonitis virus (FIPV), feline enteric coronavirus (FECoV), PEDV, and human CoV (HCoV 229E). CoVs within Alphacoronavirus 1 (TGEV, PRCV, CCoV, FECoV) are antigenically related, based on cross-reactivity in virus neutralization (VN) and immunofluorescence tests and with mAbs to the S, N, or M proteins, and all share the antigenic subsite Ac on the S protein (Enjuanes and Van der Zeijst 1995). As members of the same species, these viruses likely represent host-range mutants of an ancestral virus strain (Carstens 2010).

Since the emergence in 2002 of the severe acute respiratory syndrome (SARS) CoV, a new species in the Betacoronavirus genus (Carstens 2010), investigators have searched for animal reservoirs for SARS CoV based on serology. Several reported that SARS CoV cross-reacts with antibodies to Alphacoronavirus 1 species (TGEV, PRCV, CCoV, FIPV, FECoV) are antigenically related, based on cross-reactivity in virus neutralization (VN) and immunofluorescence tests and with mAbs to the S, N, or M proteins, and all share the antigenic subsite Ac on the S protein (Enjuanes and Van der Zeijst 1995). As members of the same species, these viruses likely represent host-range mutants of an ancestral virus strain (Carstens 2010).

Although TGEV, CCoV, and FIPV are antigenically closely related, TGEV and CCoV could be distinguished
in vitro by two-way cross-neutralization tests and other biological differences (Reynolds et al. 1980), that is, both TGEV and CCoV grow in canine kidney cells and an established feline cell line, neither CCoV nor FIPV grows in swine testicle or porcine thyroid cells, both of which support the growth of TGEV.

In vivo biological differences in pathogenicity for neonatal pigs are evident among TGEV, CCoV, and FIPV strains. Whereas virulent FIPV caused diarrhea and intestinal lesions similar to those of virulent TGEV, CCoV caused no clinical signs and slight villous atrophy. CCoV shed by acutely infected dogs, infected baby pigs and induced serum virus-neutralizing antibodies to CCoV and TGEV (Woods and Wesley 1992). However, baby pigs and pregnant gilts infected with FIPV did not produce TGEV-neutralizing antibodies, but did develop some immunity to TGEV challenge.

Reverse transcription-polymerase chain reaction (RT-PCR) and mAbs are used to detect and differentiate among the antigenically related alphacoronaviruses. For the S glycoprotein gene that confers host range specificity, the 300 amino acid residues at the N terminus are the most variable. In this domain, CCoV and FIPV are more similar to each other than to TGEV (Wesseling et al. 1994). RT-PCR is used to differentiate U.S. strains of PRCV from prototype strains of TGEV (Kim et al. 2000a). Differentiation of the TGEV-related CoVs was also possible using certain specific mAbs to the S glycoprotein of TGEV that recognized TGEV strains but failed to react with PRCV, FIPV, or CCoV (Callebaut et al. 1989; Laude et al. 1993; Sanchez et al. 1990; Simkins et al. 1992, 1993).

PRCV strains have been characterized and partially or fully sequenced (Britton et al. 1991; Costantini et al. 2004; Kim et al. 2000b; Rasschaert et al. 1990; Vaughn et al. 1995; Zhang et al. 2007). Two striking features typify the PRCV genome: (1) a large deletion (621–681 nucleotides) near the N terminus of the S gene producing a smaller S protein; and (2) a variable region with deletions that often compromise the open reading frame (ORF) downstream of the S gene. These genetic changes may account for the altered tissue tropism of PRCV (Ballesteros et al. 1997; Sanchez et al. 1999). An overall nucleotide and amino acid sequence homology of 96–98% between TGEV and PRCV suggests that PRCV evolved from TGEV and that this occurred on a number of independent occasions. Based on the complete genomic sequences, PRCV Iowa State University (ISU)-1 was more closely related to Miller than to the Purdue TGEV strain (Zhang et al. 2007).

SARS CoV, a new species in the genus Betacoronavirus, causes pneumoenteric disease in humans (Ksiazek et al. 2003; Peiris et al. 2003). Bats are a suspect reservoir for SARS. Transmission of SARS CoV to 6-week-old pigs (PRCV seropositive) failed, although pigs seroconverted to SARS and shed virus detected by RT-PCR (Weingartl et al. 2004). Preexisting PRCV antibodies may affect susceptibility of pigs to SARS since SARS CoV was isolated from a pig in China (Chen et al. 2005).

Although swine CoVs commonly share biological and molecular features, the epidemiology, clinical aspects, and pathogenesis of the infections induced by these viruses differ, requiring distinct strategies to control and prevent each disease. Disease outbreaks are endemic or variable in swine-producing countries. Nevertheless, the diseases induced by these CoVs have resisted eradication in swine and the viruses may continue to evolve in domestic pigs and other potential reservoir (bats) or secondary hosts, such as carnivores, via interspecies transmission.

### TRANSMISSIBLE GASTROENTERITIS VIRUS AND PORCINE RESPIRATORY CORONAVIRUS

#### Relevance

Transmissible gastroenteritis (TGE) is a highly contagious, enteric viral disease of swine characterized by vomiting, severe diarrhea, and high mortality (often 100%) in piglets less than 2 weeks of age. TGE was first described by Doyle and Hutchings (1946) in the United States and subsequently reported worldwide. Although swine of all ages are susceptible to TGEV or PRCV infection, the mortality in TGEV- and/or PRCV-seropositive herds and in swine over 5 weeks of age is generally low.

The appearance and widespread prevalence of PRCV, a naturally occurring deletion mutant of TGEV, lessened the clinical impact of TGE in the United States (Yaeger et al. 2002). However, TGE remains a cause of severe diarrhea in piglets in TGEV/PRCV-seronegative herds in North America. In most European countries, more than 95% of herds are seropositive for TGEV antibodies because of the appearance of PRCV in 1984 and its rapid spread throughout Europe (Brown and Cartwright 1986; Laude et al. 1993; Pensaert 1989; Pensaert et al. 1986, 1993). Economic losses from TGE can be severe (Pritchard 1987), but the establishment of endemic PRCV ameliorated the impact of TGE and outbreaks in Europe have become uncommon (Laude et al. 1993; Pensaert 1989). In Asia, TGEV and PEDV frequently cocirculate, a combination that results in significant economic loss and requires differential diagnosis (Kim et al. 2001).

#### Etiology

TGEV is in the Alphacoronavirus 1 species, Alphacoronavirus genus, and Coronavirinae subfamily (Carstens 2010). TGEV antigen can be demonstrated by immunofluorescence in the cytoplasm of infected cells 4–5 hours postinfection (Pensaert et al. 1970). Maturation of virus occurs in the cytoplasm by budding through the endoplasmic reticulum, and viral particles (65–90 nm in diameter) are observed within the cytoplasmic vacuoles (Figure 35.2A) (Pensaert et al. 1970; Thake
1968; Wagner et al. 1973). Virus may line host cell membranes after exiting from infected cells (Figure 35.2B). TGEV glycoproteins are also evident on the surface of infected swine testicle cells (Laviada et al. 1990).

TGEV is stable when stored frozen, but somewhat labile at room temperature or higher. Infectious virus persisted in liquid manure slurry for more than 8 weeks at 5°C (41°F), 2 weeks at 20°C (68°F), and 24 hours at 35°C (95°F) (Haas et al. 1995). Recent studies used TGEV as a surrogate for SARS CoV (Casanova et al. 2009). TGEV remained infectious in water and sewage for several days at 25°C (77°F), whereas at 4°C (39°F), it retained infectivity for several weeks.

TGEV is highly photosensitive. Fecal material containing $1 \times 10^5$ pig infectious doses was inactivated within 6 hours when exposed to sunlight or when exposed to ultraviolet light (Cartwright et al. 1965; Haelterman 1962). TGEV is inactivated by exposure to 0.03% formalin, 1% Lysovet (phenol and aldehyde), 0.01% beta-propiolactone, 1 mM binary ethylenamine, sodium hypochlorite, NaOH, iodine, quaternary ammonium compounds, ether, and chloroform (Brown 1981; VanCott et al. 1993). TGEV field strains are trypsin resistant, relatively stable in pig bile, and stable at pH 3 (Laude et al. 1981), allowing virus to survive in the stomach and small intestine. However, properties of attenuated and field strains of TGEV vary. Most studies failed to show a correlation between sensitivity to these various treatments and cell-culture passage level or degree of virulence (Laude et al. 1981).

### Role in Public Health

Pigs are the only species naturally susceptible to TGEV and PRCV with the exception of the suspect cases noted. However, no human infections have been reported.

### Epidemiology

On a herd basis, two epidemiological forms of TGE are recognized: epidemic and endemic. In addition, infections with the TGEV deletion mutant PRCV present a different pattern and greatly complicate seroprevalence studies of the epidemiology of TGEV (Pensaert 1989).

#### Epidemic versus Endemic Transmissible Gastroenteritis

Epidemic TGE occurs when most of the animals in a herd are TGEV/PRCV seronegative and seropositive.
susceptible. After introduction, the disease spreads rapidly to swine of all ages, especially during winter. Inappetence, vomiting, or diarrhea occurs in most animals. Suckling pigs show marked clinical signs and rapidly dehydrate. Mortality is very high in pigs under 2–3 weeks of age, but decreases in older pigs. Lactating sows often develop anorexia and agalactia, which further contribute to piglet mortality.

The history and severe clinical signs aid in the diagnosis of epidemic TGE in the United States, since diseases with similar signs have not been reported. However, in Europe and Asia, porcine epidemic diarrhea (PED) had similar clinical signs (Pensaert and de Bouck 1978). Likewise, the presence of PRCV appears to have greatly reduced the incidence of TGE in Europe (Brown and Paton 1991; Laude et al. 1993; Pensaert et al. 1993) where diagnosis of TGE has not been reported in recent years.

Endemic TGE refers to the persistence of the virus and disease in a herd perpetuated by the continual or frequent influx of susceptible swine. Endemic TGE is limited to seropositive herds that have frequent farrowings (Stepanek et al. 1979), herd additions, or commingling of susceptible pigs. Endemic TGE is a common sequel to a primary outbreak in large breeding herds. In endemic infected herds, TGEV spreads slowly among adult swine, particularly herd replacements (Pritchard 1987). Sows are frequently immune and will transfer a variable degree of passive lactogenic immunity to their progeny. Infected sows usually do not show clinical signs. In these herds, mild TGEV diarrhea occurs, and mortality is usually under 10–20% in pigs from ~6 days of age until ~2 weeks after weaning. The age-related effects are influenced by the management system and the degree of passive immunity from the sow.

Endemic TGE in suckling or recently weaned pigs can be difficult to diagnose and must be differentiated from other types of endemic diarrheal pathogens common in young pigs, such as rotavirus and *Escherichia coli*. Endemic TGE persists in the herd as long as susceptible or partially immune swine are exposed to TGEV. It is unclear whether the source of virus is from reactivation of virus shedding in carrier swine or reintroduction of virus into the herd from an external source.

**Porcine Respiratory Coronavirus.** PRCV is a TGEV variant that infects the respiratory tract with limited or no shedding in feces (Pensaert 1989). However, PRCV-infected pigs produce antibodies that neutralize TGEV. Serological surveys of sows or slaughterhouse pigs tested for international trade in Europe led to the discovery of PRCV. The first isolation of PRCV was in Belgium in 1984 (Pensaert et al. 1986). In 1989, two herds in the United States were unexpectedly seropositive for TGEV antibodies, although the herds had been neither vaccinated for TGE nor experienced clinical disease (Hill et al. 1990; Wesley et al. 1990).

Swine population density, distance between farms, and season influence PRCV epidemiology (Henningsen et al. 1989; Pensaert 1989). PRCV infects pigs of all ages by contact or airborne transmission. In areas of high swine density, the virus can spread several kilometers. The risk of a farm becoming infected increases as neighboring herds increase in size.

PRCV infections are often subclinical. The virus has spread rapidly and extensively in pigs in Europe (Brown and Cartwright 1986; Henningsen et al. 1989; Laude et al. 1993; van Nieuwstadt et al. 1989) and even into countries that were previously free of TGEV (Pensaert 1989). A limited serological survey in 1995 in the United States suggested that many asymptomatic herds in Iowa were seropositive for PRCV (Wesley et al. 1997).

PRCV has become endemic in many European swine herds (Laude et al. 1993; Pensaert 1989; Pensaert et al. 1993). The virus circulates in the herd, infecting pigs before the age of 10–15 weeks after passively acquired maternal antibodies have declined. Introduction of pigs into fattening units and commingling of PRCV-negative and PRCV-positive pigs from diverse sources results in seroconversion to PRCV in pigs shortly after introduction into most units.

Susceptible pigs experimentally infected with PRCV shed virus from nasal secretions for less than 2 weeks (Onno et al. 1989; VanCott et al. 1993; Wesley et al. 1990). There is no evidence for the fecal–oral transmission of PRCV. Pensaert et al. (1993) have shown that PRCV persists in closed breeding farms by regularly infecting newly weaned pigs, even in the presence of maternal antibodies. PRCV can persist in the herd throughout the entire year, or it can disappear temporarily from a herd in summer and reappear in the nursery and fattening units in winter. Waves of infection, without clinical disease, coincide with the rainy season in Europe (Laude et al. 1993). Coincident with the widespread dissemination of PRCV, the seroprevalence of TGEV in Europe has decreased, with low prevalence (0.0–7.6%) in Spain and the United Kingdom (Brown and Paton 1991; Pensaert et al. 1993).

**Emergence of Other Transmissible Gastroenteritis Virus-related Coronaviruses.** Outbreaks of fatal acute gastroenteritis associated with TGEV-related CCoV type II (CCoV-II) were recently reported in European dogs (Decaro et al. 2009; Erles and Brownlie 2009). The close genetic relatedness (>96% amino acid identity) in the key replicase domains suggested that the recently emerged CCoV-II strains are host-range variants of TGEV (Carstens 2010). Based on the analysis of the S protein, the CCoV-Ilb strains are referred to as TGEV-like strains (Decaro et al. 2010) and may represent emergence of novel recombinant viruses of mixed (TGEV/CCoV) origin.
Transmission and Reservoirs. An epidemiological feature of TGE is its seasonal appearance during winter. Haelterman (1962) suggested that this is because the virus is stable when frozen and more labile when exposed to heat or to sunlight. This would allow virus transmission between herds in winter on fomites or animals. Haelterman (1962) proposed at least three possible reservoirs for TGEV between seasonal epidemics: (1) associated pig farms in which the virus spreads subclinically, (2) hosts other than swine, and (3) carrier pigs. There is evidence for the existence of TGEV in nonporcine hosts. Cats, dogs, and foxes have been suggested as possible carriers of TGEV from one herd to another, since they can shed virus in their feces for variable periods (Haelterman 1962; McClurkin et al. 1970) and virus excreted by dogs was infectious for pigs (Haelterman 1962; Reynolds and Garwes 1979).

The concentration of starlings (Sturnus vulgaris) in winter in feeding areas of swine may foster mechanical spread of TGEV among farms. Pilchard (1965) reported that TGEV was detected in the droppings of starlings for up to 32 hours after they were fed TGEV. Houseflies (Musca domestica) have also been proposed as possible mechanical vectors for TGEV. TGEV antigen was detected in flies within a swine herd and experimentally inoculated flies excreted TGEV for 3 days (Gough and Jorgenson 1983). According to surveys conducted in central Europe, antibodies against TGEV are also present in approximately 30% of the feral pig population (Sedlak et al. 2008).

A third possibility relating to TGE transmission is the length of time infected swine shed viable TGEV and the role of the carrier pig. Nasal shedding of PRCV in experimentally infected pigs occurs through postexposure day 10 (Onno et al. 1989; Wesley et al. 1990). However, how long pigs recovered from TGEV and PRCV infection in the field remain infectious is unknown. One report indicated chronic and/or persistent TGEV fecal shedding for up to 18 months, suggesting a possible role for the long-term carrier hog in transmitting TGEV (Woods and Wesley 1998). Although TGEV has been detected in the intestinal and respiratory tracts for periods of up to 104 days postinfection (Underdahl et al. 1975), it is unknown whether such virus is shed or transmitted in a viable state. Addition of sentinel pigs to a herd at 3, 4, and 5 months after a previous TGE outbreak resulted in no infections in the introduced pigs, as determined by serological tests (Derbyshire et al. 1969).

Pathogenesis

Intestinal and Extraintestinal Replication of Transmissible Gastroenteritis Virus. Jejunal enterocytes undergo massive necrosis within 12–24 hours after infection, resulting in marked reduction in enzymatic activity (alkaline phosphatase, lactase, etc.) in the small intestine. This disrupts digestion and cellular transport of nutrients and electrolytes, thereby causing an acute maldigestive and malabsorptive diarrhea (Moon 1978) and ultimately leading to severe and fatal dehydration in piglets. Additional mechanisms contributing to diarrhea include altered intestinal sodium transport with accumulation of electrolytes and water in the intestinal lumen (Butler et al. 1974) and loss of extravascular protein. Dehydration is also related to metabolic acidosis coupled with abnormal cardiac function due to hyperkalemia.

The severe villous atrophy in the jejunum (Figure 35.3) and, to a lesser extent, the ileum is often absent in the proximal duodenum (Hooper and Haelterman 1966). Villous atrophy is more severe in newborn pigs than in 3-week-old pigs (Moon 1978), suggesting higher susceptibility of neonates to TGEV infection.

Mechanisms to account for age-dependent susceptibility to clinical disease include the slower replacement in newborn pigs of infected villous epithelial cells by migration of cells from crypts (Moon 1978). These newly replaced villous enterocytes are reportedly resistant to TGEV infection, possibly due to induction of innate immunity and intestinal IFN (La Bonnardiere and Laude 1981) or the inability of the regenerating cells to support virus growth.

The exposure dose of infectious virus plays a major role in age-dependent susceptibility. That is, the infectious dose of TGEV needed to infect a 6-month-old market hog was $10^4$ times greater than that needed to infect a 2-day-old piglet (Witte and Walther 1976).

Moreover, the severity of clinical signs due to TGEV increased when pigs were injected with a synthetic corticosteroid, dexamethasone (Shimizu and Shimizu 1979a). The latter treatment also led to increased lung lesions in mid-to-late stages of PRCV infection (Jung et al. 2007; Zhang et al. 2008), indicating the possible effect of stress on TGEV/PRCV disease severity. In addition, TGEV in combination with infections with other enteric pathogens, such as E. coli or porcine rotavirus, caused more severe enteritis than either infection alone (Underdahl et al. 1972). Likewise, PRCV respiratory infection and lung lesions were exacerbated by preexisting porcine respiratory and reproductive syndrome virus (PRRSV) infection (Jung et al. 2009; Van Reeth et al. 1996).

Extraintestinal sites for TGEV replication include the lungs (alveolar macrophages) and mammary tissues. Oronasal infection of pigs with TGEV caused pneumonia (Underdahl et al. 1975). Cell culture-attenuated, but not virulent, TGEV replicated in cultures of alveolar macrophages in vitro, suggesting a possible role for these cells in lung infection (Laude et al. 1984). Moreover, TGEV was detected in nasal secretions of infected piglets (VanCott et al. 1993) and lactating sows exposed to infected piglets (Kemeny et al. 1975). Cell-cultured strains of TGEV generally showed reduced virulence in pigs, with less replication in the gut and higher levels
of lymphocytic inflammation. Thereafter, pulmonary lesions and clinical signs resolved concurrently with increased virus-neutralizing antibody titers (Atanasova et al. 2008; Jung et al. 2009).

Depending on the experimental conditions and the virus strains used, PRCV may be detected in the blood, tracheobronchial lymph nodes, and occasionally, the small intestines of infected pigs. However, virus in infected enterocytes does not spread to adjacent cells (Cox et al. 1990a,b), and fecal shedding is low or undetectable. The limited intestinal replication of PRCV may be related to the deletion in the S gene. When fecal and nasal isolates of PRCV from the same pigs were compared genetically, only point mutations, but not additional deletions, were noted in the S gene (Costantini et al. 2004).

Clinical Signs

Epidemic Transmissible Gastroenteritis. Typical clinical signs of TGE in seronegative piglets are vomiting and profuse watery, yellowish diarrhea, with rapid loss of weight, dehydration, and high morbidity and mortality in pigs under 2 weeks of age. The severity of clinical signs, duration of disease, and mortality are inversely related to the age of the pig. Most pigs under 7 days of age will die in 2–7 days after onset of clinical signs. Most suckling pigs over 3 weeks of age will survive, but may remain stunted. Clinical signs of TGE in finishing swine and in sows include inappetence, transient diarrhea, and vomiting.

The incubation period is short, usually 18 hours to 3 days. Infection generally spreads rapidly through the
Entire group and most swine are affected in 2–3 days, but this is more likely to occur in winter than summer (Haelterman 1962).

**Endemic Transmissible Gastroenteritis.** Endemic TGE occurs in large herds that farrow frequently and in TGEV- or PRCV-seropositive herds. Clinical signs are usually less severe than those in seronegative pigs of the same age. Mortality is low, especially if pigs are kept warm. The clinical signs in suckling pigs can resemble rotavirus diarrhea (Bohl et al. 1978). In some herds, endemic TGE is manifested primarily in weaned pigs and may be confused with *E. coli*, coccidia, or rotavirus infections (Pritchard 1987).

**Porcine Respiratory Coronavirus.** Experimentally, PRCV infection of pigs is mostly subclinical with self-limiting respiratory infection. Very possibly, the early antiviral effects of innate immune responses to PRCV infection, followed by cell-mediated and antibody responses, effectively control the infection (Atanasova et al. 2008; Jung et al. 2007, 2009; O’Toole et al. 2010). Clinical signs include (1) respiratory signs, for example, coughing, abdominal breathing, and dyspnea; (2) depression and/or anorexia; and (3) slightly decreased growth rates (Lanza et al. 1992; Van Reeth et al. 1996; Wesley and Woods 1996).

The severity and frequency of clinical signs are influenced by the presence of other bacterial or viral pathogens in the herd. For example, coinfection with PRRSV can alter the severity of either PRRSV or PRCV infections. Inoculation with PRRSV followed by PRCV resulted in prolonged fever with respiratory disease, reduced weight gain, and prolonged severe pneumonia (Jung et al. 2009; Van Reeth et al. 1996). Ongoing or preexisting PRRSV infection significantly suppressed innate immune responses, that is, reduced IFN-α levels in the lung and blood natural killer (NK) cell cytotoxicity, during early PRCV infection, which may exacerbate PRCV pneumonia (Jung et al. 2009). PRCV coinfection also induces more severe PRRSV-related pulmonary alveolar macrophage apoptosis in the lungs of dually infected pigs.

**Transmissible Gastroenteritis Virus Lesions**

TGE gross lesions are usually confined to the gastrointestinal tract. The stomach is distended with curdled milk and may have petechial hemorrhages (Hooper and Haelterman 1969). The small intestine is distended with yellow fluid and curdled, undigested milk. The wall is thin and transparent, due to the villous atrophy.

A major lesion of TGE is markedly shortened villi of the jejunum and ileum (Figure 35.3), usually more severe and extensive than that seen in rotavirus diarrhea (Bohl et al. 1978). Infections with some strains of *E. coli* and coccidia may produce similar lesions (Hornich et al. 1977). Transmission EM of TGEV-infected enterocytes of the small intestine has revealed alterations in the microvilli, mitochondria, endoplasmic reticulum, and other cytoplasmic components. Virus particles, primarily in cytoplasmic vacuoles, were observed in villous enterocytes and in M cells, lymphocytes, and macrophages in the dome regions of Peyer’s patches (Chu et al. 1982; Thake 1968; Wagner et al. 1973).

Pathological findings and the extent of villous atrophy are highly variable in pigs from endemically infected herds (Pritchard 1987). Moxley and Olson (1989b) showed that the level of passive immunity in TGEV-infected pigs influenced both the degree of villous atrophy and its segmental distribution. Villous atrophy was minimal in pigs nursing sows previously infected with virulent TGEV, compared with pigs nursing seronegative sows or sows given live attenuated vaccines. In partially protected pigs, villous atrophy was primarily in the ileum, and not the jejunum. Similar observations were noted in pigs from herds with endemic TGE.

**Porcine Respiratory Coronavirus Lesions**

PRCV primarily causes upper and lower respiratory tract disease. The PRCV-induced macroscopic and microscopic lesions are generally limited to the lungs and commonly observed as consolidation of the lung and bronchointerstitial pneumonia, with frequent peribronchial and perivascular lymphohistiocytic cuffing (Atanasova et al. 2008; Cox et al. 1990a; Halbur et al. 1993; Jabrane et al. 1994; Jung et al. 2007, 2009; O’Toule et al. 1989). PRCV-induced bronchointerstitial pneumonia is characterized by (1) thickening of the alveolar septa by infiltration of inflammatory leukocytes, principally macrophages and lymphocytes; (2) type 2 pneumocyte hypertrophy and hyperplasia; (3) accumulation of necrotic cells and inflammatory leukocytes in alveolar and bronchiolar lumina due to airway epithelial necrosis; and (4) peribronchial or perivascular lymphohistiocytic inflammation. Within 10 days of PRCV infection, the virus simultaneously induces inflammatory (cell necrotizing) and proliferative (alveolar septa thickening) chronic-active bronchointerstitial pneumonia (Jung et al. 2007, 2009).

**Diagnosis**

The collection and preservation of appropriate clinical specimens is necessary for reliable diagnosis. Because clinical signs and atrophic enteritis caused by TGEV are frequently observed in other enteric infections (rotavirus, PEDV, and coccidia), laboratory diagnosis of TGE must be accomplished by one or more of the following procedures: detection of viral antigen or nucleic acids in feces or lesions, virus isolation from specimens, or detection of TGEV- or PRCV-specific antibodies.

Diagnosis of PRCV requires similar procedures, but with a focus on respiratory specimens. Evaluation of
Hybridization was employed for the rapid differential diagnosis of eight CoVs including TGEV (Chen et al. 2010).

Electron Microscopy. TGEV can be demonstrated in the intestinal contents and feces of infected pigs by negative-contrast transmission electron microscopy (EM) (Figure 35.5). Immune electron microscopy (IEM) has advantages over conventional EM in being more sensitive for detecting TGEV and distinguishing it from enveloped membranous debris, as well as concurrently detecting the presence of other enteric viruses (Saif et al. 1977).

Virus Isolation. Primary and secondary pig kidney (PK) cells (Bohl and Kumagai 1965) or PK cell lines (Laude et al. 1981), porcine thyroid cells (Witte 1971), and the McClurkin swine testicle cell line (McClurkin and Norman 1966) are recommended for the isolation of TGEV from feces or gut contents of infected pigs. Distinct cytopathic effects (CPEs) may be negligible upon primary isolation of field strains, requiring additional passages before CPE is evident. The CPE consists of enlarged, rounded cells with a balloon-like appearance (Bohl and Kumagai 1965). For detecting viral CPE or hybridization was employed for the rapid differential diagnosis of eight CoVs including TGEV (Chen et al. 2010).

Detection of Viral Antigen or Nucleic Acids. Detection of TGEV antigen in small intestinal enterocytes is commonly used to diagnose TGE. Either immunofluorescence (Pensaert et al. 1970) or immunohistochemical (Shoup et al. 1996) techniques using mAb against the highly conserved N protein of TGEV may be used in formalin-fixed or frozen tissues (Figure 35.4), but they require pigs in the early stage of infection.

An enzyme-linked immunosorbent assay (ELISA) using mAb or polyclonal antibodies to TGEV is used to detect TGEV antigens in cell culture, feces, and intestinal contents (Lanza et al. 1995; Sestak et al. 1996, 1999a; van Nieuwstadt et al. 1988) or PRCV antigen in cell culture, nasal swabs, or lung homogenates (Lanza et al. 1995).

Currently RT-PCR or real-time reverse transcription-polymerase chain reaction (qRT-PCR) is commonly used for the diagnosis of TGEV and differentiation of TGEV and PRCV (Costantini et al. 2004; Kim et al. 2000a). The latter is accomplished using PCR primers targeting the region of the S gene spanning the deletion region in PRCV strains. Multiplex RT-PCR has been developed for the simultaneous detection of major porcine viruses including TGEV and PEDV (Ogawa et al. 2009). These assays permit detection of up to nine viruses in a sample. Moreover, multiplex microarray
plagues, the sensitivity of swine testicle cells can be further enhanced by adding pancreatin or trypsin to cell culture media (Bohl 1979) and using older cells.

PK and swine testicle cells are preferred for isolating PRCV. Nasal swab fluids or lung tissue homogenates from affected swine are used for the isolation of PRCV. The CPE produced by PRCV resembles that produced by TGEV strains, with syncytia frequently observed for PRCV similar to that reported for SARS CoV grown in Vero cells (Ksiazek et al. 2003; Peiris et al. 2003). Identification of cell culture virus can be done by VN, immunofluorescence, or IEM using specific TGEV antisera or differential mAbs (Garwes et al. 1988) and RT-PCR using virus-specific primers (Enjuanes and Van der Zeijst 1995; Kim et al. 2000a; Laude et al. 1993).

Serology. The detection of TGEV antibodies can assist in diagnosis and control. However, TGEV serology is complicated by the fact that both TGEV and PRCV induce virus-neutralizing antibodies that are qualitatively and quantitatively similar (Pensaert 1989). A blocking ELISA test (described below) can differentiate between these antibodies. The entrance of only serologically negative swine will help maintain a herd free of TGEV and PRCV.

A rise in antibody titer between acute and convalescent serum samples provides retrospective evidence for epidemic TGE or infection with PRCV. To determine the presence of endemic TGE or PRCV, serum samples from 2- to 6-month-old swine can be tested for antibodies. At this age, passively acquired antibodies should be absent (Derbyshire et al. 1969); thus, positive results suggest endemic TGEV or PRCV.

TGEV antibodies can be detected by several serological tests. The VN test using cell culture-adapted viruses has been the most widely used (Bohl 1979; Bohl and Kumagai 1965). Virus-neutralizing antibodies to TGEV are detectable in serum by 7–8 days postinfection and persist for at least 18 months. Little is known regarding the persistence of virus-neutralizing antibodies to PRCV within a herd. Antibody ELISA tests (Bernard et al. 1989; Berthon et al. 1990; Callebaut et al. 1989; Garwes et al. 1988; Sestak et al. 1999a,b; van Nieuwstadt et al. 1993) have been reported, but they require concentrated purified virus or S protein for coating ELISA plates.

Blocking Enzyme-linked Immunosorbent Assay for Differentiation of Porcine Respiratory Coronavirus and Transmissible Gastroenteritis Virus. Studies using mAbs to TGEV have shown that certain antigenic sites present on TGEV have been deleted from the PRCV S protein (Callebaut et al. 1989; Delmas and Laude 1990; Sanchez et al. 1990; Sestak et al. 1999b; Simkins et al. 1992, 1993). This difference between TGEV and PRCV serves as the basis for blocking ELISAs differentiating TGEV and PRCV infections (Bernard et al. 1989; Callebaut et al. 1989; Garwes et al. 1988; Simkins et al. 1993).

Blocking ELISAs should only be applied on a herd basis because some pigs with low TGEV or PRCV antibody titers may not be detected (Callebaut et al. 1989; Sestak et al. 1999b; Simkins et al. 1993). In addition, the accuracy of commercial ELISAs for differentiating U.S. strains of PRCV and TGEV is low, and therefore appears to be applicable on a herd, but not individual pig basis (Sestak et al. 1999b). Recently, new differentiation ELISA systems were used to differentiate not only between TGEV and PRCV antibodies, but also between TGEV and TGEV-like CCoVs and/or classical CCoV-II antibodies (Elia et al. 2010; Lopez et al. 2009).

Immunity

Active Immunity to Transmissible Gastroenteritis Virus. The duration of active immunity in swine after oral infection with virulent TGEV has not been well characterized. Intestinal infection of breeding-age swine results in detectable serum antibodies that persist for at least 6 months and possibly several years (Stepanek et al. 1979). Although the serum antibodies provide serological evidence of TGEV or PRCV infection, they afford little indication of the degree of active immunity to TGEV. Swine that have recovered from TGE are immune to subsequent short-term challenge, presumably due to local immunity within the intestinal mucosa (Brim et al. 1995; Saif et al. 1994; VanCott et al. 1993, 1994). The age and immune status of the animal at initial infection and the severity of the challenge influence the completeness and duration of active immunity.

The mechanism of active immunity in the gut probably relates to stimulation of the secretory immunoglobulin A (sIgA) immune system with production of intestinal sIgA antibodies by lymphoid cells within the lamina propria (Saif et al. 1994; VanCott et al. 1994; VanCott et al. 1993). IgATGEV antibodies and antibody-secreting cells (ASCs) have been detected in the intestine and serum of pigs after oral, but not parenteral, inoculation with TGEV (Kodama et al. 1980; Saif et al. 1994; VanCott et al. 1993, 1994). Kodama et al. (1980) proposed that detection of IgA antibody in the serum, presumably intestinally derived, might serve as an indicator of active immunity to TGE.

Enzyme-linked immunospot (ELISPOT) was used to investigate the kinetics of IgA and IgG TGEV antibody production by the pig’s systemic and local gut-associated lymphoid tissues (GALTs). High numbers of IgA ASCs were induced in GALT only by virulent TGEV. In contrast, live attenuated (vaccine) TGEV or PRCV strains induced significantly fewer IgA ASCs (Berthon et al. 1990; Saif et al. 1994; VanCott et al. 1993, 1994). These and other studies indicated that the pig is immunocompetent at birth, but additional maturation time
may be required for intestinal antibody responses to reach adult levels.

Besides local antibody-mediated immunity, cell-mediated immune (CMI) responses may also be important in active immunity against TGEV infections. However, only indirect evidence exists concerning the role of CMI in resistance to TGEV infection. CMI was demonstrated with lymphocytes obtained from GALT of swine orally infected with virulent TGEV (Brim et al. 1995; Frederick et al. 1976; Shimizu and Shimizu 1979b), whereas swine parenterally or oronasally inoculated with attenuated TGEV or PRCV developed CMI mainly in systemic sites. Lymphoproliferative responses to TGEV persisted within GALT, but not systemic lymphocytes, for at least 110 days after oral infection of 6-month-old swine (Shimizu and Shimizu 1979b), but for only about 14–21 days after infection of younger (7- to 11-day-old) pigs (Brim et al. 1995). CD4 T-helper cells are involved in lymphoproliferative responses to TGEV (Anton et al. 1995). Potent production of antiviral IFN-α by plasmacytoid dendritic cells derived from TGEV-infected swine was observed upon stimulation of these cells in vitro with TGEV antigens (Calzada-Nova et al. 2010).

A correlation between lymphoproliferative responses and lactogenic immunity to TGEV was described in sows vaccinated with attenuated or recombinant TGEV vaccines (Park et al. 1998). Although T-cell epitopes were identified by lymphoproliferation studies for each of the three major proteins of TGEV, a dominant functional T-helper epitope was defined on the N protein (N321) (Anton et al. 1995). The N321 peptide-induced T-cells collaborated in the in vitro synthesis of TGEV-neutralizing antibodies specific for the S protein. Maximal responses were induced by native S protein combined with recombinant N protein. Such findings have important implications for design of TGEV subunit or other recombinant TGEV vaccines.

Because lymphocyte cytotoxicity was absent in newborn piglets and decreased in parturient sows, it was proposed that a lack of NK cell activity against TGEV-infected cells might correlate with the increased susceptibility of newborn piglets and parturient sows to TGEV infection (Cepica and Derbyshire 1984). Thus, CMI or innate immunity may play a role in either recovery from TGEV infection or resistance to reinfection via the rapid elimination of TGEV-infected epithelial cells.

Porcine Respiratory Coronavirus-induced Active Immunity to Transmissible Gastroenteritis Virus. The dramatic decline in epidemic outbreaks of TGE in Europe following the widespread dissemination of PRCV prompted researchers to examine if respiratory PRCV infection could induce protective intestinal immunity against TGEV. The consensus from several studies was that prior infection of nursing or weaned pigs with PRCV provided partial immunity against TGEV challenge, as evidenced by a reduced duration and level of virus shedding and diarrhea in most pigs studied (Brim et al. 1995; Cox et al. 1993; VanCott et al. 1994; Wesley and Woods 1996).

The mechanism of this partial immunity presumably is related to the rapid increase in TGEV-neutralizing antibodies (Cox et al. 1993; Wesley and Woods 1996) and numbers of IgG and IgA ASCs in the intestines of PRCV-exposed pigs after TGEV challenge (Saif et al. 1994; VanCott et al. 1994). The altered tissue tropism of PRCV was also linked to a shift in antibody responses; that is, in TGEV-infected pigs, more IgA ASCs were found in the gut, whereas PRCV predominantly induced IgG ASCs in the lungs (VanCott et al. 1994). Migration of PRCV-specific IgG and IgA ASCs from the bronchus-associated lymphoid tissues (BALTs) to the gut of the PRCV-exposed pigs after TGEV challenge might explain the rapid anamnestic response and the partial protection induced (VanCott et al. 1994). However, neonatal pigs required at least 6–8 days after PRCV exposure to develop partial immunity to TGEV challenge (Wesley and Woods 1996), suggesting that induction of active immunity might be too late to protect seronegative newborn piglets from epidemic TGE.

Passive Immunity to Transmissible Gastroenteritis Virus. Passive lactogenic immunity is critical to provide newborn piglets with immediate protection against TGEV infection. Circulating passive antibodies, acquired after absorption of colostral immunoglobulin (primarily IgG), protect the neonate against systemic, but not intestinal infection (Hooper and Haelterman 1966; Saif and Sestak 2006). During the first week of lactation, IgA becomes dominant in milk and IgG decreases. Cells seeded from the intestine produce IgA locally in the mammary gland; IgA is secreted into milk and plays a key role in passive intestinal immunity of the suckling piglet.

Mechanisms of passive immunity to TGEV infections have been reviewed (Saif and Bohl 1979; Saif and Jackwood 1990; Saif and Sestak 2006). Swine recovered from TGE transmit passive immunity to their suckling pigs by the frequent ingestion of colostrum or milk (lactogenic immunity) that contains TGEV virus-neutralizing antibodies (Hooper and Haelterman 1966). Such antibodies in the lumen of the intestine neutralize the ingested TGEV and protect the susceptible small intestinal enterocytes. This is accomplished naturally when piglets suckle immune sows frequently or is accomplished artificially by continuous feeding of antisera to piglets.

TGEV antibodies in colostrum and milk of sows are primarily associated with IgA or IgG (Abou-Youssef and Ristic 1972; Bohl et al. 1972; Saif et al. 1972). IgA TGEV antibodies in milk are stable in the gut and provide the most effective protection, but IgG antibodies are also
Porcine Respiratory Coronavirus-induced Passive Immunity to Transmissible Gastroenteritis Virus. The incidence and severity of TGE in countries with PRCV has declined since PRCV has become widespread. This suggests that prior exposure of swine to PRCV imparts partial immunity to TGEV (Laude et al. 1993; Pensaert 1989).

Prior natural exposure of sows to PRCV induced a variable degree of passive protection (44–53% mortality) against experimental TGEV challenge of suckling pigs (Bernard et al. 1989; Paton and Brown 1990). Variable protection in the field during TGE outbreaks was also noted among litters of PRCV-exposed sows (Callebaut et al. 1990; Pensaert 1989). Similar variable levels of protection (30–67% mortality) were reported after TGEV challenge of piglets suckling sows that had been experimentally infected or reinfected with PRCV during pregnancy (Callebaut et al. 1990; De Diego et al. 1992; Lanza et al. 1995; Sestak et al. 1996; Wesley and Woods 1993). In the latter two studies, litter mortality was lowest (range = 0–27%), and IgA and IgG milk antibody titers were highest, in sows multiply exposed to PRCV during two subsequent pregnancies. These experimental findings agreed with field reports that naturally PRCV-exposed sows reinfected with PRCV during pregnancy secreted IgA TGEV antibodies in milk and provided a high degree of protection (0–12.5% mortality) to TGEV challenge (Callebaut et al. 1990). Besides PRCV IgA antibodies in milk, a hallmark of protection in these and other studies (Wesley and Woods 1993) was induction of active immunity to TGEV in the sow preventing clinical disease or agalactia.

Besides quantitative differences in the levels of IgA antibodies induced in milk of sows after exposure to TGEV or PRCV, researchers have investigated potential differences in virus epitopes recognized by the IgA milk antibodies (De Diego et al. 1992, 1994). In TGEV-infected sows, antigenic subsite A (Aa, Ab, Ac), followed by antigenic subsite D (Madrid), was the best inducer of IgA antibodies, while after PRCV infection, antigenic site D and subsite Ab were immunodominant. Thus, only IgA recognizing at least antigenic sites A and D conferred protection in vivo, whereas any immunoglobulin isotype reactive to one antigenic site neutralized virus in vitro.

Prevention and Control Treatment. No antiviral drugs have been developed for treatment of TGE. After the discovery of SARS CoV, studies were conducted with various surrogate viruses, including TGEV, to develop efficient anti-CoV agents. Ortego and colleagues (2007) used TGEV deletion mutants to show that absence of the E protein blocks virus trafficking in the endoplasmic reticulum and prevents virus maturation. RNA interference (RNAi) targeting the viral RNA polymerase was studied in vitro using swine testicle cells as a strategy to prevent TGEV infection (Zhou et al. 2007). Although protective in vitro, the results of analogous in vivo experiments were less convincing (Zhou et al. 2010).

Although high levels of type 1 IFN were detected in the intestine of pigs in the early phase of TGEV infection, its role in the recovery or pathogenesis of TGE is undetermined (La Bonnardiere and Laude 1981). Studies suggest that IFN may activate NK cells in newborn pigs, contributing to resistance to challenge with TGEV (Lesnick and Derbyshire 1988; Loewen and Derbyshire 1988). In addition, during a field outbreak of TGE, 1- to 12-day-old piglets treated orally for 4 days with 1–201IU of human IFN-α had significantly greater survival rates than placebo-treated piglets (Cummins et al. 1995).

The only available treatment for TGE is to alleviate starvation, dehydration, and acidosis. Parenteral treatment with fluids, electrolytes, and nutrients are effective in treating young pigs, but not practical under farm conditions. Oral therapy with balanced electrolyte or glucose solutions is contraindicated in young pigs (Moon 1978). The following measures are suggested: provide a warm (above 32°C [90°F]), draft-free, dry environment and provide water or nutrient solutions freely to TGEV-infected pigs. Such measures reduced mortality in pigs that were infected at more than 3–4 days of age. Antibacterial therapy is beneficial in 2- to 5-week-old pigs, if there is concurrent infection with bacterial pathogens. Cross-fostering of infected or susceptible litters onto TGE-immune sows was useful in some field outbreaks (Stepanek et al. 1979).

Management Biosecurity. Swine in the incubative or viral shedding stage of the disease or possibly carriers can transmit TGEV. Precautions are needed to introduce swine into a herd that originate from herds free of TGE, are serologically negative, and/or have been placed in isolation.
on the farm for 2–4 weeks before being added to the herd. After a TGE outbreak, at least 4 weeks should elapse from the last sign of disease before introducing such animals into a “clean” herd. Feces from TGEV-infected swine can be carried on boots, shoes, clothing, truck beds, feeds, and so on, and can be a source of infection to other herds, requiring strict disinfection regimes, especially in winter.

After Onset of Transmissible Gastroenteritis and Endemic Transmissible Gastroenteritis Virus. When TGE occurs on a farm and pregnant animals have not yet been exposed, two procedures may minimize losses of newborn pigs: (1) If the sows are due to farrow in at least 2 weeks, orally expose them to virulent autogenous virus, such as a slurry of minced intestines of infected pigs, so that they will be immune at farrowing; and (2) if the sows will farrow in under 2 weeks, attempt to provide facilities and management procedures to avoid exposure to TGEV until at least 3 weeks postfarrowing.

Some success has been achieved in elimination of TGEV from epidemiologically infected closed breeder herds without depopulation by the following procedures (Harris et al. 1987): (1) Bring in breeding stock replacements for the next 4–6 months; (2) in the face of an outbreak, feed TGEV-infected minced intestines simultaneously to all pigs in the herd (including replacement stock) to eliminate susceptible hosts, shorten the time the disease progresses through the herd, and ensure more uniform exposure levels in all pigs; (3) maintain strict all-in/all-out production in farrowing and nursery units; and (4) add sentinel seronegative pigs about 2 months after clinical signs of TGE disappear and monitor these pigs for seroconversion to TGEV. Potential hazards associated with feedback control of TGE include possible spread of other pathogens to pregnant sows and throughout the herd.

Other approaches to control or terminate endemic TGE include the following. First, pregnant seropositive sows can be vaccinated intramuscularly (IM) late in gestation or shortly after farrowing with live attenuated TGEV to boost immunity, increase milk antibody levels, and maintain longer passive immunity in suckling pigs (Saif and Sestak 2006; Stepanek et al. 1979). Although this procedure may only delay onset of TGE in exposed pigs, the delay itself can reduce mortality. Second, break the cycle of infection by eliminating reservoirs of susceptible pigs in a unit: prevent the continual influx of susceptible animals into the herd temporarily (alter farrowing schedule as possible), utilize other facilities, and create smaller farrowing and nursing units to achieve an all-in/all-out system.

Immunoprophylaxis
Vaccines and Vaccinations. There are several licensed TGEV vaccines. All contain inactivated or live attenuated TGEV and are approved for use in pregnant or neonatal swine. These vaccines and their efficacy have been reviewed and will be briefly summarized (Saif and Sestak 2006).

Many variables complicate the evaluation of both experimental and commercial TGEV vaccines, resulting in conflicting data. These include the challenge dose and strain of TGEV, the age of the pig at challenge, environmental conditions (especially temperature), the milking efficiency of the vaccinated sow, and the immune status (for TGEV or PRCV antibodies) of the dam at vaccination. If previously infected sows were unknowingly used in vaccine challenge studies, this could account for some of the discrepant results seen in immune responses and piglet protection. This possibility can only be eliminated by using sensitive tests (such as VN) to measure TGEV/PRCV antibodies and by knowing the herd history of test animals since occurrence of PRCV in herds further complicates TGEV vaccine studies.

Results using attenuated strains orally and/or via intranasal (IN) route have been inconsistent and disappointing (Henning and Thomas 1981; Moxley and Olson 1989a; Saif and Bohl 1979; Saif and Sestak 2006). The generally poor results for oral or IN vaccination of sows using attenuated TGEV strains may be attributed to the superficial or limited replication of most attenuated strains in the sow’s intestine (Frederick et al. 1976; Hess et al. 1977). Consequently, this results in little antigenic stimulation of underlying intestinal IgA plasma cells and correspondingly little IgA antibody secretion in milk. Thus, the dilemma is how to develop commercial vaccines to prevent epidemic TGEV that are capable of stimulating IgA in the gut of sows, but sufficiently attenuated so as not to produce disease in newborn pigs.

Parenteral TGEV vaccines induced even lower or inconsistent protection rates in TGEV/PRCV seronegative swine. They have two major disadvantages: (1) Vaccinated swine develop little or no gut immunity and often get sick when exposed to TGEV, depriving their suckling pigs of milk; and (2) the low titer IgG and no IgA TGEV antibodies in milk of vaccinated sows fail to provide optimal passive protection to suckling pigs.

Currently available parenterally administered TGEV vaccines may be more effective in boosting immunity in pregnant swine previously infected with TGEV or PRCV than in initiating immunity in seronegative pregnant swine. These vaccines may be especially useful in herds in which endemic TGE is a problem (Stepanek et al. 1979).

Transmissible Gastroenteritis Vaccination of Neonatal or Weaned Pigs. Active immunization of suckling or feeder pigs could be important for control of endemic infections, especially in newly weaned pigs, in which
Transmissible Gastroenteritis Vaccination of the Seronegative Pregnant Dam. A variety of viral vaccines (virulent, attenuated, inactivated, and subunit) and routes of administration (oral, IN, IM, subcutaneous, and intramammary) (Bohl and Saif 1975; Moxley and Olson 1989a; Saif and Bohl 1979; Saif and Jackwood 1990; Saif and Sestak 2006) have been tested for induction of lactogenic immunity. Only oral administration of live virulent virus to pregnant sows consistently stimulated high levels of protective immunity for the sow and persisting IgA TGEV antibodies in milk associated with passive protection of piglets.

Recombinant Vaccine Approaches. During the last two decades, the emphasis has been on the development of TGEV protein subunit vaccines. Among the major structural proteins of TGEV, the S protein contains immunodominant epitopes that are recognized by virus-neutralizing antibodies. Some of these epitopes were continuous domains (Delmas and Laude 1990), prompting the design of antigenic synthetic peptides derived from the S protein (Posthumus et al. 1991). However, a peptide containing the major T-helper cell epitope derived from the N protein cooperated with the S protein for in vitro induction of TGEV-specific antibody (Anton et al. 1996).

To express the TGEV S, M, or N proteins, several prokaryotic and eukaryotic systems such as E. coli, Salmonella, adenovirus, vaccinia virus, baculovirus, and plants were used (Enjuanes et al. 1992; Godet et al. 1991; Gomez et al. 2000; Park et al. 1998; Shoup et al. 1997; Smerdou et al. 1996; Torres et al. 1996; Tuboly et al. 2000). In some studies (Torres et al. 1996), but not others (Gomez et al. 2000; Smerdou et al. 1996; Tuboly et al. 2000), protective antibodies were induced in inoculated animals correlating with partial protection (Park et al. 1998; Shoup et al. 1997). A novel approach to passive immunization of newborn piglets was suggested by feeding the recombinant immunoproteins capable of neutralizing TGEV in vitro to confer protective immunity in vivo (Bestagno et al. 2007). The concept of using immunoproteins may be cost-effective by expressing these proteins in plants (Monger et al. 2006). Various levels of virus-neutralizing antibodies and protection were induced using eukaryotic vectors to express the TGEV S glycoprotein encoding the glycosylation-dependent antigenic determinants (sites A, B) with or without sites C and D. The S glycoprotein of TGEV expressed in vaccinia virus induced low titers of virus-neutralizing antibodies, but no protection (Hu et al. 1985). The baculovirus-expressed S protein induced IgG virus-neutralizing antibodies to TGEV in serum, colostrum, and milk (Shoup et al. 1997; Tuboly et al. 1995), but the protective ability of these systemic antibodies was low (Godet et al. 1991; Shoup et al. 1997; Tuboly et al. 1995). Only S glycoprotein constructs containing antigenic site A induced high virus-neutralizing antibody titers. Sites C and D induced only low titer virus-neutralizing antibodies, but interestingly, they primed pigs for secondary serum antibody responses after challenge (Shoup et al. 1997).

Similar findings were evident in studies using the same baculovirus-expressed S constructs administered IM to boost antibody responses in sows vaccinated orally with attenuated TGEV vaccines: the partial protection rates were comparable with IM boosting with attenuated TGEV vaccine (Park et al. 1998). In studies using baculovirus-expressed TGEV structural proteins (S, N, and M) coadministered IP with E. coli mutant heat-labile toxin (LT)-adjuvant, IgA antibody responses to TGEV were associated with reduced TGEV shedding in challenged pigs (Sestak et al. 1999a).

Live vectored vaccines expressing TGEV S protein fragments have also been evaluated. Most were based on human adenovirus engineered to express the TGEV or PRCV S proteins (Callebaut et al. 1996; Torres et al. 1996; Tuboly and Nagy 2001). The vaccines elicited variable protection against TGEV mortality and little protection against TGEV or PR CV infection.

Since the pathology of TGEV remains localized in the intestine, an effective vaccine should primarily elicit an intestinal immune response (Saif and Jackwood 1990; Saif and Sestak 2006; VanCott et al. 1993). Further improvements of TGEV vaccines might be achieved by the use of supplementary mucosal delivery systems such as immunostimulating complexes (ISCOMs), biodegradable microspheres, or infectious recombinant TGEV clones engineered to enhance TGEV immunogenicity and reduce pathogenicity (Enjuanes et al. 2005). Studies of TGEV infectious cDNA minigenomes indicate that this approach can also be used for targeted delivery of immunogens derived from other pathogens to the intestine or respiratory tract.

PORCINE EPIDEMIC DIARRHEA VIRUS

Relevance

In 1971, previously unrecognized acute outbreaks of diarrhea were observed in feeder pigs and fattening swine in England (Oldham 1972). Suckling pigs did not
become sick. The disease spread to other European countries and the name “epidemic viral diarrhea” (EVD) was adopted. In 1976, similar outbreaks of acute diarrhea were still observed, but now in swine of all ages, including suckling pigs (Wood 1977). TGEV and other known enteropathogenic agents were ruled out as the cause of this newly emerged disease not only at its first appearance, but also at this later stage. In 1978, a CoV-like agent was associated with these latter outbreaks in piglets (Chasey and Cartwright 1978; Pensaert and de Bouck 1978). Experimental inoculations with the Belgian isolate, designated CV777, revealed its enteropathogenic character for both piglets and fattening swine (Debouck and Pensaert 1980). The names “porcine epidemic diarrhea” (PED) and PED virus (PEDV) were proposed (Pensaert et al. 1982) and are still used.

In Europe, PEDV has caused widespread epidemics with severe losses in suckling pigs in several swine-raising countries during the 1970s and 1980s. Since then, epidemics have become rare and PEDV has been more often associated with single outbreaks and recurrent diarrheic problems in weaned and feeder pigs. However, epidemics may occur at any time, as was observed in Italy in 2005–2006, since the immunity in the sow population is now presumed to be low.

In Asia, epidemics with important losses in suckling pigs were first reported in 1982 and continued to occur in the 1990s and 2000s. Thus, PED appears to be of more significance to swine health in Asia than in Europe, but estimates on its economic impact are not available. There are no indications that PEDV is present in the western hemisphere or in Australia.

**Etiology**

Morphological and physicochemical properties of PEDV are similar to those of the members of the family *Coronaviridae*. Based on genetic and antigenic criteria, PEDV was included in the genus *Alphacoronavirus* together with TGEV, FCoV, CCoV, and HCoV 229E. The nucleotide sequence and amino acid identity of the entire N gene of Korean strain Chinju99 and Chinese strain LBJ/03 showed more than 96% homology with those of the Belgian CV777 strain (Junwei et al. 2006; Lee and Yeo 2003). The entire S gene of Chinju99 showed 94.5% nucleotide and 92.8% amino acid sequence identity with CV777 (Yeo et al. 2003). Similar nucleotide sequences of the N gene were detected in both Korean and Japanese strains (Kubota et al. 1999).

Vero (African green monkey kidney) cells support the serial propagation of PEDV in the culture medium if supplemented with trypsin. CPEs consist of vacuolation and formation of syncytia with up to 100 nuclei (Hofmann and Wyler 1988). PEDV was also successfully grown in porcine bladder and kidney cells in Japan (Shibata et al. 2000). While the virus did not show hemagglutinating activity in early studies, such activity was demonstrated recently with rabbit erythrocytes after trypsin treatment (Park et al. 2010).

PEDV isolates from different countries or continents do not differ when compared by classical serological methods. Differences in virulence of geographically diverse field isolates have not been reported.

**Role in Public Health**

PEDV is only infectious for swine and does not play a role in public health.

**Epidemiology**

Although PED regularly caused epidemics with pig mortality in Europe from 1971 until the late 1980s, reports after 2000 are rare. In the Czech Republic, 27 out of 219 fecal samples from diarrheic pigs less than 21 days old were positive for PEDV, often in combination with other enteric viruses (Rodák et al. 2004). In Italy, an epidemic occurred in 2005 and 2006 involving 63 herds with disease in pigs of all ages, but mortality was largely restricted to suckling piglets (Martelli et al. 2008). Because of the low clinical importance of the disease, serological surveys have not been conducted recently in Europe and, therefore, the prevalence of the virus in the swine population is currently unknown.

In Asia, PED first occurred in Japan in 1982 (Takahashi et al. 1983) and continued into the 1990s with mortality ranging from 30% to 100% in suckling pigs (Kuwahara et al. 1988; Sueyoshi et al. 1995). PED was first recognized in Korea in 1993 (Kweon et al. 1993), with disease in pigs of all ages. It accounted for more than 50% of the enteric viral infections in suckling piglets diagnosed in the 1990s (Chae et al. 2000; Hwang et al. 1994). A serosurvey in slaughterhouse pigs from seven provinces in Korea in 1994 showed 17.6–79% (average 45%) positive animals (Kweon et al. 1994). A recent serosurvey in Korea found that 754 of 1024 sows from 48 commercial farms were positive (Oh et al. 2005). In the northeastern part of India, 21.2% of 528 serum samples from pigs (2–6 months old) were positive for PEDV antibodies (Barman et al. 2003). In Thai-land, between 2007 and 2008, PED occurred in 24 farms from eight provinces. Pigs of all ages were affected, and mortality in the newborn piglets was 100% (Puranaveja et al. 2009). These data indicate that PEDV has been widespread in some countries or regions in Asia. In China, PEDV infections occur frequently in swine farms, despite the use of vaccines, and damage is continuous and serious in many provinces.

Direct or indirect fecal–oral transmission is probably the main, if not the only route of PEDV transmission. Acute outbreaks in nonimmune farms often occur within 4–5 days after sale or purchase of pigs. Virus chiefly enters farms via infected pigs, but also by contaminated trucks, boots, or other fomites. Virus
excretion in infected pigs lasts 7–9 days, and long-time virus carriers have not been reported.

After an outbreak on a breeding farm, the virus may either disappear or persist. PEDV can become endemic status if sufficient litters of pigs are produced and weaned so that, after the acute phase of the outbreak has passed, the virus is maintained through infection of consecutive litters that have lost their lactogenic immunity at weaning.

Pathogenesis
The pathogenesis of PED has been studied in piglets orally inoculated with the CV777 isolate at 3 days of age (Debouck et al. 1981). Clinical signs were observed after 22–36 hours. Viral replication occurred in the cytoplasm of villous epithelial cells throughout the small intestine as early as 12–18 hours postinoculation (PI) and a maximum effect was reached at 24–36 hours. Infection resulted in the degeneration of enterocytes leading to a reduction in the villous height: crypt depth ratio from the normal 7:1 to 3:1. The pathogenic features of PEDV in the small intestine of piglets are very similar to those of TGEV, but somewhat less pronounced. PEDV replication was also observed in the colonic epithelium, but no cell degeneration was seen. Shibata et al. (2000) showed that SPF pigs, inoculated with field PEDV between the ages of 2 days and 12 weeks, developed age-dependent resistance and deaths occurred only in 2- to 7-day-old pigs. Pathogenic features of PED described in Korea and Japan are very similar to those reported in Europe (Kim and Chae 2003; Sueyoshi et al. 1995).

Clinical Signs
The main and often the only obvious clinical sign of PED is watery diarrhea. On breeding farms, pigs of all ages become sick. Morbidity approaches 100% in piglets, but can vary in sows. Piglets up to 1 week of age may die from dehydration after the diarrhea has lasted 3–4 days. Mortality averages 50% but may reach 100%. Older pigs recover after about 1 week. Sows may or may not have diarrhea and only show depression and anorexia. They often give the impression of experiencing bellyache. In fattening pigs, all the pigs in the unit will show diarrhea within a week. The animals have watery feces and often show severe anorexia and depression.

The disease on a breeding farm is self-limiting and stops when the pregnant sows have become immune and protect their offspring by lactogenic immunity. The interval between onset and cessation of the disease is generally 3–4 weeks, but may be much longer in large breeding farms with multiple, separated units.

After the acute outbreak has passed, diarrhea may persist on the farm in pigs after weaning and become recurrent weaning diarrhea (Martelli et al. 2008; Pijpers et al. 1993). PEDV may also be involved in a multietio-

logical diarrhea syndrome in feeder pigs, appearing 2–3 weeks after entering the fattening units, particularly when the pigs originate from different sources and when new pigs are continuously added to the fattening unit (Van Reeth and Pensaert 1994).

PED has several clinical features in common with TGE, but the virus spreads more slowly within and between the different units on closed breeding and/or fattening farms. It may take 4–6 weeks before the virus infects the different sites. Some units may even remain or deliberately be kept free of infection.

Lesions
Lesions have been described in experimentally and naturally infected suckling piglets (Coussement et al. 1982; Kim and Chae 2003; Pospischil et al. 1981; Sueyoshi et al. 1995). Lesions are confined to the small intestine that, early after the start of diarrhea, is distended with watery yellowish fluid. Neonatal pigs are severely dehydrated. Microscopically, vacuolation and exfoliation of small intestinal enterocytes occur mainly on the upper part of the villi. The small intestinal villi become reduced to two-thirds of their original length, and the enzymatic activity of the intestine is markedly decreased. This pathology is very similar to that observed in TGE, but is somewhat less extensive. No histopathological changes have been observed in the colon.

Diagnosis
Diagnosis can be made by direct demonstration of PEDV and/or its antigens using direct immunofluorescence or immunohistochemical tests applied to sections of the small intestine of baby pigs sacrificed within 1 day after the onset of diarrhea and prior to the total desquamation of enterocytes (Bernasconi et al. 1995; Debouck et al. 1981; Guscetti et al. 1998; Sueyoshi et al. 1995).

PEDV particles can be demonstrated using direct EM of feces of pigs collected the day after the onset of diarrhea. Virus particles are often difficult to recognize when the virion spikes are lost or not clearly visible. Furthermore, IEM must be applied to differentiate PEDV from TGEV, because both viruses have identical morphology.

Isolation of field strains of PEDV from feces is done in Vero cells or in other cell types. Trypsin treatment and blind passages may be needed before CPE appears, but early detection can be done by immunofluorescence (Hofmann and Wyler 1988; Shibata et al. 2000).

A number of ELISA techniques have been developed for the detection of PEDV antigens in feces using polyclonal antibodies and mAbs (Callebaut et al. 1982; Carvajal et al. 1995; van Nieuwstadt and Zetstra 1991). ELISA is reliable for diagnosis when testing fecal samples collected at the acute stage diarrhea. Several animals in
the group must be sampled for detection of PEDV in diarrheal samples from weaned pigs or feeder pigs.

Other diagnostic tests for the detection of PEDV and/or differentiation of PEDV from TGEV in intestines and feces are RT-PCR (Ishikawa et al. 1997; Kubota et al. 1999) and in situ hybridization (Kim and Chae 2000).

Paired serum samples are required for a serological diagnosis of PEDV. PEDV antibodies have been demonstrated with ELISAs using antigens consisting of cell-cultivated virus (Carvajal et al. 1995; Kweon et al. 1994) or S and N viral proteins extracted from infected Vero cells (Knuchel et al. 1992). Antibodies detected by blocking ELISA appear at 7 days postinfection. The VN test in Vero cells can also be used (Oh et al. 2005).

Possible PEDV infection must be differentiated from TGE, which in the case of acute diarrhea in swine of all ages can only be done with certainty through laboratory testing. In neonatal colibacillosis or rotavirus diarrhea, adult animals are not affected and sick pigs are usually born from gilts or young sows. Laboratory techniques must be used to differentiate PED from other causes of diarrhea in weaned or feeder pigs.

Immunity
Immune responses to PEDV infection are very similar to those described above for TGEV (also Saif and Sestak 2006). Neutralizing antibodies appear in the serum, but may not play an important role in protecting against the enteric disease since protection is primarily dependent on the presence of secretory IgA antibodies in the intestinal mucosa. Immunity may not be long lasting, but a rapid anamnestic response upon reexposure generally prevents reappearance of disease.

While PED occurs in pigs of all ages, piglets up to 1 week of age may experience high mortality and need to be protected by transfer of maternal antibodies, especially IgA, via colostrum and milk from immune dams. The mechanisms of lactogenic protection described for TGEV infection apply to PED as well (Saif and Sestak 2006). Lactogenic immunity is induced in sows by intestinal infection with PEDV, which then activates the gut mammary link. Pigs lose lactogenic protection at weaning and soon become susceptible to the virus. PEDV may persist on the farm as part of recurring weaning diarrhea after an acute outbreak.

Prevention and Control
Suckling pigs suffering from PED should have free access to water to diminish dehydration. In fattening swine, it is advisable to withhold feed.

Since PEDV does not spread very quickly, measures to prevent virus entrance into farrowing units with newborn piglets may postpone the infection until a later age and avoid mortality. In the meantime, intentional exposure of pregnant sows to virus using watery feces from infected piglets will stimulate rapid lactogenic immunity and shorten the outbreak on the farm. If the virus is detected in successive litters of weaned piglets, the transmission cycle can be interrupted by moving pigs immediately postweaning to another site for 4 weeks.

In Europe, the disease has been of insufficient economic importance to develop a vaccine. However, outbreaks in Asia have been so severe that attenuated virus vaccines are being developed.

Bernasconi et al. (1995) reported that cell culture adaptation of the CV777 virus lowered its virulence for newborn cesarean-derived piglets and histopathological changes were decreased in severity. A Korean strain (KPEDV-9) showed reduced pathogenicity for neonatal pigs and was found to be safe for pregnant sows after 93 passages in Vero cells (Kweon et al. 1999). Another Korean strain (DR13), attenuated by 100 serial passages in Vero cells, induced lactogenic immunity in sows after oral administration (1 × 10^7.0 TCID₅₀) at 4 and 2 weeks prior to farrowing. Mortality in the piglets born from these sows was 13% after challenge with a virulent PEDV, whereas mortality in the piglets born from unvaccinated control sows was 100% (Song et al. 2007). Although partial protection by these live vaccines is recognized, their efficacy in the field still needs to be proven.

In China, a bivalent live vaccine consisting of the attenuated TGE H and CV777 strain is routinely used against TGE and PED since 1997.

HEMAGGLUTINATING ENCEPHALOMYELITIS VIRUS (VOMITING AND WASTING DISEASE)

Relevance
In 1962, Greig and coworkers isolated a previously unrecognized viral pathogen from the brains of suckling pigs with encephalomyelitis in Canada. Designated HEV, the virus was later classified as a CoV (Greig et al. 1971). In 1969, an antigenically identical virus was isolated in England from suckling pigs showing anorexia, depression, vomiting, and stunting, but without clear signs of encephalomyelitis (Cartwright et al. 1969). The condition was called vomiting and wasting disease (VWD). Both forms of the disease were experimentally reproduced by Mengeling and Cutlip (1976) using isolates from the same farm. HEV is widespread among swine, but the infection is generally subclinical, although some outbreaks may cause considerable losses (Alsop 2006; Quiroga et al. 2008).

Etiology
HEV belongs to the Betacoronavirus genus of the Coronaviridae family. The virus spontaneously agglutinates erythrocytes of mice, rats, chickens, and several other kinds of animals. The natural host of HEV is the pig. Although HEV may show different clinical manifestations, only one serotype exists. Age-related
susceptibility of the pigs, possible strain differences in virulence, and variation in pathogenic course may influence clinical signs. HEV shows a strong tropism for neural tissues in pigs. The virus has been adapted to mice (Yagami et al. 1986) and Wistar rats (Hirano et al. 1993), in which its neurotropism is also apparent (Hirano et al. 2004; Yagami et al. 1993).

In vitro, only porcine cells are susceptible to HEV. HEV was first isolated in primary cultures of PK cells and produced a CPE characterized by syncytia (Greig et al. 1962). HEV was also shown by immunofluorescence to propagate in several other porcine cell cultures, for example, adult thyroid gland, embryonic lungs, and cell lines such as swine testicle, PK-15, IBRS2, SK, SK-K, and KSEK6 swine embryo kidney.

Role in Public Health
Pigs are the only species known to be naturally susceptible to HEV, and HEV has no public health significance.

Epidemiology
Serological surveys carried out between 1960 and 1990 revealed that infection with HEV in swine occurs worldwide and that HEV is endemic in both breeding and fattening swine (Pensaert 2006). The presence of the virus, as detected by isolation or serology, was reported in Europe (Belgium, England, Germany, Northern Ireland, France, Austria, Denmark), in the western hemisphere (United States, Canada, Argentina), in Asia (Japan, Taiwan), and in Australia.

The virus is believed to maintain itself in the herd or in the swine populations by infecting successive groups of pigs after replacement or weaning. The virus is excreted oronasally (Hirahara et al. 1989; Pensaert and Callebaut 1974) for 8–10 days. The mode of transmission is through nasal secretions, nose-to-nose contact, and aerogenic means. Persistent virus carriers are not known to exist.

Generally, pigs will only develop disease when they become infected oronasally prior to 3–4 weeks of age and if originating from nonimmune mothers (Alexander 1962; Appel et al. 1965). Pigs with maternally derived HEV antibodies are clinically unaffected when exposed to HEV (Appel et al. 1965), as circulating antibodies prevent the virus from reaching neural target tissues. Pigs infected at later ages normally do not develop clinical disease. Since HEV is endemic in most swine populations, sows are often immune and protect their offspring by maternal antibodies. For this reason, clinical outbreaks are rare and usually occur in litters from nonimmune mothers, often first-parity sows. Three recent outbreaks are worth mentioning. In 2001, HEV was isolated from newborn and early-weaned pigs with vomiting and posterior paralysis on a Canadian farm (Sasseville et al. 2001). Alsop (2006) described a clinically diagnosed outbreak of VWD that occurred in 2002 in a 650-sow genetic nucleus herd. Quiroga et al. (2008) described an outbreak with vomiting and wasting, as well as motor disorders, in Argentina in 2006. This outbreak occurred in a three-site herd with 6000 sows where the breeder stock consisted of 55% gilts and first- or second-parity sows.

Pathogenesis
The type and severity of clinical signs can vary within a litter and are primarily related to age at the time of infection and possible differences in virus virulence (Mengeling and Cutlip 1976), but may also depend on the course of the pathogenesis.

The primary site of replication of HEV in pigs is the respiratory tract (Andries and Pensaert 1980a; Hirahara et al. 1987; Mengeling et al. 1972). Immunofluorescence revealed that the epithelial cells of nasal mucosa, tonsils, lungs, and some unidentified cells in the small intestine were infected. Primary replication may result in mild or subclinical signs.

Experimental studies in colostrum-deprived piglets inoculated oronasally with HEV provided insight into HEV pathogenesis (Andries and Pensaert 1980b). From the primary sites of replication, the virus spread via the peripheral nervous system to the CNS using several different pathways. One pathway led from the nasal mucosa and tonsils to the trigeminal ganglion and the trigeminal sensory nucleus in the brain stem. A second pathway occurred along the vagal nerves via the vagal sensory ganglion to the vagal sensory nucleus in the brain stem. A third pathway led from the intestinal plexuses to the spinal cord, also after replication in local sensory ganglia. Viremia was of little or no importance in the pathogenesis of the disease (Andries and Pensaert 1980a).

In the CNS, the infection started in well-defined nuclei of the medulla oblongata, but progressed into the entire brain stem, the spinal cord, and sometimes also the cerebrum and cerebellum. Immunofluorescence in the brain was always restricted to the perikaryon and processes of the neurons. Vomiting was induced by viral replication in the vagal sensory ganglion (ganglion distale vagi) or by impulses to the vomiting center produced by infected neurons at different sites (Andries 1982). To elucidate the pathogenesis of wasting, Andries (1982) suggested that virus-induced lesions in the intramural plexi of the stomach may contribute to gastric stasis and delayed stomach emptying.

Clinical Signs
Upon infection, sneezing or coughing may first appear because of primary HEV replication in the upper respiratory tract. Body temperature can be elevated at the beginning of the disease, but returns to the normal range in 1–2 days. The incubation period for the appearance of more specific signs is 4–7 days. Two main clini-
clinical manifestations are possible in pigs below 3–4 weeks of age and both are a consequence of neurotropism of the virus: (1) the typical VWD with frequent vomiting leading to death or subsequent wasting and (2) acute encephalomyelitis with motoric disorders. However, signs of both clinical forms can be observed in the same herd during an outbreak.

For VWD, clinical signs are repeated retching and vomiting. Pigs start suckling, but soon stop, withdraw from the sow and vomit the milk they have consumed. The persistent vomiting and decreased food intake results in constipation and a rapid decline of condition. Neonatally infected pigs become severely dehydrated after a few days, exhibit dyspnea and cyanosis, lapse into coma, and die. Older pigs lose their appetite and become emaciated. They continue to vomit, although less frequently than in the early stage of the disease. Wasting, often with large distension of the cranial abdomen, may appear. This “wasting” state persists for several weeks and may be most prominent during the postweaning period. During the acute stage of outbreaks of VWD, some pigs may show neurological signs, such as abnormal gait, dullness, tremors, and nystagmus.

At herd or farrowing unit level, morbidity varies greatly and probably depends on the proportion of nonimmune neonatal litters present at the time of infection. In litters without maternal protection, morbidity is litter dependent and may approach 100% when the infection occurs soon after birth. Morbidity markedly decreases with increasing age at the time of infection. Mortality is variable, but may be 100% in neonatally infected litters. Pigs that show wasting often need to be euthanized.

In an outbreak in Argentina (Quiroga et al. 2008), only suckling pigs were involved. Vomiting and wasting were the main signs, although slight motor disorders were observed. Ten of 19 farrowing barns were affected. Disease occurred in 27.6% of pigs less than 1 week old and declined to 1.6% in pigs 3 weeks of age (mean 13.6%). In this outbreak, an estimated 12.6% of the suckling pigs in the affected farrowing units died from HEV infection. After weaning, 29% of the pigs coming from affected farrowing units showed wasting, although the percent varied among nursing units (15–40%). In all, 3683 pigs died or were euthanized on the farm.

Outbreaks of the so-called motor encephalomyelitic disease in suckling pigs may start with sneezing, coughing, and vomiting as early as 4–7 days after birth. Vomiting continues intermittently for 1–2 days, but is rarely severe. In some outbreaks, the first sign is acute depression and a tendency to huddle. After 1–3 days, pigs exhibit various combinations of nervous disorders. Generalized muscle tremors and hyperesthesia are common. Pigs that are able to stand usually have a jerky gait and tend to walk backward, often ending in a dog-sitting position. They soon become weak, unable to rise, and paddle their limbs. Blindness, opisthotonus, and nystagmus may also occur. Finally, the animals become dyspnic and lie prostrate on their sides. In most cases, coma precedes death.

Morbidity and mortality in neonatal pigs is usually 100%, but older pigs show a mild transient illness in which posterior paralysis may be the most common sign. Outbreaks described in Taiwan (Chang et al. 1993) in 30- to 50-day-old pigs were characterized by fever, constipation, hyperesthesia, muscular tremor, progressive anterior paresis, posterior paresis, prostration, recumbency, and paddling movements with a morbidity of 4% and a mortality approaching 100% in the pigs that were clinically affected. The pigs died 4–5 days after the onset of clinical signs.

Lesions
The only gross lesions reported in natural HEV infections are cachexia, stomach dilatation, and distension of the abdomen in some chronically affected pigs (Schlenstedt et al. 1969).

Microscopic lesions of epithelial degeneration and inflammatory cell infiltration are found in the tonsils and respiratory system of acutely diseased pigs (Cutlip and Mengeling 1972; Narita et al. 1989a). A nonsuppurative encephalomyelitis was reported in 70–100% of pigs with nervous signs and in 20–60% of pigs showing the VWD syndrome. The lesions are characterized by perivascular cuffing, gliosis, and neuronal degeneration (Alexander 1962; Chang et al. 1993; Narita et al. 1989b; Richards and Savan 1960). They are most pronounced in the gray matter of the pons Varoli, medulla oblongata, and the dorsal horns of the upper spinal cord.

Microscopic changes in the stomach wall were found only in pigs showing the VWD syndrome. Degeneration of the ganglia of the stomach wall and perivascular cuffing were present in 15–85% of diseased animals. The lesions were most pronounced in the pyloric gland area (Schlenstedt et al. 1969).

Diagnosis
Diagnosis can be made by virus isolation, immunohistochemistry or RT-PCR (Quiroga et al. 2008). Tonsils, brain stem, and lungs are dissected aseptically from young diseased piglets as soon as possible after initial signs of infection. It is difficult to isolate the virus from pigs that have been sick for more than 2–3 days. For virus isolation, suspensions are inoculated onto primary PK cells, secondary pig thyroid cells, or porcine cell lines. The presence of HEV is shown by the presence of syncytia, by hemadsorption, or by hemagglutination. One or more blind passages may be needed since specimens from sick pigs often contain very small amounts of infectious virus.

Antibodies to HEV can be detected by plaque reduction, VN, or hemagglutination inhibition (HI) tests.
The HI and VN tests were almost equally diagnostic in swine sera, but VN is more specific (Sasaki et al. 2003). Antibody titer results must be evaluated carefully because subclinical infections with HEV are very common. Moreover, a significant rise in antibody titer can be detected only if acute sera are taken very soon after the appearance of clinical signs. Pigs may develop considerable antibody titers as early as 6–7 days postinfection, which often coincides with early disease, making an interpretation of paired serology difficult.

Differential diagnosis must be made between HEV encephalomyelitis, Teschen–Talfan disease, and pseudorabies (Aujeszky’s) disease. In the latter infections, clinical signs of encephalomyelitis, including motor disorders, are more severe and may appear among older pigs and in piglets. Aujeszky’s disease in nonvaccinated animals also induces respiratory signs in older pigs and abortions in sows. All these viruses can be grown in PK cells and pig thyroid cells, but the type of CPE differs, and only HEV causes hemadsorption and hemagglutination. They can be further differentiated by virus-specific tests.

Immunity

After infection, pigs develop protective circulating antibodies (HI, VN) to HEV that are detectable after 7–9 days. The duration of antibodies has not been determined. The duration of immunity is less important in HEV because of the resistance to disease that develops with age. Neonatal pigs born to immune mothers are fully protected by maternally derived antibodies that persist until the age of 4–18 (mean 10.5) weeks (Paul and Mengeling 1984).

Prevention

On most breeding farms, HEV infection persists endemically by pig-to-pig transmission and through subclinical respiratory infections. Sows usually contract the virus before their first farrowing and thereafter provide protection to their offspring via colostral antibodies, as discussed above. When sows are not immune at farrowing, for example, in newly populated farms, well-isolated gilts, or small farms in which the virus is not maintained, infection of pigs within the first weeks after birth results in clinical signs. Promoting virus circulation in the farm so that gilts are immune at farrowing prevents disease in piglets.

Once clinical signs are evident, the disease will run its course; spontaneous recoveries are rare. Litters born 2–3 weeks after the onset of disease are usually protected because nonimmune gestating sows should have become infected and immune by farrowing. Piglets born to nonimmune sows early in the outbreak can be protected by parenteral inoculation with specific immune serum shortly after birth. A rapid herd diagnosis is then required. Hyperimmune serum is not commercially available, but pooled serum collected from several old sows (possibly at the slaughterhouse) will likely contain anti-HEV antibodies. The serum should be sterile and examined for the presence of such antibodies prior to administration. No vaccines against HEV are currently available.

PORCINE TOROVIRUS

Relevance

ToV particles were initially detected by EM in the feces of a 3-week-old piglet with diarrhea in England (Scott et al. 1987). Subsequent studies revealed a high seroprevalence (81–100%) in adults or young nursing piglets and high detection rates (50–75%) among subclinically infected weaned pigs (Kroneman et al. 1998; Pignatelli et al. 2010). Latter reports from Europe, North America, and South Africa suggested that ToV was endemic in the swine herds tested. In contrast, in Korea, only 6.4% (19 of 295) of diarrheic feces from 3- to 45-day-old piglets were positive for porcine ToV (Shin et al. 2010). Of these, about 74% also contained other enteric pathogens. Consequently, the link between porcine ToV and enteric disease is unclear, and there are no reports confirming porcine ToV pathogenicity or gut lesions.

Etiology

Porcine ToV represents one of four proposed species within the Torovirus genus of the Torovirinae subfamily in the Coronaviridae family (Carstens 2010). The genomic organization, replication strategy, and properties resemble those of other Coronaviridae (de Groot 2008). Like some betacoronaviruses, porcine ToVs also possess an HE protein. Notable differences from CoVs included a smaller N protein (∼18.7 kDa) and a tubular nucleocapsid leading to differences in ToV particle morphology (spherical, elongated, or kidney shaped) (Kroneman et al. 1998). Multiple clusters of porcine ToVs have been identified based on gene sequence analysis (de Groot 2008; Pignatelli et al. 2010; Shin et al. 2010).

Epidemiology and Immunity

Based on serological and shedding data from clinically normal pigs or sows in European herds, ToVs were endemic in 14 farms tested. High seroprevalence rates (81%) were detected in sows on 10 Dutch farms by testing for cross-reactive virus-neutralizing antibodies to equine ToV (Kroneman et al. 1998). Similarly, 100% of sows, nursing piglets (<1 week), and older piglets (>11 weeks) in three farms in Spain were seropositive for ToV antibodies using an ELISA based on porcine ToV N protein (Pignatelli et al. 2010). Longitudinal studies revealed fecal shedding of ToVs (RT-PCR or qRT-PCR) postweaning at 4–14 days for 1–9 days (80%) (Kroneman et al. 1998) or at 4 and 8 weeks postweaning (50–75%) (Pignatelli et al. 2010). In both studies,
maternal antibody titers were initially high in piglets (<1 week), titers declined at weaning and then increased postinfection at 11 or 15 weeks of age.

Because most pigs become infected with ToVs postweaning, maternal antibodies apparently provide at least partial protection. However, the immune correlates of protection to porcine ToV infection are not known. In one of four farms, it was postulated that ToV infection of suckling piglets in the presence of maternal antibodies delayed development of active immune responses such that these pigs, but not pigs from the other farms, shed the same ToV strain pre- and postweaning (Pignatelli et al. 2010). Genetically diverse ToV strains were detected within herds in the latter study and in Korean farms. The porcine ToVs were associated with sporadic infections among diarrheic pigs from 65 Korean farms surveyed (6.2% of farms positive) (Shin et al. 2010). Based on phylogenetic analysis of the S and N genes, the Korean ToV strains formed distinct branches with clusters corresponding to the farm of origin.

**Diagnosis**

Methods to propagate porcine ToVs in cell culture have not been described. For serological studies, a cell culture-adapted equine ToV has been used to assess cross-reactive virus-neutralizing antibodies in swine (Kroneman et al. 1998; Pignatelli et al. 2010). Recently, an indirect ELISA using recombinant purified porcine ToV N protein as antigen was developed (Pignatelli et al. 2010). In most, but not all cases, there was a good correlation between ELISA and VN tests. Discrepancies observed could reflect the use of heterologous antigen in VN compromising detection of low-titer antibodies to primary infections, or more broadly reactive antibodies in colostrum leading to longer detection of persisting cross-reactive virus-neutralizing antibodies in young pigs.

Porcine ToVs has been detected in feces using IEM to identify antibody-aggregated ToV particles and differentiate them from other fecal porcine CoVs (TGEV, PEDV) (Kroneman et al. 1998). For detection of ToV-specific viral RNA, RT-PCR and qRT-PCR targeting conserved regions of the porcine ToV N gene or the 3′ nontranslated region of the genome have been described (Kroneman et al. 1998; Pignatelli et al. 2010; Shin et al. 2010).

**Prevention and Control**

Based on the limited data available, the stress of transport, movement, and redistribution of pigs even within multisite farms could precipitate porcine ToV infection with similar or distinct cocirculating strains (Pignatelli et al. 2010). Thus, management practices applicable to control of other enteric CoV infections should be implemented for control of porcine ToVs.

**REFERENCES**

RELEVANCE

In October 2008, severe disease outbreaks suspected to be caused by porcine reproductive and respiratory syndrome virus (PRRSV) were reported in swine herds in the Philippines. Diagnostic samples were submitted to the United States Department of Agriculture (USDA) Plum Island Foreign Animal Disease Diagnostic Laboratory (FADDL) and PRRSV was isolated. However, cytopathic effects (CPEs) were observed in Vero cells inoculated with diagnostic material, a response not attributable to PRRSV. Panviral DNA microarray analysis revealed Reston ebolavirus (REBOV) in Vero cell cultures (Barrette et al. 2009) and a follow-up study demonstrated REBOV-specific antibodies in farm workers.

The broader significance of this outbreak of REBOV remains to be determined. At a minimum, it showed that domestic swine are susceptible to infection with REBOV and raised the possibility that domestic and/or feral swine could function as reservoirs for filoviruses.

ETIOLOGY

A comprehensive review of filoviruses is found in Sanchez et al. (2007). REBOV is classified in the order Mononegavirales, family Filoviridae. The filoviruses are further divided into two genera, Marburgvirus and Ebola virus. The Marburgvirus genus contains a single species, Lake Victoria marburgvirus. The Ebola virus genus contains five species: Bundibugyo ebolavirus (BBOV), Sudan ebolavirus (SBOV), Zaire ebolavirus (ZEBOV), Ivory Coast or Cote d’Ivoire ebolavirus (ICEBOV), and REBOV. The swine Ebola virus isolates (Reston-08 A, C, and E), were about 97% identical at the nucleotide level to the original 1989 REBOV isolate (Barrette et al. 2009) and, therefore, were not assigned a new species designation.

In general, the Mononegavirales are enveloped, nonsegmented, negative-stranded RNA viruses. In particular, the Filoviridae possess a characteristic pleomorphic, filamentous shape (Figure 36.1). The REBOV genome (~19 kilobases) encodes seven structural proteins (NP, VP35, VP40, GP, VP30, VP24, and L) and two nonstructural proteins (“secreted glycoprotein” and delta peptide).

The Ebola virus RNA genome is packaged within a helical nucleocapsid composed of major (NP) and minor (VP30) nucleoproteins. The ribonucleoprotein complex is surrounded by envelope matrix proteins VP40 and VP24. L and VP35 proteins are also associated with the ribonucleoprotein complex. The envelope contains peplomers composed of a single glycoprotein (GP). During infection, the host antibody response is primarily directed against NP and GP. The functions of the nonstructural proteins are not known, but may be involved in pathogenesis.

After viral attachment and uncoating, the nucleocapsid is released into the cytoplasm, the site of virus replication. Individual mRNAs are transcribed from the negative-sense genome. Once viral proteins accumulate, RNA synthesis switches to the production of negative-sense genomes copied from positive-sense templates. The eventual accumulation of nucleocapsid proteins results in the formation of inclusion bodies, which are visible by light microscopy. After assembly, the mature virions bud from the plasma membrane.
Like other Ebola viruses, REBOV can be cultivated on a variety of mammalian cell lines, but Vero cells are commonly used for virus isolation and propagation. Productive infection produces CPE, which appears as light-refractive inclusions in the cytoplasm followed by cell death. The presence of REBOV may be confirmed by reverse transcription-polymerase chain reaction (RT-PCR), antigen-capture enzyme-linked immunosorbent assay (ELISA), and/or immunocytochemistry using REBOV-specific monoclonal and polyclonal antibodies. Diagnostic and experimental work with REBOV is restricted to Biosafety Level 4 (BSL4) laboratory conditions.

**EPIDEMIOLOGY AND ROLE IN PUBLIC HEALTH**

Ebola viruses are geographically restricted to Africa and the Philippines. Rare appearances of Ebola viruses outside of these regions are associated with the importation of infected monkeys. For example, in 1989, REBOV-infected cynomolgus monkeys (*Macaca fascicularis*) were transported from the Philippines to a Reston, Virginia (USA) nonhuman primate facility (Jahrling et al. 1990). Macaques were also the suspected source of the 2008 Philippine REBOV outbreak because the swine farms were located near the source of REBOV-infected monkeys transported to Reston, Virginia, in 1989.

In addition to humans and nonhuman primates, Ebola viruses are infectious for pigs, laboratory mice, guinea pigs, bats, and possibly domestic canines (Allela et al. 2005; Swanepoel et al. 1996). Bats are considered a principal wildlife reservoir (Leroy et al. 2005, 2009; Swanepoel et al. 1996). Establishing the role of wildlife and feral swine as reservoirs of REBOV infection is important because most domestic swine in the Philippines are raised in the open and under conditions with low biosecurity.

The incidence and prevalence of REBOV in swine in the Philippines is unknown. Limited information is available on domestic pig populations and no data are available on feral swine. Barrette et al. (2009) tested samples from five groups of pigs at four separate locations and found REBOV-positive swine at two of the four locations. All pigs positive for REBOV were also positive for PRRSV, and in some instances porcine circovirus type 2 (PCV2), but were negative for classical swine fever virus, foot-and-mouth disease virus, African swine fever virus, and swine vesicular disease virus.

The mechanism of transmission of REBOV to pigs or between pigs is unknown. For nonhuman primates and other species, Ebola virus infection occurs through contact with breaks in the skin, needle sticks, or contact with mucosal surfaces. This includes the consumption and handling of virus-contaminated raw meat, fomites, and possibly respiratory inhalation of aerosolized virus.

Routes of REBOV shedding by pigs are also not known. In nonhuman primate models, REBOV is shed in all body fluids, including semen (Bausch et al. 2008). Whether REBOV is shed by boars in semen or transmitted to recipient females is unknown, but is an important consideration because of the extensive use of artificial insemination in pig breeding.

Generally, filoviruses are stable at room temperature, but inactivated at 60°C (140°F) for 30 minutes (Sanchez et al. 2007). All filoviruses are susceptible to commonly used viricidal agents, that is, hypochlorite, lipid solvents, and phenolic compounds. The stability of virus in a desiccated state depends on the temperature and type of surface. For example, desiccated virus maintained at 4°C (39°F) on a plastic surface lost only 1–2 logs of infectivity over a 2-week period (Piercy et al. 2010).

**PATHOGENESIS, CLINICAL SIGNS, AND LESIONS**

Descriptions of REBOV in humans are primarily based on observations following laboratory or occupational exposures. In all documented cases, REBOV-infected humans seroconverted, but showed no symptoms. Among 141 individuals employed in facilities with REBOV-infected swine in the Philippines in 2009, six were positive for anti-REBOV serum antibodies. Serocconversion in farm workers suggests the possibility that they acquired the infection directly or indirectly from REBOV-infected pigs (Barrette et al. 2009).
The clinical signs and pathogenesis of REBOV infection of swine have not been described, but preliminary reports indicated that REBOV did not cause clinical signs. Rather, the available information suggests that REBOV may function as a cofactor in polymicrobial infections involving other pathogens of swine, for example, PRRSV.

After preparation of this chapter, Kobinger et al. (2011) reported the experimental infection of pigs with ZEBOV in the absence of PRRSV. Pigs were mucosally infected and produced respiratory clinical signs. Virus replication was primarily localized to the respiratory tract. Kobinger et al. (2011) reported that ZEBOV was easily transmitted from infected to naïve pigs.

In nonhuman primates, intranasal administration of Ebola virus resulted in virus replication in the upper respiratory tract, primarily targeting macrophages and other cells of monocyte lineage. Whether or not inhalation is a common route for infection is unclear. Infected macrophages efficiently spread infection to endothelial cells in lung, lymph nodes, liver, and other organs. The original field cases from the Philippine outbreak described the presence of PRRSV and REBOV antigens in lung lesions in association with inflammatory cells and the accumulation of necrotic debris in alveoli of infected pigs (Barrette et al. 2009). The lesions were consistent with interstitial pneumonia, a common outcome of PRRSV infection. PRRSV and REBOV antigens were also found in lymph nodes.

DIAGNOSIS

Saijo et al. (2001) and Sanchez et al. (2007) have reviewed laboratory diagnosis of Ebola virus infection. An important consideration in the diagnosis of Ebola virus is the availability of BSL-4 containment facilities for processing samples and handling live virus.

Because of the absence of a characteristic clinical picture, isolation and/or detection of virus in swine should be taken into consideration in geographic regions where Ebola virus species are endemic. Laboratory confirmation of REBOV infection is based on virus isolation, serology, and RT-PCR. Virus isolation is performed on Vero or Vero E6 cells. Observations for CPE are carried out for as long as 14 days or cells can be stained with virus-specific antibodies. Because of its distinctive pleomorphic filamentous morphology, electron microscopy is a useful diagnostic tool (Figure 36.1). Indirect immunofluorescence assay (IFA) is a common approach for the detection of antibodies in serum and other body fluids. Substitutes for IFA are ELISA-based techniques that incorporate NP as the target antigen. As described by Barrette et al. (2009), detection of REBOV nucleic acid in pigs can be performed by RT-PCR amplification within a region of the L gene.

PREVENTION AND CONTROL

The only method used in the control of REBOV infection in swine is the closure and depopulation of affected farms. Thereafter, the infection status of animals and caretakers can be monitored serologically.

The features of innate and acquired immunity of swine to REBOV infection are unknown and swine vaccines are not available. Inactivated and subunit vaccines have not proved effective in the protection of nonhuman primates. The most promising efforts have focused on recombinant virus vector vaccines based on vesicular stomatitis virus (VSV) and human recombinant adenovirus 5 (rAd5). Both vector systems express Ebola virus GP as the vaccine antigen (Geisbert et al. 2010). VSV, rAd5 and other vaccine approaches developed for humans could potentially be adapted for use in swine.

REFERENCES

**OVERVIEW**

The *Flavivirus* genus belongs to the *Flaviviridae* family and comprises over 70 members, including several important pathogens of significance to human and animal health. Yellow fever virus is the type species of the genus and the source of its name (Latin *flavus* means “yellow”) and that of the family. Flaviviruses are divided into three groups based on vector association: tick-borne, mosquito-borne, and no known vector. This chapter will consider three of the mosquito-borne flaviviruses that can infect swine, namely Japanese encephalitis virus (JEV), West Nile virus (WNV, including the subtype Kunjin [KUNV]), and Murray Valley encephalitis virus (MVEV). These viruses belong to the JEV taxonomic group, which is defined by the antigenic and genetic relatedness of its members. This group also includes Cacipacore, Koutango, St. Louis encephalitis, Usutu, and Yaounde viruses (Thiel et al. 2005), none of which have been associated with infections or disease in swine. The JEV group members share a similar transmission cycle, between avian vertebrate hosts and *Culex* species mosquitoes.

Flaviviruses have a single-stranded positive-sense RNA genome approximately 11 kilobases in length. The genome itself is infectious, and encodes a single open reading frame (ORF) flanked by noncoding regions. Three structural proteins (capsid, premembrane [prM], envelope [Env]) and seven nonstructural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, NS5) are encoded by the ORF (Lindenbach et al. 2007). The viral genome is enclosed by the nucleocapsid, which is surrounded by a host-derived lipid envelope containing the prM/M and Env proteins. The Env protein is involved in cell attachment and entry, and is an important determinant of neurovirulence and neuroinvasion (McMinn 1997). The M protein is produced prior to release of mature virions from the cell via cleavage of prM. The NS proteins have a range of functions required for polyprotein processing, viral RNA replication, and modulation of the host immune response. The noncoding regions contain conserved elements and secondary structures that are also involved in viral RNA translation and replication.

The flavivirus particle has icosahedral symmetry and is 40–50 nm in diameter. Viruses are readily inactivated in serum by incubation at 56°C for 30 minutes and this is recommended prior to serological testing using cell-based assays (Roehrig et al. 2008). Several methods for WNV inactivation in blood products have been reported, including photochemical treatment, pasteurization, treatment with solvents/detergents or caprylate, and low pH (Lin et al. 2005; Remington et al. 2004). Flavivirus cultures can be inactivated chemically with binary ethylenimine or enzyme-linked immunosorbent assay (ELISA) wash buffer containing Tween 20 (Mayo and Beckwith 2002; Pyke et al. 2004a). An evaluation of the thermostability of WNV in cell culture medium demonstrated that incubation at 37°C for 3 days reduced virus titers by 3 logs, while no reduction was found in virus held at 4°C (Mayo and Beckwith 2002).

In infected mosquitoes, WNV or JEV RNA was detected by reverse transcription-polymerase chain reaction (RT-PCR) up to 2 weeks after mosquito death under a variety of conditions, including high temperatures and humidity (Johansen et al. 2002; Turell et al. 2002). In contrast, the ability to culture virus was virtually lost after 24–48 hours. These studies emphasize the importance of appropriate sample handling for...
diagnostic testing and highlight the need for timely collection and cold chain storage of samples, where applicable.

The recent development of an arbovirus surveillance system that uses honey-soaked preservation filters to detect virus expectorated by trapped mosquitoes circumvents the need for a cold chain and mosquito processing (Hall-Mendelin et al. 2010). Using this system, WNV RNA was preserved for at least 7 days, allowing its subsequent detection by RT-PCR.

**JAPANESE ENCEPHALITIS VIRUS**

**Relevance**

JEV was first isolated in 1933 in Japan from humans with encephalitis by Fujita (1933), and subsequently isolated from *Culex tritaeniorhynchos* mosquitoes (Mita-mura et al. 1938, cited by Burke and Leake 1988). JEV is the major cause of human viral encephalitis and childhood viral neurological infection and disability in southern, eastern, and southeastern Asia (Burke and Leake 1988; Gould and Solomon 2008; Mackenzie et al. 2007). JEV is also an important pathogen of horses causing fatal encephalitis, and an economically important disease of swine causing reproductive failure with abortion and stillbirths.

**Etiology**

JEV exists as a single serotype, although two major immunotypes can be recognized by various serological methods. JEV can be classified genetically into four, and possibly five, distinct genotypes based on prM and/or Env gene or full-length genome sequences (Solomon et al. 2003; Uchil and Satchidanandam 2001; Williams et al. 2000). Genotype 1 comprises strains from Cambodia, Korea, northern Thailand, Vietnam, Japan, and Australia; genotype 2 is comprised of strains from Indonesia, Malaysia, southern Thailand, and Australia; genotype 3 contains isolates from the known geographic range of JEV—with the exception of the Australasian region—and includes strains from Japan, China, Taiwan, the Philippines, and South and Southeast Asia; and genotype 4 includes isolates found only in Indonesia. Based on full-length genome phylogeny, the Muar strain, isolated in Singapore in 1952, has been identified as the sole member of the fifth genotype (Mohammed et al. 2011).

**Role in Public Health**

JEV is believed to be responsible for more than 40,000 cases of encephalitis annually, with at least 10,000 deaths. These figures are generally regarded as a significant underestimate, with the true disease burden suggested to be closer to 175,000 cases annually (Tsai 2000). Between 1 in 300 and 1 in 25 JEV infections result in clinical disease (Burke and Leake 1988; Vaughan and Hoke 1992); thus, most human infections are asymptomatic. Approximately 25% of clinical Japanese encephalitis (JE) cases are fatal, 50% have some form of neurological sequelae, such as quadriplegia or mental retardation, and 25% recover fully.

**Epidemiology**

JEV is a zoonotic virus maintained in nature by transmission cycles involving *Culex* sp. mosquitoes and certain species of wild and domestic birds and pigs as the vertebrate hosts (Endy and Nisalak 2002). Humans become infected when bitten by an infected mosquito, but they are incidental, dead-end hosts. Of particular importance in the ecology of JEV is the interplay between rice cultivation, vector densities, and pig rearing in close proximity to human habitation (Gajanan et al. 1997; Kanojia et al. 2003).

Pigs are the principle amplification hosts of JEV, especially in epidemic areas, and the maintenance hosts in endemic areas. They develop a high and prolonged viremia following natural infection, with JEV viremia lasting 2–4 days and capable of infecting various mosquito species. In serological surveys, pigs consistently display higher geometric mean virus titers than other domestic or wild animals. They are particularly attractive to the major mosquito vectors as the source of blood meals, providing a sensitive indicator of virus transmission in endemic areas (Burke et al. 1985a). Despite the potential danger to humans if raised in open pens near human habitation, pigs have been used as sentinel animals to monitor for virus activity in a number of countries, essentially serving as an early warning system.

Horses, cattle, buffaloes, and goats are dead-end hosts (Mackenzie et al. 2007), but because they attract a number of the major vector species, especially *C. tritaeniorhynchos*, they make good potential hosts for surveillance and may act as “dampers” in an outbreak situation (Johnsen et al. 1974; Peiris et al. 1993). Other animals may have relatively high seroprevalence rates, such as sheep and dogs, but viremia levels are believed to be too low to infect mosquitoes (Banerjee et al. 1979; Johnsen et al. 1974).

JEV has been isolated from a number of bats belonging to the orders *Microchiroptera* and *Megachiroptera* (Mackenzie et al. 2008; Sulkin and Allen 1974), but most studies of their potential role in maintenance and transmission have been carried out in *Microchiroptera*, in which viremia lasts as long as 25–30 days at a level sufficiently high to infect mosquitoes.

Wild birds, particularly ardeid species, are believed to be important maintenance hosts of JEV, and may act as amplifiers in epidemics (Scherer et al. 1959; Soman et al. 1977). JEV isolates have been obtained from a number of species, but the most important is undoubtedly the Black-crowned night heron (*Nycticorax nycticorax*). Chickens appear to be rarely infected and may have a limited role in either transmission or surveillance.
Wild birds have been implicated as the source of virus in the spread of JEV to new areas (Scott 1988; Solomon et al. 2003).

JEV has been isolated from a wide range of mosquito species, but not all are believed to be able or competent to transmit the virus to new hosts. There is a consensus that C. tritaeniorhynchus is the major vector throughout most of Asia, but other species may be locally important, such as Culex geltidus, Culex vishnui, Culex fuscocephala, Culex pseudovishnui, Culex bitaeniiorhynchus, Culex annulirostris, Culex quinquefasciatus, and Mansonia uniformis (Burke and Leake 1988; Rosen 1986; van den Hurk et al. 2009; Vaughn and Hoke 1992). The major Culex vectors of JEV are rice field-breeding species that bite during the night, particularly in the period shortly after sunset and in the early morning, and are zoophilic, preferring animals to humans for obtaining blood meals.

There has been much speculation about how JEV survives between epidemics and over the winter months in its northern range. There are four major hypotheses: (1) that the virus overwinters in mosquito eggs after vertical transmission; (2) that the virus survives in hibernating mosquitoes; (3) that the virus survives in hibernating animals, such as cold-blooded animals or bats; or (4) that the virus is reintroduced each year by migratory birds or even by mosquitoes blown by prevailing wind patterns from endemic areas. Possibly, each of the four methods contributes to overwintering in different situations. Vertical transmission occurs in several Aedes and Culex species mosquitoes in nature and under laboratory conditions (Rosen et al. 1989).

Pathogenesis
There is little direct information available on the pathogenesis of JEV in pigs; most of our knowledge comes from rodent model systems or from human infections (Chambers and Diamond 2003; Solomon and Vaughn 2002). Following infection by the bite of a mosquito, the virus is believed to initially replicate in the skin and regional lymph nodes. This leads to a primary viremia, by which a number of tissues are infected, especially muscle (skeletal, cardiac, and smooth), lymphoreticular cells, and cells of the endocrine and exocrine systems. Virus excreted from these cells and tissues causes secondary viremia, usually within 1–3 days postinfection (DPI), that persists for about 4 days. Virus can reach the central nervous system (CNS) as early as 3 DPI (Yamada et al. 2004), and can cross the placenta to reach the fetus by 7 DPI. How the virus breeches the blood–brain barrier is not known, but is believed to be either via the olfactory mucosa where there is no blood–brain barrier (Yamada et al. 2009) or via hematogenous spread (Solomon and Vaughn 2002). Usually neonates and young animals are more susceptible to CNS infection than adults.

It is not known whether transplacental transmission is a consequence of the level of viremia. However, the fact that affected litters of piglets can include stillborns, mummified fetuses, weak neonates, and normal live-born animals suggests that there is a sequential infection of the neonates in utero.

Clinical Signs
Adult pigs do not generally show overt signs of infection. The most common disease manifestation in infected pregnant sows or gilts is reproductive failure leading to abortion and litters that contain stillborn or mummified fetuses or live, weak piglets (Daniels et al. 2002; Platt and Joo 2006). Reproductive failure occurs in sows infected with JEV before 60–70 days of gestation; infections later in gestation do not appear to affect the piglets. Natural infection of piglets with JEV is normally inapparent, but recent reports suggested that infection of young animals between 2 and 40 days old might very occasionally lead to a wasting syndrome with histological evidence of meningoencephalitis, and with varying degrees of depression and hind limb tremors (Yamada et al. 2004).

JEV has also been associated with infertility in boars. Infection of boars may lead to edematous, congested testicles resulting in lowered motile sperm counts and abnormal spermatooza. These effects are usually temporary and complete recovery occurs in most instances.

Lesions
There are no characteristic gross lesions of JEV infection in sows. However, accumulation of mucous fluid in the cavity of the tunica vaginalis may be observed in infected boars, as well as fibrous thickening of the epididymis and visceral lamina of the tunica vaginalis (Platt and Joo 2006). Stillborn fetuses or live, weak piglets often present with hydrocephalus, subcutaneous edema, cerebellar hypoplasia, and spinal hypomyelogenesis (OIE 2009). Hydrothorax, ascites, serosal petechiae, necrotic foci in the liver and spleen, and congestion in the lymph nodes, meninges, and spinal cord may also be found (Burns 1950). Microscopic lesions of infected boars may include edema and inflammation of the epididymis, tunica vaginalis, and testis (Platt and Joo 2006). Infected piglets may present with diffuse nonsuppurative encephalitis, characterized by neuronal necrosis, neuronophagia, glial nodules, and perivascular cuffing in the brain and spinal cord (Yamada et al. 2004).

Diagnosis
Agents causing clinical diseases characterized by abortion, fetal mummification or stillbirth, and encephalitis in animals up to 6 months of age should be considered in the differential diagnosis. These should include pseudorabies (Aujeszky’s disease) virus, classical swine fever virus, hemagglutinating encephalomyelitis
virus, rubulavirus (blue eye paramyxovirus), Menangle virus, porcine brucellosis, porcine teschovirus, porcine parvovirus, porcine reproductive and respiratory disease virus, and salt poisoning (OIE 2009).

The reference standard in the laboratory diagnosis of JEV is isolation and identification of the isolate. Viremia lasts only a few days and virus can rarely be isolated from either blood or cerebrospinal fluid (CSF), thus isolation of virus is best achieved from the infected brain, spleen, liver, or placental tissues from fetuses, stillborns, and neonates. The methods of choice for virus isolation are inoculation of tissue homogenates into suckling mice or onto a range of susceptible cell substrates. The continuous Aedes albopictus cell line C6/36 is particularly useful, but other cell lines, for example, African green monkey kidney (Vero), baby hamster kidney (BHK), and porcine kidney (PSEK) are usually susceptible. Unlike vertebrate cells, JEV does not normally cause cytopathic effect (CPE) in C6/36 cells. Hence, confirmation may require further culture in vertebrate cells and/or detection of viral antigen or RNA.

Molecular methods of virus detection are also routinely used in diagnostic laboratories. RT-PCR can be used to detect and identify JEV in CSF, sera, and tissue culture supernatants. Both conventional and real-time tests have been reported (Pyke et al. 2004b; Tanaka 1993). A reverse transcription-loop-mediated isothermal amplification (RT-LAMP) assay for detection of JEV in CSF has also been developed and offers a simple method of nucleic acid testing without the need for sophisticated equipment or skilled personnel (Parida et al. 2006).

JEV antigen can be detected in serum specimens using flavivirus-specific monoclonal antibodies (MAb) and in fetal tissues using immunohistochemistry (Iwasaki et al. 1986; Yamada et al. 2004).

Diagnosis of JEV infection, like other flaviviruses, is often based on the detection of serum antibodies using ELISA, hemagglutination inhibition (HI), immunofluorescent antibody (IFA), and virus neutralization (VN) assays (Beaty et al. 1995; Burke et al. 1987; Clarke and Casals 1958; OIE 2010a). Immunoglobulin M (IgM) capture (MAC-)ELISA is frequently the method of choice.

In older pigs, interpretation of serological tests needs to take a number of factors into consideration, for example, vaccination history and age. Maternal antibody can persist in some pigs for as long as 8 months (Hale et al. 1957). IgM antibody appears 2–3 days after infection and persists for at least 2 weeks (Burke et al. 1985b) and, in some animals, several months.

There is a high degree of serological cross-reactivity between flaviviruses, especially among members of the JEV group, and care must be taken in interpretation of serological results (Williams et al. 2001). In locations where related flaviviruses are known to circulate, these should be included in parallel testing. It is more reliable if there are no other possible infecting flaviviruses, which is unusual in JEV endemic areas, or where it can be demonstrated that there is IgM only to JEV and not to other flaviviruses. Immunoglobulin G (IgG) is even more broadly cross-reactive than IgM and identifying the virus-specific antibody is challenging and requires more specific tests. Classical this is done using virus-neutralizing antibody titer, but this is technically difficult, time consuming, and may not always yield clear results (Mackenzie et al. 2007). An epitope-blocking (B-)ELISA using a JEV Env-specific MAb has been developed that can identify IgG, though antibodies to very closely related flaviviruses, such as MVEV, can still cross-react (Williams et al. 2001). Tests for antibodies directed to the prM protein may show better specificity (Cardosa et al. 2002). Measurement of the magnitude of antibody response and relative differential of serum antibody titers against different flaviviruses can also be used to evaluate the significance of serological results (Pant et al. 2006). An ELISA to detect NS1 antibodies, produced only following natural infection, can be used for differentiation of antibody responses to natural infection and inactivated vaccine (Konishi et al. 2004).

**Immunity**

The flavivirus proteins Env, prM, and NS1 are immunogenic and the most important for inducing protective immune responses (Gubler et al. 2007). The Env protein is a major component of the virion surface and is immunodominant during infection. Although prM is a part of immature virus particles, in certain circumstances incomplete proteolytic cleavage occurs during maturation, providing an additional target on the virus surface for host immunity. NS1 is secreted from and expressed on the surface of infected cells and anti-NS1 antibodies can protect against infection in vivo via the complement pathway (Lindenbach et al. 2007).

Cross-protective antibody responses to JEV have been observed in pigs following experimental infection with closely related flaviviruses. Prior exposure to MVEV prevented or suppressed the level and duration of viremia following challenge with JEV and correlated with boosted levels of existing cross-reactive virus-neutralizing antibodies to JEV (Lunt et al. 2001; Williams et al. 2001). Similar booster responses occurred if infection with WNV was followed by challenge with JEV. However, levels of JEV viremia varied, ranging from undetected or low (Ilkal et al. 1994; Williams et al. 2001) to virus titers equivalent to primary infected control animals (Lunt et al. 2001). This variation may reflect differences in virus strains, breed of pigs, and/or methodologies used. Cumulatively, these findings suggested that in regions hyperendemic for flaviviruses, cross-reactive immunity in pigs from prior infection with related flaviviruses may inhibit subsequent infection with JEV; thus, amplification and spread of the
virus may be restricted, along with a reduced risk of porcine reproductive disease.

Prevention and Control
The current JEV vaccines and future directions have been extensively reviewed (Monath 2002; WHO 2006; Wilder-Smith and Halstead 2010). Most information and effort is directed at human vaccine development, rather than vaccines for pigs.

Inactivated, mouse brain-derived or live-attenuated vaccines have been used to prevent virus amplification or reproductive disease in pigs in Japan (Igarashi 2002), Taiwan, and Nepal (Daniels et al. 2002). The live attenuated vaccine has been shown to be more efficacious than the inactivated vaccine against natural and experimental infection (Daniels et al. 2002; Ueba et al. 1978). Vaccination of swine against JEV is not widely used for two reasons: (1) immunization of large numbers of newborn animals each year would be costly and (2) the period for effective immunization using live-attenuated JE vaccine is limited by the presence of maternal antibodies (Igarashi 2002).

Konishi and colleagues have explored the use of DNA vaccines for pigs (Konishi et al. 1992, 2000). While effective in inducing high antibody titers, they have not been taken further. However, the development of live virus vectors and DNA vaccines raises the possibility of new approaches to JEV control through oral immunization of pigs. Oral vaccines would be advantageous in the places in Southern and Southeast Asia where pigs are not husbanded in an organized fashion or are feral (Monath 2002).

JEV can also be controlled by preventing exposure of pigs to JEV-infected mosquitoes, but this is usually not practical unless pigs are maintained in a mosquito-free environment.

WEST NILE VIRUS
Relevance
WNV was first isolated in Uganda in 1937 from the blood of a febrile woman (Smithburn et al. 1940). It is one of the most widely distributed flaviviruses, with a geographic range encompassing Africa, the Middle East, parts of Western and Eastern Europe, and Asia (Hubalek and Halouzka 1999; Murgue et al. 2002). The virus is endemic to Australia, where it exists as the subtype KUNV (Hall et al. 2002). WNV was detected in North America in 1999 when its introduction caused an outbreak in New York City (Nash et al. 2001). Since then, it has rapidly spread throughout the United States, southern Canada, and to parts of Central America, South America, and the Caribbean.

Historically, WNV infections were associated with sporadic cases or occasional outbreaks of mild disease in humans, birds, and horses, and these were rarely associated with neurological disease. However from the mid-1990s, large outbreaks of severe disease involving high incidences of neurological infection in humans occurred in Europe, the Mediterranean Basin, and the United States (Hayes and Gubler 2006; McLean et al. 2002; Murgue et al. 2002), and were accompanied by large numbers of equine encephalitis cases and avian mortalities (Castillo-Olivares and Wood 2004; CDC 2002). In equines, ~10% of infected animals develop neurological disease, with high mortality rates (40–60%) (Castillo-Olivares and Wood 2004). Large numbers of mortalities in wild birds and commercial flocks have also occurred during epidemics (Komar 2003; Weinberger et al. 2001). Although WNV is a significant human and veterinary pathogen, no apparent disease association has been observed following infection of pigs (Platt 2004; Teehee et al. 2005).

Etiology
Based on full-length genomes and partial genes, two distinct lineages of WNV were initially proposed (Lanciotti et al. 2002; Scherret et al. 2001). Lineage 1 was divided into three clades: lineage 1a, comprising strains from Africa, the Middle East, Europe and the Americas, including recent outbreak strains; lineage 1b, made up of the Australian KUNV subtype; and lineage 1c, composed of human and mosquito isolates from India. Lineage 2 consists of the prototype Ugandan strain and strains from Africa and Madagascar. Recent investigations have provided evidence for additional lineages of WNV comprising isolates from Europe, Southern and Southeast Asia, and Western Africa (Bondre et al. 2007; Mackenzie and Williams 2009).

Role in Public Health
Approximately 20% of human infections are symptomatic, typically manifesting as a self-limited West Nile (WN) fever (Mostashari et al. 2001). Less than 1% of infections develop into neuroinvasive disease (meningitis, encephalitis or acute flaccid paralysis), and associated case fatality rates range from 10% to 50%, depending on disease severity (Sejvar 2007). Sequelae can persist in up to half of survivors for as long as 1.5 years.

Epidemiology
WNV exists in endemic transmission cycles involving ornithophilic mosquitoes and birds. The virus can infect a remarkable range of vectors and vertebrate hosts, thus facilitating its rapid spread throughout the world. In the United States alone, the virus has been isolated from ~60 species of mosquitoes, >300 species of birds, and >30 species of nonavian hosts (Gubler 2007). *Culex* species mosquitoes are the most common vector, but WNV has also been isolated from species of *Aedes, Anopheles, Coquillettidia, Culiseta, Mimomyia*, and *Ochlerotatus* (Hall et al. 2002; Hubalek and Halouzka 1999; Komar 2003). Evidence from serological surveys...
and experimental infections has established birds as the primary vertebrate hosts of WNV (Komar 2003). Passerine species such as the house sparrow, blue jay, and common grackle are thought to be important for epidemic transmission in urban settings, while migratory species such as storks (Ciconiiforms) may be important for long-range virus transmission and spread.

The majority of nonavian species infected by WNV, including humans and horses, are incidental hosts and are thought to play only minor roles, if any, in transmission. Although serological evidence of WNV infection in domestic and feral pigs has been reported (Gard et al. 1976; Geevarghese et al. 1987; Gibbs et al. 2006; Pant et al. 2006), there are limited reports of WNV isolation from pigs in the field (Ilkal et al. 1994). Furthermore, experimental infections indicate that pigs are generally poor amplifying hosts of WNV (Ilkal et al. 1994; Lunt et al. 2001; Platt 2004; Teehee et al. 2005). Viremic responses can vary from undetectable to moderate grade and appear to depend on the age of test animals and virus strain. In weanling pigs experimentally infected with a NY99 strain, virus titers reached levels sufficient to infect blood-feeding Culex pipiens mosquitoes, that is, $1 \times 10^6$–$10^7$ median tissue culture infectious dose (TCID$_{50}$) per milliliter. However, the duration of peak viremia was relatively short, ranging from 0.2 to 1.1 days (Platt 2004). This contrasts with that observed in passerine birds infected with a NY99 strain, in which titers exceeded $1 \times 10^8$ plaque-forming units per milliliter for 4 days (Komar et al. 2003). Adult pigs fed WNV-infected mice failed to produce antibody and viremic responses, indicating that pigs, unlike cats and birds, cannot be readily infected by ingestion (Platt 2004). Transmission between infected and noninfected control pigs housed together in experimental conditions was not found (Platt 2004; Teehee et al. 2005), suggesting that WNV is not shed from infected pigs.

Similar to JEV transmission, WNV occurs year-round in tropical and subtropical regions, but is epidemic in temperate regions. WNV can overwinter in hibernating adult mosquitoes (Nasci et al. 2001) and evidence of vertical transmission of the virus in Culex species mosquitoes has also been reported (Anderson and Main 2006), indicating potential mechanisms by which the virus can survive in temperate areas. Reintroduction of WNV by viremic migratory birds or perpetuation in persistently infected birds has also been proposed (Kramer et al. 2008). Ticks may also play a role in WNV maintenance and overwintering (Lawrie et al. 2004; Mumcuoglu et al. 2005).

**Pathogenesis, Clinical Signs, and Lesions**

There is no clear evidence for clinical signs attributable to WNV infection in swine (Platt 2004; Teehee et al. 2005), and consequently there is little information available on its pathogenesis in pigs. Platt (2004) observed moderate perivascular cuffing in brain and spinal cord tissue of infected weanling pigs; however, viral antigen was not detected in these tissues. In a proportion of pigs with perivascular cuffing, meningoencephalitis was also evident. In this study, a pregnant sow was also infected during the second trimester of gestation. Fetus development was unaffected by infection and no abnormal histological features were observed. Viral antigen was also undetected in fetal CNS tissue. Additional experimentation will be necessary to fully evaluate WNV as a reproductive pathogen.

**Diagnosis**

Serological diagnosis is the primary means of determining WNV infections (Dauphin and Zientara 2007; Shi and Wong 2003). ELISA (IgM capture, blocking, and IgG), HI, and plaque-reduction neutralization (PRNT) tests are used for testing human and veterinary specimens. In many instances, this involves ELISA screening followed by PRNT for confirmation of positive samples. Microsphere flow immunoassays that offer rapid, high-throughput testing for IgM and IgG antibodies to WNV have been described (Johnson et al. 2005; Wong et al. 2003). HI and VN assays, as well as specific blocking ELISAs, have been reported for detecting serum antibodies to WNV taken from domestic and feral pig populations in serosurveillance studies or experimental infections (Blitvich et al. 2003; Gibbs et al. 2006; Ratho et al. 1999). As described above for JEV, serological diagnosis of WNV can be complicated by the presence of cross-reactive antibody to closely related flaviviruses.

WNV can be detected in CSF, blood, or tissues by isolation in cell culture, conventional or real-time RT-PCR, and immunoassay. Several cell lines are suitable for isolation, including Vero, rabbit kidney (RK-13), Chinese hamster ovary (CHO), and the Aedes pseudocutellaris clone AP-61. Inoculation of embryonated eggs or sucking mouse brain can be done, but the latter is considered less sensitive than cell culture (OIE 2010b). Confirmation of WNV isolation is performed by immunoassay with WNV-specific antibodies or by RT-PCR of culture extracts.

Antigen capture assays can be used to detect WNV in field-collected avian swabs and mosquito pools (Burkhalter et al. 2006; Hunt et al. 2002). Immunohistochemical staining of fixed tissue samples from infected birds and mammals has also been used to detect WNV antigen. Caution should be exercised when testing mammalian tissues using this method, as false-negative results have been found in equine CNS specimens (OIE 2010b), and nonspecific binding of primary antibody to mammalian tissues has been reported (Kauffman et al. 2003).

Several RT-PCR assays have been described for the detection of WNV RNA in cell culture, mosquito pools, and in human, equine, and avian samples. These
include conventional primary and nested RT-PCRs, real-time RT-PCR, and RT-LAMP assays (Jimenez-Clavero et al. 2006; Johnson et al. 2001; Lanciotti et al. 2000; Parida et al. 2004; Shi et al. 2001). Prevalence of circulating WNV lineages and assay specificity are important regional considerations. For example, an RT-PCR has been developed for the specific detection of WNV–KUNV (lineage 1b) (Pyke et al. 2004b), found only in the Australasian region and where other WNV lineages are exotic.

**Prevention and Control**

There is currently no specific treatment available for WNV infection. Vaccine development has predominantly focused on preventing disease in humans, horses, and geese (Arroyo et al. 2004; Dauphin and Zientara 2007; Hall and Khromykh 2004). The only veterinary licensed vaccines are for use in horses and geese.

Vector control activities include reducing mosquito populations through source reduction and the use of insecticides and public education to limit exposure to mosquito bites (Kramer et al. 2008). WNV surveillance involves identifying infection in humans, horses, wildlife, and bird populations (especially corvids and sentinel chickens) by either passive or active means, as well as mosquito trapping to monitor vector abundance and isolate the virus (CDC 2010; Drebot et al. 2003).

**MURRAY VALLEY ENCEPHALITIS VIRUS**

**Relevance**

MVEV is the most important cause of arboviral neurological disease in humans in Australia. MVEV can also cause fatal encephalitis in horses (Gard et al. 1977; Williamson 2008). Although pigs can be infected with MVEV, no disease association has been reported (Kay et al. 1985; Lunt et al. 2001). Pigs may, however, play a role in maintenance of virus transmission.

**Etiology**

Isolates of MVEV are closely related antigenically and cannot be differentiated by standard serological techniques. Four genotypes of MVEV have been recognized from phylogenetic analysis of partial Env and NS5 genes (Johansen et al. 2007; Mackenzie and Williams 2009). Genotype 1 is the dominant type on mainland Australia and the most recent strains from Papua New Guinea (PNG) also belong to this type. Genotype 2 consists only of mosquito isolates from the Kimberley region of northwestern Australia. The remaining genotypes each comprise single isolates from PNG made in 1956 (G3) and 1966 (G4).

**Role in Public Health**

Epidemics of severe encephalitis were reported between 1917 and 1925 in eastern and southeastern Australia, but no etiology could be determined. MVEV was isolated for the first time from fatal cases during a major outbreak of encephalitis in the Murray-Darling river system of southeastern Australia in 1951 (French 1952). A second major outbreak occurred in 1974, and although 58 cases with 13 deaths were reported from all mainland Australian states, the majority of cases were observed again in southeastern Australia (Marshall 1988). Approximately 107 human cases have been recorded subsequently, but they have almost all occurred in northern and northwestern Australia, where the virus is believed to be endemic. Most human infections are asymptomatic or cause mild febrile illness, and only ~1:1000 infected individuals develop encephalitis, with fatality and morbidity rates similar to those for JE (Spencer et al. 2001).

**Epidemiology**

Although the major epidemics of MVE occurred in southeastern Australia in the last century, the majority of human cases are now found in northern Australia, where MVEV exists in endemic cycles between mosquitoes and water birds. The principle vector of MVEV is *C. annulirostris*, and this species accounts for >90% of isolates. Based on serosurveys and experimental evidence, the main vertebrate hosts are thought to be water birds belonging to the orders Ciconiiformes, Pelecaniformes, and Anseriformes (Marshall 1988). In particular, herons have been implicated as having a prominent role in MVEV transmission.

Experimental evidence indicated that other vertebrates might also contribute to the transmission cycle. Grey kangaroos and rabbits produced relatively high levels of viremia following inoculation with MVEV, suggesting that they could serve as ready amplifiers of the virus (Kay et al. 1985). Dogs and chickens responded to infection with a relatively moderate viremic response and may play a minor role in virus amplification, but it is notable in this regard that MVEV has been isolated from sentinel chickens (Campbell and Hore 1975).

The role of pigs in the ecology of MVEV remains unclear. Serosurveys demonstrated a high prevalence of seropositive feral pigs in eastern Australia (Gard et al. 1976). However, following experimental infection with MVEV, pigs were found to produce only moderate to low-grade viremia (Kay et al. 1985). Since feral pig densities are extremely high in parts of northern and eastern Australia (Choquenot et al. 1996), it has been proposed that even if only a small proportion respond to infection with viremia capable of infecting mosquito vectors, a significant contribution may be made to the maintenance or amplification of MVEV (Marshall 1988). However, a study of host-feeding preferences of *C. annulirostris* in northern Australia showed that the majority of blood meals were obtained from marsupials (>60%), suggesting that host-seeking vectors are diverted away from feral pigs (van den Hurk et al.
MVEV is endemic in northern Western Australia and epidemic in the southern regions of this state. A similar scenario is likely for the Northern Territory. Virus activity is epidemic in the eastern and southeastern states of Australia. MVEV is also found in New Guinea and probably the eastern Indonesian archipelago. It is not known precisely how MVEV activity resurfaces in regions where occasional or rare activity is found. The virus may be reintroduced by migratory viremic water birds or wind-blown mosquitoes. There is also evidence that MVEV can be maintained over dry periods in desiccation-resistant eggs of *Aedes normensis* (Broom et al. 1989). Thus, environmental factors such as wind, rainfall, and temperature are likely to affect transmission and maintenance.

**Pathogenesis and Clinical Signs**

No disease association or clinical signs following MVEV infection of pigs have been reported. There is also no evidence available in the literature that infection leads to secondary amplification in reproductive organs or fetuses. Kay et al. (1985) performed experimental infections on domestic and feral pigs aged between 6 and 20 weeks. These animals responded with low-to-moderate, but variable, levels of viremia lasting from 1 and 20 weeks. These animals responded with low-to-moderate, but variable, levels of viremia lasting from 1 and 20 weeks. These animals responded with low-to-moderate, but variable, levels of viremia lasting from 1 and 20 weeks. These animals responded with low-to-moderate, but variable, levels of viremia lasting from 1 and 20 weeks. These animals responded with low-to-moderate, but variable, levels of viremia lasting from 1 and 20 weeks. These animals responded with low-to-moderate, but variable, levels of viremia lasting from 1 and 20 weeks. 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The pestiviruses are small (approximately 40–60 nm), enveloped, roughly spherical, positive-sense, single-stranded RNA viruses in the genus *Pestivirus* of the family *Flaviviridae* (Becher et al. 1999). Currently the genus *Pestivirus* includes four formally recognized species: classical swine fever virus (CSFV), bovine viral diarrhea viruses 1 and 2 (BVDV-1 and BVDV-2), and border disease virus (BDV).

Molecular characterization of some viruses and the recent identification of new viruses suggest that there could be other species in the *Pestivirus* genus. These include the “pestivirus of giraffe” (Becher et al. 1999; Harasawa et al. 2000), the “HoBi” and related viruses isolated from fetal bovine serum and cattle (Kreutz et al. 2000; Schirrmeier et al. 2004), a virus isolated from pronghorn antelope in the United States (Vilcek et al. 2005), and most recently, Bungowannah virus identified in an outbreak of disease in pigs (Kirkland et al. 2007). Interspecies transmission of the pestiviruses is well known and the possibility that pigs could be infected with any of the other pestiviruses, although as yet unrecognized in the field, cannot be excluded.

Among the pestiviruses, the most important in pigs is CSFV. Infection of pigs with pestiviruses such as BVDV or BDV can result in disease, especially reproductive loss, but without major clinical impact. Bungowannah virus has been shown to be a significant pathogen. In addition to severe reproductive disease, in utero infection with Bungowannah virus can result in chronic infections that are clinically indistinguishable from low virulence strains of CSFV. However, the known geographical distribution of Bungowannah virus is extremely limited, having only been found in two herds in Australia.

The majority of pestiviruses are noncytopathogenic in cell culture, but some CSFV strains and some BVDV isolates from cases of mucosal disease are cytopathogenic in vitro (Gallei et al. 2008). Cytopathogenicity of BVDV is correlated with the expression of the nonstructural protein NS3, which is generated by processing the fusion protein NS2-3 (Kummerer and Meyers 2000; Zhang et al. 2003).

The pestivirus genome is 12.5–16.5 kilobases in size and encodes a single polyprotein (Meyers et al. 1989) encompassing all of the structural proteins (C, E*ms*, E1, and E2) and the nonstructural proteins (N(pro), p7, NS2-3, NS4A, NS4B, NS5A, and NS5B) as follows: NH₂-(N(pro)-C-E*ms*-E1-E2-p7-NS2-3-NS4A-NS4B-NS5A-NS5B)-COOH. The single polyprotein is co- and posttranslationally converted to mature proteins by a combination of virus and host cell proteases (Rumenapf et al. 1993). The structure and function of some envelope proteins has been studied in detail, but the nonstructural proteins are not well characterized. Little is known about mechanisms of viral RNA replication, packaging, or assembly of viral particles. Virions are released from the host cell by exocytosis, usually without morphological cell damage, the exception being the cytopathogenic strains that are relatively uncommon in nature and arise from mutations of the predominant noncytopathogenic populations.

As enveloped viruses, the pestiviruses are very susceptible to treatment with detergents and lipid solvents. Although they are relatively susceptible to the effects of either high or low pH and/or temperature above 60°C (140°F), inactivation can be variable among viruses and markedly influenced by the medium in which the virus is contained. A protein-rich environment usually enhances the stability of pestiviruses.
Role in Public Health
Although interspecies transmission of pestiviruses does occur, in nature this is limited to spread between the artiodactyls (even-toed ungulates). There is no evidence of human infection with pestivirus and they are not of any significance for public health or food safety.

CLASSICAL SWINE FEVER (HOG CHOLERA)

Relevance
Classical swine fever (CSF), formerly known as “hog cholera,” is a highly contagious viral disease of worldwide importance and one of the World Organisation for Animal Health (OIE)-listed diseases. Clinical outbreaks suggestive of CSF were reported in the early 19th century (Fuchs 1968; Kernkamp 1961; USDA 1889) and CSF was recognized as viral in nature by 1903 (Wise 1981). Wild and domestic pigs are the only natural reservoirs of CSFV. CSFV is endemic in parts of Eastern Europe, Southeast Asia, Central America, and South America. Although eradicated from domestic pigs in Western Europe, CSFV remains endemic in some populations of wild boar and farms in these areas are at risk of reinfection.

Etiology
CSFV is relatively stable for an RNA virus (Vanderhallen et al. 1999), but is antigenically and genetically diverse. Recombination between strains may be possible (He et al. 2007). Antigenic variability among CSFV isolates can be characterized using monoclonal antibodies (Edwards et al. 1991) and genetic variability evaluated using genomic sequencing. For example, two panels of monoclonal antibodies, directed against E2 and Erns using genomic sequencing. For example, two panels of monoclonal antibodies, directed against E2 and Erns using genomic sequencing. For example, two panels of monoclonal antibodies, directed against E2 and Erns using genomic sequencing. For example, two panels of monoclonal antibodies, directed against E2 and Erns using genomic sequencing.

Genetic characterization of new CSFV isolates has become standardized in terms of the genomic fragment sequenced, the algorithms used in constructing phylogenetic trees, and the classification of the genetic groups. Three regions of the viral genome are usually evaluated: the 3’ end of the polymerase gene (NS5B), 150 nucleotides of the 5’ nontranslated region (NTR), and 190 nucleotides of the gene encoding E2. Genetic typing is most commonly based on the E2 glycoprotein because abundant sequence data are available.

CSFV is divided into three major genetic groups (Lowings et al. 1996), each with three or four subgroups: 1.1, 1.2, 1.3; 2.1, 2.2, 2.3; and 3.1, 3.2, 3.3, and 3.4 (Paton et al. 2000). The phylogenetic analyses of the last decade have demonstrated a link between genotype and geographic origin (Greiser-Wilke et al. 2000a). Group 1 isolates are present in South America (Pereda et al. 2005) and Russia (Vlasova et al. 2003). Most viruses belonging to group 2 were isolated from outbreaks in Western, Central, or Eastern Europe (Blome et al. 2010), and some Asian countries (Blacksell et al. 2004; Kamakawa et al. 2006; Pan et al. 2005). Group 3 viruses are apparently confined to Asia (Parchariyanon et al. 2000). The Community Reference Laboratory for CSF in Hanover, Germany, has developed a web-accessible database of sequences from isolates throughout the world. This database is a useful tool for identifying possible viral sources for outbreaks occurring in previously uninfected areas (Greiser-Wilke et al. 2000b).

Epidemiology
Domestic pig populations in Australia, New Zealand, North America, and Western Europe are free of CSF (Paton and Greiser-Wilke 2003). In South America, Chile and Uruguay have been declared CSF free. Argentina, free of outbreaks since 1999, stopped vaccination in April 2004 (Vargas Teran et al. 2004). Extensive areas of Central and South America continue to control the disease by vaccination (Morilla and Carvajal 2002). CSF remains endemic in Asia (Paton et al. 2000) and, although the situation in Africa is not well defined, the disease has been identified in Madagascar and South Africa (Sandvik et al. 2005).

The reemergence of CSF is always a risk and several areas previously free of CSF have had incursions in recent years. For example, CSF reemerged in Cuba in 1993 after an absence of more than 20 years. The outbreaks in western Cuba were apparently caused by strain Margarita (group 1.2) used in vaccine potency trials, but outbreaks in eastern Cuba were of uncertain origin and with no demonstrated relationship to Caribbean strains. The outbreaks were exacerbated by the presence of a highly susceptible (unvaccinated) swine population, the result of a worsening economy on the island (Frias-Lepoureau 2002; Pereda et al. 2005). In spite of a campaign of total depopulation carried out in the Caribbean, new infections have occurred, leading the authorities to turn to vaccination as the method of control.

Under natural circumstances, the primary routes of transmission are oronasal by direct or indirect contact with infected wild or domestic pigs, or oral by ingestion of contaminated foodstuffs (Edwards 2000; Fritzemeier et al. 2000; Horst et al. 1997). In finishing units and in areas with small pig farms, transport and introduction of infected pigs accounts for the majority of outbreaks and for the spread of the disease (Ribbens et al. 2004).

Airborne spread of CSFV has been demonstrated under experimental conditions (Dewulf et al. 2000; Weesendorp et al. 2009), although its importance under field conditions is uncertain. Nevertheless, in a fully susceptible population and in a densely populated region, even a minor route of transmission can have major consequences. Analysis of data from the 1997–1998 CSF epidemic in The Netherlands suggested that airborne transmission did not occur over long distances, but did occur within a holding or within a
The survival and inactivation of CSFV has been reviewed (Edwards 2000). Typical of enveloped viruses, CSFV is inactivated by organic solvents (ether or chloroform) and by detergents. Sodium hydroxide (2%) is still considered the most suitable for disinfection of contaminated premises.

In spite of the fact that CSFV is an enveloped virus, CSFV survives for prolonged periods under certain conditions, that is, cool, moist, protein-rich conditions, such as those found in meat, but in liquid manure CSFV can survive for 2 weeks at 20°C (68°F) and more than 6 weeks at 4°C (39°F). CSFV is relatively stable over pH ranging from 5 to 10. The rate of inactivation under pH 5 is dependent on the temperature. Thermal and pH stability may vary by strain, but inactivation of the virus is primarily dependent on the medium. For example, CSFV in cell culture loses its infectivity after 10 minutes at 60°C (140°F), but survives for up to 30 minutes at 68°C (154°F) in defibrinated blood. The inactivation of CSFV in feces and urine from pigs intranasally inoculated with a highly or moderately virulent CSFV strain was inversely related to the storage temperature. Average half-life values were 2–4 days at 5°C (41°F) and 1–3 hours at 30°C (86°F). Significant differences were observed in survival between virus strains in feces, but not in urine (Weesendorp et al. 2008). For these reasons, it is difficult to give guidelines for the survival of CSFV in the environment.

Pathogenesis
Transmission of CSFV is most commonly oroanal, with primary virus replication in the tonsils. From the tonsils, it spreads to the regional lymph nodes, then via the peripheral blood to the bone marrow, visceral lymph nodes, and lymphoid structures associated with the small intestine and spleen. The spread of the virus within the pig is usually complete in less than 6 days.

Within the pig, CSFV replicates in monocyte-macrophage cells and vascular endothelial cells. CSFV is immunosuppressive and neutralizing antibodies may not appear until 2–3 weeks after infection. Leukopenia, in particular lymphopenia, is a classic early event (Susa et al. 1992). CSF leukopenia affects leukocyte subpopulations unequally, with B-lymphocytes, helper T cells, and cytotoxic T cells the most affected. Depletion of lymphocyte subpopulations occurs shortly before virus can be detected in serum by reverse transcription-polymerase chain reaction (RT-PCR).

The severity of the changes in bone marrow and circulating leukocytes suggest that effects of the virus on uninfected cells are induced indirectly, for example, by a soluble factor or by cell-to-cell contact and are not a direct effect of the virus or viral protein(s). Research has shown that the glycoprotein Em1 at a high concentration induces apoptosis in lymphocytes in vitro (Bruschke et al. 1997). However, exposure to supernatant from infected cells did not induce apoptosis in
target cells. Although the process is still not fully understood, such a mechanism could also account for the delay in cellular and humoral immune responses (Summerfield et al. 2001).

In cell culture, most CSFV strains grow without producing cytopathic effect and without inducing interferon alpha secretion by infected cells. Indeed, CSFV infection causes cells to acquire a greater capacity to resist apoptosis (Ruggli et al. 2003). These observations provide evidence that CSFV interferes with cellular antiviral activity and suggests the possibility that the lesions seen in pigs have an immunopathological basis.

Interactions between CSFV and the monocyte–macrophage system result in the release of mediator molecules that promote the progression of the disease. Changes in hemostatic balance are thought to be caused by proinflammatory and antiviral factors that mediate the thrombocytopenia and hemorrhage characteristics of CSFV infection (Knoetig et al. 1999). The production of inflammatory cytokines by infected endothelial cells could play a role in immunosuppression and facilitate virus dissemination by attracting monocytic cells (Bensaude et al. 2004). It has recently been recognized that CSFV can replicate in dendritic cells. It is possible that these highly mobile cells disseminate CSFV to various sites in the body, especially lymphoid tissues (Jamin et al. 2008). Of themselves, interactions between CSFV-infected dendritic cells and lymphocytes are not sufficient to induce lymphocyte depletion without other interactions within the environment of the lymphoid follicles (Carrasco et al. 2004; Jamin et al. 2008).

The expression of differences in virulence between strains is a result of the CSFV–host interactions. The evasion of the host's innate immune responses delays the onset of acquired immunity and produces the resultant pathogenic effects. Results from a comparative microarray analysis strongly suggest that CSFV subverts the interferon response, leading to bystander killing of lymphocytes and lymphopenia, the severity of which might be due to the host's loss of control of interferon production (Renson et al. 2010).

**Clinical Signs**

In the acute form of CSF, the initial clinical signs include anorexia, lethargy, conjunctivitis, respiratory signs, and constipation followed by diarrhea (Cariolet et al. 2008; Floegel-Niesmann et al. 2009). In the chronic form, the same clinical signs are observed, but the pigs survive for 2–3 months before dying. Nonspecific signs—for example, intermittent hyperthermia, chronic enteritis, and wasting—may also be seen.

Historically, peracute, acute, chronic, or prenatal forms of CSF were attributed to distinct levels of virus virulence. However, virus strain virulence is difficult to define because clinical signs also depend on pig age, breed, health status, and immune status (Depner et al. 1997; Floegel-Niesmann et al. 2009; Moennig et al. 2003).

Since the early 1980s, diagnosis of CSF based on clinical signs has been problematic and resulted in the belated recognition of CSF outbreaks, thereby giving time for the virus to spread (Durand et al. 2009). CSF is one of several diseases characterized by cutaneous hyperemia or cyanosis and nonspecific clinical signs. Particularly when CSFV strains of low virulence are involved, it may be difficult to differentiate CSF from African swine fever (ASF), porcine reproductive and respiratory syndrome (PRRS), postweaning dermatitis and nephropathy syndrome (PDNS), salmonella, or cumarin poisoning. The one constant sign in CSF is hyperthermia usually >40°C (>140°F) (Floegel-Niesmann et al. 2003), with piglets often piled in a corner. Clinical signs are more marked in piglets than adults, in which hyperthermia may be lower (39.5°C, 103°F).

CSFV is able to cross the placenta and infect fetuses at any stage of pregnancy. Depending on the strain and the time of gestation, infection can cause abortion and stillbirths. However, infection at 50–70 days of gestation can lead to the birth of persistently viremic piglets. Such piglets initially appear clinically normal, but subsequently begin to waste or develop congenital tremors (Vannier et al. 1981). This course of infection has been described as “late-onset CSF” (Van Oirschot and Terpstra 1977). Similar to BVDV in ruminants, these animals shed high levels of virus for several months and are important reservoirs of CSFV.

Depending on the virulence of the strain of virus and the host response, infected pigs can show clinical signs within 3–6 days of exposure to the virus and die rapidly, recover, or develop chronic disease, which is invariably fatal. Animals may start to shed virus within a few days of infection and before the onset of clinical signs. With less virulent strains, the time to the onset of disease can be as long as 13–19 days (Durand et al. 2009). However, due to the nonspecific nature of clinical signs, especially with strains of moderate or low virulence, the virus may remain undetected in a herd for 4–8 weeks, which increases the risk of further dissemination.

**Lesions**

CSF lesions vary in severity and distribution, depending on the course of the disease. In acute forms, the pathological picture is often hemorrhagic. Leucopenia, thrombocytopenia, petechiae, and ecchymoses in the skin, lymph nodes, larynx, bladder, kidney (Figure 38.1), and ileocecal junction are often described. Multifocal infarction of the margin of the spleen is characteristic of CSF, but is not always present (Figure 38.2). Swollen or hemorrhagic lymph nodes or tonsils are common (Figure 38.3). In chronic forms, button ulcers in the cecum or large intestine may be present (Figure 38.4), as well as a generalized depletion of lymphoid.
tissues. Hemorrhagic and inflammatory lesions are less common, or even absent, despite the degeneration of endothelial cells. Congenital CSF can result in abortion, fetal mummification, stillbirths, and congenital malformations, such as central dysmyelogenesis, cerebellar hypoplasia, microencephaly, and pulmonary hypoplasia (van der Molen and van Oirschot 1981).

Floegel-Niesmann et al. (2009) compared the clinical signs and lesions produced by six field strains isolated during the last decade from domestic pigs or wild boars in Europe to a reference strain (Alfort 187). Comparing lesions in skin, subcutis and serosae, tonsil, spleen, kidney, lymph nodes, ileum and rectum, brain, and respiratory system, they found that lymph nodes were the tissues most severely affected by all isolates, followed by necrotic lesions in the ileum and hyperemia of the blood vessels of the brain. Thus, these tissues were the most reliable for diagnosis of CSF. Infarction of the spleen and necrotic lesions of the tonsil, although commonly described in the earlier literature, were infrequent. Likewise, respiratory signs were absent or mild.

**Diagnosis**
Recent CSF epidemics in Europe have shown that early recognition of CSF and prompt elimination of CSFV-infected animals is the key to control. The longer CSF remains undetected, the greater the opportunity for the virus to spread. It should be recognized that farmers and veterinarians detected 75% of the recent CSFV epidemics based on clinical observations. The need to establish a standardized protocol for evaluating herds for CSF has been recognized (Elbers et al. 2002; Floegel-Niesmann et al. 2009; Mittelholzer et al. 2000).
However, the list of clinical criteria cannot be overly complex if it is to be used in the field. Average daily gain and feed consumption are two quantifiable measures that may be of use (Cariolet et al. 2008). Body temperature may also be useful, since hyperthermia is consistently associated with CSF and appears before, or concurrently, with the first clinical signs.

Because CSF has no pathognomonic clinical signs, laboratory diagnosis is always required. Since CSFV, BVDV, and BDV share common antigens, it is of the utmost importance to discriminate among these. A variety of methods for the detection of CSFV, components of the virion (antigens or nucleic acid), or specific antibodies against viral antigens, are available. While pan-pestivirus diagnostic assays are useful for screening specimens, positive results must be confirmed with CSFV-specific assays. Real-time, reverse transcription-polymerase chain reaction (qRT-PCR) assays are now widely used to detect nucleic acid from pestiviruses, and both pan-reactive and CSFV-specific assays are available. Monoclonal antibodies that distinguish between pestiviruses are used in a variety of techniques to specifically identify the virus, for example, virus isolation (VI), fluorescent antibody test (FAT), or enzyme-linked immunosorbent assay (ELISA) tests.

It is not feasible to perform all available diagnostic assays during an outbreak. Therefore, it is important to perform the assay(s) most appropriate to the situation and intended purpose. Since the key to controlling an outbreak is prevention of virus spread among farms, the assay of choice in terms of diagnostic sensitivity, diagnostic specificity, and speed is qRT-PCR. However, since the period of viremia is short, antibody detection assays are also useful, especially in herds where clinical signs have been present for more than 2 weeks (Greiser-Wilke et al. 2007).

Detection of Classical Swine Fever Virus. Depending on the virulence of the strain, the tests, and the specimens, the virus can be detected within 24 hours after infection. Virus may be isolated from tissue homogenates, serum, plasma, buffy coat, and whole blood collected in heparin or ethylenediaminetetraacetic acid (EDTA) (Greiser-Wilke et al. 2007). The tissues most likely to contain virus are tonsils, spleen, kidney, ileocecal lymph node, and retropharyngeal lymph node (Narita et al. 2000). These specimens are also good sources of virus for RNA or antigen detection.

Although VI is the reference method in most CSFV eradication programs, it is labor intensive, time consuming, and incompatible with the rapid response required to prevent further spread of the virus. The purpose of VI, at least in reference laboratories, is to isolate viruses for detailed characterization and for use in vaccination studies. CSFV can be isolated on porcine kidney cell lines (PK-15 or SK6). It is critical that all cells, media, and reagents have been previously determined to be free of pestiviruses or antibodies against pestiviruses.

qRT-PCR assays have many advantages over other methods and are now the preferred method for the detection of virus or RNA. These assays have both high sensitivity (diagnostic and analytical) and specificity, particularly probe-based assays (Hoffmann et al. 2009; Le Potier et al. 2006). Hybridization probes may be slightly more specific than hydrolysis probes and are also preferred over SYBR green-based assays (Hoffmann et al. 2005; Jamnikar Ciglenecki et al. 2008). When the same protocol is used, qRT-PCR assays can be standardized between laboratories to achieve high levels of agreement. Several CSFV-specific qRT-PCR kits are commercially available (Le Dimna et al. 2008).

A wide variety of samples are suitable for testing by qRT-PCR, but mainly whole blood samples, swabs, and tissue samples are used for the diagnosis of CSF. Besides whole blood, serum, plasma, or leukocytes can be used. Preferable tissue samples are those collected for VI: tonsil, spleen, ileum, and lymph nodes. Kidney samples may be less suitable.

Good quality, fresh specimens are preferred, but viral RNA can still be detected in specimens in which virus is inactivated or VI is no longer possible due to bacterial contamination or autolysis, for example, wild boar samples (Depner et al. 2007). qRT-PCR is not affected by the presence of antibodies, so specimens from animals of any age can be tested.

Viral RNA can be detected for a long time in certain tissues from animals that are fully recovered from an infection. In tonsils from pigs that had recovered after an infection, viral RNA was detectable for at least 9 weeks (Blome et al. 2006).

Depending on the segment of RNA that is targeted for amplification, qRT-PCR can be designed to differentiate between virus species (CSFV, BVDV, and BDV) and even strains of CSFV. Depending on the vaccine and the sample to be tested, qRT-PCR can be used as a genetic DIVA (differentiate infected from vaccinated animals) test (Beer et al. 2007). That is, if the vaccine does not contain viral genome, for example, E2-subunit vaccines, or if the vaccine has deletions or substitutions on the primer sites, for example, deletion mutants or chimeric vaccines, a qRT-PCR-positive result would be proof of infection with field virus (Koenig et al. 2007). Newly developed C-strain-specific qRT-PCRs (Leifer et al. 2009) can be used to test vaccinated animals for the presence of modified live virus (MLV) vaccines, but in the case of a positive result, infections with wild-type virus cannot be ruled out. PCR assays that are specific for wild-type virus (Liu et al. 2009; Zhao et al. 2008) and can be used to detect or rule out wild-type virus infections, independent of the vaccination status of the animal, are of considerable value.

The high sensitivity of qRT-PCR will support the testing of pooled samples (Depner et al. 2007; Le Dimna
et al. 2008), which can significantly increase throughput. There are, however, other considerations, including the time to prepare sample pools and the need to retest all individual samples in a positive pool. To avoid a loss of sensitivity, detailed knowledge of the performance characteristics of the assay and the levels of RNA likely to be detected, for example, clinical cases versus screening in vaccinated populations, need to be established prior to pooling.

In general, it can be concluded with a high degree of confidence that a negative RT-PCR result indicates that the tested animal or tissue sample is not infectious to other pigs. In contrast, a positive RT-PCR result does not necessarily imply that the animal is infectious (Dewulf et al. 2005; Haegeman et al. 2006).

Antigen-capture ELISAs may be used for early diagnosis of CSFV in live pigs. Double-antibody sandwich ELISAs are based on monoclonal and/or polyclonal antibodies directed against a variety of viral proteins. Serum, buffy coat fraction, whole blood in heparin or EDTA, or tissue homogenates can be tested in these assays. The technique is relatively simple to perform, does not require tissue culture facilities, is suitable for automation, and provides results within 36 hours (Depner et al. 1995). However, it is important to recognize the diagnostic limitations of antigen-capture ELISAs. All currently available commercial ELISAs are less sensitive than VN on cell culture (Blome et al. 2006). In addition, their diagnostic sensitivity is significantly better on blood samples from piglets compared to samples from adult pigs or samples from mild or subclinical cases (Anonymous 2002). To compensate for the lack of diagnostic sensitivity, all pigs showing pyrexia in suspect herds should be tested. These tests also have lower diagnostic specificity and false-positive reactions may occur. For these reasons, the use of antigen-capture ELISAs is only recommended on samples from animals with clinical signs or pathological lesions compatible with CSF and for screening herds suspected to have been recently infected.

Although the direct FAT on frozen sections was the method of choice for detecting viral antigen, given the high sensitivity, throughput, and rapid turnaround provided by qRT-PCR, it is unlikely that this would be the assay of choice in a future outbreak.

### Detection of Antibodies to Classical Swine Fever Virus

Virus neutralization (VN) has been considered the reference assay for the detection of CSFV-specific antibodies. CSFV neutralizing antibody levels are determined by end-point titration of serum. However, VN requires good quality serum samples and the use of a cell culture system. As VN is relatively time consuming and takes 3–5 days to obtain results, it is not the assay of choice for routine large-scale testing.

Because of antibody cross-reactions among pestiviruses, VN can be used to identify the virus with which an animal has been infected. Samples are tested in dual or multiple VN tests and the CSFV neutralizing antibody titers are compared to neutralizing antibody titers against BVDV or BDV reference strains. A difference of 4-fold or more between the end points of the two titrations is considered conclusive for infection by the virus species yielding the highest neutralizing antibody titer (Anonymous 2002). This method is frequently used to screen neighboring herds around an outbreak prior to lifting control measures.

ELISAs for the detection of anti-CSFV antibodies are useful for conducting epidemiological surveys and for monitoring CSFV-free areas. Competitive ELISAs are based on competition between anti-CSFV serum antibodies and a CSFV-specific monoclonal antibody directed against the viral glycoprotein E2 (gp55). Cross-reactions with antibodies against other pestiviruses are reduced in the competitive ELISA format. A baculovirus recombinant E2 protein is often used as the antigen in the system. ELISA-detectable antibodies appear 10–15 days postinfection, similar to the period described for the appearance of neutralizing antibodies.

The E\textsuperscript{\textsubscript{\textnu}}-ELISAs were developed as differential companion tests for use in E2-subunit vaccinated populations (Van Rijn et al. 1999), but some of the commercially available assays lack sensitivity and specificity and sometimes cross-react with antibodies to other pestiviruses (Floegel-Niesmann 2001).

New combinations of protein-specific ELISA and subunit, deletion mutant, or chimeric vaccines may become available in future. Therefore, during an outbreak, the currently available serological assays should be reviewed in parallel with the type of vaccine to be used. The capacity to distinguish between antibodies produced in response to infection or vaccination (DIVA) can be invaluable.

### Immunity

Even though CSFV can induce immunosuppression, pigs that recover develop neutralizing antibodies to the virus. Neutralizing antibodies are induced by the envelope glycoprotein E2 while the envelope protein E\textsuperscript{\textnu} and the nonstructural protein NS3 induce non-neutralizing antibodies. Therefore, clearance of the virus is possible after the onset of production of neutralizing antibodies between 10 and 20 days after infection.

Both CSFV-specific neutralizing activity and specific killer cell activity are important for an effective immune response (Piriou et al. 2003). The dual combination of cellular immunity and neutralizing antibody is optimal for providing fast and complete protection that results in sterilizing immunity. However, each component in itself has the potential to protect pigs from a lethal CSFV infection. E2-subunit vaccines protect pigs by inducing high titers of neutralizing antibodies (Bouma et al. 1999), but experimental infection with related pestiviruses or chimeric constructs were also protective,
even though they did not induce detectable neutralizing activity (Beer et al. 2007; Reimann et al. 2004; Voigt et al. 2007).

Pigs that have been either vaccinated or infected are resistant to subsequent virus challenge. CSFV is quite stable for an RNA virus and cross-protection exists between different genogroups and even between BVDV and CSFV (Leforban et al. 1992). Maternal antibodies can confer protective immunity to piglets for 8–12 weeks, depending on the level of neutralizing antibodies in the colostrum (Kaden and Lange 2004). However, these antibodies can also interfere with the response to vaccination (Vandeputte et al. 2001).

**Prevention and Control**

CSF is endemic in many parts of the world and remains a disease of worldwide importance. Although some regions are free of CSFV, it is still present at the borders between free and endemic areas and in some wild boar populations (Laddomada 2000). At present, the risk of reintroducing CSFV into free areas is high. Producers and veterinarians are in the best position to detect CSF outbreaks in free areas, but early detection will require both vigilance and training in the recognition of clinical signs.

For purposes of international trade, free areas maintain a “no vaccination” policy against CSF. That is, control is based on stamping out infected or suspected herds, with the implementation of concomitant quarantine measures (Anonymous 2001). However, the eradication of CSF outbreaks in Europe by stamping out has raised concerns regarding the “no vaccine” policy. This is especially true in pig-dense areas, where a variety of factors increase the risk of disease spread (Koenen et al. 1996; Mintiens et al. 2003). In certain situations, like the 1997 outbreak in The Netherlands, movement restrictions that prevented the movement of pigs to slaughter resulted in the unnecessary euthanasia of large numbers of animals. While the use of vaccine has economic consequences, that is, vaccinated areas are banned from international trade for at least 1 year, emergency vaccination in the face of an outbreak is more likely to be considered in the future.

Various CSFV vaccines are available, including the well-known live “Chinese” C strain, the Thiverval strain, and newer marker vaccines that allow differentiation of field virus-infected from vaccinated animals (Beer et al. 2007; van Oirschot 2003). The traditional live vaccines induce a high level of protection against clinical disease and neutralizing antibodies are detectable at 2 weeks postchallenge (Dahle and Liess 1995; Vandeputte et al. 2001). Duration of immunity is 6–10 months, regardless of the route of administration (intramuscular or oronasal) (Kaden and Lange 2001; Kaden et al. 2008). The primary drawback of live vaccines is that it is impossible to differentiate vaccine antibodies from field virus-induced antibodies.

E2 recombinant protein subunit vaccines are commercially available and provide the means to differentiate infected from vaccinated animals (DIVA). The efficacy of two E2 marker vaccines has been evaluated in vaccination-challenge and transmission trials, but with variable results. A single dose of vaccine prevented clinical signs and mortality due to a CSFV challenge 3 weeks after vaccination (Bouma et al. 1999), but at least 14 days were needed to obtain clinical protection (Bouma et al. 2000; Uttenthal et al. 2001). If challenged earlier, no protection against clinical disease and no reduction of virus shedding were observed (Uttenthal et al. 2001).

Evaluation of the E2 marker vaccines showed that transplacental transmission of CSFV occurred even when two doses of vaccine were administered and challenge occurred 14 days after the second dose. The two-dose vaccination protocol protected pregnant gilts from clinical disease, but did not prevent horizontal or vertical spread of the CSFV (Dewulf et al. 2001b).

Thus, transplacental infection would not be prevented in most vaccinated animals under the conditions of emergency vaccination. Vaccination could not prevent the “carrier sow syndrome” and, subsequently, the late-onset form of CSF (Depner et al. 2001).

Currently, the development CSF vaccine has focused on five strategies, mainly based on genetically engineered constructs: immunogenic CSFV peptides, DNA vaccines, viral vectors expressing CSFV proteins, chimeric pestiviruses, and trans-complemented deleted CSFV genomes (replicons) (Beer et al. 2007).

**Classical Swine Fever in Wild Boars.** Vaccination in domestic pigs was prohibited in European Union member states in 1990, but the virus is periodically reintroduced into domestic pigs via contact with wild boars. In some areas of Germany, an increase in the number of wild boars has led to endemic CSFV within these populations (Fritzemeier et al. 2000; Moennig 2000). CSFV infection is seemingly harmless for wild boars, as little mortality has been reported in recent outbreaks. In the presence of natural or man-made barriers, CSFV in wild boar populations can be confined to a defined area until it is eliminated (Pol et al. 2008).

Classic measures for CSFV control in wild boars involved reduced hunting to allow the virus to spread within the susceptible population and induce mortality or immunity, followed by targeted hunting of the most susceptible animals, that is, juveniles and the young sows. In some areas where it has been difficult to eradicate CSFV using standard methods, oral vaccination using live C strain distributed in oral bait has been attempted (Kaden et al. 2000). The safety of this vaccine has been demonstrated for other wild animal species (Chenut et al. 1999).

In pigs, the oral vaccine can induce strong protective immunity in 10 days (Kaden and Lange 2001). However,
recent analysis of the data from oral vaccination field campaigns has shown that three doses were required to induce immunity in the field. Due to poor access to baits, young boars did not acquire sufficient protective immunity in most field studies (Kaden et al. 2002). Vaccination seems more effective if it is used on a preventative basis and delivered at least 1 year before the virus enters the area, giving time for a high level of population immunity to be achieved. Routine vaccination around an outbreak has recently been proposed to prevent the spread of disease (Rossi et al. 2010).

When vaccine is used, the only method to differentiate between infected and vaccinated boars is detection of the virus by DIVA qRT-PCR (Leifer et al. 2009). There is a need for an oral DIVA vaccine that would permit serological monitoring of a vaccinated population for the spread of wild-type virus. Subunit E2 marker vaccines are not indicated for oral vaccination and require more than one parenteral application to be effective. A chimeric pestivirus is currently the most promising live vaccine candidate for oral application. It was shown to be safe and to induce an effective protective immunity (Reimann et al. 2004).

**BUNGOWANNAH VIRUS**

**Relevance**

In June 2003, a severe disease outbreak characterized by an increase in stillbirths, preweaning mortality, and mummified fetuses occurred on two properties in southern New South Wales, Australia. Because the cause was unknown at the time of the outbreak, the term “porcine myocarditis syndrome” (PMC) was used to describe the disease (McOrist et al. 2004). Subsequently, a novel pestivirus, named Bungowannah virus, was identified as the cause of PMC (Finlaison et al. 2009, 2010; Kirkland et al. 2007).

Bungowannah virus has only been recognized on two farms in Australia and its origin remains obscure. Bungowannah virus is highly pathogenic for the porcine fetus, a feature consistent with its distant genetic relatedness to CSFV. Clinically, fetal infection with Bungowannah virus could be confused with a low virulence strain of CSFV. It is of concern that this virus is not detected by diagnostic assays that were considered to react with all members of the pestivirus genus. Until a suite of diagnostic assays becomes available and global pig populations are surveyed, the full significance of this new pestivirus will remain unknown.

**Etiology**

Bungowannah virus is genetically distinct from CSFV, but molecular characterization supports its inclusion in the genus *Pestivirus*. Phylogenetic analysis and limited antigenic cross-reactivity indicate this is the most divergent pestivirus identified to date (Kirkland et al. 2007). Bungowannah virus is noncytopathic in cell culture and replicates to the highest titers in strains of the continuous porcine kidney cell line, PK15 (Kirkland et al. 2007).

**Epidemiology**

Bungowannah virus has only been identified in two related piggeries in New South Wales, Australia. The source of the virus is not known. The virus was eradicated from one site and efforts to eradicate the virus at the second site commenced in 2010. Other piggeries connected to the affected farms were free of infection and there is no evidence of infection on other farms in Australia. Serological surveillance in Germany has not identified antibodies to this virus. While the PMC syndrome is readily detected in a fully susceptible population, it is possible that it could remain unrecognized in countries where CSFV is present or pigs are affected with similar clinical entities.

Under field conditions, disease caused by Bungowannah virus has only been reported in pigs (McOrist et al. 2004). Experimentally, nonpregnant sheep and cattle have also been infected. It is not known whether this virus could infect the ruminant fetus.

Due to the large amount of virus shed into the environment by persistently infected pigs, postnatal transmission presumably occurs following oronasal exposure. Intranasal exposure of pigs under experimental conditions readily established infection. The risk of transmission by embryos or semen has not yet been evaluated.

If an animal is first infected during pregnancy, there is a high risk of transplacental transmission and fetal infection. During experimental studies fetal infection occurred in the litters of 87% of sows that became infected.

Following the experimental infection of 5- to 6-week-old pigs by the intranasal route, viral shedding was detected by qRT-PCR from 3–10 days postchallenge in oropharyngeal and nasal secretions. The duration of shedding and amount of virus shed in feces and conjunctival secretions was markedly lower. Limited experimental studies suggested that transmission is poor from transiently infected animals.

Experimental inoculation of pregnant pigs suggested that the epidemiology of Bungowannah virus was similar to in utero infections with BVDV, BDV, and low virulence CSFV (D. Finlaison, unpublished data). High quantities of Bungowannah virus RNA were detected in placenta and fluids (fetal and/or vaginal) at farrowing, irrespective of the stage of gestation at which infection occurred. Thus, fetal fluids and placenta are an important source of environmental contamination.

All infected piglets shed high quantities of Bungowannah virus RNA at birth, regardless of whether the dam was challenged before or after the approximate age of immunocompetence in the fetal pig (70 days). The duration of postnatal virus shedding was
inversely related to the stage of gestation at which infection occurred. Thus, fetuses infected in late gestation had the shortest period of shedding (1–2 weeks of age). Most pigs from sows infected at 55 or 75 days gestation had Bungowannah virus-specific antibody at birth.

Animals persistently infected with Bungowannah virus have not been identified in the field, but they were observed during experimental studies. These pigs shed high quantities of Bungowannah virus in oropharyngeal secretions, urine, and feces, and infection was readily transmitted to naïve pigs.

Specific studies on the persistence of Bungowannah virus in the environment and its susceptibility to disinfectants have not been carried out, but its characteristics are probably similar to that of CSFV and BVDV. Successful eradication from the affected farm described above was carried out with a combination of measures including depopulation, disinfection (detergent/Virkon® (Antec International, London, UK)/sodium hypochlorite), and site biosecurity measures.

Pathogenesis
The primary route of viral entry is presumed to be oronasal, with primary replication in the tonsils, like CSFV. Although this site of replication is yet to be confirmed, virus has been detected in oropharyngeal secretions at the earliest sampling time of 3 days postinoculation and in high quantities in tonsil tissue.

Unlike CSFV, there appears to be no appreciable clinical effects following postnatal infection with Bungowannah virus, regardless of the dose of virus (Finlaison et al. 2012). Thus, disease appears to be wholly the result of in utero infection of the fetus with the clinical outcome and lesions dependent on the stage of gestation at which infection occurs. Direct intrauterine transmission appears likely and, during experimental studies, most litters were completely infected by 20 days postinfection (Finlaison et al. 2010).

Litters were most severely affected when sows were infected at approximately 35 days of gestation, with approximately 40% of fetuses either stillborn or mummified and 70% of the piglets born alive dying before 3 weeks of age. Of the small group of animals that survived to weaning, over 80% were persistently infected.

Infection of the sow at approximately 55, 75 or 90 days gestation resulted in stillbirth percentages of 10–15% with the number of mummified piglets close to normal production targets. Preweaning losses were also elevated (29%) in piglets from sows infected at 90 days of pregnancy. Persistently infected animals were immunotolerant and most failed to mount a humoral immune response. The pig fetus appears to be first able to mount a humoral immune response to Bungowannah virus between 57 and 73 days gestation (Finlaison et al. 2010).

Clinical Signs
To date, there has been only one known disease outbreak due to Bungowannah virus in pigs (McOrist et al. 2004). The most significant effects of Bungowannah virus infection are the consequence of in utero infection. Sudden death in healthy 2- to 3-week-old pigs triggered the initial disease investigation, but the nature of the losses altered within 2–3 weeks of the commencement of the outbreak. Sudden death became less common and increased numbers of stillborn pigs and preweaning mortalities in the early postnatal period were noted. Piglets born alive from affected litters showed greatly reduced viability. Evidence from the field outbreak suggested that litter size remained relatively unaffected. At the peak of the outbreak, preweaning mortality reached 50%, 40% of fetuses were stillborn, and 13% were mummified in some production units. In a combined herd consisting of approximately 30,000 sows, it is estimated that 50,000 pigs were lost from production due to PMC over a 15-month period (Finlaison et al. 2009).

Pigs persistently infected with Bungowannah virus were not identified during the outbreak of PMC, but have subsequently been produced following experimental infection of pregnant sows up to 56 days gestation. Those resulting from sow challenge at around 35 days of gestation experienced poor viability and high mortality in the early postnatal period. The few piglets that appeared clinically normal soon after birth developed a syndrome similar to “late-onset CSF” in the weeks following weaning, becoming severely stunted with high morbidity and mortality compared to unaffected cohorts of the same age. A small proportion of pigs born following infection of the sow at around 55 days appeared to be persistently infected and exhibited stunting compared with age-matched cohorts, but had better long-term survival. Some of these animals seroconverted and appeared to clear the infection, cease viral shedding, and grow better after this occurred. The term “chronically infected” may therefore be more appropriate for these than “persistently infected” pigs, as the latter exhibit life-long virus excretion.

Postnatal infection with Bungowannah virus is associated with few clinical effects. An incubation period of 3–5 days prior to the detection of viremia has been observed following experimental intranasal infection of weaner-aged pigs. While mild transient temperature rises were recorded, no other evidence of disease was noted (Finlaison et al 2012). Likewise, on the piggeries affected by PMC, dams of the affected piglets were clinically normal with normal feed intake and body temperature (McOrist et al. 2004). Once the virus becomes endemic in a population, if animals are infected prior to first breeding, clinical disease may not be recognized despite ongoing transmission.
Lesions
Field cases of PMC were associated with increased stillbirths (both recent and longstanding with autolysis), fetal mummification, and preweaning mortalities (McOrist et al. 2004). A range of gross lesions were observed, including subcutaneous edema spread particularly over the head and thorax of some stillborn piglets, a dilated heart, irregular areas of myocardial pallor, increased pericardial, thoracic, and abdominal fluid, and occasional fibrin tags on the thoracic and abdominal viscera. The placentas appeared normal. Up to 50% of stillborn pigs may have elevated immunoglobulin G (IgG) levels. During experimental studies, some piglets born to sows infected with Bungowannah virus at 35 days of pregnancy exhibited purpura that appeared to resolve over approximately 7 days, an absence of facial whiskers, and white focal lesions in the cerebrum. Gross lesions were not observed in weaner pigs at necropsy 6 weeks postinoculation following experimental infection.

Histopathological findings in affected piglets consistently included acute to subacute multifocal, nonsuppurative myocarditis with myonecrosis occasionally observed. Inflammation was frequently mild and localized and myofiber destruction minimal. A minority of cases in older neonatal piglets had early myocardial fibrosis. Occasional affected piglets had nonsuppurative interstitial pneumonia, encephalitis, hepatitis, and lymphadenitis (McOrist et al. 2004).

Diagnosis
While Bungowannah virus has to date only been identified in Australia, its origin remains unknown and it should be included within the differential diagnoses of viral pathogens causing reproductive disease in the pig. In addition, the clinical presentation has similarities to in utero infection with CSFV, BVDV, and BDV and with “late-onset” CSF. For laboratory diagnosis, samples should be collected for qRT-PCR, histopathology, and serology.

Rapid identification of infected fetuses is best achieved with detection of Bungowannah virus RNA by qRT-PCR (Finlaison et al. 2009). Viral RNA can be readily detected in fetal fluids, pharyngeal swabs, and tissues of infected fetuses. The virus can be isolated from a number of tissues, including lung and lymphoid tissues, although it is not routinely used for diagnostic purposes because qRT-PCR has higher sensitivity. Additionally, the virus is not cytopathic in cell culture so immunoperoxidase staining is required to detect virus replication in cell cultures (Kirkland et al. 2007).

Serology by peroxidase-linked immunoassay (PLA) or VN is best utilized for surveillance and monitoring purposes. For diagnostic applications, PLA may be performed on poor quality samples that may not be suitable for testing by VN. This is of particular use for fetal specimens. Approximately 50% of stillborn piglets will have elevated IgG levels and specific antibodies can be detected in the serum and fluids from body cavities of stillborn piglets. Pericardial and thoracic fluids are usually of better quality than peritoneal fluid.

Immunity
To date, only humoral immune responses to Bungowannah virus have been studied. During experimental studies, antibody was detected from 10 days postinoculation in weaner pigs challenged intranasally (Finlaison et al 2012) and from 17 days in fetuses infected by direct inoculation (Finlaison et al. 2010). Postnatally, the production of specific antibody is associated with clearing of the viremia and cessation of viral shedding. In contrast, although the pig fetus is immunocompetent and able to produce detectable antibodies to Bungowannah virus from around 70 days of gestation (Finlaison et al. 2010), the humoral immune response of the fetal piglet was not sufficient to clear the infection prior to birth. Following suckling, these animals developed high antibody titers that were maintained for at least 2–3 months. It is not known how long this naturally acquired antibody persists postnatally. Maternally derived antibody waned by approximately 2 months of age in persistently infected piglets and uninfected piglets from infected dams.

Prevention and Control
There is no specific treatment for PMC or vaccine against Bungowannah virus. As the origin of the virus remains unknown, only general biosecurity measures can be adopted to prevent introduction of the virus into a population. If the virus is detected early in an outbreak, measures can be taken to attempt to reduce the impact of the disease. Evidence of poor postnatal transmission suggests that the most significant spread will occur following the farrowing of affected litters or by persistently infected piglets. In the face of an outbreak, it will be important to maximize exposure of breeding animals prior to mating to reduce the duration of the outbreak and to prevent possible exposure of pregnant animals.

Bungowannah virus eradication from an endemically infected piggery has been achieved through a combination of measures including depopulation, cleaning, disinfection, site biosecurity, and repopulation with previously unexposed animals.

BOVINE VIRAL DIARRHEA AND BORDER DISEASE VIRUSES

Relevance
Pigs are susceptible to pestiviruses other than CSFV and Bungowannah virus. Cross-species transmission among artiodactyls has been reported for strains of both BVDV and BDV (Carbrey et al. 1976; Terpstra and Wensvoort 1988).
Infection with either BVDV or BDV can complicate CSFV control or eradication programs. Cross-neutralization tests and tests using monoclonal antibodies (Leforban et al. 1990a; Wensvoort 1989) suggest that BVDV may have been isolated from pigs in the past and misidentified as CSFV by tests based on polyclonal antibodies. Likewise, serum antibodies against BVDV or BDV have the potential to cross-react in assays for the detection of CSFV antibodies. Because of cross-reactions among pestiviruses, it is essential to identify the specific etiological agent when pestivirus antibodies are detected in CSF eradication programs.

Natural infection of swine with BVDV was first reported in Australia in 1964, but BVDV was not isolated from a naturally infected pig until 1973 (Fernelius et al. 1973). The teratogenic properties of pestiviruses are well established (Terpstra and Wensvoort 1988; Vannier et al. 1988; Wensvoort and Terpstra 1988) and infection of pregnant sows with BVDV or BDV may induce a pathology resembling congenital CSF.

Although natural infection of pigs with BVDV or BDV is relatively uncommon, interspecies transmission may occur in countries where pigs are raised in close contact with ruminants. Disease is most likely to occur when pregnant sows are infected, but may only affect a small number of litters until piglets are born and further transmission occurs. If the source of virus is a contaminated vaccine, the initial impact is likely to be much greater.

**Etiology**

BVDV and BDV are typical pestivirus species and cannot be differentiated morphologically or structurally from CSFV (Laude 1979). However, they can be readily differentiated using monoclonal antibodies and molecular assays targeting the viral genome.

**Epidemiology**

The prevalence of BVDV antibodies in the pig populations of CSFV-free countries (Australia, Ireland, Great Britain, Denmark) have been estimated at 1.6–43.5%, depending on the age of the animals and possibly on the degree of contact with cattle (Jensen 1985). In countries where CSFV is present, the situation with regard to BVDV antibodies seems to be about the same.

Cattle are the most common source of BVDV infection in pigs. In units with dairy farming, one potential source of infection is BVDV-contaminated whey or milk fed to sows (Terpstra and Wensvoort 1988). In some cases, pigs have had contact with cattle recently vaccinated with BVDV (Stewart et al. 1971). In other reports, pigs and cattle were kept in separate lots and buildings, but personnel and equipment moved freely between the different farm units (Carbrey et al. 1976).

The prolonged presence of a persistently infected litter of pigs is the most likely source of BVDV or BDV to susceptible, pregnant sows (Terpstra and Wensvoort 1988; Vannier et al. 1988). Persistent BDV infection of piglets occurs when sows are infected during early pregnancy, that is, fetuses are transplacentally infected and piglets become immunotolerant and persistently infected (Vannier et al. 1988). The course of the infection is quite similar to that described for BVD infection in pregnant cows (Baker 1987). When a pregnant sow is infected under experimental conditions, the litter may consist of a mixture of virus-positive and antibody-positive piglets. This suggests variability in the time at which individual fetuses became infected (Edwards et al. 1995). Congenitally BDV-infected piglets appear to excrete large amounts of virus, since susceptible young animals kept in contact rapidly seroconvert and produce high antibody titers. Conversely, when piglets are infected after birth, spread of infection to in-contact animals does not occur; suggesting low or perhaps no excretion of virus (Vannier et al. 1988).

Pigs may also become infected through the use of modified live virus vaccines (CSF or Aujeszky’s disease) or other biologicals contaminated with virus (Vannier et al. 1988; Wensvoort and Terpstra 1988). While contaminants could be of either bovine or ovine origin, bovine are far more likely to be involved as most pestiviruses found in bovine serum products are strains of BVDV.

**Pathogenesis**

BVDV and BDV are pathogenic for fetal pigs, but relatively nonpathogenic for pigs after birth, apart from a slight increase in body temperature and a slight leukopenia and/or thrombocytopenia in some pigs (Makoschey et al. 2002). The ability of BVDV and BDV to establish intrauterine infections in swine is well established (Stewart et al. 1980; Vannier et al. 1988; Wrathall et al. 1978). The degree of clinical disease depends upon the stage of gestation at which the infection occurred. Clinical signs are more severe if sows are infected during the first trimester of pregnancy. The most severe clinical signs and lesions in fetuses or piglets are observed when sows are infected 25–41 days postbreeding (Leforban et al. 1990b; Mengeling 1988). Under experimental conditions, piglets infected in utero with BVDV or BDV became persistently infected and were immunotolerant. After the disappearance of maternal antibodies, no active humoral response was detected in the majority of piglets. Furthermore, the virus was isolated from piglets and was shed by some, as evidenced by infection in young animals placed in contact.

In some experimental infections of pregnant sows with BDV, the onset of the clinical signs in the piglets was delayed until 13–14 days after birth. The reason for the delayed response is unknown, but colostral antibodies ingested by piglets would presumably block the replication of the virus and/or delay disease in transplacentally infected piglets (Leforban et al. 1990b; Mengeling 1988; Vannier et al. 1988).
The pathogenicity of BVDV or BDV seems to depend on the strain used in the experiment. BVDV seems to be more consistently pathogenic for fetuses, whereas variable results are obtained with BVDV viral strains. The Singer strain, adapted to replicate in porcine cells and BVDV strain 87/6, can infect and cause mortality in porcine fetuses, whereas the NADL strain does not induce clinical disease in piglets (Edwards et al. 1995; Leforban et al. 1990b; Mengeling 1988). Dahle et al. (1993) intranasally inoculated weaned pigs with BVDV strain Osloss/2482, then 4 weeks later challenged with decreasing doses of CSFV. After the CSFV challenge, the only clinical sign observed was fever in one animal, although most animals became viremic.

Clinical Signs
In the field, infection of pigs with BVDV usually occurs without clinical signs. In some cases, however, natural infection of pig herds with pestiviruses other than CSF has been associated with breeding problems, for example poor conception rates, small litters, and a few abortions. Hyperthermia and colic spasms have also been described (Carbrey et al. 1976). In The Netherlands and France, signs compatible with congenital CSF infection were described in piglets born to sows vaccinated 4 months earlier with CSF or Aujeszky’s disease (psororabies) vaccines contaminated with a ruminant pestivirus (Vannier et al. 1988; Wensvoort and Terpstra 1988). Clinical signs in piglets included anemia, rough hair coats, growth retardation, wasting, congenital tremors, conjunctivitis, diarrhea, polyarthritis, petechiae in the skin, and blue ear tips (Terpstra and Wensvoort 1988).

Natural infection of sows with BDV has been reported to result in reproductive signs, for example, repeat breeding and mummified and stillborn pigs at farrowing (Vannier et al. 1988). A high proportion of piglets from infected sows showed eyelid edema, locomotor disorders, and occasionally, diarrhea and arthritis. The mortality rate in affected litters at 2 days of age ranged from 30% to 70%.

Experimental inoculation of pregnant sows with BDV field strains at 30–32 days of gestation produced transplacental infection of fetuses and newborn piglets with low body weights and short body lengths (Wrathall et al. 1978). Leforban et al. (1990b) reported an increase in perinatal mortality and eyelid edema, hyperthermia, and anemia in survivors during the second week of life. Slow growth rates, respiratory signs, and diarrhea developed in pigs; some of which died by 2 months of age. Pigs without respiratory and enteric signs survived and had normal growth despite marked snout deformations, including prognathism in one individual. BDV was isolated from blood and organs of all dead piglets, but not from survivors. When 40-day-old specific pathogen-free (SPF) pigs were placed in contact with BDV transplacentally infected piglets, they did not show clinical signs, but developed high levels of antibody to BDV that was able to completely protect them against challenge with a virulent strain of CSFV.

Lesions
When infected postnatally with BVDV or BDV, no or very mild lesions are observed in pigs. Hyperemia of the small intestine was seen in one pig 11 days after being placed in contact with calves infected with the NADL strain of BVDV (Stewart et al. 1971). A transient leucopenia was detected during the first week following experimental infection of pigs with a pig isolate of BVDV (Carbrey et al. 1976). In utero infection of fetuses by transplacental transmission is followed by consistent pathological disorders in fetuses or piglets. In 13 naturally occurring BVDV outbreaks in The Netherlands, chronic gastroenteritis and septicemia with hemorrhages in lymph nodes, epicardium, and kidneys were the most consistent lesions reported. Inflammation of the digestive tract was frequently characterized by catarrh, hypertrophy, or ulceration of the mucosa. Necrotic tonsillitis, icterus, polyserositis, polyarthritis, and atrophy of the thymus were also noted (Terpstra 1987). A porcine BVDV isolate administered to gilts at 42–46 days of gestation produced significant microscopic lesions in the leptomeninges and the choroid plexus of the fetus characterized by collections of lymphocytes, histiocytes and cellular accumulation in the vascular adventitia and perivascular spaces (Stewart et al. 1980).

In the case of BDV, experimental inoculation of sows on day 34 of gestation produced cerebellar hypoplasia in 9 of 19 live-born piglets, with a small meningocele in one of the nine (Wrathall et al. 1978). The French BDV isolate Aveyron (Chappuis et al. 1984) inoculated into sows at day 30 of gestation produced lesions in lymphoid tissues in some piglets. Marked hemorrhages in lymph nodes and other lymphoid tissues were found in stillborn fetuses or in piglets that died shortly after birth. Histological examination of lymph nodes, spleen, and tonsil revealed marked subacute inflammatory lesions characterized by accumulations of lymphocytes, plasmacytes, and eosinophilic polymorphonuclear leukocytes, numerous secondary follicles, increased populations of reticulocytes, and lymphoid hypoplasia with pyknosis and karyorrhexis. Thymus, liver, and nervous tissues were normal (Leforban et al. 1990b).

Diagnosis
BVDV or BDV may be isolated using the same methods described for CSFV and from the same tissues submitted for CSFV diagnosis, that is, tonsils, spleen, kidney, and whole blood collected in heparin or EDTA. However, if BVDV or BDV is isolated from pigs, these viruses grow better and to a higher titer in cells of
ruminant origin, and of the homologous species, rather than in porcine cells (Wensvoort et al. 1989). In CSF-free countries, BVDV and BDV must be considered in the differential diagnosis of CSFV and all CSF suspect cases should be tested for BVDV and BDV.

Pestiviruses have some shared antigens and serological tests for the detection of antibodies against CSFV may cross-react with antibodies to ruminant pestiviruses. The practical importance of this is that the presence of ruminant pestivirus antibodies in pig sera often causes false-positive reactions in serological surveys for CSFV. This presents problems in CSFV eradication campaigns and in epidemiological surveys for CSFV (Jensen 1985).

Immunity
There is little practical interest in establishing protection by vaccination in pigs against BVDV or BDV. Consequently, there has been little done to study the immune response of pigs to these viruses. Nevertheless, the characteristics of the immune response are considered similar to that induced in pigs by CSFV.

Prevention and Control
To prevent BVDV or BDV infection in pigs, it is necessary to avoid direct or indirect contact with cattle or sheep. Natural infection with BVDV often occurs when pigs are fed with cow’s milk or bovine offal and these practices should be avoided.

Inadvertent transmission of these viruses is a risk when live virus vaccines are used because of contamination of media and/or cells used in production of the vaccine. Cells used for multiplication of master seed virus used to prepare vaccine can be contaminated by BVDV or BDV. Indeed, some batches of CSF and Aujeszky’s disease vaccines were contaminated by a pestivirus (probably BDV) because secondary lamb kidney cells were used to propagate the vaccine strain virus (Vannier et al. 1988; Wensvoort and Terpstra 1988). Both bovine and nonbovine cell lines can be contaminated with pestiviruses and all cell cultures need to be monitored carefully for their presence. The primary source of contamination of cells is usually bovine serum added to the nutrient medium. Fetal infection with BVDV is extremely common. As commercial batches of fetal bovine serum usually involve pooling of serum from many calves or fetuses, the likelihood of BVDV contamination is high (Rossi et al. 1980). Hobi virus, one of the novel pestiviruses, has also been detected in some batches of fetal bovine serum (Stahl et al. 2007) and may also present a possible risk, although nothing is known about its capacity to infect the pig. Therefore, to avoid interspecies transfer of these viruses, the systematic testing and treatment of bovine serum and of biological products used for the preparation of vaccines is strongly recommended.

REFERENCES
Hepatitis E Virus
Xiang-Jin Meng, Patrick G. Halbur, and Tanja Opriessnig

RELEVANCE
Hepatitis E virus (HEV), the causative agent of human hepatitis E, is an important public health problem in many developing countries in Asia and Africa. Hepatitis E is also endemic in industrialized countries. As a fecal–orally transmitted disease, contaminated water or water supplies are major sources of HEV infections in humans. The mortality rate associated with HEV infection in humans is generally low (<1%), but can be up to 25% during pregnancy. Meng et al. (1997) isolated and characterized the first animal strain of HEV, swine hepatitis E virus (swine HEV), from piglets in the United States. Although swine HEV only causes microscopic lesions of hepatitis in pigs with no sign of clinical disease (Halbur et al. 2001), it poses a zoonotic risk to humans via direct contact with infected pigs (Meng et al. 2002; Withers et al. 2002) or through consumption of undercooked pork (Feagins et al. 2007, 2008; Yazaki et al. 2003).

ETIOLOGY
HEV is classified in the family Hepeviridae (Meng 2010a,b). At least four major genotypes of HEV have been recognized in mammalian species: genotypes 1 and 2 HEV are restricted to humans, whereas genotypes 3 and 4 HEV are zoonotic. Thus far, all swine HEV strains identified from pigs worldwide belong to either genotype 3 or 4. HEV is a spherical, nonenveloped, virus particle of approximately 32–34 nm in diameter. Swine HEV cannot be efficiently cultivated in cell culture.

The genome of swine HEV is a polyadenylated, single-stranded, positive-sense RNA molecule of approximately 7.2 kilobases (Meng et al. 1998). The genome consists of three open reading frames (ORFs), a short 5’ noncoding region (NCR), and a short 3’ NCR. ORF1 encodes nonstructural proteins, ORF2 encodes an immunogenic capsid protein, and ORF3 encodes a small multifunctional protein. The ORF2 and ORF3 are translated from a single bicistronic mRNA, and overlap each other but neither overlaps ORF1 (Huang et al. 2007).

ROLE IN PUBLIC HEALTH
Hepatitis E is a zoonotic disease for which pigs are a reservoir (Meng 2010b). Concerns for zoonotic infections include occupational exposures, food safety, and transmission of swine HEV from pig xenografts to human recipients.

Genotypes 3 and 4 strains of swine HEV infected both rhesus monkeys and a chimpanzee. Conversely, the genotypes 3 and 4 strains of human HEV infected pigs (Meng 2010a,b; Meng et al. 1998). Pig caretakers and swine veterinarians in both developing and industrialized countries are at an increased risk of acquiring HEV infection. For example, swine veterinarians in the United States were 1.51 times more likely to be positive for anti-HEV antibodies than other blood donors (Meng et al. 2002). Withers et al. (2002) reported that swine workers in North Carolina (a major swine-producing state) had a 4.5-fold higher anti-HEV antibody prevalence rate (10.9%) than control subjects (2.4%).

Infected pigs excreted large amounts of HEV in feces, thus posing a concern for environmental safety. HEV-containing pig manure and feces could contaminate irrigation or coastal water with concomitant contamination of produce or shellfish. HEV strains of swine origin have been detected in sewage water (Pina et al. 2000).
Sporadic cases of acute hepatitis E have been linked to the consumption of contaminated raw and undercooked pig liver (Yazaki et al. 2003). Approximately 2% of the pig liver sold in grocery stores in Japan and 11% in the United States tested positive for swine HEV RNA. Furthermore, the contaminating virus present in pig liver from the grocery stores in the United States was fully infectious. These data provided compelling evidence for zoonotic HEV transmission via direct contact with infected pigs or via consumption of contaminated pig meats (Meng 2010b).

**EPIDEMIOLOGY**

Swine HEV infection is ubiquitous in pigs worldwide, regardless of whether HEV is endemic in the respective human population. Besides domestic pigs, swine HEV also infects wild boars. Under experimental conditions, rhesus monkeys and chimpanzees are susceptible to infection by both genotypes 3 and 4 swine HEV (Meng et al. 1998).

HEV seroprevalence in pigs is age dependent: most pigs younger than 2 months of age are seronegative, whereas the majority of pigs older than 3 months of age are seropositive (Meng et al. 1997). The infection generally occurs at 2–3 months of age, shortly after maternally derived antibodies wane. Infected pigs generally have a transit viremia lasting for 1–2 weeks, and shed viruses in feces for 3–7 weeks (Meng et al. 1997; Takahashi et al. 2003).

Transmission of swine HEV among pigs is presumably fecal–oral, with feces from infected pigs probably the primary source of virus. It is believed that pigs acquire infection through direct contact with infected pigs or through ingestion of feces-contaminated feed or water. However, experimental reproduction of swine HEV infection in pigs via the oral route of inoculation proved to be difficult (Kasorndorkbua et al. 2003). Therefore, other routes of transmission cannot be ruled out.

The in vivo infectivity of swine HEV present in commercial pig livers is completely inactivated by adequate cooking, for example, frying or boiling for 5 minutes. However, incubation of the contaminated pig liver homogenates at 56°C (133°F) for 1 hour did not abolish virus infectivity (Feagins et al. 2008). HEV is thought to resist inactivation by acidic and mild alkaline conditions in the intestinal tract.

**PATHOGENESIS**

The pathogenesis of swine HEV is largely unknown. It is assumed that HEV replicates in the gastrointestinal tract after ingestion and subsequently spreads to its target organ, the liver. Virus replication in the liver has been demonstrated (Meng et al. 1998). Extrahepatic sites of swine HEV replication have also been identified in various tissues of pigs, including small intestines, colon, and hepatic and mesenteric lymph nodes (Williams et al. 2001).

**CLINICAL SIGNS**

Pigs naturally and experimentally infected with swine HEV are asymptomatic. The incubation period, from the time of infection to virus shedding in feces, ranged from 1 to 4 weeks (Halbur et al. 2001). The percentage of HEV-infected pigs within a herd is very high (up to 80–100% in some herds); however, the morbidity and mortality attributable to swine HEV infection is not known.

**LESIONS**

Specific pathogen-free (SPF) pigs experimentally infected with swine HEV remained clinically normal, but mild-to-moderate enlargement of hepatic and mesenteric lymph nodes was observed from 7 to 55 days postinoculation (DPI) (Halbur et al. 2001). Microscopic lesions characterized by mild-to-moderate multifocal lymphoplasmacytic hepatitis and focal hepatocellular necrosis are commonly observed in infected pigs (Figure 39.1). Hepatic inflammation and hepatocellular necrosis peaked in severity at 20 DPI (Halbur et al. 2001). Pregnant gilts infected with swine HEV had mild multifocal lymphohistiocytic hepatitis, and individual hepatocellular necrosis was observed in some gilts. Evidence of HEV-associated lesions in the reproductive tract or fetuses was lacking (Kasorndorkbua et al. 2003).

**DIAGNOSIS**

Swine HEV is difficult to work with because it does not grow in cell culture or cause clinical disease in pigs. Currently, the diagnosis of swine HEV infection is based on polymerase chain reaction (PCR) and enzyme-linked immunosorbent assay (ELISA). However, the diagnostic sensitivity and specificity of these assays is largely unknown. The results of an ELISA based on swine HEV capsid antigen correlated well with those obtained with a genotype 1 human HEV capsid antigen (Meng et al. 2002). Specific RT-PCR and real-time PCR assays have also been developed (Meng 2010a).

**IMMUNITY**

The capsid protein of swine HEV is immunogenic and induces protective immunity. The capsid protein of swine HEV shares common antigenic epitopes with human and avian HEVs. All HEV strains identified thus far, including swine HEV, appear to belong to a single serotype. Cross-challenge experiments in primates have demonstrated cross-protection following infection with different genotypes of human HEV strains.
SECTION III  
VIRAL DISEASES

Piglets born to seropositive sows had maternal antibodies lasting 7–9 weeks (Meng et al. 1997). It is believed that the maternal antibodies confer protective immunity to the piglets against swine HEV infection.

PREVENTION AND CONTROL

The major concern for swine HEV is its zoonotic potential. A vaccine against HEV is not yet available, but when a vaccine becomes available it may be advantageous to vaccinate pigs in order to minimize potential zoonotic transmission and eliminate food safety concerns. Adequate personal and public hygiene can minimize HEV transmission.

REFERENCES


39.1. Histological liver lesions in pigs experimentally infected with swine HEV or human HEV. (A) Liver of a pig experimentally infected with the swine HEV, showing mild focal infiltration of lymphocytes, plasma cells, and macrophages and mild diffuse inflammation in hepatic sinusoids at 14 days postinoculation (DPI). Hepatocytes are mildly swollen and vacuolated. (B) Liver of a pig experimentally infected with the US-2 strain of human HEV, showing moderate lymphoplasmacytic and histiocytic hepatitis and severe vacuolar degeneration and swelling of hepatocytes at 14DPI. Hematoxylin and eosin stain (from Halbur et al. 2001, by permission of the American Society for Microbiology).
Influenza Virus
Kristien Van Reeth, Ian H. Brown, and Christopher W. Olsen

RELEVANCE
The first reports of influenza-like disease in pigs occurred in both the United States and Europe in conjunction with the 1918 human influenza pandemic. Contemporary genetic analyses have confirmed that the early H1N1 swine viruses, which are progenitors of the “classical” H1N1 lineage of swine influenza viruses (SIVs), and the human viruses of 1918 were closely related, with both derived from a wholly avian ancestral virus (reviewed in Taubenberger and Palese 2006).

Influenza viruses are one of the major causes of acute respiratory disease outbreaks in pigs (Loeffen et al. 1999; Terebuh et al. 2010), but infections are also frequently subclinical. The epidemiology of influenza in pigs encompasses a complex interplay of viruses of human, avian, and swine evolutionary origins. Conversely, pigs are postulated to play important roles as intermediate hosts in the reassortment and/or adaptation events leading to the development of influenza viruses of pandemic potential for people (Subbarao et al. 2006; Webster et al. 1992).

Genetic reassortment among human, avian, and/or swine viruses in pigs has been documented repeatedly over the past two decades. These reassortant viruses have fundamentally altered the epidemiology of influenza in pigs in many parts of the world. Further, the 1957 and 1968 human influenza pandemic viruses arose through reassortment (Kawaoka et al. 1989; reviewed in Van Reeth 2007), although it cannot be proven that such reassortment occurred in pigs. Most recently, the 2009 pandemic H1N1 (pH1N1) virus arose as the result of reassortment between SIVs spanning North American and Eurasian lineages (Garten et al. 2009; Smith et al. 2009), though once again it cannot be proven whether the actual reassortment event occurred in pigs, people, or some other influenza virus host.

ETIOLOGY
Influenza viruses are members of the virus family Orthomyxoviridae. They are polymorphic, enveloped viruses ∼80–120 nm in diameter (Figure 40.1). The viral lipid envelope renders the viruses highly susceptible to detergents and most commonly used antiviral disinfectants. Influenza viruses encode 10 or 11 viral proteins on eight separate segments of negative-sense RNA. The segmented nature of the influenza genome allows two viruses that coinfect a single host to exchange RNA segments during viral replication, a process known as genetic “reassortment.”

Influenza viruses are classified by type, subtype, and genotype. Classification by type refers to common core proteins, the matrix (M) and nucleoprotein (NP), with types A, B, and C recognized. Only influenza A viruses are of routine clinical significance as swine pathogens. Influenza A virus subtypes are defined by the nature of the hemagglutinin (HA or H) and neuraminidase (NA or N) spike-like glycoproteins that project from the surface of the viral envelope. There are 16 different forms of HA and 9 different forms of NA that can be distinguished antigenically and genetically. The combination of HA and NA forms in a virus defines its subtype, for example, H1N1, H3N2.

Influenza A virus isolates are named using the following convention: A/species of origin/location of isolation/isolate number/year of isolation, for example, A/swine/Wisconsin/125/98. If no species is designated, it is, by default, a human isolate. A detailed review of
and geographic region of the world, from which each
defines the evolutionary lineages, based on host species
phylogenetic analysis. The output of these analyses
viral RNA segment and then subjecting the sequences
ducted by determining the genetic sequence of each
ing (H, N) but also genotyping. Genotyping has been an
epidemiology and evolution requires not just subtyp-
epidemiology and evolution requires not just subtyp-
epidemiology and evolution requires not just subtyp-
owns to birds, and, to a more limited extent, birds to pigs.

Zoonotic infections with SIVs have been reported
worldwide. Serological studies for SIV anti-
nosic SIV infections, as well as interspecies trans-
mation of influenza viruses from humans to pigs, pigs
to birds, and, to a more limited extent, birds to pigs.

ROLE IN PUBLIC HEALTH

Martin Kaplan at the World Health Organization pro-
epidemiology of influenza in humans at the time of the
Asian influenza pandemic in 1957 and initiated a cross-
disciplinary network of investigators (Kaplan 1969,
80) as an early example of a “one-health” approach
to influenza. Dr. Bernard Easterday first conclusively
demonstrated transmission of swine influenza (SI) to
farm personnel in 1976 (Easterday 1980; Pawlisch et al.
1976). Many more data now support the occurrence of
zoonotic SIV infections, as well as interspecies trans-
mation of influenza viruses from humans to pigs, pigs
to birds, and, to a more limited extent, birds to pigs.

Zoonotic infections with SIVs have been reported
worldwide, including classical H1N1 swine
viruses, as well as avian lineage viruses and reassortant
viruses of multiple subtypes and genotypes (as reviewed
in Myers et al. 2007; Newman et al. 2008; Sancho et al.
2009; Shinde et al. 2009; Van Reeth 2007). The number
of infections formally documented in these reports is
small compared to the number of people in contact
with pigs worldwide. Serological studies for SIV anti-
nodies (Abs) in humans suggest more widespread, but
undiagnosed, SIV infections (Gray et al. 2007; Myers et
al. 2006, 2007; Olsen et al. 2002; Terebuh et al. 2010).
However, the interpretation of serological studies is
limited by the difficulty of accurately differentiating
swine versus human influenza virus infection. Histori-
cally, with the exception of the SI outbreak at Fort Dix,
New Jersey, in 1976 (Top and Russell 1977), evidence
for person-to-person spread of swine lineage viruses is
limited (Myers et al. 2007). The pandemic of 2009–
2010 presents a uniquely different circumstance in this
regard.

Pandemics require the emergence of an influenza
virus with two properties: (1) the virus must be suffi-
ciently antigenically unique to be able to evade host
immunity in the human population and (2) it must
become sufficiently adapted to infection of humans to
be able to spread from person to person. Such viruses
can develop through in toto transmission from an
animal host, for example, emergence of a wholly avian
virus to create the 1918 pandemic strain (Taubenberger
and Palese 2006) or they appear through genetic reas-
sortment between a virus with an immunologically
unique HA and another virus(es), ideally, one already

structure and genetics may be found

elsewhere (Wright et al. 2007).

The HA viral glycoprotein mediates attachment to
sialic acid-containing receptors on the host cell and is
the principal target for the induction of neutralizing
antibodies (Abs). At the molecular level, sialic acids are
bound to galactose residues by α2,3 or α2,6 linkages.
In binding tests in the laboratory, human influenza
viruses prefer to bind to 2,6-linked (“human-type”)
receptors, whereas avian viruses prefer 2,3-linked
(“avian-type”) receptors. It was once believed that
humans were not susceptible to avian influenza viruses
(AIVs) because they do not express avian-type receptors
in their respiratory tract, but this is an oversimplifica-
Sialic acid binding is also responsible for the agglutina-
tion of red blood cells and this property is used in some
diagnostic applications (hemagglutination and hemag-
glutination inhibition [HI] assays—see below). The NA
viral glycoprotein enables virus release from the infected
cell by breaking the bonds between sialic acids and
adjacent sugar residues.

Increasingly, an understanding of influenza A virus
epidemiology and evolution requires not just subtyp-
ing (H, N) but also genotyping. Genotyping is con-
ducted by determining the genetic sequence of each
viral RNA segment and then subjecting the sequences
to phylogenetic analysis. The output of these analyses
defines the evolutionary lineages, based on host species
and geographic region of the world, from which each

viral gene originated. A phylogenetic lineage refers to
a group of viruses that share a common genetic origin
for a particular gene. It is possible for a virus to com-
prise genes from distinct lineages reflecting different
origins for individual genes. Genotyping has been an
extremely important tool in recent years for under-
standing the origins and continued evolution of influ-
enza viruses isolated from pigs across the globe.

40.1. Structure of an influenza virus. The hemagglutinin (HA),
neuraminidase (NA), and part of the matrix2 (M2) protein are on
the outer surface of the virion and protrude through the envelope.
The viral RNA segments are coated with nucleoprotein (NP) and
bound by a complex of three polymerases (PB2, PB1, PA). The eight
segments are arranged according to their length, which puts the HA
gene at place 4 and the NA gene at place 6. NS1 is the single
nonstructural protein and is not part of the viral particle (from
Wright and Webster 2001 by permission of the publisher).
adapted to human infection. The “Asian flu” virus of the 1957 pandemic and the “Hong Kong” virus of the 1968 pandemic both arose via the latter mechanism, with reassortment between a novel avian virus (providing new HA, PB1 [and NA in 1957] genes) and the previously circulating seasonal human influenza virus (Kawaoka et al. 1989; Webby and Webster 2001; Webster et al. 1992).

Influenza viruses are substantially restricted by host range factors that limit interspecies infections (Landolt and Olsen 2007; Neumann and Kawaoka 2006), in particular between birds and mammalian hosts. Thus, only a small subset of virus subtypes has ever become endemically established in mammals, for example, H1, H2, and H3 in humans; H1 and H3 in pigs; and H3 and H8 in horses. In contrast, influenza viruses of all HA and NA subtypes have been found in wild waterfowl, the global reservoir hosts of influenza viruses (Hinshaw et al. 1980; Webby and Webster 2001; Webster et al. 1992). Depending on the strain of virus and species of bird, influenza viruses in waterfowl can infect and be shed from the respiratory and/or the enteric tract (Webster et al. 1978). Enteric infection leads to virus excretion in feces and contamination of lake water for weeks to months (Hinshaw et al. 1980; Staliknecht et al. 1990). This feature can play a role in AIV outbreaks (Rohani et al. 2009) and possibly in avian-to-swine transmission (Karasin et al. 2000b, 2004).

Evidence exists for contributions from all eight influenza genomic segments to host range, that is, “multigenic” species specificity (Landolt and Olsen 2007; Neumann and Kawaoka 2006), but the HA plays a dominant part because of its role in receptor binding and the presumed differences in sialic acid receptors between different hosts (Ito 2000; Landolt and Olsen 2007; Matrosovich et al. 2000; Nicholls et al. 2008).

The long-standing question in influenza virology has been, “In what species can reassortment between avian and mammalian viruses occur to produce a novel pandemic strain?” “Pig” was presumed to be the correct answer because swine tissues express both avian-like 2,3-linked and human-like 2,6-linked sialic acid receptors (Ito et al. 1998; Nelli et al. 2010; Van Poucke et al. 2010). Indeed, pigs are susceptible to infection with influenza viruses of both avian and human origins and, therefore, were denoted the “mixing vessel” host for genetic reassortment (Brown 2000; Ito et al. 1998; Karasin et al. 2000b, 2004; Kida et al. 1994; Pensaat et al. 1981; Scholtissek et al. 1998; Van Reeth 2007; Webster et al. 1992). However, the species specificity of sialic acids is far more complex than once believed (Bateman et al. 2010; Gambaryan et al. 2005; Nicholls et al. 2008; van Riel et al. 2010). For instance, virus binding to a given sialic acid may not lead to productive infection of cells (Bateman et al. 2008, 2010); secondary receptors may be required (Chu and Whittaker 2004). Furthermore, humans and some land-based poultry species also express both types of sialic acids (Gambaryan et al. 2002; Landolt and Olsen 2007; Wan and Perez 2006; Webby and Webster 2001).

Nonetheless, pigs remain the focus, in particular because of the genetic origins of the 2009 pH1N1 virus. Genetic analyses clearly demonstrated that the 2009 pH1N1 virus originated evolutionarily from a unique “intercontinental” reassortment between swine-like influenza viruses of North American and Eurasian lineages (Garten et al. 2009; Smith et al. 2009). It must be stressed, however, that one cannot a priori assume that this reassortment event occurred in pigs or that the virus circulated in pigs in the world during the 10–17 years since its presumed reassortant origin (Smith et al. 2009; Figure 40.2).

**EPIDEMIOLOGY**

**Susceptible Species**

Beyond domestic pigs and humans, SIVs have also been shown to infect wild boar (Saliki et al. 1998), domestic turkeys (Choi et al. 2004; Hinshaw et al. 1983; Ludwig et al. 1994; Olsen et al. 2006; Suarez et al. 2002; Tang et al. 2005; Wood et al. 1997; Wright et al. 1992), and, on rare instances, free-ranging waterfowl (Olsen et al. 2003; Ramakrishnan et al. 2010).

Pigs can experimentally be infected with a wide range of subtypes of AIVs (Kida et al. 1994) and naturally acquired infections of pigs with AIVs have also been documented throughout the world. As an example, H1N1, H3N2, H3N3 and H4N6 viruses have been isolated from pigs in Canada or Asia (Brown 2000; Guan et al. 1996; Karasin et al. 2000b, 2004; Kida et al. 1988), and H5N1 and H9N2 viruses from pigs in Asia (Choi et al. 2005; Peiris et al. 2001; Shi et al. 2008; Takano et al. 2009). In general, these avian-to-swine transmissions have been epidemiologically dead-end events without maintenance in the swine population. This is consistent with experimental studies showing that AIVs are restricted in their replication in pigs and transmission between pigs (Choi et al. 2005; De Vleeschauwer et al. 2009a,b; Lee et al. 2009; Lipatov et al. 2008; Manzoor et al. 2009). Thus, AIVs must likely mutate or reassort with swine-adapted viruses to replicate efficiently in pigs, and reassortants with avian and swine virus genes have become established in swine. The European avian-like swine H1N1 virus is, in fact, the single example of a wholly avian virus that became swine-adapted (Brown et al. 1997; Donatelli et al. 1991; Kyriakis et al. 2011; Scholtissek et al. 1983; Van Reeth et al. 2008).

Human influenza viruses have also occasionally been isolated from pigs. In particular, human H3N2 viruses have frequently been recovered from pigs in Asia (see below) and occasionally from pigs in Europe and North America (Brown 2000; Hinshaw et al. 1978; Karasin et al. 2000c). As with AIVs, efficient
transmission of human viruses among pigs seemingly requires adaptation to the new host species. Thus, the H3N2 viruses that have been maintained in swine populations in North America and Europe are reassortants with a mix of human and swine-adapted genes.

Antigenic drift in the HA is usually slower in swine than in human beings. As an example, the current European swine H3N2 viruses are still antigenically related to the human viruses from the 1970s and 1980s, while the current human viruses have drifted away
from their precursors (de Jong et al. 1999, 2007; Kyriakis et al. 2011). Interestingly, human and swine viruses seem to show a similar rate of genetic evolution in their HA, but swine viruses apparently mutate in regions that are less important for the immune response (de Jong et al. 2007; Nerome et al. 1995). However, comprehensive genetic and antigenic analyses of various SIV lineages and comparisons with human lineages are needed to fully understand drift in SIVs and its significance for the immune response.

Transmission
Historically, outbreaks of SI in northern climates occurred most frequently during the late fall through early winter months, often in association with the onset of colder temperatures and cold autumn rains. However, studies have demonstrated that SIVs circulate year round (Hinshaw et al. 1978; Kyriakis et al. 2011; Olsen et al. 2000; Van Reeth and Pensaert 1994) and, as swine production has moved to confinement systems, disease seasonality has become less prominent.

The exact modes of transmission of SIV and its population dynamics have been defined only to a limited degree. Influenza viruses are most commonly introduced into herds through the movement of animals. The primary route of virus transmission is thought to be pig-to-pig contact via nasopharyngeal exposure, with virus titers in nasal secretions of ≥1 × 10^7 infectious particles/mL at the peak of shedding (De Vleeschauwer et al. 2009a,b; Landolt et al. 2003; Larsen et al. 2000; Van Reeth et al. 2003a). Aerosol transmission has been demonstrated for human influenza viruses and equine influenza virus transmission appears to have occurred over distances of several kilometers between premises in Australia (reviewed in Tellier 2009). Aerogenic transmission may thus contribute to the spread of SIVs in areas densely populated with large herds of pigs and will likely account for SIV infections on farms with high biosecurity standards. In most cases, the virus will disappear from specialized finishing herds, especially those with all-in/all-out systems of management, to be reintroduced at a later time. On farrow-to-finish farms, where young susceptible piglets with declining maternal immunity may be continuously available, the virus seems to be able to persist within the population in some cases (Loeffen et al. 2009).

Geographic Distribution
SIVs are present in pigs throughout large parts of the world, and probably wherever pigs are raised. However, the distribution of various subtypes and genotypes varies widely in different geographic regions.

Swine Influenza in North America. Classical H1N1 lineage viruses were the dominant cause of influenza among pigs in North America from their first isolation by Shope in 1930 through the 1990s (Chambers et al. 1991; Hinshaw et al. 1978; Olsen et al. 2000). The classical H1N1 SIVs in the United States remained antigenically and genetically highly conserved from 1965 through the 1980s (Luoh et al. 1992; Noble et al. 1993; Sheerar et al. 1989), but antigenic and genetic variants were isolated during the 1990s (Olsen et al. 1993, 2000; Rekik et al. 1994).

In 1998, the epidemiology of influenza among pigs in North America changed dramatically with the isolation of two genotypes of reassortant H3N2 viruses: (1) a double reassortant containing genes from classical swine and seasonal human influenza viruses (Zhou et al. 1999), which did not persist in the population and (2) “triple reassortant” (tr) H3N2 viruses containing HA, NA, and PB1 genes of human influenza virus phylogenetic lineage; M, NP, and NS genes of classical swine H1N1 virus lineage; and PA and PB2 genes of North American avian virus lineage (Karasin et al. 2000c; Zhou et al. 1999). The latter viruses have spread widely throughout the North American swine population and evolved over time into four distinct phylogenetic clades (I–IV) (Olsen et al. 2006; Richt et al. 2003; Webby et al. 2000, 2004).

Cocirculation and subsequent reassortments of the trH3N2 and the existing classical swine H1N1 viruses led to the emergence and spread of trH1N2 (Choi et al. 2002a,b; Karasin et al. 2000a, 2002) and trH1N1 (Subbarao et al. 2006) viruses in which the H1 (trH1N2) or H1 and N1 (trH1N1) genes were of classical swine H1N1 virus lineage and the remaining genes were of the classical swine/human/avian tr genotype. This swine/human/avian tripartite mix of M, NP, NS, PA, PB1, and PB2 genes is now referred to as the “TRIG” (triple reassortant internal gene) gene cassette (Vincent et al. 2008). More recently, an additional genotype of trH1 virus has emerged and spread within the swine population in the United States. These viruses contain the TRIG cassette, but the H1 and/or N1 or N2 genes are derived from seasonal human influenza viruses (Vincent et al. 2009b).

For clarity, the H1 HA genes among swine viruses in the United States are now designated in four phylogenetic clades: α (the classical H1N1 wholly swine viruses); β and γ (trH1N1 and H1N2 viruses with genetic drift variants of the classical swine H1 HA genes); and δ (trH1N1 and H1N2 viruses with seasonal human H1 HA genes, two subclades) (Vincent et al. 2009b,c).

Additional TRIG-based reassortants have been recovered during limited outbreaks from pigs in the United States and Canada. These include trH2N3 and H3N1 viruses, as well as double reassortant H1N2 and H1N1 viruses (Karasin et al. 2006; Ma et al. 2006, 2007), but none of these viruses have become established in swine populations.
Swine Influenza in Europe. In Europe, the predominant H1N1 SIVs have an entirely avian genome introduced from wild ducks to pigs in 1979 (Pensaert et al. 1981; Scholtissek et al. 1983). These avian-like H1N1 viruses have established a stable lineage and have been endemic on the European mainland since 1979. They are antigenically and genetically distinct from the classical swine H1N1 viruses, which no longer exist in Europe (Brown et al. 1997; Donatelli et al. 1991).

The European swine H3N2 viruses were derived from descendants of the 1968 “Hong Kong” pandemic human virus, but they have evolved further through reassortment with the avian-like H1N1 virus. This has resulted in H3N2 viruses with human-like HA and NA glycoproteins and avian-like internal proteins (Campitelli et al. 1997; Castrucci et al. 1993; de Jong et al. 1999; Haesebrouck et al. 1985).

Since the mid-1990s, H1N2 viruses have also become established in European pigs. The dominant H1N2 viruses retained the genotype of these reassortant H3N2 viruses, but they have acquired a 1980s-era human lineage H1 gene (Brown et al. 1998; Marozin et al. 2002; Schrader and Süss 2003; Van Reeth et al. 2004).

Thus, the three major subtypes share common internal genes, but they have clearly distinguishable HAs. All three subtypes are endemic in regions of Europe with high swine density and unvaccinated sows frequently have Abs to two or all three subtypes (Van Reeth et al. 2008). However, regional variation exists and H3N2 viruses appear to be absent in some areas (Kyriakis et al. 2011). Novel H1 and H3 reassortants with different HA/NA gene combinations are found sporadically, but have not persisted in swine populations (Bálint et al. 2009; Brown et al. 1994; Marozin et al. 2002; Moreno et al. 2009).

Swine Influenza in Asia. The epidemiology of SIVs in Asia is more complex than that in Europe or North America. H3N2 viruses have repeatedly been transmitted from people to pigs in Asia since the 1970s, and variants of the Hong Kong/68 pandemic virus cocirculate in pigs with more contemporary human H3N2 viruses (Kida et al. 1988; Nerome et al. 1995; Peiris et al. 2001; Sun et al. 2009; Yu et al. 2007, 2008b). Human H1N1 viruses appear to be less widespread in Asian swine and have not been reported beyond China (Lu et al. 2010; Sun et al. 2009; Yu et al. 2007, 2009b).

While there has been little, if any, spread of SIVs from North America to Europe or vice versa, viruses from both continents have been introduced in Asia. In South China, for example, there is cocirculation of classical swine H1N1 viruses, which were dominant for a long time, European avian-like H1N1 viruses, and North American H1N2 viruses (Guan et al. 1996; Lu et al. 2010; Qi et al. 2009; Vijaykrishna et al. 2010; Yu et al. 2009a). Various H3N2 reassortant viruses occur throughout Asia and some resemble European or North American virus lineages (Takemae et al. 2008; Yu et al. 2008b). Finally, other SIVs have emerged locally and are clearly unique for Asia (Nerome et al. 1995; Shu et al. 1994; Sun et al. 2009). The concurrent circulation of so many genetically diverse SIVs has resulted in multiple, very complex, reassortant viruses (Lu et al. 2010; Vijaykrishna et al. 2010; Xu et al. 2011; Yu et al. 2008b, 2009a).

To further complicate matters, the situation varies markedly within Asia, although there are no data for some regions. In Japan, for example, H1N2 viruses with an H1 from classical swine viruses and an N2 from early human H3N2 viruses have been a major lineage from the 1980s (Ouchi et al. 1996; Saito et al. 2008; Sugimura et al. 1980). These H1N2 viruses seem to be absent from other parts of Asia. Conversely, trH1N2 viruses, which are common in China and Korea, have not been detected in Japan (Jung and Song 2007; Vijaykrishna et al. 2010).

In the last decade, avian H5N1, H5N2, and especially H9N2 influenza viruses crossed the species barrier to pigs on multiple occasions (Cong et al. 2008; Lee et al. 2009; Peiris et al. 2001; Yu et al. 2008a). This is not surprising, because these viruses are endemic in certain Asian poultry populations. In some instances, these viruses have spread between pigs on a limited scale, but have not (so far) formed a stable lineage in swine, as have viruses of H1N1, H3N2, or H1N2 subtypes (Choi et al. 2005; Jung et al. 2007; Nidom et al. 2010; Santhia et al. 2009). A variety of reassortants between the latter swine-adapted viruses and the avian viruses have been isolated from pigs in recent years, a few with an avian H5 or H9 (Bi et al. 2010; Cong et al. 2007, 2010; Lee et al. 2009; Shi et al. 2008). It is unknown whether such viruses will become endemic in swine.

2009 Pandemic H1N1 Virus Infection in Pigs. Following the emergence of the 2009 pH1N1 among humans worldwide, the same virus was isolated from pigs in multiple areas of the world, often without remarkable clinical signs (e.g., Forgie et al. 2011; Hofshagen et al. 2009; Howden et al. 2009; Pereda et al. 2010; Vijaykrishna et al. 2010; Welsh et al. 2010). Infections in pigs were apparently initially due to transmission from infected humans (amphixenotic spread), but subsequently through pig-to-pig spread. Despite the fact that the 2009 pH1N1 virus was termed “swine flu,” there is no evidence to suggest that infection in pigs epidemiologically contributed to widespread human transmission. H1N1 viruses from swine in Thailand in 2000 and 2003 represent the only sequenced examples, prior to the pH1N1 virus, of reassortants combining the HA segment from classical H1N1 swine and the NA segment from the Eurasian H1N1 swine lineage, but their composition was still different from that of the pandemic virus (Kingsford et al. 2009; Takemae et al. 2008). A reassortant virus possessing only the HA gene...
of avian-like swine H1N1 virus into the background of the pandemic virus has been detected in Hong Kong in 2010 (Vijaykrishna et al. 2010).

PATHOGENESIS

The pathogenesis of influenza in pigs is well characterized and very similar to that in humans (De Vleeschauwer et al. 2009a; Khatri et al. 2010; Van Reeth et al. 1998). Virus replication is limited to epithelial cells of the upper and lower respiratory tract of pigs (nasal mucosa, ethmoid, trachea, and lungs) and virus excretion and transmission occur exclusively via the respiratory route. Infectious virus can thus be isolated from these tissues, as well as tonsils and lymph nodes in the respiratory tract, bronchoalveolar lavage (BAL) fluid, and nasal, tonsil, or oropharyngeal swabs (Brown et al. 1993; De Vleeschauwer et al. 2009a,b; Heinen et al. 2001b; Khatri et al. 2010; Landolt et al. 2003; Richt et al. 2003; Vincent et al. 2009a). In most experimental studies, the virus can be isolated from day 1 postinoculation onwards and becomes undetectable after 7 days. SIV clearly has a preference for the lower versus the upper respiratory tract (De Vleeschauwer et al. 2009a; Khatri et al. 2010). This is shown by virus titration and by immunohistochemical studies, which reveal massive numbers of viral antigen-positive cells in bronchial, bronchiolar, and alveolar epithelia, compared to fewer positive cells in the nasal mucosa (Figure 40.3). Viral nucleic acid or antigen have also been found in alveolar macrophages, but there is no proof for a productive infection of these cells (Brookes et al. 2010; Jung et al. 2002; Weingartl et al. 2010).

SIV is unlikely to spread beyond the respiratory tract. The brain stem is the single extra-respiratory tissue from which low amounts of virus are occasionally isolated (De Vleeschauwer et al. 2009a). Only one study describes a low titered and transient viremia (Brown et al. 1993). In a few studies, feces, intestines, or spleen occasionally tested positive by polymerase chain reaction (PCR), but virus-positive cells have never been demonstrated outside the respiratory tract (Brookes et al. 2010; De Vleeschauwer et al. 2009a). Recent pig infection studies with the 2009 pH1N1 virus have confirmed the absence of the virus in pork and muscle tissue (Brookes et al. 2010; Vincent et al. 2009a).

Infection with SIV is easily reproduced by experimental inoculation of influenza naive pigs via intranasal (IN), aerosol, or intratracheal (IT) exposure, but the kinetics of virus replication in the respiratory tract, and the severity of lung inflammation and disease, are markedly dependent upon the inoculation route and dose.

IT inoculation produces the characteristic infiltration of the lungs with neutrophils and the typical lower respiratory tract disease (with high lung virus titers, which may exceed $1 \times 10^8$ infectious particles per gram of tissue), plus high fever ($\geq 41^\circ C$, $\geq 106^\circ F$) and lethargy (De Vleeschauwer et al. 2009a; Van Reeth et al. 1998, 2002).

Less intensive methods (IN inoculation, or IT inoculation of a lower virus dose) produce a slower increase of the viral load in the lungs, milder lung inflammation, and less specific signs, that is, mainly nasal discharge, sneezing, a low-to-moderate fever, or subclinical infection (Brown et al. 1993; Larsen et al. 2000; Richt et al. 2003).

Cytokines produced by the host during the acute stage of infection appear to determine the difference between subclinical infection and disease. Titers of interferon-α and -γ, tumor necrosis factor-α, and the interleukins-1, -6, and -12 in BAL fluids are considerably higher after IT than after IN inoculation. Experimental studies support the concept that a heavy viral load in the lungs is required to induce high levels of these cytokines, which in turn induce the typical lung inflammation and disease (Barbé et al. 2011; Kim et al. 2009; Van Reeth et al. 1998, 2002). Any factors likely to reduce the extent of virus replication in pigs in the field, for example, partial active or passive immunity or sanitary measures to reduce infection pressure, are thus likely to reduce the severity of clinical signs. Many cytokines, however, also have antiviral and immunostimulating effects, and thus may contribute to clearance of influenza viruses.

Experimental infection studies have not yielded convincing evidence for differences in the pathogenesis or virulence between SIV lineages or strains. The
occasional differences reported seem to be due to biological variation between pigs, experimental variation, or differences in replication competency between viruses (Landolt et al. 2003; Richt et al. 2003; Sreta et al. 2009; Vincent et al. 2006, 2009b). Pig infection studies with avian or human influenza viruses typically result in a mild or subclinical infection, consistent with low-to-moderate virus titers in the porcine respiratory tract (Choi et al. 2005; De Vleeschauwer et al. 2009a; Landolt et al. 2003; Lipatov et al. 2008). The 2009 pH1N1 virus was shown to have a similar pathogenetic course in pigs as the SIVs that are endemic in swine populations (Brookes et al. 2010; Lange et al. 2009; Vincent et al. 2009a; Weingartl et al. 2010).

**CLINICAL SIGNS**

Typical swine flu outbreaks are characterized by high fever (40.5–41.5°C, 105–106.7°F), anorexia, inactivity, huddling, reluctance to rise, tachypnea, and, after a few days, coughing. Labored abdominal breathing and dyspnea are most typical. The onset of the disease is sudden, after an incubation period of 1–3 days. Morbidity is high (up to 100%), but mortality is low (usually less than 1%) in uncomplicated infections. Generally, rapid recovery begins 5–7 days after onset. Acute outbreaks of clinically typical SI are generally limited to fully susceptible, seronegative pigs, whether immunologically unprotected nursery pigs or older pigs (Loeffen et al. 1999).

Infections with H1N1, H1N2, and H3N2 subtype viruses are clinically similar and viruses of all subtypes and lineages have been associated with acute respiratory episodes (Karasin et al. 2000a,b,c; Loeffen et al. 1999; Zhou et al. 1999). Experimental studies have failed to demonstrate convincing differences in virulence among SIV strains (see above).

Several other factors beyond immune status may also determine the clinical outcome of SIV infection, including age, infection pressure, climatic conditions, housing conditions, and concurrent infections. It is well established that secondary infections with bacteria, such as *Actinobacillus pleuropneumoniae*, *Pasteurella multocida*, *Mycoplasma hyopneumoniae*, *Haemophilus parasuis*, and *Streptococcus suis* type 2, may enhance the severity and course of infections with SIVs.

Other respiratory viruses, such as porcine respiratory coronavirus (PRCV) and porcine reproductive and respiratory syndrome virus (PRRSV) frequently infect pigs around the same age as SIV (Van Reeth and Pensaert 1994). Among these pathogens, PRRSV, *M. hyopneumoniae*, and SIV are most frequently detected in 10- to 22-week-old pigs with clinical signs of the “porcine respiratory disease complex (PRDC)” (Thacker et al. 2001). Several experimental dual infection studies with SIV in combination with *M. hyopneumoniae*, PRCV, or PRRSV showed more severe disease in dual infections as compared to single virus-infected pigs (Brockmeier et al. 2002). However, it is difficult to reliably reproduce disease with combinations of any two infectious agents and other studies failed to show enhancement of disease. Thus, the pathogenesis of the PRDC remains elusive.

Subsequent to an influenza outbreak in a herd, producers and veterinarians occasionally report reduced reproductive performance, for example, increased infertility, abortion, small weak litters, and stillbirths. However, little data exist to suggest that influenza viruses infect the reproductive tract of pigs or directly induce reproductive disease.

**LESIONS**

The gross lesions found in uncomplicated SI are mainly those of a viral pneumonia. Abnormalities are most often limited to the apical and cardiac lobes of the lung. The percent of lung tissue with grossly visible consolidation varies greatly within and between experimental infection studies, but more than 50% of the lung may be affected by 4–5 days postinoculation (Khatri et al. 2010; Landolt et al. 2003; Richt et al. 2003). Generally, there is a sharp line of demarcation between the affected and normal lung tissue and the involved areas will be purple and firm. Some interlobular edema may be evident, airways may be filled with blood-tinged, fibrinous exudates, and the associated bronchial and mediastinal lymph nodes are usually enlarged. In naturally occurring SI, these lesions may be complicated or masked by intercurrent, especially bacterial, infections.

Microscopically, the hallmarks of SI include necrosis of lung epithelia, desquamation/denudation of the bronchial epithelial cell layer, and airway plugging with necrotic epithelial and inflammatory cells, mainly neutrophils (Haeboul and Pensaert 1986; Haeboul et al. 1985) (Figure 40.4). Neutrophils may account for up to 50% of the cell population in BAL fluids collected 24 hours after IT inoculation, while macrophages are the dominant cells in uninfected, healthy pigs (Barbé et al. 2011; Khatri et al. 2010; Van Reeth et al. 1998). The neutrophils not only cause obstruction of the airways, but also contribute to lung damage by release of their enzymes. After a few days, peribronchial and perivascular infiltration of lymphocytes occurs (Landolt et al. 2003; Richt et al. 2003). Similar pathological lesions have been observed in clinically typical SIV outbreaks in the field (Loeffen et al. 1999). Like clinical signs, however, lung lesions can also be mild or unremarkable.

**IMMUNITY**

The adaptive immune response to SIV infection includes both the production of Abs and cell-mediated immu-
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Khatri et al. 2010; Larsen et al. 2000). HI Abs in serum can be detected by 7–10 days postinoculation and peak by 2–3 weeks postinoculation (Heinen et al. 2000; Larsen et al. 2000). Ab titers remain high for several weeks before beginning to decline by 8–10 weeks postinoculation. (Van Reeth et al. 2004, 2006). Pig sera also contain Abs to the NA and NP (Heinen et al. 2000, 2001a; Van Reeth et al. 2003a), moderate Ab levels to M1, and low-to-variable Ab levels to the external part of M2 (Kitikoon et al. 2008). Whole virus Abs and NP-specific Abs have also been found in nasal and lung lavage fluids (Heinen et al. 2000; Khatri et al. 2010; Kitikoon et al. 2006, 2009; Larsen et al. 2000; Vincent et al. 2008).

As would be expected, immunoglobulin M (IgM), and later, immunoglobulin G (IgG), were the dominant isotypes in serum, whereas immunoglobulin A (IgA) was the main isotype in nasal washes (Heinen et al. 2000; Larsen et al. 2000). Larsen et al. (2000) demonstrated Ab-secreting cells in nasal mucosal tissue, which proved that Abs are locally produced in the respiratory tract of pigs. Abs at the lung level are, at least in part, transudated from serum, as suggested by the dominance of virus-specific IgG in BAL samples of pigs (Heinen et al. 2000). However, substantial IgA levels have also been found in lung lavage fluids of SIV-infected pigs (Kitikoon et al. 2006; Larsen et al. 2000) and local Ab production in the lung parenchyma cannot be excluded.

After a primary infection with SIV, there is solid protection against reinfection with the same or a similar virus strain (De Vleeschauwer et al. 2011; Larsen et al. 2000; Van Reeth et al. 2003a). Based on findings in humans, it is assumed that this immunity may last for several years, but its exact duration has not been studied in the pig. HA-specific VN Abs are likely the primary mediators of this “homologous” immunity. Protection studies to prove the role of other immune mechanisms in the pig are still lacking.

Because of the concurrent circulation of different subtypes and lineages of influenza viruses, pigs may be exposed to multiple, antigenically different SIVs during their lifetime. There is no serological cross-reaction between H1 and H3 subtype SIVs in the HI test, nor in some cases between viruses within H1 and H3 lineages. Still, several experimental infection studies have shown cross-protection between influenza viruses in the absence of cross-reactive serum HI Abs. Pigs that had been infected first with a classical swine H1N1 virus (α clade) were protected against challenge with a trH1N2 virus (γ clade) 5 weeks later (Vincent et al. 2008).

Prior infection with European avian-like H1N1 SIV also protected against subsequent infection with a North American trH1N1 SIV (De Vleeschauwer et al. 2011), as well as against the 2009 pH1N1 virus (Busquets et al. 2010). More limited cross-protection was observed between any two European SIV subtypes.
(Heinen et al. 2001a; Van Reeth et al. 2003a, 2004). For example, pigs that were first infected with H1N1 still shed H1N2 challenge virus in nasal swabs, but for a 1- to 2-day shorter duration than influenza naive control pigs. However, the extent of cross-protection against H1N2 was dramatically enhanced in pigs that had been infected with both H1N1 and H3N2.

Despite the absence of cross-reactive serum HI Abs between the viruses used in all these studies, VN and/or neuraminidase inhibiting (NI) Abs, as well as CMI responses, were cross-reactive in some instances. The relative contribution of these immune mechanisms to the “broad” protection needs further study. In addition, apparent cross-protection may be less pronounced in the field.

The immune response after intramuscular (IM) administration of killed SIV vaccine is fundamentally different from that after infection with live virus. Vaccination immunity relies largely on the induction of high titer of serum HI and VN Abs to the HA of the vaccine strain(s), which can reach the lungs by diffusion. In contrast, mucosal Abs or CD8+ T cells are not efficiently induced by killed virus vaccination.

Maternally derived serum IgG Abs to SIV can protect young pigs against antigenically related viruses, but they will also interfere with the development of an active Ab response to vaccination or infection. Maternal SIV Ab levels in newborn pigs are dependent on Ab levels in the dam, which decline in the pig over a period of about 4–14 weeks (Loeffen et al. 2003). In experimental studies, none of the pigs with maternal Abs were completely protected from nasal virus shedding upon challenge, but some pigs showed complete clinical protection (Loeffen et al. 2003; Renshaw 1975).

**DIAGNOSIS**

A clinical diagnosis of SI is only presumptive because there are no pathognomonic signs and SI must be differentiated from a variety of respiratory diseases of swine. A definite diagnosis is only possible by isolation of virus, detection of viral proteins or nucleic acid, or demonstration of virus-specific Abs. Diagnostic techniques for SIV are described in detail elsewhere (Swenson et al. 2008).

Detection of virus, its nucleic acid, or viral proteins provides definitive evidence for the presence of the etiological agent. The sensitivity of these approaches will be dependent on the specialized reagents used within the assay and their “degree of match” to circulating field strains. SIV isolations from live animals are typically undertaken on samples of mucus obtained by swabbing the nasal passages or, in the case of very small pigs, the oropharynx. The virus is most likely to be found in nasal and pharyngeal secretions during the febrile period of illness. Samples should be collected on polyester (e.g., Dacron®, Invista, Wichita, KS), not cotton, swabs (Figure 40.5). Swabs should be suspended in a suitable transport medium, such as cell culture medium or phosphate-buffered saline at neutral pH, and kept cold. If the samples for virus isolation can be tested within 48 hours after collection, they should be maintained at refrigerator temperature (4°C, 39°F). If the samples must be held longer, storage at −70°C (−94°F) is recommended because SIVs are not stable at −20°C (−4°F). Virus may also be isolated/detected from tracheal or lung tissues of pigs that die or are euthanized during the acute stage of the disease. The tissue material should be held under the same conditions as swab material until ready for culturing.

The virus can be cultivated in embryonated fowl's eggs, generally by allantoic cavity inoculation of 10- to 11-day-old embryonated eggs and subsequent incubation at 35–37°C (95–98.6°F) for 72 hours. SIVs do not typically kill the embryo, but the virus can be detected in allantoic fluid using the HA assay (generally using chicken or turkey erythrocytes), which provides presumptive evidence for the presence of an influenza virus. Up to two blind passages may be required to confirm the absence of virus. An alternative to the use of embryonated eggs for SIV isolation is cell culture. Cell lines of several different origins support the growth of influenza viruses (Swenson et al. 2008), with Madin–Darby canine kidney (MDCK) cells being most frequently used.

The HA and NA subtypes of influenza viruses have historically been determined using conventional techniques, that is, HI and NI tests (Swenson et al. 2008). These methods subtype viruses using Abs that are specific for the different HA and NA subtypes. Increasingly, however, molecular-based methods are being used to detect and subtype influenza viruses utilizing tools such as PCR, gene sequencing, and microarray...
(Heil et al. 2010) that are based upon detection of gene signatures unique to different subtypes. These approaches are in their infancy with respect to widespread, commercial diagnostic application for SIV and may have limitations for use in the detection of a highly variable virus population, even within the same HA subtype. However, such approaches have been used successfully for the detection of the predominant subtypes in pigs.

Reverse transcription-polymerase chain reaction (RT-PCR) assays (traditional and real-time technologies) also provide highly sensitive and specific detection of viral nucleic acid extracted from clinical sample preparations. Well-validated methods are at least equivalent to virus isolation (Landolt et al. 2005), with greater speed, reduced cost, and scalability as inherent advantages. It should, however, be noted that due to increased sensitivity in these assays, “weak” positive samples may contain degraded rather than infectious virus.

RT-PCR assays can be classified into two broad types. The first type allows generic detection of any influenza A virus and is applicable to all SIVs, but does not provide information on the virus subtype. These assays usually offer high levels of sensitivity and specificity and are most suitable for initial screening of clinical samples. The second are subtype-specific assays designed to detect specific HA subtypes and often able to further discriminate strains within the same HA subtype, that is, classical, avian-like swine, or 2009 pH1N1 viruses. These are usually of slightly lower sensitivity and less useful for primary screening of samples from clinical cases than the pan-influenza A assays. The occurrence of 2009 pH1N1 virus in pigs has required that any method be demonstrably applicable for both endemic SIVs and this virus (Hiromoto et al. 2010; Lorusso et al. 2010; Slomka et al. 2010).

Other methods for detecting virus or viral antigen can be applied to fresh, nonautolysed tissues of the respiratory tract including lung and trachea. These include direct or indirect immunofluorescence techniques (Onno et al. 1990) and immunohistochemical detection in fixed tissue (Swenson et al. 2001; Vincent et al. 1997). In addition, nasal swabs can be tested even without specialist laboratory facilities using commercially available enzyme immunoassay membrane tests. Although easy to perform, this approach generally lacks the analytical sensitivity necessary to reliably detect SIV at the levels shed from the nasal cavity (Ryan-Poirier et al. 1992). These methods detect influenza A antigens, but do not differentiate between virus subtypes.

Serological tests are used to demonstrate the presence of influenza-specific Abs. Diagnosis of acute SIV infection by serology requires the use of paired acute and convalescent (3–4 weeks later) serum samples. Serology is most useful to determine the immune status of a herd, levels of maternally derived Ab in the young piglets and their kinetics, postvaccination Ab titers, and for premovement testing of pigs.

The HI test remains the most common serological test for anti-SIV Ab detection. A number of enzyme-linked immunosorbert assays (ELISAs) for SIV are commercially available. Broadly, ELISAs can be separated into two groups. The first detect Ab to a highly conserved core antigen of influenza A. These tests generally have good sensitivity (Ciacci-Zanella et al. 2010) and are useful as a screening assay to determine, for example, herd status, but they do not differentiate between virus subtypes. The second detect subtype-specific Abs. These ELISAs generally offer much reduced sensitivity compared to the HI test (Barbé et al. 2009; Leuwerke et al. 2008), but may have application in studies where status to a specific virus subtype/strain is required. Finally, VN assays are increasingly used and show similar performance characteristics to the HI test (Leuwerke et al. 2008; Van Reeth et al. 2006), but they are more appropriate for use in specialized laboratories.

The interpretation of serological data is often complex and may be further confounded by concurrent circulation of different virus subtypes and gene lineages. This is especially problematical with cocirculation of strains within the same subtype, for example H1, possessing variable cross-reactivity in tests that detect Abs to the HA, such as HI and VN, as well as subtype-specific ELISAs (Barbé et al. 2009; Leuwerke et al. 2008). The emergence of the 2009 pH1N1 virus in pigs has further complicated interpretation through a broad spectrum, and sometimes unpredictable range, of Ab responses with endemic strains in HI and VN tests (Dürrwald et al. 2010; Kyriakis et al. 2010b; Vincent et al. 2010b). Therefore, where H1N1 is endemic, infection with the 2009 pH1N1 virus may not be identified with certainty by serological tests alone (Kyriakis et al. 2010b).

PREVENTION AND CONTROL

Vaccination remains the primary means of preventing SI in pigs. The commercial SIV vaccines are traditional inactivated vaccines for IM administration. Most vaccines are whole virus products with a potent oil-based adjuvant. Primary vaccination should consist of two injections 2–4 weeks apart, with biannual booster vaccinations recommended for sows. Routine prefarrow booster vaccination of sows results in higher and longer lasting maternal SIV Ab levels in their piglets and may protect pigs through the nursery phase. Vaccination of feeder pigs is less commonly performed and may be difficult to combine with vaccination of sows because prolonged passive immunity may interfere with effective vaccination of piglets. However, this strategy may be beneficial in herds where influenza is a problem in growers/finishers.
In keeping with the antigenic and genetic differences between SIVs circulating in Europe and the United States, the vaccines for each region are produced locally and contain entirely different strains. Even within a continent, there is no standardization of vaccine strains and the antigenic mass and adjuvants can also differ between different commercial products.

In Europe, SIV vaccines were first licensed during the mid-1980s and they are available in most, but not all, countries. Most of the current vaccines contain older strains of the subtypes that have been circulating since the 1980s, H1N1 and H3N2, without updating. Only one vaccine is trivalent and contains H1N2 along with more recent H1N1 and H3N2 strains.

In the United States, a monovalent H1N1 SIV vaccine was first introduced in 1993. After the emergence of H3N2 influenza viruses in the U.S. swine population in 1998, monovalent H3N2 and ultimately multivalent H1/H3 SIV vaccines became available. The seven vaccines fully licensed by the United States Department of Agriculture (USDA) Center for Veterinary Biologics (CVB) for nationwide use at the time of this writing include products ranging from those with one H1N1 and one H3N2 strain, to products with up to five strains representing multiple H1 and H3 clades. In contrast to the situation in Europe, most manufacturers have updated SIV vaccine strains in recent years, which is in part related to more flexible licensing procedures for the substitution or addition of new strains in the United States. Some commercial vaccines from North America and Europe are also available in some Asian countries. A vaccine for the 2009 pH1N1 influenza virus for use in pigs is available in the United States, but not in Europe.

In the United States, “autogenous vaccines” are also used extensively. These vaccines are custom-made vaccines that are legally used within a single pig production system. They are purity and safety tested, but are not required to undergo efficacy testing. All are killed virus preparations and typically contain adjuvants.

Most SIV vaccine efficacy data are from experimental vaccination-challenge studies in which SIV-seronegative pigs are vaccinated twice with commercial vaccine and challenged with heterologous SIV 2–6 weeks after the second vaccination. Such studies use different vaccines, challenge strains, routes of inoculation, and experimental designs. Complete, sterilizing immunity is rarely observed. Vaccination is unlikely to block virus transmission completely, but it generally reduces virus titers in the lungs, BAL fluids, nasal or oropharyngeal swabs, as well as gross and histopathological lung lesions (Haesebrouck and Pensaert 1986; Heinen et al. 2001b; Kittkoon et al. 2006, 2009; Kyriakis et al. 2010a; Larsen et al. 2001; Lee et al. 2007; Van Reeth et al. 2001, 2002).

Only a few studies have used an intensive IT challenge with a high virus dose and allowed critical evaluation of clinical protection (Haesebrouck and Pensaert 1986; Van Reeth et al. 2001, 2002). Even if the vaccines in these studies reduced lung virus titers only moderately, they were still very effective at preventing disease. Virus titers in the lungs were inversely correlated with prechallenge H1 Ab titers against the challenge virus. These Ab titers were usually several times lower than those against the homologous virus(es) in the vaccine, and they declined rapidly between 2 and 6 weeks after the second vaccination (Kyriakis et al. 2010a). However, prior infection with an influenza virus within the same subtype can dramatically boost the serum Ab response upon a single vaccination (Van Reeth et al. 2006). The latter scenario resembles the field situation, as sows have often been primed by infection with one or more SIVs at the time of their first vaccination.

SIV vaccine design has become much more complex in recent years because of the emergence of several novel SIV subtypes and lineages. Consistent criteria to justify replacement or addition of vaccine strains remain to be determined.

Several commercial vaccines in Europe have clearly shown the ability to provide protection against H1N1 and H3N2 SIVs isolated over the course of many years and with considerable genetic drift compared to the H1 (78–93% amino acid identity between vaccine and challenge strains) and H3 vaccine strains (86% amino acid identity) (Heinen et al. 2001b; Kyriakis et al. 2010a; Van Reeth et al. 2001). In the United States, commercial vaccines offered partial protection against challenge with H3N2 viruses from a genetic cluster different than that in the vaccine (Lee et al. 2007).

The existing North American and European vaccines also induced partial serological cross-reaction and cross-protection against the 2009 pH1N1 virus, even though there was only 72–75% amino acid homology between the pandemic H1 and most European H1 vaccine strains (Dürrwald et al. 2010; Kyriakis et al. 2010b; Vincent et al. 2010a). Yet specific monovalent vaccines based on 2009 pH1N1 virus showed superior protection (Dürrwald et al. 2010; Vincent et al. 2010a).

On the other hand, a European bivalent H1N1- and H3N2-based vaccine failed to induce Abs or protection against challenge with a European H1N2 virus. This was in line with the very low amino acid homology (70.5%) between the H1 of vaccine and challenge strains (Van Reeth et al. 2003b). Paradoxically, however, commercial vaccines with strains with more heterologous HA genes may offer better protection in experimental studies than those with the closest homology to the challenge virus (Kyriakis et al. 2010a). In fact, all data indicate that the adjuvant and antigen mass, which differ between commercial products, is at least as important for the potency of SIV vaccines as the SIV strain(s) in the vaccine. The oil-based adjuvants in
most SIV vaccines can also explain the much broader protection compared to that observed with unadjuvanted influenza vaccines for humans.

So-called new-generation vaccines for SIV have only been tested experimentally and the results have been disappointing. These vaccines include DNA vaccines based on the influenza HA, NP, or M genes or their combinations, recombinant vaccines expressing the M2 protein, and a human adenovirus S vector expressing the HA and/or NP of SIV (Heinen et al. 2002; Kitikoon et al. 2010; Larsen et al. 2001; Macklin et al. 1998; Olsen 2000; Wesley et al. 2004; Wesley and Lager 2006). Better results have been obtained when such vaccines were used in combination with conventional killed virus vaccines in prime-boost regimens, and such strategies may hold promise for the future. A double IT/IN vaccination with a live attenuated vaccine with a modified NS1 gene partially protected pigs from heterosubtypic challenge (Richt et al. 2006).

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Paramyxoviruses

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OVERVIEW

The family Paramyxoviridae contains viral pathogens of international significance in most animal species and humans. Until relatively recently, there were no major paramyxovirus pathogens of pigs, the single possible exception being the rubulavirus that causes blue eye (BE) disease (blue eye paramyxovirus; BEP). However, BEP is confined to Mexico, where its economic impact remains low at present. During a 3-year period commencing in 1997, several new paramyxoviruses were identified and two (Menangle and Nipah viruses) were found to be serious pathogens of both pigs and humans.

The family Paramyxoviridae encompasses a group of large (150–400 nm in diameter), pleomorphic viruses. The genome consists of a long, single strand of RNA within a herringbone-like nucleocapsid. The nucleocapsid is surrounded by a lipid envelope that usually contains an outer fringe of surface projections or “spikes.”

The paramyxoviruses are currently organized in two subfamilies and seven genera. There are five genera in the subfamily Paramyxovirinae: Avulavirus, Henipavirus, Morbillivirus, Respirovirus, and Rubulavirus. There are major pathogens of animals and humans in each of these genera; for example, Newcastle disease virus of poultry in the genus Avulavirus; canine distemper and human measles viruses in the genus Morbillivirus; several parainfluenza viruses of animals and humans in the genus Respirovirus; and in the genus Rubulavirus, human mumps virus, BEP, and Menangle virus. Both of the latter two viruses are infectious for pigs. The genus Henipavirus was created for two of the most recently recognized and closely related paramyxoviruses, Hendra and Nipah, reflecting their morphological and genetic differences from other paramyxoviruses.

Because of their tissue tropisms, there are broad similarities in the diseases caused by paramyxoviruses. Typically, pathogenic paramyxoviruses are associated with diseases of the central nervous system (CNS) (canine distemper and Newcastle disease) and respiratory systems (parainfluenza infections, Hendra, Nipah, and Newcastle disease). Some, especially Menangle virus and BEP, are also important reproductive pathogens.

The paramyxoviruses that cause significant disease in pigs are BEP, Menangle virus, and Nipah virus. There have been occasional reports of other paramyxoviruses associated with respiratory and CNS disease in pigs in Japan (Sasahara et al. 1954), Canada (Greig et al. 1971), Israel (Lipkind et al. 1986), and the United States (Paul et al. 1994), but none has proven to be of significance. Hendra virus is not known to have infected pigs naturally, but experimental infections of pigs have been reported (Weingartl 2010). This chapter will provide an overview of BEP, Menangle, and Nipah viruses and the diseases they cause.

RUBULAVIRUS (BLUE EYE) DISEASE

Relevance

BE is a disease of swine caused by infection with BEP (Stephano et al. 1988b) or porcine rubulavirus, also known as La Piedad-Michoacán virus (LPMV). The disease was first reported in 1980 in central Mexico, with numerous outbreaks of encephalitis and corneal opacity in piglets (Stephano et al. 1982). A hemaglutinating virus was isolated, characterized, and
identified as a serologically distinct member of the family Paramyxoviridae (Stephano and Gay 1983, 1984, 1985a; Stephano et al. 1986b).

The first reported outbreak of BE was observed on a commercial farm with 2500 sows located in La Piedad, Michoacán, Mexico (Stephano et al. 1982). Thereafter, BE was recognized as an important pathogen in central Mexico, with serological evidence of BEP in at least 16 states in Mexico (Stephano et al. 1988b). The disease is still recognized in central Mexico, but its economic impact has lessened. BE has not been reported outside of Mexico.

Etiology
Extensive molecular characterization of BEP (Berg et al. 1991, 1992; Sundqvist et al. 1990, 1992), combined with its morphology and biological properties, support its placement in the Rubulavirus genus.

BEP particles are similar to other paramyxoviruses, measuring 135–148 nm by 257–360 nm (Figure 41.1). The virion is pleomorphic, but usually more or less spherical. No filamentous forms have been observed. Nucleocapsids from disrupted virus particles have a diameter of 20 nm and a length of 1000–1630 nm (Figure 41.2) or more (Stephano and Gay 1985a).

In the laboratory, BEP replicates and produces cytopathology in a wide range of cell cultures from many different animal species, including both continuous cell lines and primary cell cultures. Changes consist of individual rounded cells, cytoplasmic vacuoles, and syncytium formation. Some cells also contain viral inclusion bodies (Moreno-Lopez et al. 1986; Stephano and Gay 1985a; Stephano et al. 1986a). The chick embryo also supports BEP replication.

BEP agglutinates erythrocytes from a wide range of animal species as well as humans. Spontaneous elution occurs at 37°C after 30–60 minutes. Hemadsorption of chicken erythrocytes has also been described (Stephano and Gay 1985a; Stephano et al. 1986b).

BEP is not known to share any antigens with other paramyxoviruses (Stephano et al. 1986b) and no antigenic differences have been observed between different isolates of BEP (Gay and Stephano 1987).
Role in Public Health
Unlike some of the other paramyxoviruses, BEP does not have any public health significance and is not infectious for humans.

Epidemiology
Pigs are the only species known to be affected clinically by BEP following natural exposure. Experimentally, BEP affects mice, rats, and chick embryos. Rabbits, dogs, cats and peccaries do not show clinical signs, but rabbits, cats and peccaries produce antibodies (Stephano and Gay 1985a; Stephano et al. 1988a).

Subclinically infected pigs from affected farms are the primary source of BEP. The virus is apparently transmitted by nose-to-nose contact between infected and susceptible pigs. The virus is mainly disseminated in nasal secretions and urine. Transmission through semen is possible and the virus can be recovered from semen, testis, epididymis, prostate, seminal vesicles, and the bulbo-urethral glands 10–45 days after inoculation (Solis et al. 2007). The virus may also be disseminated by fomites, for example, people and vehicles, and possibly by birds and wind. Other sources of infection have not been demonstrated.

The disease is self-limiting in closed herds. Sentinel pigs introduced to a farm 6–12 months after an outbreak remain asymptomatic and do not seroconvert. Although there was evidence of persistence of BEP RNA in the brain, lung, lymph nodes, pancreas, and epididymis up to 57 and 277 days in experimentally infected pigs, neither infectious virus nor viral antigen was detected, even after immunosuppression (Cuevas et al. 2009; Wiman et al. 1998). Further, positive animals moved to seronegative herds did not spread the virus (Stephano and Gay 1986b; Stephano et al. 1986a). However, the disease can recur if a susceptible group of pigs is introduced onto a farm. Large farms with a continuous system of production may have cases periodically.

Pathogenesis
It is believed that BEP infection is acquired by inhalation. Experimentally, intratracheal or intranasal exposures are effective routes of infection. The initial site of BEP replication is the nasal mucosa and tonsils. From the initial site of replication, BEP spreads quickly to the brain through the olfactory and trigeminal nerves, to the lung by air, and later, to many other organs by viremia.

Variation in the viral neuraminidase is associated with differences in virulence among BEP isolates (Sánchez-Betancourt et al. 2008). CNS manifestations occur early in the disease. Nervous signs have been induced in 1-day-old piglets 20–66 hours after inoculation, some weaned pigs (21–50 days old) developed a nervous syndrome at 11 days postinoculation, and pregnant sows developed reproductive failure when inoculated during pregnancy. Corneal opacity was occasionally observed in these cases. The disease was also reproduced in susceptible pigs placed in contact with experimentally infected pigs as long as 19 days after experimental infection.

Dissemination by viremia is indicated by the interstitial pneumonia that is observed and by isolation of BEP from different organs in naturally and experimentally infected pigs (brain, lung, tonsil, liver, turbinate, spleen, kidney, mesenteric lymph node, heart, pancreas, ovary, etc.) and blood (Allan et al. 1996; Stephano and Gay 1983; Stephano et al. 1988b).

The cause of the corneal opacity is unknown, but anterior uveitis is commonly observed microscopically in the cornea (Stephano and Gay 1986b). The opacity usually occurs late in the course of the disease and is thought to be due to an immunological reaction similar to that observed in dogs after adenovirus-induced hepatitis.

Infection of pregnant sows and gilts results in reproductive failure due to embryonic mortality. BEP virus can cross the placenta. In the first one-third of gestation, affected sows usually return to estrus. When infection occurs later in gestation, the result is stillbirths and fetal mummification. Abortion can also occur (Hernández-Jáuregui et al. 2004; Stephano and Gay 1984).

Intranasal infection of young boars results in inflammation and edema of the testis and epididymis by 15 days after inoculation. By 30 days, there is necrosis of the seminiferous tubules and rupture of the epithelial wall of the epididymis with leakage of spermatozoa from the lumen, leading to abscess formation. Boars sacrificed 80 days after infection showed fibrosis and granuloma formation in the epididymis as well as testicular atrophy (Ramirez et al. 1995).

Clinical Signs
An outbreak of BE may start in any area of a pig farm, but is usually first observed in the farrowing house, with CNS signs and high piglet mortality. At about the same time, corneal opacity may be observed in some weaned or fattening pigs (Stephano and Gay 1985a, 1986a; Stephano et al. 1988a). The mortality rate increases rapidly and then declines within a short time. Once the initial outbreak is over, no new clinical cases appear, unless susceptible pigs enter the population. Clinical signs are variable and depend primarily on the age of the pig. However, corneal opacity, the sign that gives the disease its name, frequently occurs in pigs of all ages without other signs and resolves spontaneously.

Piglets 2- to 15-days-old are the most susceptible and the onset of clinical signs is sudden. Healthy piglets may suddenly become prostrate, generally in lateral recumbency, or show nervous signs. However, the
disease usually runs a course that starts with fever, a rough hair coat, and an arched back, sometimes accompanied by constipation or diarrhea. These signs are followed by progressive nervous signs, including ataxia, weakness, rigidity (mainly of the hind legs), muscle tremor, and abnormal posture, such as a sitting position. Anorexia does not occur as long as piglets can still walk. Some piglets are hyperexcitable and may squeal and show paddling movements when handled. Other signs include lethargy, with some involuntary movements, dilated pupils, apparent blindness and, occasionally, nystagmus. Some piglets suffer from conjunctivitis, with swollen eyelids and lacrimation. Often the eyelids are closed and adherent with exudate. In up to 10% of affected piglets, either unilateral or bilateral corneal opacity is present.

Of the litters farrowed during an outbreak, about 20% are affected. In these litters, piglet morbidity is 20–50% and mortality in affected piglets is about 90%. In the first cases observed, piglets usually died within 48 hours of the appearance of clinical signs, but in later cases, death occurred after 4–6 days. During an initial outbreak, deaths occur for 2–9 weeks, depending on the management system.

Pigs older than 30 days show moderate and transient clinical signs such as anorexia, fever, sneezing, and coughing. Nervous signs are less common and less obvious, but when present, consist of listlessness, ataxia, circling and, rarely, swaying of the head. Unilateral or bilateral corneal opacity and conjunctivitis continue to appear on the farm for another month without other signs. Fewer than 2% of pigs older than 30 days are affected and the mortality is generally low. Outbreaks with 20% mortality and severe CNS manifestations have been observed in 15–45 kg pigs. Corneal opacity was present in up to 30% of these pigs (Stephano and Gay 1985b).

Most of the sows suckling affected litters are clinically normal. Some show moderate anorexia 1 or 2 days before the appearance of clinical signs in piglets. Corneal opacity has also been observed in the farrowing house during outbreaks.

In pregnant sows, reproductive failure lasting 2–11 months (usually 4 months) is observed. Reproductive signs during outbreaks include an increase in the number of animals returning to estrus, a reduction in farrowing rate, and an increase in the weaning-to-service interval and nonproductive sow days. The rate of stillbirths and mummified fetuses also increases, and consequently, there is a reduction in the number of pigs born alive. Later, there is also a reduction in the total number of pigs born. Abortion is not a cardinal feature, but has sometimes been observed during an acute outbreak. Gilts and other adult pigs occasionally develop corneal opacity.

Boars, like other adult animals, generally do not show clinical signs, but mild anorexia and corneal opacity have been recorded. Semen evaluation demonstrated that about 30% of boars in herds infected by BEP showed temporary or permanent infertility, with a decrease in concentration, an increase in abnormalities, and a decrease in motility and viability of spermatozoa. In some boars there is azoospermia, the ejaculate becomes clear and resembles coconut water. Some boars develop swollen testicles. The testis and epididymis become turgid with marked edema. Later, some develop a granular texture and most atrophy (generally unilateral) or become soft and flabby with or without granular epididymitis. Boars with severe lesions lose libido (Campos and Carbajal 1989; Stephano et al. 1990).

Differences in clinical signs became evident a few years after the virus was discovered. In 1980, piglets were primarily affected. Mortality and CNS disorders in pigs older than 30 days were uncommon. In 1983, severe outbreaks of encephalitis with high mortality in pigs weighing 15–45 kg were observed on badly managed farms, always with concomitant viral and bacterial diseases (Stephano and Gay 1985b, 1986a). Also in 1983, reproductive failure in sows and transient infertility in boars were identified (Stephano and Gay 1984, 1985a). In 1988, severe orchitis, epididymitis, and testicular atrophy in boars became evident (Campos and Carbajal 1989; Stephano et al. 1990).

**Lesions**

There are no specific gross changes in cases of BE. In piglets, a mild pneumonia is frequently observed at the ventral tips of the cranial lung lobes. There is mild distension of the stomach with milk, distension of the urinary bladder, and a slight accumulation of peritoneal fluid with fibrin. The brain is often congested and there is an increase in the quantity of cerebrospinal fluid. Conjunctivitis, chemosis, and varying degrees of corneal opacity (Figure 41.3), usually unilateral, are observed. Vesicle formation, ulcers, and keratoconus have been observed in the cornea as well as exudate in the anterior chamber. Pericardial and renal hemorrhages are occasionally observed (Stephano and Gay 1985a, 1986b).

Boars develop swollen testicles and epididymes. These changes are frequently unilateral. There is orchitis, epididymitis, and, later, atrophy of the testicle, with or without granulomatous formation in the epididymis. Hemorrhages are occasionally observed in the tunica albuginea, epididymis, or testis (Campos and Carbajal 1989; Ramirez et al. 1995; Stephano et al. 1990).

The main histological changes are seen in the brain and spinal cord. These reflect a nonsuppurative encephalomyelitis affecting mainly the gray matter of the thalamus, midbrain, and cerebral cortex and include a multifocal and diffuse gliosis, perivascular cuffing with
lymphocytes, plasma cells and reticular cells, neuronal necrosis, neuronophagia, meningitis, and choroiditis (Ramirez and Stephano 1982). Intracytoplasmic inclusion bodies are found in neurons (Stephano and Gay 1986b; Stephano et al. 1988a).

The lungs have localized areas of interstitial pneumonia characterized by thickened septa with mononuclear cell infiltration.

Changes in the eye are mainly corneal opacity, characterized by corneal edema and anterior uveitis. Neutrophils, macrophages, or mononuclear cells infiltrate the iridocorneal endothelium, corneoscleral angle, and cornea (Stephano and Gay 1986b; Stephano et al. 1988a).

In boars, the affected testes show degeneration and necrosis of the germinal epithelium. The interstitial tissue shows Leydig cell hyperplasia, mononuclear cell infiltration, hyalinization of the vascular wall, and fibrosis. The epididymis shows vesicle formation, loss of epithelial cilia, rupture of the epithelial wall, presence of spermatozoa in the intertubular space, and severe infiltration of inflammatory mononuclear cells with macrophage phagocytosis of fragmented sperm. Fibrosis and spermatic granuloma are organized.

Diagnosis
Clinical signs such as encephalitis, corneal opacity, and reproductive failure in the sow and orchitis and epididymitis in the boar are consistent with a diagnosis of BE. Histological lesions, such as nonsuppurative encephalitis, anterior uveitis, keratitis, orchitis, and epididymitis provide additional diagnostic evidence. The presence of intracytoplasmic inclusions in neurons and corneal epithelium in conjunction with these clinical signs and histological findings provides strong support for a diagnosis of BE.

Other causes of encephalitis and reproductive disease must also be considered, for example, pseudorabies (Aujeszky’s disease) virus and porcine reproductive and respiratory syndrome virus (PRRSV). Only BEP produces corneal opacity along with orchitis and epididymitis in boars (Campos and Carbajal 1989; Stephano and Gay 1985b; Stephano et al. 1988a, 1990).

Paired serum samples, 15 days apart, are recommended for serological confirmation by hemagglutination inhibition (HI), virus neutralization (VN), or enzyme-linked immunosorbent assay (ELISA). HI is the most frequently used test, but false-positive titers up to 1:16 have been detected when chicken erythrocytes were used or when the antigen was grown in chicken embryos (Ramirez et al. 1996). Therefore, bovine erythrocytes are recommended. Naturally infected pigs develop antibodies that usually persist for life.

The brain is the best tissue for virus isolation and antigen detection, but lung and tonsil are also suitable (Stephano et al. 1988a). PK-15 cells or primary pig kidney cells are preferred for virus isolation. Virus replication induces syncytium formation. Direct immunofluorescence and the polymerase chain reaction (PCR) have been used to detect antigens in tissue sections and monolayers ( Cuevas et al. 2009; Stephano and Gay 1985a; Stephano et al. 1988a).

Prevention and Control
As with most viral diseases of swine, there is no specific treatment. Pigs with corneal opacity frequently recover spontaneously, whereas pigs with central nervous disease generally die. Antimicrobial therapy is commonly used to treat and prevent secondary infections. At present, there are two commercial inactivated virus vaccines approved for use in pregnant sows, gilts, boars and piglets.

Herd health programs are the most reliable method of preventing the introduction of BEP to a farm. Replacements should be selected from a healthy herd and quarantined prior to introduction. Standard biosecurity measures provide insurance against infection, for example, perimeter fencing; separate load-out areas; changing rooms and showers; control of birds, rats, and mice; prompt waste removal and disposal of dead pigs; and control of the movement of personnel, visitors, and vehicles. Serological screening of replacement animals is recommended.

Elimination of BEP from infected herds has been accomplished by management practices, for example, herd closure, cleaning and disinfecting, all-in/all-out production, elimination of clinically affected animals, and disposal of dead pigs. Serological testing of the population and sentinel animals (BEP seronegative pigs) should be used to confirm the elimination of BEP (Stephano et al. 1986b).
MENANGLE VIRUS

Relevance
Menangle virus was first identified during a disease outbreak in New South Wales, Australia, in 1997. This virus causes reproductive disease and congenital defects in pigs, occasionally causing moderately severe disease in humans, and has fruit bats (*Pteropus* sp., flying foxes) as a reservoir host. It has been eradicated from the affected farms. There are no records of its occurrence in any other country.

Etiology
Like BEP, Menangle virus is a member of the genus *Rubulavirus* within the family *Paramyxoviridae* (Bowden et al. 2001). Other well-known viruses in this genus are human parainfluenza viruses types 2 and 4 and human mumps virus.

Menangle virus has typical paramyxovirus morphology. Virions are pleomorphic with both spherical and elongated forms that range in size from 100 to 350 nm. Virions possess a single layer of surface spikes approximately 17 nm in length. Ruptured particles reveal long herringbone-shaped nucleocapsids of approximately 19 nm in diameter (Philbey et al. 1998).

Menangle virus induces pronounced cytopathology in cell culture, including prominent vacuolation of cells and the development of large syncytia. The virus replicates and produces cytopathology in a wide range of cell types from many animal species, including birds and fish. There is no evidence of hemadsorption or hemagglutinating activity (Philbey et al. 1998). Menangle virus is not related antigenically to any other paramyxovirus.

Role in Public Health
In contrast to Nipah virus, Menangle virus does not appear to be highly infectious for humans. However, care should be taken when working with potentially infected pigs or suspect reproductive specimens. While only two out of more than 30 humans directly exposed to infected pigs were infected with the virus, both experienced a severe febrile illness associated with a macular rash followed by prolonged debility (Chant et al. 1998).

There was no evidence of infection in a large number of other people, including veterinarians, abattoir workers, and laboratory workers, who had less direct and less protracted contact with potentially infectious material. Transmission to humans may require the contamination of cuts and abrasions with infectious body fluids or tissues or possibly splashing of material onto the conjunctivae (Chant et al. 1998).

Epidemiology
Studies of archival and newly collected sera suggested that the Menangle virus was not highly contagious among the pigs on the affected farm (Kirkland et al. 2001). This was deduced by the relatively slow spread of infection in a building that contained pens of sows, that is, it took several weeks for all of the sows to become infected. Nevertheless, the virus was widely dispersed through the pig population on the affected farm. About 6 months after the estimated time of entry of the virus to the farm, a high proportion (>90%) of sera collected from pigs of all ages contained high levels of VN antibody. Positive VN antibody titers ranged from 1:16 to 1:4096 and remained high for at least 2 years after infection. In contrast, all samples collected prior to the estimated time of entry of the virus into the pig population were negative. All serum samples collected at the two grower-fattening farms were positive (Kirkland et al. 2001). Testing of 1114 swine sera from other pig farms throughout Australia indicated that infection was confined to the affected pig farm and the two associated grow-out facilities.

Following the initial spread of the infection through the herd, the virus was maintained by infection of young pigs at about 10–12 weeks of age as they lost the protection provided by maternally derived antibodies. In a large pig population, the constant availability of susceptible animals was sufficient to ensure persistence of the virus. In smaller pig herds, such persistence would be much less likely. Almost all selected replacement breeding pigs on the farm had been exposed to the virus and were seropositive before mating at around 28–30 weeks of age, preventing further reproductive failure.

It appeared that close contact between pigs was required for spread of infection and that the virus did not survive in the environment for long. Susceptible sentinel pigs moved into an uncleaned area occupied 3 days previously by infected pigs did not become infected.

It is believed that flying foxes are a reservoir of Menangle virus (Kirkland et al. 2001; Philbey et al. 1998). During the summer–autumn period, when the virus was thought to have entered the pig farm, there was a large breeding colony of gray-headed fruit bats (*Pteropus polocephalus*), as well as little red fruit bats (*Pteropus scapulatus*), roosting within 200 meters of the affected pig farm. Sera collected from gray-headed fruit bats (*Pteropus alecto*), and spectacled fruit bats (*Pteropus conspicillatus*), but not in little red fruit bats. These results indicated that Menangle virus was endemic in the fruit bat population and preceded the infection in pigs.

Except for spread to humans, there is no evidence that this virus has spread naturally to other animal
species. Samples collected from rodents, birds, cattle, sheep, cats, and a dog in the vicinity of the affected pig farm were all seronegative.

Pathogenesis
The route of transmission of Menangle virus and the mechanism of spread are not known, although fecal–oral or urinary–oral transmission is suspected (Love et al. 2001). Infection in pigs appears to be of short duration (10–14 days) and results in strong immunity. Virus was not detected in surviving piglets born during the outbreak, suggesting that persistent infection is unlikely. There is also strong circumstantial evidence that adult pigs do not become persistently infected.

The principal cause of reproductive loss associated with Menangle virus appears to be in utero infection, often resulting in fetal death. In many sows, there was early death of the whole litter, resulting in a delayed return to estrus or sometimes a state of pseudopregnancy.

At parturition, affected litters sometimes contained piglets of varying size and with a range of abnormalities. Some piglets were mummified and were of different gestational ages, some piglets were stillborn and had congenital malformations, and there were a few normal piglets (Figure 41.4). These findings indicated that, as with parvovirus, transplacental infection of a few fetuses can occur early in gestation followed by progressive spread of the virus from fetus to fetus within the uterus. The teratogenic defects observed are the direct result of virus replication and cell destruction in rapidly developing fetal tissues.

Clinical Signs
To date, there has only been one known disease outbreak due to Menangle virus in pigs (Love et al. 2001; Philbey et al. 1998). In 1997, over a 5-month period (mid-April to early September), sows in a 3000-sow, intensive farrow-to-finish pig farm near Sydney, New South Wales, Australia experienced severe reproductive failure (Love et al. 2001). There was a marked increase in the incidence of mummified fetuses and stillborn piglets. After a period, some of the stillborn piglets were born with severe malformations. Sows in all four breeding units on the farm were affected. There were some weeks when the farrowing rate decreased from an expected 82% to as low as 38%. Many sows showed delayed returns to estrus at around 28 days after mating, while others remained in a state of pseudopregnancy until more than 60 days postmating. The disease occurred sequentially in all four breeding units at the pig farm, affecting the progeny of sows of all parities. In the weeks of low farrowing rates, up to 45% of sows farrowed litters with reduced numbers of live piglets and an increase in the proportion of mummified and stillborn piglets, some of which had congenital deformities.

Individual litters contained mummified fetuses of varying size, ranging upwards in gestational age from 30 days, together with stillborn piglets (some with malformations) and a few normal piglets (figure 41.4). Teratogenic defects, including arthrogryposis, brachygнатia, and kyphosis were frequently seen in stillborn piglets and there were occasional cases of artiodactyla (Love et al. 2001). The cranium of some piglets was slightly domed.

Although the virus was also detected on two associated growing farms, there were no breeding animals held on these farms and no clinical disease was recognized. The virus had apparently spread to these farms (separated from the main farm and each other by several hundred kilometers) when young growing pigs were moved. There were no clinical signs evident in growing pigs of any age and the only clinical signs in sows on the main farm were those associated with reproductive failure. It is not known whether Menangle virus can be spread in the semen of acutely infected boars.

Following the isolation of Menangle virus, two seropositive workers were identified (Chant et al. 1998).
During subsequent medical investigations, it was found that both had experienced a severe febrile illness with headaches. Extensive testing failed to identify any other possible cause of the illness and it was concluded that the disease was due to Menangle virus infection (Chant et al. 1998). Both workers recovered fully after a prolonged period of convalescence.

Lesions
Affected litters usually consist of a mixture of mummified fetuses, autolysed and fresh stillborn piglets, and a few normal live piglets (Love et al. 2001; Philbey et al. 1998). Congenital defects, including arthrogryposis, brachygnathia, kyphosis, and, occasionally, artiodactyly are only seen in dead piglets. Affected stillborn piglets frequently have slight-to-severe degeneration of the brain and spinal cord (Figure 41.5). Gross defects ranging from porencephaly to hydranencephaly are most common in the cerebrum. Occasionally, there may be fibrinous body cavity effusions and pulmonary hypoplasia.

Histological changes are most marked in the CNS (Love et al. 2001; Philbey et al. 1998). There is extensive degeneration and necrosis of gray and white matter of the brain and spinal cord associated with infiltrations of macrophages and other inflammatory cells. Intranuclear and intracytoplasmic inclusion bodies may be observed in neurons of the cerebrum and spinal cord. These bodies are eosinophilic to amphophilic and consist of aggregates of nucleocapsids. Nonsuppurative multifocal meningitis, myocarditis, and, occasionally, hepatitis may be present in some cases.

Diagnosis
As Menangle virus is a recently recognized agent that is pathogenic for pigs and only one outbreak has been recorded, most pig populations should be fully susceptible. The birth of litters with a marked reduction of normal live piglets and a number of stillborn piglets with teratogenic defects is suggestive of Menangle virus infection. The most rapid method of excluding Menangle virus infection is to test affected sows for the presence of specific antibody to the virus.

The birth of litters containing mummified fetuses of varying size together with stillborn piglets is indicative of in utero viral infection. By far the most common cause of similar losses is porcine parvovirus, but a variety of other viral infections, for example, encephalomyocarditis, the pestiviruses, that is, classical swine fever (hog cholera) and Bungowannah virus, pseudorabies (Aujeszky’s disease), Japanese encephalitis, porcine reproductive and respiratory syndrome (PRRS), and porcine rubulavirus (BEP) may cause significant fetal death. A feature that distinguishes Menangle virus infection from all but Japanese encephalitis infection is the presence of congenital malformations in piglets. However, it should be noted that these are only evident in approximately one-third of affected litters. In addition, many of these other viral infections also cause disease in both piglets and adults. BEP is the only other paramyxovirus to cause significant fetal loss as a presenting sign, but differs from Menangle virus in that neurological and other signs are usually observed in young piglets and the virus can be readily distinguished by its ability to agglutinate erythrocytes from mammals and birds (Moreno-Lopez et al. 1986). Reproductive disease is not a major feature of infection with Nipah virus.

For laboratory diagnosis, fetal specimens should be collected for virus isolation, serology, and pathology. The virus can be isolated from a number of organs from stillborn piglets, especially brain, lung, and myocardium. A wide range of cell cultures support replication of Menangle virus, but baby hamster kidney cells (BHK-21) have been used for the isolation of the virus from field specimens. Three to five passages may be necessary before suggestive cytopathology is observed. As the virus does not hemagglutinate, identification will depend on electron microscopy and neutralization of an isolate with specific antiserum. Specific antibodies may be detected in body cavity fluids of some stillborn piglets.

Prevention and Control
There is no specific treatment for Menangle virus. By the time clinical signs are observed, the virus is likely to be widely disseminated in the affected population, negating measures to limit spread.

Fruit bats (Megachiroptera) are the primary source of infection for the pig population. Megachiroptera are not found in North America, but are present in Africa, the Middle East, South Asia, Australia, and many Pacific Islands. It is not known whether small bats (Microchiroptera) are susceptible to Menangle virus, but it is important to restrict direct and indirect contact between pigs and bats to prevent introduction of this virus to
the pig population. Fruit bats do not normally enter pig farm buildings, but they defecate and urinate during flight over and around buildings and occasionally inadvertently drop their young in flight. Cover all outside areas, for example, outside walkways, to prevent contamination of the facilities and infection of the herd. Flowering trees and fruiting trees should not be grown in the immediate vicinity of pig farm buildings as these may attract fruit bat activity.

In an outbreak of reproductive disease, the infection will probably have already spread through the entire population of a pig farm by the time the first affected litters are farrowed. In small piggeries, there would be insufficient numbers of susceptible animals available to maintain a cycle of infection as there is no carrier state and, unlike parvovirus, environmental survival of this virus is poor. In large piggeries, infection may become endemic, with the infection being maintained in groups of pigs as they lose their maternally derived protection. In such a situation, it is important to maximize the opportunity for infection of all selected replacement breeding stock prior to mating.

The eradication of Menangle virus from an endemic infected pig population can be achieved by moving all the age groups in which infection is active, for example, pigs between 10 and 16 weeks of age, to another site (Love et al. 2001). If affected facilities are cleaned, vacated for a few weeks, and then restocked with unexposed pigs or pigs known to be immune to the virus, the cycle of endemic infection in the herd should be broken and the virus eliminated naturally.

NIPAH VIRUS

Relevance
Nipah virus is a zoonotic virus that first caused a major epidemic in pigs in 1998–1999 in a relatively small geographical area in Southeast Asia, but has since been detected in other countries in Asia. In this instance, it is believed that the virus jumped from a wildlife reservoir to domestic pigs and then spread to humans and to other domestic animals including cats, dogs, and horses. The virus was not contagious in cats or dogs, but was associated with a high case fatality rate.

Etiology
Nipah virus is a negative-stranded RNA virus in the family Paramyxoviridae. Nipah and Hendra viruses are the sole members of the genus Henipavirus (Chua et al. 2000). Unlike Nipah virus, serological surveys have not detected evidence of natural Hendra virus infections in pigs (Black et al. 2001), but Hendra virus was infectious for swine and caused clinical signs similar to Nipah virus infection under experimental conditions (Li et al. 2010).

Nipah is a large pleomorphic virus similar to most paramyxoviruses. Virus particles vary in size, but their average diameter is 500 nm. Surface spikes on the envelope are approximately 10 nm in length. The typical long “herringbone-shaped” nucleocapsids have a diameter of approximately 21 nm and an average length of 1.67 μm.

Nipah virus replicates readily in several continuous cell lines, especially Vero and BHK-21 and produces cytopathic effects in 3–4 days. In Vero cells, virus replication induces large syncytia in which the cell nuclei are arranged around the periphery of the multinucleated cell. (Daniels et al. 2002). Antigenically, Nipah virus is closely related to Hendra virus and diagnostic reagents for Hendra virus have been utilized to assist Nipah virus investigations.

Role in Public Health
Nipah virus presents a serious threat to public health. Although the first outbreak of Nipah virus was confined to a relatively small geographical area, it resulted in numerous human infections and deaths in pig farmers and other people who had close contact with pigs, including abattoir workers in a neighboring country. More recently, disease outbreaks occurred in human populations without the known involvement of pigs (Chadha et al. 2006; Gurley et al. 2007; Hsu et al. 2004; Luby et al. 2006). If there is a risk of an outbreak, it is essential to prevent the movement of all animals and minimize human contact with potentially infected animals.

Epidemiology
The disease outbreak observed in Malaysia is assumed to be the result of the virus “jumping” species into farmed domestic pigs (Chua et al. 2000; Daniels 2000; Field et al. 2001; Yob et al. 2001). There is strong evidence that pteropid bats are a reservoir of this virus. A high prevalence of neutralizing antibodies was detected in both of the wild bat species (Pteropus vampyrus and Pteropus hypomelanus) present in Malaysia (Yob et al. 2001). Nipah virus has also been isolated from wild P. hypomelanus in Malaysia (Chua et al. 2001). In Australia, the fruit bat population remains a potential source of Menangle virus infection for pigs and possibly other species. Likewise, Nipah virus poses a continuing threat in Bangladesh, India, and Malaysia. The risk these viruses pose to pigs and other animal species in other countries has not been determined, but should not be ignored, especially in countries where pteropid bats are present.

Observations made during the outbreak in 1998–1999 in Malaysia indicated that Nipah virus was highly contagious among swine (Nor et al. 2000). Direct, and possibly airborne, exposure to secretions from infected animals was the presumed modes of transmission of Nipah virus among pigs. This was supported by detection of Nipah virus in the epithelium of the upper and lower respiratory tract, as well as airways and oral/nasal
swabs. The pattern of human infection suggested spread via sputum or large droplets, rather than by fine aerosol. Coughing, a frequent clinical sign in infected pigs, would facilitate such a mode of transmission. Subcutaneous, oral, and nasal routes of inoculation, as well as contact with infected animals (Hooper et al. 2001; Middleton et al. 2002; Weingartl et al. 2006), may result in transmission.

In addition to pigs, other domestic animals are susceptible to Nipah virus. Large numbers of dogs died on infected farms and clinically affected dogs were identified during investigations of outbreaks on swine farms (Chua et al. 2000; Daniels et al. 2000). There was no evidence of lateral transmission between dogs (Asiah et al. 2001). Cats were also reported by farmers to have been infected. Under experimental conditions, cats were susceptible to infection and excreted Nipah virus in urine (Muniandy 2001). Nipah virus was isolated from the kidney of a clinically affected dog in Malaysia (Chua et al. 2000), which suggests that urinary excretion by domestic carnivores may be a mode of spread. There was no evidence of transmission of Nipah virus among horses, and serological studies of rodents on infected farms and various other wildlife species showed no evidence of Nipah virus infection.

In Malaysia, the movement of infected pigs was the primary means by which Nipah virus spread between farms, between states, and internationally to Singapore, where abattoir workers processing Malaysian pigs became infected (Nor 2001; Nor et al. 2000; Nor and Ong 2000; Paton et al. 1999). The outbreak probably originated from a point source (Lye et al. 2001), implying that the virus moved from its wildlife reservoir on only one occasion.

Human infection was associated with close contact with infected pigs (Parashar et al. 2000). Feeding or handling pigs, assisting with farrowing, treatment, and/or removal of sick or dead pigs were most likely to result in infection. Living on an infected pig farm was not a significant risk factor.

More recent outbreaks in Bangladesh and India had higher case fatality rates than the first outbreak in Malaysia and Singapore and human-to-human transmission of the virus was reported. Significantly, drinking date palm sap, apparently contaminated by flying foxes, was associated with some of the index cases, while contact with pigs was not reported (Chadha et al. 2006; Gurley et al. 2007; Hsu et al. 2004; Luby et al. 2006).

**Pathogenesis**

Pathogenesis studies with Nipah virus in swine have been limited to pigs 4–10 weeks of age due to the constraints of working under Biosafety Level 4 (BSL4) conditions. Nipah virus primarily targets three systems: vascular, nervous, and lymphoreticular. Upon oronasal inoculation, Nipah virus infects a number of cell types present in the oronasal mucosa: epithelial cells, cells of the immune system, and presumably extracellular peripheral nerve endings of the cranial nerves, leading to direct invasion of the brain in some animals (Weingartl et al. 2005). Endothelial cells of small blood and lymph vessels are an early characteristic target for Nipah virus. Infection of endothelial cells and cells of the immune system leads to viremia and subsequent spread of Nipah virus, as the virus appears to prefer endothelial cells of specific organs and tissues (Meisner et al. 2009). Infection of endothelial cells results in vasculitis and recruitment of immune cells that can also be productively infected (Berhane et al. 2008). Infiltration of these cells into parenchyma, as well as direct infection of cells adjacent to the endothelial cells, further increases the virus load in tissues. Thus, Nipah virus can alternatively infect the CNS by crossing the blood–brain barrier (Weingartl et al. 2005).

Infection of the lower respiratory tract most likely occurs by viremia and the associated vascular damage and infiltration of infected mononuclear cells. However, direct infection of epithelial cells due to inhalation of the virus, or spread of the virus along the respiratory epithelium from the upper respiratory tract, or a combination of all routes, cannot be excluded.

Cells of the lymphoreticular system are an important target. The virus productively infects monocytes and a subset of T lymphocytes (Berhane et al. 2008), and viral antigen was detected in macrophages and dendritic cells. In addition, lymphocyte necrosis and depletion were observed, especially in lymph nodes. Reduction of specific populations of immune cells may be a contributing factor in secondary infections.

**Clinical Signs**

Nipah virus differs from most paramyxoviruses in that it causes a severe, often fatal disease in a number of species. Disease and death in people may be the first indication of an outbreak. Nipah virus infection of humans presents as an encephalitis. Patients may show fever, headache, dizziness, and vomiting. The condition progresses in over 50% of cases to impaired consciousness accompanied by brain stem dysfunction (Goh et al. 2000). A full clinical description of Nipah virus-induced disease in humans has been provided elsewhere (Chua et al. 1999; Goh et al. 2000).

Nipah virus infection in pigs may be asymptomatic or result in acute febrile disease with respiratory and/or CNS signs, for which reason it was initially termed “porcine respiratory and encephalitis syndrome.” Clinical signs of Nipah virus infection in pigs vary by age (Bunning et al. 2000; Nor et al. 2000) and vary in severity from self-limiting to occasionally fatal. There are no pathognomonic clinical signs of Nipah virus infection in pigs, although a “barking” cough is considered characteristic.
In field outbreaks, an acute febrile illness was described in weaner and grower pigs, with temperatures of $\geq 40^\circ C$ ($104^\circ F$). Respiratory signs ranging from increased or forced respiration to a harsh, paroxysmal, nonproductive cough (a loud barking cough) or open-mouth breathing were prominent, especially if animals were forced to move. Neurological signs, such as muscle fasciculation, rear leg weakness, and varying degrees of spastic paresis and uncoordinated gait when driven and hurried were observed. Clinical signs may progress to lateral recumbency accompanied by thrashing of the limbs or tetanic spasms. Mortality in this age group is low (<5%). Animals that die may show blood-tinged discharge from the nose. However, infection is frequently asymptomatic.

Acute death was occasionally observed in sows and boars, either with no prior clinical signs or with 24 hours of onset of clinical disease. Sudden death in sows and boars is considered an unusual outcome of Nipah virus infection. A bloody nasal discharge was frequently apparent after death. Neurological signs were frequently observed, which included head pressing, agitation displayed as biting the bars of the pen, tetanic spasms or seizures, and an apparent pharyngeal muscle paralysis, resulting in the inability to swallow, frothy saliva, and drooping of the tongue. Abortions were also reported.

Suckling pigs showed open-mouth breathing, leg weakness with muscle tremors, and neurological twitches. Mortality was high in this age group, but whether from primary disease or because of disease in the sow was not clearly established.

Under experimental conditions, the majority of infected piglets did not develop apparent disease, although they all showed a transient increase in rectal temperatures between 3 and 6 days postinoculation. Some developed mild respiratory signs and about 20% of piglets that were inoculated nasally or subcutaneously developed CNS signs requiring euthanasia (Berhane et al. 2008; Middleton et al. 2002; Weingartl et al. 2006; Weingartl et al. 2005). However, clinical signs observed after the first week postinoculation were suspected to be partially due to secondary infection and bacteria such as Enterococcus faecalis, Streptococcus suis, and Staphylococcus hyicus were isolated from piglets with different signs (Berhane et al. 2008; Middleton et al. 2002; Tanimura et al. 2004).

Lesions
The most common clinical sign in pigs is respiratory disease characterized by severe coughing. However, there is no pathognomonic gross pathology, and concurrent pulmonary disease from other causes may be present. Macroscopic lesions were consistently observed in lungs and meninges of pigs infected with Nipah virus, both naturally and experimentally. Mild-to-severe pulmonary consolidation was also observed in subclinical cases, as well as distended interlobular septa on cut surfaces. Occasionally, dark depressed lobules were notable, mostly in the diaphragmatic lobes. In the outbreak, bronchi and trachea of pigs were frequently filled with exudate or frothy fluid sometimes tinged with blood. Enlarged lymph nodes, most frequently bronchial, submandibular, and mesenteric were also observed. In cases with neurological disease, the meninges were congested and edematous. In experimentally infected animals, Nipah virus-related gross pathological lesions resolved by around 3 weeks postinoculation (Berhane et al. 2008; Daniels et al. 2000; Hooper et al. 2001; Middleton et al. 2002; Nor et al. 2000; Shahirudin 2001; Weingartl et al. 2005).

Microscopically, the presence of multinucleated alveolar macrophages and syncytial cells in respiratory epithelium may indicate a Nipah virus infection. Infrequently, syncytia may be found in the endothelium of small blood and lymphoid vessels (Figure 41.6), mainly in lung, spleen and lymph nodes. In the absence of fresh tissue, this suspicion could be confirmed by immunohistochemistry. Syncytia in the endothelium stain especially strongly for Nipah virus antigen (Tanimura et al. 2004).

Other microscopic lesions in lungs include interstitial pneumonia with peribronchial, peribronchial, and perivascular infiltration of mononuclear cells, and vasculitis often with fibrinoid necrosis. In some instances, alveolitis with infected macrophages, cellular debris (also in bronchioles), and proteinaceous fluid has been observed. Viral antigen has been detected in the endothelial cells, in the smooth muscle cells of the tunica media, as well as in macrophages and bronchial and, less frequently, alveolar epithelial cells (Berhane et al. 2008; Hooper et al. 2001; Middleton et al. 2002; Tanimura et al. 2004; Weingartl et al. 2005).

41.6. Submandibular lymph node of a pig experimentally infected with Nipah virus showing multinucleated syncytial cells with pyknotic nuclei (arrows) (bar = 20µm, hematoxylin and eosin [H&E]). Photo courtesy of Dr. Carissa Emburry-Hyatt.
In cases with neurological disease, a nonsuppurative meningitis or meningoencephalitis was observed more frequently than encephalitis. Prominent perivascular cuffing was observed in the meninges and sometimes also in the brain (Hooper et al. 2001; Middleton et al. 2002; Weingartl et al. 2005). Virus antigen was detected in neurons and glial cells, and endothelial and smooth muscle cells of the tunica media of blood vessels, infiltrating mononuclear cells, ependyma, choroid plexus, and the meninges (Hooper et al. 2001; Middleton et al. 2002; Weingartl et al. 2005).

Important pathological changes occur in the lymphoid organs and tissues. Beside the presence of syncytia in endothelial cells of blood and lymphoid vessels and a vasculitis, necrosis of lymphocytes and their depletion from the lymph nodes are another important feature of Nipah virus infection in swine. Nipah virus antigen is detected mainly in endothelial cells, multinucleated giant cells, and dendritic cells (Berhane et al. 2008; Hooper et al. 2001; Middleton et al. 2002; Weingartl et al. 2005). (Figure 41.7). Other organs, such as the kidney (Middleton et al. 2002; Tanimura et al. 2004), can also be affected, most likely due to vascular damage, but the extent and frequency of lesions are much less significant than those described for lung, brain, lymph nodes, and spleen.

**Diagnosis**

Specimen collection and diagnostic assays available for Nipah and Hendra viruses are reviewed elsewhere (Daniels and Narasiman 2008). Nipah virus is a BSL4 agent and extreme care must be taken in the diagnosis of cases suspected to involve Nipah virus. Some aspects of laboratory diagnosis should only be conducted in a BSL4 laboratory. Conduct antemortem and postmortem sampling in a manner that will exclude human contact with body fluids from affected animals. Respiratory protection is advisable (Daniels et al. 2000).

Nipah virus may be suspected if a clinical syndrome consistent with Nipah virus disease occurs on a pig farm in an area where there is an opportunity for contact with pteropid bats. The list of differential diagnoses should include those that cause sudden death in boars and/or sows; reproductive failure characterized by abortion; respiratory disease in any age group characterized by severe coughing; and neurological disease characterized by tremors, muscle fasciculation, and agonal thrashing of the limbs or tetanic spasms in lateral recumbency.

Nipah virus does not produce pathognomonic clinical signs and clinical signs vary with the age and reproductive status of the animals affected. Thus, the differential diagnosis may vary with the age and class of pigs affected. In addition, secondary infections with other microorganisms can contribute to a wide range of nonspecific clinical signs. Animals coinfected with other pathogens, such as classical swine fever virus and opportunistic bacteria, are likely to display clinical signs most compatible with the coinfectants (Berhane et al. 2008). This may partially explain how it was possible for Nipah virus to be present, but unidentified, in pigs in Malaysia since 1996, as indicated in retrospective studies of archival specimens (Chua et al. 2000).

Nipah virus is a high-risk zoonotic agent and diagnostic procedures that do not amplify the virus and minimize the handling of infectious material are preferred. Detection of viral RNA by reverse transcription-polymerase chain reaction (RT-PCR) is rapid, usually more sensitive than virus isolation, and offers greater biosafety for the operator. Real-time reverse transcription-polymerase chain reaction (qRT-PCR) assays specific for Hendra virus or Nipah virus have been described (Guillaume et al. 2004; Li et al. 2010). The high sensitivity of qRT-PCR allows the reliable detection of viral RNA in a wide range of tissues and serum, making serum a sample suitable for both virus and antibody detection. It is likely that a retrospective confirmation could be achieved even with formalin-fixed tissues (Chua et al. 1999; Hooper and Williamson 2000).

Demonstration of viral antigens in formalin-fixed postmortem samples is also a rapid and safe option for confirmation of a diagnosis. Nipah virus antigens have been demonstrated in formalin-fixed tissues, especially the lung and upper airways, meninges, spleen, olfactory bulb, trigeminal ganglion, lymph nodes, and kidney (Daniels 2001; Middleton et al. 2002; Weingartl et al. 2005).

When virus isolates are required, either for confirmation of a diagnosis or for additional research, procedures should be conducted in a laboratory with a high level of biosecurity. Nipah virus has been isolated from lung, spleen, kidney, tonsil, meninges, and lymph...
nodes collected postmortem and from nasal and throat swabs, cerebrospinal fluid, or urine collected from live animals (Daniels 2001; Middleton et al. 2002; Weingartl et al. 2005). Nipah virus is detectable in oral and nasal swabs at 2 days postinoculation, prior to the onset of clinical signs, and shedding can last up to 3 weeks postinfection. Vero cells are preferred for virus isolation. Cytopathology characterized by the formation of large syncytia may be observed in 2–3 days, but several passages of at least 5 days are usually conducted before declaring an isolation attempt unsuccessful.

ELISA is the preferred method for routine screening for antibody because of the availability of inactivated or recombinant reagents and ELISA’s high throughput capacity (Daniels 2001). The potential for serum samples to contain an infectious virus should not be overlooked. Testing of suitable numbers of pig sera by ELISA provides a rapid method for evaluating the presence of Nipah virus in swine populations. Any ELISA reactors should be confirmed by VN (Daniels 2001), but because VN requires the use of live virus in cell culture, secure laboratory facilities should be used.

Immunity
Neutralizing antibodies provide protection against Nipah virus disease in pigs (Weingartl et al. 2006) and appear 7–10 days postinoculation, reaching maximum titers (∼1:1280) by 14–16 days postinoculation (Berhane et al. 2008; Middleton et al. 2002). The humoral arm of the immune response, for example, the development of neutralizing antibodies, does not appear to be affected and the majority of pigs recover from Nipah virus infection. There is currently no evidence that persistent infections occur, although the possibility cannot be excluded.

Prevention and Control
Nipah virus is a dangerous zoonotic agent, and treatment of affected animals should not be considered. In countries where pteropid bats may be a reservoir of Nipah virus, pig farms should be managed to ensure that infection cannot reach the population (Choo 2001; Daniels 2001). That is, farms should be devoid of fruit trees and other vegetation that may attract the bats to the proximity of the animal housing. Strict biosecurity should be in place to preclude the importation of infected animals. Herd replacements should be isolated and quarantined prior to introduction.

Control measures in cases of confirmed Nipah virus infection will reflect its extreme hazard as a zoonotic agent. It is essential to prevent the spread of infection among domestic animals and to preclude the possibility of infection of humans. Rapid eradication is the recommended response. This was achieved in Malaysia by quarantine of infected premises and the culling of all susceptible animals on those premises. It is essential that quarantine and associated movement controls be enforced during this period of culling (Mangat 2001).

REFERENCES


OVERVIEW

The Picornaviridae family is one of five families in the order Picornavirales, the others being Dicistroviridae and Iflaviridae (both infecting invertebrates), Marnaviridae (infecting algae), and Secoviridae (infecting plants) (Le Gall et al. 2008). There are currently 12 genera in the Picornaviridae, seven of which contain viruses that infect pigs: Aphthovirus, Cardiovirus, Enterovirus, Kobuvirus, Sapelovirus, Senecavirus, and Teschovirus (Table 42.1; Figure 42.1; Knowles et al. 2011).

Picornaviruses for which genome sequence data have become available, but which have not yet been assigned to specific taxa, include two new groups of human picornaviruses, as well as picornaviruses of bats, seals, turkeys, wild birds, reptiles, and fish. None of these are known to infect pigs (Knowles et al. 2011).

Picornaviruses infect vertebrate hosts and enter host cells using receptor-mediated endocytosis or possibly, for the enteroviruses, by direct entry of RNA across the plasma membrane following changes induced in the virus particle by receptor binding. They replicate in the cytoplasm of infected cells.

Molecular Biology

Picornaviruses are small, roughly spherical viruses with a protein shell (capsid) ~30nm in diameter (Ehrenfeld et al. 2010). The capsid contains a single copy of positive-sense RNA ~7–9kb in length with a small, virus-encoded, polypeptide (VPg) covalently linked to the 5’ terminus (Figure 42.2).

The picornavirus genome includes two untranslated regions (UTRs), one preceding and one following the single open reading frame (ORF). Various RNA elements in the long (500–1300 nucleotide) 5’ UTR are involved in controlling virus replication and the initiation of viral protein synthesis and include an internal ribosome entry site (IRES). There are four different IRES types (I to IV; Belsham 2009). These differ in their secondary structure and mechanism of action, for example, requirement for cellular factors. IRES type IV is also found in some members of the virus family Flaviviridae, such as hepatitis C virus and classical swine fever virus. The 3’ UTR is usually much shorter (~35–300 nucleotides) and also contains structures involved in virus replication. It is followed by a poly (A) tail.

The ORF is translated into a polyprotein that is usually processed by one or two virus-encoded proteases. Two or three primary cleavages take place during translation, while others occur later to produce the various mature proteins.

The leader polypeptide (L) is not present in all genera; for example, there is no leader protein in the enteroviruses, and it is quite diverse among the other picornavirus species. In some, including aphthoviruses and erboviruses, it is a papain-like cysteine proteinase. The P1 (or P1-2A) capsid precursor includes the three structural proteins (VP0, VP3, and VP1) that form the basic capsid subunit (protomer). Five of these subunits come together to form a pentameric unit and 12 of these can self-assemble to form the complete capsid. In most genera, when the RNA is packaged, there is maturation cleavage within VP0 that produces VP4 (located on the inside of the capsid) and VP2.

The P2 region consists of 2A (except when 2A is part of the P1-2A precursor), 2B, and 2C. 2A is highly variable between picornavirus species, not only in primary
of the processing intermediates, for example, 3CD, also have distinct functional roles.

### Diseases of Picornaviruses

The range of diseases caused by picornaviruses ranges from acute and sometimes fatal paralysis—for example, poliomyelitis in humans—to mild respiratory disease—for example, rhinoviruses in humans and equine rhinitis viruses in horses—to inapparent infections in many hosts. The principal picornaviral diseases of pigs are acute vesicular disease caused by foot-and-mouth disease virus (FMDV) and swine vesicular disease virus (SVDV); acute fatal myocarditis caused by encephalomyocarditis virus (EMCV) and FMDV; reproductive failure caused by EMCV; and teschovirus encephalomyelitis caused by porcine teschovirus (PTV).

It has been recognized in the last few years that some recently characterized picornaviruses are infectious for swine, including Seneca Valley virus (SVV) and porcine kobuvirus (PKV). Nucleotide sequence analyses (Krumholz et al. 2002; Zell et al. 2001) showed that the viruses initially described as “porcine enteroviruses” (PEVs) actually included a variety of different types of picornaviruses, many of which are teschoviruses (PEV-1–7 and 11–13). The group also includes two true enteroviruses (PEV-9 and PEV-10) belonging to the species *Porcine enterovirus B*. PEV-8, formerly classified in the species *Porcine enterovirus A*, now belongs to...
42.1. Midpoint-rooted neighbor-joining tree showing the relationships between the polymerase sequences of 37 picornaviruses. The tree was constructed using MEGA 4.0 (Tamura et al. 2007). The evolutionary distances were computed using the JTT matrix-based method. Formally recognized taxa are indicated in bold italics. Picornaviruses that infect pigs are highlighted in gray.

42.2. Alternative picornavirus genome organizations showing the UTRs, polyprotein coding region, and the location of the mature polypeptides. The presence and form of the L and 2A proteins are quite variable between genera.

genus Sapelovirus and is known as porcine sapelovirus (PSV). SVDV is also a true enterovirus, but is not included among the PEVs because it is closely related to the human coxsackievirus B5.

Diagnostic reverse transcription-polymerase chain reaction (RT-PCR) assays that distinguish between the different types of enteric porcine picornaviruses have been described (Krumbholz et al. 2003; Palmquist et al. 2002). An unexpected cross-reaction of specific primers designed to recognize the 5′ UTR of the teschoviruses with PSV was observed by Palmquist et al. (2002), but the products detected were of a different size. Recent
work has shown that the teschoviruses and PSV all have a shared type of RNA structure within the 5′ UTR (Chard et al. 2006) termed the IRES, which is required for the initiation of protein synthesis on the viral RNA. There are four different classes of IRES within the picornavirus family (Belsham 2009). The IRES elements within the teschoviruses and PSV are of the same class and have some highly conserved motifs that are presumably responsible for the cross-recognition.

The clinical importance of SVV, PSV, PKV, and PEVs 9/10 is not well characterized, but there is some evidence that they are pathogenic for pigs, as discussed later in the chapter.

FOOT-AND-MOUTH DISEASE VIRUS

Relevance

Foot-and-mouth disease (FMD) is a severe, clinically acute, vesicular disease of cloven-hoofed animals, including domesticated and wild swine and ruminants (Alexandersen and Mowat 2005; Thomson 1994). Because of its potential for rapid and extensive spread within and between countries and its severe economic consequences, including its effect on trade (Leforban and Gerbier 2002), FMD is on the World Organization for Animal Health (OIE) list of diseases.

FMD has been known in Europe for many centuries and was probably one of the diseases described by the Italian physician Hieronymi Fracastorii in his work *De Contagione et Contagiosis Morbis et Eorum Curatione* in 1546 (English translation in Wright 1930). FMD spread from Europe to the Americas in the 1860s, initially to Argentina, with subsequent spread from there (Olascoaga 1984). At some time, FMD has been present in most countries and, with the exception of North and Central America, Australia, New Zealand, Chile, and the European Union (EU), remains endemic to many areas of the world.

The scientific study of the disease and FMDV, its etiological agent, began at the end of the 19th century when Loeffler and Frosch demonstrated that a filterable agent could cause FMD (Brown 2003). The existence of FMDV serotypes was discovered in the 1920s (serotypes O, A, and C), followed by the recognition of Southern African Territories (SAT) 1, 2, and 3 serotypes in the 1940s, and serotype Asia 1 in the 1950s.

In 1947, Frenkel showed that large amounts of FMDV could be produced in cattle tongue epithelium harvested at slaughter. This discovery formed the basis of the vaccination programs initiated in Europe in the 1950s. These programs intensified in the 1960s and 1970s when continuous cell cultures made it possible to produce the large amounts of FMDV needed to produce many million doses of vaccine per year (Brown 2003; Sutmoller et al. 2003). Vaccination programs primarily focused on cattle, except when particularly aggressive strains of type C, and to a lesser extent type O, made it necessary to vaccinate swine, as occurred in the 1960s (Sutmoller et al. 2003).

Thousands of outbreaks of FMD occurred in Europe in the 20th century, with larger epidemics every 5–10 years (Leforban and Gerbier 2002). This situation continued until 1970–1973, after which the situation improved significantly due to the availability of high quality vaccines. FMD was eradicated from the EU and, in 1991, prophylactic vaccination was stopped.

In North America, FMD was last reported in 1929 in the United States, in 1952 in Canada, and in 1954 in Mexico. Stamping out was used to eradicate the infection in the United States and Canada, while a combination of vaccination and stamping out was used in Mexico (Sutmoller et al. 2003). Despite significant vaccination efforts, FMDV serotypes O and A are still present in several countries in South America (Sutmoller et al. 2003).

Etiology

FMDV, a member of genus *Aphthovirus* in the family *Picornaviridae* (Belsham 1993), is a nonenveloped, icosahedral virus, 26–30 nm in diameter, containing a single positive-sense RNA ~8300 nucleotides in length. The complete viral capsid consists of 60 copies of each of the four structural proteins (VP1-4), with many critical determinants for infection and immunity inherent in the VP1 protein.

FMDV can be propagated in continuous cell cultures such as baby hamster kidney (BHK) cells (Mowat and Chapman 1962) and IB-RS-2 pig kidney (PK) cells, as well as porcine, bovine, ovine, and caprine primary cells. Kidney cells or primary bovine thyroid (BTY) cells are particularly susceptible to FMDV infection (Alexandersen et al. 2003c; Snowdon 1966). Field samples are most successfully cultured in porcine or ruminant cells, depending on the species from which the sample is derived. Thus, the usual diagnostic procedure for swine samples is to inoculate both swine and ruminant cell cultures, while samples from ruminants may either follow the same procedure or only be inoculated onto ruminant cells. FMDV can also be grown in unweaned mice (Skinner 1951).

Role in Public Health

Although there are historical sources in the scientific literature that report the occurrence of FMD in humans, the evidence indicates that FMDV is not zoonotic and is not a public health concern. In the vast majority of human cases described, the appearance of blisters or vesicular lesions was due to other agents, including viruses such as the human enteroviruses in the *Picornaviridae* family that cause “hand, foot, and mouth disease” in humans. There have also been reports of mild clinical signs of disease in humans after the ingestion of untreated milk or after close contact with acutely infected animals.
Proof of causality requires the isolation and identification of the agent, its successful transmission to a known susceptible species, and subsequently serological evidence in the infected individual. Most of the reports of FMD in people lack this evidence and probably did not involve FMDV. The one case in which FMDV was confirmed (Armstrong et al. 1967) involved a man that lived on an FMD-affected farm in the United Kingdom in 1966 and consumed milk from a cow that later showed signs of FMD. Vesicular lesions developed in his mouth, on his hands, between his toes, and FMDV serotype O was isolated. His serum, collected 30 days after infection, had high type O antibody titers. Thus, there was evidence that this man had been infected with FMDV. In contrast, in the U.K. 2001 FMD epidemic, in which 2030 outbreaks occurred among livestock, none of the 15 human suspect cases was positive for FMDV by RT-PCR (Turbitt 2001). Thus, the infection of humans with FMDV cannot be totally excluded, but it is extremely rare and in the one documented case had only mild and transient consequences (Bauer 1997; Donaldson and Knowles 2001).

It follows that human infection does not have any significant role in the epidemiology of FMDV. However, people may play a significant role in passive transport of the virus from infected animals or contaminated surfaces to susceptible animals. Humans may even passively carry the virus in their respiratory tract for a day or more (Sellers et al. 1970; Sellers et al. 1971). Thus, the role of people in FMDV transmission is an important consideration in control programs.

**Epidemiology**

**Geographic Distribution.** FMDV infection is endemic in large areas of Africa (mainly the SAT types, but also type O and A), Asia and the Middle East (types O, A, and Asia 1), and South America (types O and A; type C may have disappeared). Although considerable effort has been expended on vaccination programs in South America and countries or regions have achieved the official OIE status “Free of FMD” with or without vaccination, the infection is still present in several countries.

The virus has shown an extraordinary ability to cross international boundaries and cause epidemics in previously free areas, as illustrated by the 2001 epidemic in the United Kingdom and continental Europe, as well as the outbreaks in the year 2000 in South Africa, Japan, and South Korea (Knowles et al. 2001). It has been estimated that the direct cost of the U.K. epidemic in 2001 was ∼$5 billion U.S. dollars (USD) and the indirect costs from the combined losses of agricultural exports and tourist trade an additional $10 billion USD (Alexandersen et al. 2003c).

Introductions into FMDV-free countries after 2001 included Japan, with a high number of outbreaks of serotype O in 2010, and South Korea, which was able to control the year 2000 and 2002 outbreaks, but experienced outbreaks of FMD in 2010. More significantly, both serotype A and serotype O appeared in South Korea within a period of a few months. Taiwan experienced an incursion of a porcinephilic strain of serotype O in 1997. Despite a massive vaccination effort and hopes of stopping vaccination in 2009, Taiwan is still not free of FMDV and observed new outbreaks in swine with this strain in 2010. These examples show that unless global eradication is achieved, FMDV will continue to spread and countries will need to be prepared.

**Susceptible Species.** With minor exceptions, FMDV affects members of the order Arteriodactyla (cloven-hoofed animals), including domestic and wild ruminants and pigs (Thomson 1994). When considering the hosts of FMDV, it is important to distinguish between species that play a significant role in the epidemiology of the disease versus those that do not. The latter category would include species that only play a role under certain conditions or are only susceptible to FMDV under experimental conditions. Because they are susceptible to FMDV, these species cannot be excluded as epidemiological risks, but under field conditions, they appear to be of little or no importance (Alexandersen and Mowat 2005).

The species of greatest significance in the field include cattle, pigs, small ruminants (sheep and goats), and the water buffalo, particularly in Asia and South America. The African buffalo plays an important role as the natural maintenance host of the SAT serotypes in Africa, but other wildlife such as impala, kudu, antelopes, mountain gazelle, wild boar, and so on, may also be involved in the natural epidemiology of FMDV (Thomson et al. 2003).

Species that may present some level of risk or may contribute to the transmission of virus under certain conditions include the North American bison, elk, deer, llamas, alpacas, and Bactrian camels, but not dromedary camels (Alexandersen and Mowat 2005; Alexandersen et al. 2008; Larska et al. 2008; Rhyan et al. 2008), as well as other animals of the order Arteriodactyla. Interestingly, the Indian elephant, but not the African elephant, is in this category (Bengis et al. 1984; Hedger 1972; Hedger and Brooksby 1976; Howell et al. 1973; Piragino 1970). Although these species do not appear to play an important role in the wild, they may present a risk if they come into close contact with livestock, for example when kept in zoos or under crowded conditions, such as deer farms.

A variety of other species may be infected with FMDV, but not routinely involved in the epidemiology of FMDV. However, all animals, even highly resistant animals such as horses and carnivores, can serve as mechanical vectors if they become contaminated with
the virus and subsequently come in close contact with susceptible livestock.

The susceptibility of small mammals to infection has been extensively researched (reviewed in Alexandersen and Mowat 2005). In brief, the coypu, water vole, mole, and the brown rat are susceptible to experimental FMDV infection, but like other small rodents, for example, mice, are unlikely to play a role in the epidemiology of FMD because FMDV infection is usually rapidly fatal in these species. Conversely, rats appear to survive experimental infection and could migrate considerable distances. For this reason, rodent control is considered an important component of efficient FMDV outbreak control. All the same, there is nothing to suggest that rodents could maintain the disease or cause recurrence of FMDV after it is eradicated from domestic livestock.

FMDV infection in wild Australian species was mild and clinical signs were rare, although viremia was detected and some of the animals developed antibodies. In contrast, the tree kangaroo developed tongue lesions (Snowdon 1968), and lesions were also observed in a kangaroo in a zoo in India (Bhattacharya et al. 2003). However, what role Australian wildlife species might play if FMDV were introduced into the region cannot be determined experimentally because many species that are susceptible under experimental conditions play little or no epidemiological role under field conditions.

Other species shown to be susceptible to FMDV under experimental conditions include mice, guinea pigs, rabbits, cats, dogs, mink, monkeys, snakes, birds, chickens, and embryonated eggs (Cottral and Bachrach 1968; Hyslop 1970; Skinner 1954). Interpretation of these studies must be done in the context of the experimental design. For example, in mice, susceptibility was highly dependent on age (only very young mice being highly susceptible), and the genotype of the strain of mice (Skinner 1951; Skinner 1953). Moreover, the virus had to be directly injected into the animal. Infection in mice did not produce vesicles, but produced infection and inflammation of the skeletal muscles (Platt 1956) or in older mice, the pancreas (Platt 1959). Infection of the other species listed above (Henderson 1949) required multiple passage of the virus to adapt the virus to the host. Consequently, infection of these species is possible, but they are not likely to play a role in the field because infection required forced passage of high FMDV doses, leading to virus adaptation to the new vertebrate species. Often, the virus was then less fit for the original host.

Hedgehogs have long been given a special status in regard to FMDV. This is because the hedgehog is highly susceptible to experimental infection, can easily transmit the infection to other hedgehogs or livestock, and appears to excrete the virus in respiratory exhalations when infected. Although there is a report describing lesions and isolation of FMDV from hedgehogs under field conditions (McLauchlan and Henderson 1947), hedgehogs actually appear to play no role in the epidemiology of FMD. Nevertheless, during an epidemic it may be wise to exclude the access of hedgehogs to susceptible livestock.

**Transmission.** Under field conditions, pigs usually become infected with FMDV by direct or indirect contact with infected animals, contaminated fomites, and occasionally, via consumption of FMDV-contaminated products, for example, waste food. When animals are in close proximity, the movement of FMDV in aerosols and secretions from infected animals to the respiratory tract of recipient animals is probably the most common form of transmission. Intact skin provides good protection against FMDV, but abrasions or cuts in the skin permit transmission by physical contact with excretions or secretions containing infectious virus. Such skin lesions are not uncommon in swine kept on concrete floors or as a result of aggressive interactions between animals.

Transmission of virus may occur indirectly via contact with FMDV-contaminated personnel, vehicles, products, and so forth. Husbandry or disease control activities—for example, physical handling of animals, tail or tooth clipping, vaccination, clinical examination, or collecting blood samples—increase the risk of indirect spread of the virus. Transmission has resulted from the use of contaminated instruments, medications, and FMD vaccines containing live virus before optimal inactiants were used (Beck and Strohmaier 1987).

Our understanding of the process of FMDV transmission comes primarily from experimental studies attempting to simulate natural exposure by direct or indirect contact, contaminated products or fomites, and virus aerosols. The infective dose for different routes can be calculated in such studies, but the estimates are invariably bounded by the constraints and practical considerations of performing FMDV studies under appropriate biosecurity measures (Alexandersen et al. 2003c).

Intradermal or subdermal injection of virus into the tongue, coronary bands, and heel bulbs, or application of a suspension of virus to damaged (scarified) skin, targets the highly susceptible epithelial regions (Alexandersen et al. 2003c) and simulates natural infection through damaged skin. The infectious dose by this route may be $\leq 1 \times 10^2$ tissue culture 50% infective doses (TCID$_{50}$). By comparison, a single infected animal may excrete $1 \times 10^{10}$ TCID$_{50}$ or more per day at the peak of excretion, most of it in vesicular fluid, saliva, nasal fluid, and other excretions (Alexandersen et al. 2003c). Direct entry of the virus into the circulatory system by intravenous inoculation also results in infection, but appears to be less efficient and more variable than the
routes targeting epithelia. Intramuscular inoculation is relatively inefficient and requires a dose of $\geq 1 \times 10^4$ TCID$_{50}$ (Burrows et al. 1981; Donaldson et al. 1984).

Several recent outbreaks of FMD have been linked to virus in contaminated human food waste subsequently fed to animals. For example, the South Africa 2000 and U.K. 2001 epidemics probably involved feeding unheated food waste to pigs (Alexandersen et al. 2003a; Knowles et al. 2001). The estimated infectious dose by oral exposure for pigs and ruminants is $1 \times 10^4$–$10^5$ and $1 \times 10^5$–$10^6$ TCID$_{50}$ per pig per day, respectively (Sellers 1971). It is conceivable that abrasions or damage to the epithelium of the buccal cavity by bone or other objects commonly present in waste food could reduce the dose required to achieve infection by the oral route.

Transmission via Aerosols. Airborne transmission of FMDV is a dynamic, complex process affected by the species of animals (usually swine as the source and cattle or sheep as the recipient species), the number of animals, topography of the area, and the meteorological conditions.

Airborne transmission of FMDV over significant distances becomes a significant risk when large numbers of pigs are infected because they respire large quantities of virus. Pigs aerosolize up to $1 \times 10^6$ TCID$_{50}$ per pig per day for most strains of FMDV, although up to $1 \times 10^8$–$10^9$ TCID$_{50}$ per pig per day has been recorded. In contrast, ruminants aerosolize less virus in their respirations ($1 \times 10^4$–$10^5$ TCID$_{50}$ per day), but are highly susceptible to infection by inhalation. Ruminants can be infected experimentally by airborne exposure to low concentrations of FMDV ($1 \times 10^3$ TCID$_{50}$), whereas pigs require airborne exposures of more than $1 \times 10^5$ TCID$_{50}$ and infection only occurs if the virus is delivered at a high concentration (Alexandersen and Donaldson 2002; Alexandersen et al. 2003b,c; Donaldson 1986; Donaldson et al. 1987, 1970). Therefore, the pattern of airborne spread of FMDV is most often from infected pigs to cattle and sheep located downwind.

Long distance airborne spread requires atmospheric conditions that maintain the infectivity of the virus and keep the aerosol plume (“virus cloud”) intact. For example, FMDV infectivity is dependent on relative humidity above 55%. Sunlight has little or no direct effect on infectivity. Stable atmospheric conditions with continuous steady or slight wind, cloud cover, and a level topography, for example, large tracts of water, tend to preserve the virus plume and increase the likelihood of airborne transmission (Alexandersen et al. 2003c; Donaldson et al. 2001; Gloster and Alexandersen 2004; Gloster et al. 2005). In contrast, air turbulence from wind, topography, trees, or buildings disperses the virus and reduces the probability of transmission.

Models to predict airborne spread of FMDV were used successfully in the United Kingdom in 1981 and 2001, and in Italy in 1993 (Alexandersen et al. 2003a; Donaldson et al. 1982; Gloster et al. 2003; Maragon et al. 1994). Current models suggest that most isolates of FMDV are unlikely to spread more than 20 km via aerosol, even under a “worst-case” scenario, that is, meteorological conditions optimal for maintaining the virus plume, the virus originating from a large population of infected pigs, and cattle located downwind of the pigs. However, specific factors can significantly affect the potential distance of airborne transmission. For example: (1) Some strains of FMDV, in particular C Novile, which appears to be excreted at very high levels, may have the potential for longer spread under some conditions. (2) If the source farm contains large numbers of infected cattle or sheep excreting maximal levels of FMDV, rather than pigs, the predicted distance of airborne spread would be less than 2 km (Donaldson et al. 2001). (3) Because pigs are more resistant to airborne FMDV, spread to pigs is only likely to occur at distances of 200 m or less (Alexandersen et al. 2003b,c; Donaldson et al. 2001). Thus, distances for aerosol transmission should be considered estimates, not absolutes, as some of the important parameters in the transmission model are variable or not well understood.

Aerosols are also created by splashes from infected milk and urine, by the use of high-pressure hoses to clean FMDV-contaminated animal housing and equipment, and by the process of applying infected slurry on pastures. However, the infectivity of such aerosols is probably much lower than that of aerosols exhaled by infected animals.

Duration and Routes of Shedding. All secretions and excretions from infected animals contain infectious virus and some contain significant titers before the development of clinical signs. Thus, saliva, nasal and lachrymal fluid, milk, and respiratory exhalations may contain virus during the prodromal period. Urine and feces also contain virus, but to a lesser extent. It appears that feces contain only small amounts of virus (Parker 1971), but are likely to be contaminated further by desquamated lesion material, vesicular fluid, and saliva. Since preputial lesions are sometimes present, it is possible that these are the source of infectious FMDV in urine. In sheep, virus could be detected in respiratory exhalations 1–2 days before the appearance of clinical signs (Alexandersen et al. 2002b; Sellers and Parker 1969). In contrast, the peak of airborne viral excretion in cattle and pigs occurred after early generalized lesions had developed (Alexandersen et al. 2003b,c). Virus is also excreted in milk and semen (Burrows 1968; McVicar et al. 1977) from shortly before clinical signs appear and through the clinical phase, in a pattern that largely mirrors the profile of viremia. Large amounts of virus are excreted in vesicular fluid, in desquamated vesicular epithelium, and in saliva (Hyslop 1965; Scott et al. 1966).
is similar to that in cattle, but the amounts of virus and viral RNA recovered in the blood and breath are higher in pigs (Alexandersen et al. 2001, 2003b).

A sharp decline in viral excretion and load occurs around days 4–5 of clinical disease, when a significant antibody titer can be detected. However, although all secretions and excretions (other than oropharyngeal fluid in ruminants) are free of detectable infectivity at 10–14 days postinfection, infectious virus already excreted during the preclinical and acute clinical phases is stable in the environment for weeks. Although tissues are also usually free from infectious virus by this time after infection, low levels of viral RNA may be found

The overall pattern of viral excretion for pigs is illustrated in Figure 42.3. Airborne viral excretion coincided with the appearance of vesicular lesions and occurred within the viremic phase. Viral RNA was recovered in nasal swabs from inoculated animals soon after they developed viremia and probably reflected early production and excretion of virus. The detection of infectivity and viral RNA (Alexandersen et al. 2003b) in nasal swabs from contact animals up to 3 days before they showed signs of infection, as well as in animals after the viremic phase, probably represented background environmental virus that had been inhaled and trapped in the respiratory tract. The pattern of excretion by pigs is similar to that in cattle, but the amounts of virus and viral RNA recovered in the blood and breath are higher in pigs (Alexandersen et al. 2001, 2003b).

A sharp decline in viral excretion and load occurs around days 4–5 of clinical disease, when a significant antibody titer can be detected. However, although all secretions and excretions (other than oropharyngeal fluid in ruminants) are free of detectable infectivity at 10–14 days postinfection, infectious virus already excreted during the preclinical and acute clinical phases is stable in the environment for weeks. Although tissues are also usually free from infectious virus by this time after infection, low levels of viral RNA may be found

42.3. Graphs showing the time course of infection in pigs inoculated with FMDV O UKG 2001 virus (top) or kept in contact with such pigs (bottom). Time on the x-axes is given in hours. The mean levels of FMDV RNA (log_{10} genomes per milliliter) in serum samples (indicated as mean viremia) and in mouth and nasal swabs are shown together with the development of clinical signs (score of 0–6 scaled to fit on the 0–10 axis) on the left y-axes, and the log_{10} ELISA antibody titer and the body temperature (°C) on the right. Virus in breath is given in log_{10} TCID_{50}/h and the slopes are predicted, as only levels above approximately 2 log_{10} TCID_{50}/h can be measured with the methods used. Selected data on the concentration of infectious virus in serum samples, determined by virus titration in cell culture, are shown in addition. Reprinted from Alexandersen et al. 2003b with permission from Elsevier.
in lymph nodes and tonsils up to 4 weeks after infection (Zhang and Bashiruddin 2009).

**Persistence in the Animal.** Some ruminant species exposed to FMDV become carriers, irrespective of whether they are fully susceptible or immune as a result of vaccination or recovery from previous infection. The percentage of ruminants that become carriers under experimental conditions is variable, but averages around 50%. The virus titer in oropharyngeal samples from carriers is usually low and declines over time. The maximum reported duration of the carrier state in ruminants is species dependent: cattle, 3.5 years; sheep, 9 months; goats, 4 months; African buffalo, 5 years; and water buffalo, 2 months.

In contrast, pigs do not become carriers of FMDV and do not harbor the infectious virus for more than 28 days. FMDV has been found in relatively high concentrations in soft palate, tonsil, and pharynx in early infection in pigs infected by contact exposure (Alexandersen et al. 2001; Oleksiewicz et al. 2001), but there was no detectable virus and only a low residual level of viral RNA in lymph nodes and tonsils by 3–4 weeks after infection (Zhang and Bashiruddin 2009). Why FMDV persists in the pharyngeal region of ruminants, but not of pigs, is unknown.

**Persistence in the Environment.** Quantitative data on the persistence of FMDV in the environment is sparse (Cottral 1969; Donaldson 1997; Sanson 1994) and results are not amenable to direct comparison because of differences in experimental design, procedures, and analyses. Typically, the kinetic curve for the decay of FMDV infectivity is biphasic: an initial steep decay curve followed by a prolonged, shallow tail. Residual virus may be remarkably resistant, especially in the presence of high concentrations of organic material.

FMDV can remain infectious in the environment for weeks and, for any point in time, the relevant question is whether there is sufficient residual infectivity in the material or environment to initiate infection in an exposed animal. The duration of virus infectivity in the environment will depend on the matrix, the initial concentration of the virus, and the ambient conditions, for example, relative humidity, temperature, pH, and so on.

There are isolated reports of the persistence of infectious FMDV for long periods, for example, on hay and straw for at least 20 weeks, and in fecal slurry for 6 months in winter (Hyslop 1970; Kindyakov 1938). More typical are reports of infectious FMDV for up to 4 weeks on cow’s hair at 18–20°C (64–68°F), up to 14 days in dry feces, up to 39 days in urine, 3 days on soil in summer, and up to 28 days in autumn.

Most FMDV strains are only stable at lower temperatures and at pH 7–8, becoming increasingly labile at pH values outside that range (Bachrach 1968; Bachrach et al. 1957). The acidity produced in carcass meat during rigor mortis in cattle will inactivate the virus, but such acidity is variable in pig meat. Furthermore, the pH in bone marrow, lymph nodes, certain organs, and offal does not decline during rigor mortis. Therefore, virus can be found in such material (especially if refrigerated or frozen) for a long time and may cause new outbreaks if fed to livestock as unheated waste food (Donaldson 1987).

Inactivation of virus by sunlight is indirect and occurs mainly through the effects of drying and temperature (Donaldson 1987; Donaldson and Alexander 2003). Drying will inactivate most, but not all of the virus, hence aerosolized virus is stable at relative humidity above 55–60%. The drying of fluids or organic material containing the virus will also inactivate a large proportion of the virus, but surviving virus may be more stable after drying, thereby creating a “tail” of infectivity.

Thus, the time that infectious virus will remain in the environment is difficult to predict and restocking after an outbreak has to be done with care and only after thorough disinfection of the premises. Additional information on the stability of infectious FMDV is available elsewhere (Bachrach 1968; Cottral 1969; Donaldson 1987; McColl et al. 1995).

**Susceptibility to Disinfectants.** FMDV is resistant to detergents and organic solvents, such as ether and chloroform, but can be inactivated by appropriate disinfectants and heat (Brown et al. 1963; Cunliffe et al. 1979; Dekker 1998; Fellowes 1960; Sellers 1968). Disinfectants that are either acidic or alkaline are highly effective, in particular, alkaline disinfectants such as sodium hydroxide and sodium carbonate that also disperse organic material. The dispersing effect can be further improved by adding a small amount of detergent (not in itself an effective disinfectant of FMDV) to further increase penetration of the disinfectant and the solubilization of organic material present. Oxidizing disinfectants such as sodium hypochlorite (bleach) and Virkon S (DuPont Animal Health Solutions, Sudbury, UK) are highly effective, as are aldehydes such as formaldehyde and glutaraldehyde.

**Pathogenesis**

The pharyngeal area is the primary site of infection, except when the virus directly enters via the cornified epithelia or the circulation by damage to the intact integument. In contact- or aerosol-exposed animals, virus may be detected in the pharynx 1–3 days before viremia or clinical disease (Alexandersen et al. 2003b,c). The dorsal surface of the soft palate, the adjacent nasopharynx, and perhaps the tonsil are sites of particular significance for initial virus entry and replication. Most of the oral cavity is covered by cornified/keratinized, stratified squamous epithelia, that is, a superficial layer
of dead cells. In contrast, the dorsal soft palate, the roof of the pharynx, and part of the tonsil is covered by special noncornified epithelia, and therefore, live cells are on the surface of the tissue. Easily accessible to FMDV in this anatomical location, these cells provide efficient virus entry, if the appropriate receptors are present.

FMDV entry into cells in vivo is believed to involve attachment of the viral capsid to host integrins, for example, alphaV-beta6, on the surface of target cells (Berryman et al. 2005; Duque and Baxt 2003; Jackson et al. 2000; Monaghan et al. 2005). Little is known about the role of FMDV receptors in the context of host range, target cells, or virus persistence.

After initial replication in the pharynx, or in the skin if the virus has entered directly through damaged integument, the virus spreads to the regional lymph nodes (Henderson 1948) and into the circulation (Alexandersen et al. 2003c). Viremia usually lasts 4–5 days. Viral seeding of secondary sites is followed by multiple cycles of viral replication and spread, with the main sites of viral amplification in the cornified epithelia of the skin, tongue, and mouth. Although vesicular epithelia contain the highest concentration of virus, apparently normal skin, both hairy and hairless, also contains significant amounts (Alexandersen et al. 2001). Experimental studies suggest that lymph nodes, as well as lymphocytes and macrophages, play little or no part in FMDV replication and that any virus present in lymphoid organs is produced elsewhere, that is, the epithelia of the pharynx, mouth, and skin (Alexandersen et al. 2003c; Burrows et al. 1981; Cottral et al. 1963; Murphy et al. 2010).

**Factors Affecting the Severity of Disease.** The severity of disease is affected by the virulence of the strain of FMDV involved, the inoculating dose (higher doses produce more severe clinical disease), and the physical activity of the animals (Alexandersen et al. 2003b; Murphy et al. 2010; Platt 1961; Quan et al. 2009; Quan et al. 2004). Little is known regarding host genetic factors associated with disease or resistance to disease, although indigenous cattle breeds in FMDV-endemic countries, for example, zebu breeds (*Bos indicus*), often exhibit less severe or no clinical signs compared to European breeds (*Bos taurus*). A difference among breeds has not been observed for pigs.

Activity associated with crowding, fighting, and damage to the skin and mucosa are more likely to lead to severe lesions. Trauma or intense physical stress increases the lateral or local spread of the virus to additional cells (Platt 1961), leading to larger foci of infected cells that, together with a physical separation of damaged tissue, appear as vesicles. The high vascularity of the coronary band, in combination with the severe local inflammatory response, may lead to cutaneous tension or stress and increased vascular permeability, both of which are likely to contribute to the development of visible vesicular lesions (Platt 1961).

While the temporal pattern of FMDV replication and the development of specific lesions is well described, relatively little is known about the pathogenesis of general, acute clinical signs, for example, fever, depression/dullness, and reduced feed intake. The severity of clinical signs is not necessarily correlated with the severity of the vesicular lesions. Although FMDV and SVDV cause very similar vesicular lesions in pigs, FMDV causes much more severe general clinical disease than SVDV. Possibly, FMDV causes a more severe proinflammatory host reaction, which is manifested as fever, general depression/dullness, reduced feed intake, occasional inability to maintain body temperature, and even mortality. These aspects of FMD, although not well understood, are probably the result of virus–host interactions extending beyond the observed acute cytopathology in virus-infected cells. Factors responsible may include cell death (releasing so-called danger signals), virus–antibody immune complex formation, complement activation, and the release of cytokines, prostaglandins, and acute phase proteins.

Interferons alpha and beta may have a role in the host response to FMDV infection and various FMDV isolates may differ in their ability to induce an interferon response (Chinsangaram et al. 1999; Cottral et al. 1966; Kothmann et al. 1973; McVicar et al. 1973; Seibold et al. 1964; Sellers 1963). For example, a large FMDV plaque size in porcine cells was associated with high virulence in pigs (Borgen and Schwobel 1964; Sellers et al. 1959). Studies on haptoglobin (Hofner et al. 1994) indicated that this acute phase protein is elevated in FMDV-infected cattle when viremia and clinical signs become evident, suggesting that the inflammatory response is activated.

**Clinical Signs**

**Incubation Period.** The incubation period for FMDV is highly variable and depends on the virus strain, exposure dose, the route of exposure, the animal species, and the husbandry conditions (Alexandersen et al. 2002b, 2003a,b,c; Quan et al. 2009; Quan et al. 2004). Under experimental conditions, the mean incubation period was 3.5 days for continuous, direct cattle-to-cattle contact and 2 days for intensive sheep-to-sheep contact. Pigs were readily infected by direct and intensive pig-to-pig contact exposure and had a mean incubation period of 1–3 days, but up to 9 days, depending on the intensity of contact (Alexandersen et al. 2002b, 2003b; Quan et al. 2004, 2009). These differences confirm the close relationship between exposure dose and length of incubation: the higher the dose or the intensity of contact, the shorter the incubation period. Under field conditions, the dose of FMDV will depend on several factors, including stocking density, that is,
intensive or extensive management, and how the animals are housed and handled.

The incubation period for farm-to-farm airborne spread ranges from 4 to 14 days (Sellers and Forman 1973), which is also the normal range for farm-to-farm spread by indirect contact. The incubation period for farm-to-farm spread resulting from direct contact may range from 2 to 14 days (Garland and Donaldson 1990). The period for within-farm spread is generally 2–14 days, but may be as short as 24 hours, especially in pigs and under very high challenge conditions. When spread is occurring within a herd or flock, the typical incubation period is 2–6 days, although under certain conditions it may be as short as 1 day or as long as 14 days.

**Clinical Signs.** FMD is characterized by an acute febrile reaction and the formation of vesicles in and around the mouth and on the feet (Figure 42.4). On handling, heat and local pain may be detected in the feet 1–2 days before vesicular lesions appear. Lameness or lesions may not be a consistent finding in all animals. Animals kept on soft bedding are less likely to develop severe foot lesions or show lameness.

Clinical disease is usually severe in pigs. The pain from foot lesions causes lameness, manifested by foot “flicking,” a tucked-up stance, reluctance to stand or walk, and inappetence. Early signs include acute lameness, reluctance to stand, adoption of a dog-sitting posture, depression, loss of appetite, and fever. Severely affected pigs become lethargic, huddle together, and have reduced or no feed intake (Kitching and Alexandersen 2002). Fever is often variable in degree and duration; it may be as high as 42°C (107.6°F), but often in the 39–40°C (102–104°F) range, and sometimes brief or close to normal. Body temperature in severely affected pigs may be below normal (Kitching and Alexandersen 2002). Consequently, body temperature in pigs can be used to support other clinical findings, but should not be used to exclude the possibility of FMDV infection.

**Morbidity and Mortality.** Mortality in adult animals is generally low, but may be high in young animals, especially piglets, due to acute myocarditis. Although mortality is usually not significant in animals with vesicular lesions, secondary bacterial infections in vesicular lesions may lead to chronic lameness, wasting, or mortality.

FMD may cause abortion in pregnant animals, but the pathogenesis has not been established. It is possible that the fever associated with FMDV infection may be a factor, but it is also possible that the virus crosses the placenta and infects the fetus (Ryan et al. 2008b; Ryan et al. 2007).

Stocking density may affect the expression of clinical signs at the population level. Thus, animals housed in confinement often show obvious clinical disease while, for example, infected sheep kept under extensive conditions may show no overt signs of infection (Alexandersen et al. 2003c). Similarly, vaccines will not prevent infection but may prevent development of
severe clinical disease; hence, allowing the infection to continue unrecognized for some time.

Lesions

Gross Lesions. Lesions often appear initially as blanched areas that subsequently develop into vesicles. They are found most consistently in and around the mouth, and on the feet, but may also be seen on the snout, teats, mammary gland, prepuce, vulva, and other sites. Lesions of the feet of swine may include the shedding of claws (“thimbling”). The accessory digits may be affected, as well as pressure points on the knees, hocks, and elbows, particularly if kept on concrete. Lactating sows often develop vesicles on the udder (Kitching and Alexandersen 2002). The expression of gross lesions may differ by virus isolate and species-specific virulence. For example, the O Taiwan 1997 strain caused severe lesions in pigs, but no cases were seen in ruminants (Dunn and Donaldson 1997).

If oral lesions are present in pigs, they are most often on the tongue, either far back on the dorsum or as tiny lesions at the tip. Vesicles on the feet of pigs are most often seen in the interdigital space, at the bulb of the heel, and along the coronary band. As in sheep and goats, oral lesions in pigs may heal without much exudate or subsequent scarring. However, soon after rupture, the base of vesiculated areas often becomes covered within a few days by a serofibrinous exudate. The regeneration of epithelia is usually well advanced within 2 weeks, although usually with a variable degree of scarring, in particular after the occurrence of severe lesions. The rupture of vesicles, especially on the feet or teats, may predispose the affected areas to secondary bacterial infections, which may complicate and prolong the healing processes.

The age of lesions can be assessed using the following criteria:

1. development of vesicles on days 0–2,
2. rupture of vesicles on days 1–3 (initially with fragments of epithelium attached),
3. sharply margined erosion (days 2–3), with the sharpness lost around day 3,
4. serofibrinous exudation on days 4–6, and
5. beginning of repair with a marked fibrous tissue margin at day 7 or more (Anonymous 1986).

Severe lesions of the coronary bands, as seen especially in pigs, may often lead to a separation of the horn during the acute inflammatory stage. If the horn is not shed, a ring will be formed in the horn that becomes visible below the coronary band approximately 1 week after the first appearance of clinical disease. This ring progresses down the hoof as the horn grows. Growth rate is approximately 1–2 mm per week, with faster horn growth in younger animals.

In young animals (pigs less than 8 weeks of age) that die from acute myocarditis, visual examination often reveals the heart to be soft and flaccid, with white or grayish stripes (the so-called tiger heart) or spots, seen mainly in the left ventricle and interventricular septum. In young animals dying from hyperacute disease, there may be no significant visible lesions in the heart and an absence of vesicular lesions, but the virus can usually be isolated from the myocardium or from blood and lesions can be detected by histopathological examination (Donaldson et al. 1984). Occasionally, the skeletal muscles may also be affected.

The significance of FMD acute myocarditis for the spread of the disease is not well understood. Possibly, there is little virus excretion, since death usually occurs early before the development of vesicular lesions. However, although in such cases FMDV mainly replicates in the heart, a significant viremia may occur (Donaldson et al. 1984) and virus may be present in respirations, saliva, nasal fluid, and so forth (Ryan et al. 2008b).

Microscopic Lesions. The first histopathological changes in the cornified, stratified squamous epithelium are ballooning degeneration and increased cytoplasmic, eosinophilic, staining of the cells in the stratum spinosum and the onset of intercellular edema within the dermis. These early lesions are detectable only by microscopic examination (Gailiunas 1968; Yilma 1980). This early stage may be followed by necrosis and subsequent mononuclear cell and granulocyte infiltration. The lesions, now macroscopically visible, develop further into vesicles by separation of the epithelium from the underlying tissue and filling of the cavity with vesicular fluid. In some cases, the vesicular fluid production may be high and the resulting vesicles large. In other cases, the amount of fluid may be limited and the epithelium may undergo necrosis or be torn off by physical trauma without the formation of an obvious vesicle. The variability seen is most likely due to combinations of viral strain virulence, thickness of the affected skin, and husbandry conditions, especially as they affect physical stress on different regions of the skin. In young animals dying from acute disease, there is lymphohistiocytic myocarditis with hyaline degeneration, necrosis of myocytes, and infiltration with mononuclear cells.

In pigs, it is puzzling that no lesions develop on the soft palate or the dorsal part of the pharynx, despite the presence of significant amounts of virus. Possibly, the infection causes no acute cytopathology in the transitional epithelial cells found in these areas. Alternatively, the viral cytopathology is restricted to a few cells and is, therefore, not easily detected. Or perhaps, since the epithelia of this region are noncornified, the development of distinguishable lesions is somehow prevented. Regardless, the underlying mechanisms
behind this apparent lack of cytopathology are unknown.

Diagnosis
Differential Diagnosis. The clinical diagnosis of FMD is sometimes difficult, for example in sheep and goats, in which clinical signs are often mild (Alexandersen et al. 2002b; Donaldson and Sellers 2000; Hughes et al. 2002). Moreover, certain strains of the virus may be of low virulence for some species (Donaldson 1998). In pigs, several other viral vesicular diseases, including swine vesicular disease (SVD), vesicular stomatitis, and vesivirus infection, cannot be distinguished from FMD on the basis of clinical findings. Other picornaviruses, including SVV and PEV infection, have also been implicated in vesicular diseases in swine (so-called idiopathic vesicular disease). If the disease is not noted early, any vesicles present will have ruptured and will be indistinguishable from erosive lesions, such as those induced by trauma, caustic substances, and photosensitivity. Thus, a definitive diagnosis requires urgent laboratory investigation.

Pathological Evaluation. The first step in the diagnostic evaluation is to determine whether vesicles are present. If no vesicles are present, it should be established whether there are lesions that could be consistent with older stages of vesicles, that is, after rupture and loss of the overlying lesion epithelia and thus resembling erosions. Although detailed gross examination of lesions may help in the evaluation, only laboratory testing can confirm or refute the presence of FMDV.

Virus Detection. Definitive diagnosis of FMD must be carried out at specialized laboratories. Enzyme-linked immunosorbent assays (ELISAs) have supplanted the earlier use of the complement fixation test due to their sensitivity, specificity, and ability to test large numbers of samples (throughput).

Laboratory diagnosis is usually made by ELISA detection of specific FMDV antigens in epithelial tissue suspensions, often with concurrent attempts at cell culture isolation and the application of ELISA to any samples showing cytopathogenic effect (Ferris and Dawson 1988; Hamblin et al. 1984; Roeder and Le Blanc Smith 1987). These tests are used to confirm the diagnosis and identify the FMD serotype.

Given a sample with sufficient quantities of virus, a positive result for FMDV (including serotyping) can be obtained in 3–4 hours by antigen ELISA (Ferris and Dawson 1988; Hamblin et al. 1984; Have et al. 1984; Roeder and Le Blanc Smith 1987). However, samples with low quantities of virus may yield weak, inconclusive, or negative results. Thus, the antigen ELISA is highly specific and very suitable for confirming positive cases, but a negative result requires further examination in highly susceptible cell cultures before FMD can be ruled out.

Most often, samples are routinely inoculated onto primary BTY cells (Snowdon 1966) or bovine or lamb kidney cells, as well as on an established line of PK cells (IB-RS-2 cells) (De Castro 1964). Cultures showing a cytopathogenic effect are confirmed by antigen ELISA using cell culture supernatant. For most FMDV strains, the BTY cell system is about 10 times more sensitive than other cultures (Burrows et al. 1981; Snowdon 1966). However, certain pig-adapted strains, for example, the O Taiwan 1997 strain (Dunn and Donaldson 1997), grow better in IB-RS-2 cells. Virus isolation in BTY and IB-RS cell cultures essentially detects all positive samples with more than 1–5 infectious units per milliliter or per 0.1 g. Depending upon the amount of virus present, two 48-hour passages may be required before a final result can be determined. However, if the specimens are of low quality or if the transport conditions were less than optimal, a small proportion of samples may give negative results for infectivity, but positive results by ELISA or RT-PCR.

RT-PCR assays have been developed for the detection of FMDV, but none seems to be of sufficient sensitivity, specificity, and robustness for routine diagnostics (House and Meyer 1993; Moss and Haas 1999; Reid et al. 1998, 1999). RT-PCR assays for serotyping FMDV have been described, but the procedures are labor intensive (Callens and De Clercq 1997; Reid et al. 1998, 1999; Vandgrypserre and De Clercq 1996). Alexandersen et al. (2000) developed an RT-PCR ELISA of increased sensitivity and included a SNAP (simple and aqueous phase) hybridization step for optimal specificity, speed, and ease of use. Both conventional polymerase chain reaction (PCR) techniques and the SNAP method can provide serotype-specific results, but they are not of sufficiently high throughput for use in an epidemic.

Fluorogenic “real-time” RT-PCR methods combine the total RNA extraction and reverse transcription (RT) procedures of conventional RT-PCR with fluorogenic probe PCR amplification in real-time PCR equipment. This approach is able to achieve high sensitivity and specificity for the detection of FMDV genomes of all seven serotypes (Alexandersen et al. 2003b,c; Callahan et al. 2002; Hearps et al. 2002; Moniwa et al. 2007; Rasmussen et al. 2003; Reid et al. 2002). The assay can be used for the detection of FMDV in tissue samples, serum samples, swab samples, and tissue culture supernatants and can include automated procedures for nucleic acid extraction, RT, and PCR amplification stages to increase sample throughput (Reid et al. 2003). In addition, some of these assays may be used on portable platforms (Callahan et al. 2002; Hearps et al. 2002; King et al. 2008).

Real-time RT-PCR methods are now as diagnostically effective as the combined ELISA/virus isolation system. These assays provide good agreement with virus
isolation with same-day turnaround. With experimental samples, the sensitivity of RT-PCR is higher than virus isolation. The timeliness of investigating suspect cases could be further improved by conducting the testing on or near the farm, but this type of testing, especially in a portable format, will require further evaluation.

In addition to the methods described, “pen-side” antigen detection methods have been developed based essentially on the same principle as the antigen ELISA described above, but with a pan-serotype monoclonal antibody in a format adapted for field use (Ferris et al. 2009; Reid et al. 2001; Ryan et al. 2008a). Laboratory trials indicated that the sensitivity of this approach is equal to, or probably greater than, the traditional antigen ELISA. Field validation of such tests is a high priority.

**Antibody Detection.** The liquid-phase blocking ELISA was formerly in routine use at many laboratories for FMDV antibody detection (Hamblin et al. 1986). The diagnostic sensitivity of the assay is close to 100%, but the specificity is only ∼95%. Since specimens with inconclusive ELISA results require testing by virus neutralization (VN) (Golding et al. 1976), the liquid-phase ELISA is not the optimal test for large-scale screening because numerous VNs are likely to be required because of the low specificity of the assay.

The OIE considers the VN the definitive “reference standard” for the final assessment of inconclusive ELISA results. At the World Reference Laboratory, the specificity of the VN for type O antibodies at a dilution of 1:45 was 100% (Paiba et al. 2004). However, it is likely that some of the newer tests may completely replace the VN when sufficient validation data become available.

Solid-phase competitive or blocking ELISAs (SP-C or SP-B ELISAs) possess both high sensitivity and specificity (Have and Holm-Jensen 1983; Mackay et al. 2001; Sorensen et al. 1992). These assays detect all experimentally infected animals at 5–8 days after infection and for many months thereafter (Paiba et al. 2004).

Research on vaccines from which FMDV nonstructural proteins (NSPs) have been removed offers the future promise of a DIVA (differentiate infected from vaccinated animals) vaccine. Tests to detect antibodies against the conserved NSPs of FMDV have already been reported (Berger et al. 1990; Bergmann et al. 1993; Lubroth and Brown 1995; Mackay 1998; Neitzert et al. 1991; Shen et al. 1999; Sorensen et al. 1998). In vaccinated populations, such tests could be used to differentiate vaccinates from infected animals on a herd basis. Unvaccinated populations can be screened by tests that detect antibodies to structural antigens and a negative result will exclude FMDV in a statistically robust manner.

**Immunity**

The host immune response, including antibody production detected as early as 3–4 days after the first clinical signs, usually results in the clearance of FMDV, except in those infected ruminants that develop a persistent infection of the pharyngeal region. Clearance of virus from “peripheral” or “external” sites, for example, nasal and oral surfaces, is less efficient. The virus may remain in the vesicular epithelium of foot lesions for 10–14 days, that is, longer than in oral lesions (Oliver et al. 1988).

**Humoral Response.** ELISA can detect circulating antibodies 3–5 days after the appearance of clinical signs. Detection of antibodies by VN is usually 1–2 days later than ELISA. Coincident with the first detection of antibody, there is a progressive, rapid clearance of virus from the circulation and a significant reduction of virus in most organs, excretions, and secretions. The antibody response normally remains high for many months after infection and may still be detectable after several years. However, in fast-growing young pigs, antibodies may have a half-life as short as 1 week and may remain detectable for only a few months.

Immunity to FMDV is primarily mediated by circulating antibodies and protection after infection or immunization (active or passive) is closely correlated with the antibody titer. Circulating antibodies capable of binding to the surface of virions will facilitate opsonization and uptake by phagocytes located in the liver, spleen, and elsewhere, and thus rapidly reduce or prevent viremia (McCullough et al. 1992). As viremia is an important phase in the early infection process, its reduction or prevention will have a corresponding effect on acute generalized disease. However, since circulating antibodies will not prevent primary local infection (e.g., at the sites of intradermal entry, or in the pharynx) it would seem that they prevent disease, but not infection (McVicar and Sutmoller 1976).

Infection with FMDV induces a strong mucosal immunoglobulin A (IgA) response that may protect against reinfection with the same virus, but traditional inactivated vaccines induce a very weak or no secretory IgA response. It is possible that vaccines with a high antigenic mass may induce some secretory IgA production in swine and that this response, if sufficiently high, may protect against infection (Eble et al. 2007; Pacheco et al. 2010).

**Cell-Mediated and Innate Responses.** Although it is possible to protect pigs with passively acquired antibodies and although immunity to FMDV appears to be primarily mediated by circulating antibodies, immunity after vaccination or infection must necessarily involve more than just humoral immunity. However,
little is known about the cell-mediated immune response to FMDV infection. In swine, FMDV infection results in a significant lymphopenia. T-cell activity is significantly reduced during acute infection, possibly the result of increased interleukin (IL)-10 production by dendritic cells (Diaz-San Segundo et al. 2009; Golde et al. 2008; Grubman et al. 2008).

Both a CD4 and a CD8 T-cell response can be demonstrated in infected or vaccinated animals. However, the development of neutralizing antibodies and of class switching appears to be independent of a CD4 T-cell response and the CD8 T-cell response may be of limited effect during acute infection, because acute FMDV infection rapidly results in reduced major histocompatibility complex (MHC) class I surface expression on infected cells. Moreover, the acute infection itself rapidly kills infected cells (Childerstone et al. 1999; Gerner et al. 2009; Guzman et al. 2008; Juleff et al. 2009; Sanz-Parra et al. 1998).

Likewise, the innate immune response is poorly described and some studies suggest that the innate response plays a minor or no role in protection (Alves et al. 2009; Summerfield et al. 2009). Swine can be protected by interferons, as shown using replication-defective adenovirus expressing type I or type I and II interferons, a promising tool for potential new emergency vaccines or rather, antivirals (Diaz-San Segundo et al. 2010). Although FMDV is highly sensitive to interferons, the virus has efficient mechanisms to block interferon production in infected cells by blocking cellular protein synthesis via the FMDV leader protein (Belsham 2005). Other innate immune responses may work through natural killer cells (NK cells), but FMDV also appears able to counteract this mechanism by significantly reducing NK cell function during acute infection (Toka et al. 2009). Overall, it appears that FMDV has evolved efficient mechanisms for immune evasion during the acute stages of infection (Golde et al. 2008; Grubman et al. 2008). Nevertheless, emergency vaccines may provide early protection by induction of a combination of innate and adaptive immune responses.

**Maternal Immunity.** Maternal immunity against FMDV is transferred to the offspring via colostral immunoglobulins from previously infected or vaccinated dams. Provided the levels are sufficiently high and directed toward the relevant challenge strain, this may protect piglets up to 8–12 weeks of age. Piglets respond poorly to FMD vaccination and are best protected by maternal immunity. They should not be vaccinated before 8–12 weeks of age, by which time maternal immunity is waning and they are able to respond to FMD vaccination (Francis and Black 1986; Kitching and Alexandersen 2002; Kitching and Salt 1995; Morgan and McKercher 1977).

**Prevention and Control**

The most common methods of spreading FMDV are (1) movement of infected animals, (2) feeding contaminated animal products to susceptible livestock, and (3) movement of virus on fomites or mechanical vectors (humans and animals). Transmission by these routes can be prevented by application of strict disease control measures, that is, movement restrictions and biosecurity procedures. It should be borne in mind that virtually any surface or product could be contaminated with the virus if the infection is present in the region or country. Airborne transmission of FMDV is essentially uncontrollable and not uncommon over short distances, but rarely occurs over long distances. However, when long distance transmission occurs, the consequences can be dramatic (Anonymous 1969; Donalson et al. 1982; Gloster et al. 1981, 1982).

FMDV has a wide host range, a low infectious dose, a rapid rate of replication, a high level of viral excretion, and multiple modes of transmission. Therefore, it is essential to identify suspect cases, conduct the diagnostic evaluation, and implement the appropriate response as quickly as possible. Extreme measures are required to eradicate FMDV and, if they are not rapidly and effectively applied, there is a high probability that an outbreak will reach epidemic proportions.

There is currently no way of treating FMDV-infected pigs, and once the virus has been introduced into a swine farm, it is unlikely to disappear unless all the animals, both infected and uninfected, are euthanized, the carcasses removed (buried, rendered, or incinerated), and the premises disinfected (Alexandersen et al. 2003c; Kitching and Alexandersen 2002). Therefore, the response to the positive identification of FMDV is euthanasia of all susceptible animal species on infected farms, while vaccination may be used in noninfected farms. Depending on local regulations, animals suspected of having been exposed to FMDV could be transported to an abattoir for slaughter, if it is possible to avoid the risk of transmitting FMDV to other susceptible animals. Products from such animals must be heat treated and canned.

Control of FMDV is complicated by the existence of carrier ruminants (Alexandersen et al. 2002a; Sutmoller and Gaggero 1965; Van Bekkum et al. 1959). Recognition of the carrier state and the disease risk presented by carrier animals had a major impact on the design of control and eradication strategies for FMD. Experience has shown that total stamping out, whereby all susceptible species on an infected premises are euthanized, both affected and apparently normal, is necessary to ensure the elimination of persistently infected carriers (Alexandersen et al. 2002a; Hedger and Stubbins 1971). These strategies have been shown to be effective under varying conditions in many countries. The risk posed by ruminant carriers has also had a marked influence on the safeguards taken to manage the risk of FMD.
associated with international livestock movements. These measures, mainly directed at animals originating from countries where the disease is either endemic or sporadic, range from a complete embargo to quarantine and testing.

During the U.K. 2001 epidemic, mathematical models played a major part in influencing disease control strategy. However, they had not been used previously under operational conditions (Ferguson et al. 2001) and the assumptions behind them, as well as the justification for the use of such average-based, mathematically derived, centrally controlled procedures and their actual impact on efficient disease management are still highly contentious.

**Vaccines and Vaccination.** There are seven serotypes of FMDV, and infection or vaccination with one serotype does not protect against infection or disease with any of the other serotypes. Moreover, a wide range of strains may exist within a serotype, some of which may be sufficiently divergent to reduce the efficacy of a given vaccine (Kitching 1998; Kitching et al. 1989). As a general rule, protection against heterologous strains will be lower than against the homologous strain (Goris et al. 2008). Therefore, FMDV vaccine antigens need to be tailored to strains circulating in the region or strains with the potential to be introduced. Preparedness for emergency vaccination requires accurate intelligence with respect to the strains most likely to be introduced. An alternate strategy is to be broadly prepared against a large number of FMDV antigens.

The degree of cross-protection that one vaccine strain provides a vaccinated animal when challenged with another strain of the same serotype can only be assessed in animal trials, but most trials offer only limited insight because the low number of animals typically used offers low statistical power.

The antigenic relatedness of FMDV strains can be assessed in the laboratory using ELISA and VN methods. These results help select vaccine strain(s) most appropriate for use against a given field strain. Notably, these indirect methods are highly dependent on the individual laboratory doing the tests, the actual composition of the vaccines, and other factors (Jamal et al. 2008; Maradei et al. 2008; Mattion et al. 2009; Paton et al. 2005).

Thus, although vaccines are available to control FMD, the existence of multiple serotypes of FMDV, each with multiple, continuously evolving strains, should be considered when considering a control program that includes vaccination. Further, it should be recognized that vaccine-induced protection usually only lasts 4–6 months and that susceptible animals are continuously introduced into swine populations through reproduction and animal movement. Consequently, vaccination programs often require two or more doses per year (Domenech et al. 2010). It should also be noted that, once established in the swine population, FMD is difficult to control by vaccination (Orsel and Bouma 2009; Orsel et al. 2007) and even more difficult to eradicate, as shown by the efforts in Taiwan, which, after nearly 15 years of vaccination following the 1997 introduction, has not eradicated FMD. Nevertheless, the successful eradication of FMD from the EU has clearly shown that eradication can be achieved using prophylactic vaccination with good quality vaccines, but it should be realized that this required a coordinated and efficient control effort, including stamping out of infected farms, over many years (Leforban and Gerbier 2002).

**SWINE VESICULAR DISEASE VIRUS**

**Relevance**

In 1966, a disease that appeared clinically like FMD, but was caused by an enterovirus, appeared in pigs in Italy (Nardelli et al. 1968). SVDV has only been isolated in Asia and Europe and outbreaks have been reported in a limited number of countries (Table 42.2). Portugal (2007) and Italy (2011) reported SVD outbreaks recently, but SVD could be present elsewhere since few countries test for antibodies against SVD virus.

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Based on the EAO Animal Health Yearbook (1971–1996) and information obtained from the European reference laboratory for vesicular diseases in Pirbright, UK.
SVD is on the OIE list of diseases because the vesicular lesions caused by SVDV resemble those caused by FMDV. In the past, when differentiating SVDV from FMDV using laboratory assays was more difficult, it was important to be free of SVD so as to be sure that FMD outbreaks were not missed. Current diagnostics make differentiation relatively easy and even on-farm diagnosis is possible (Ferris et al. 2009). However, the issue is still valid and SVD could mask an FMD outbreak, as was the case in 1997 in Taiwan.

**Etiology**

SVDV is classified as an enterovirus within the family *Picornaviridae*. Like other picornaviruses, SVDV is ~30 nm in diameter (Nardelli et al. 1968) and nonenveloped. Only small antigenic differences exist among SVDVs, therefore SVDV is considered a single serotype. Isolates, however, can be divided into four distinct phylogenetic groups by comparing monoclonal antibody reaction patterns or nucleotide sequences of the 1D (VP1) coding sequence (Borrego et al. 2002a,b; Brocchi et al. 1997).

The genome of SVDV consists of approximately 7400 nucleotides, and encodes a single polyprotein of 2815 amino acids (Inoue et al. 1989). This polyprotein is posttranslationally cleaved into 11 mature proteins, plus various precursors. Four of these proteins, 1A, 1B, 1C, and 1D, form the virus capsid (Fry et al. 2003), and one of these proteins, 3B (VPg), is linked directly to RNA and is, therefore, also part of the virus. The NSPs are involved in virus replication and interruption of host cell functions.

The virus can be grown on primary porcine kidney cells and a wide range of PK-derived cell lines. The virus is lethal to newborn mice (Nardelli et al. 1968), which links it to its ancestor virus Coxsackie B5 because among the enteroviruses only Coxsackie viruses can infect mice (Graves 1995). Sequence data show that SVDV has approximately 75–85% nucleotide identity with Coxsackie B5 virus (Knowles and McCauley 1997) within the coding sequence for the structural proteins. There is also high sequence identity with echovirus 9 in the coding sequence for the NSPs (Zhang et al. 1999). Phylogenetic analyses indicate that SVDV and recent Coxsackie B5 isolates probably shared a common ancestor in the period between 1945 and 1965 (Zhang et al. 1999).

**Role in Public Health**

SVD is not considered a public health risk. Infection with SVDV was suggested to have caused the hospitalization of one person (Brown et al. 1976). Since the virus is closely related to human Coxsackie B5 virus and echovirus 9 (Zhang et al. 1999), infection of human cells is not unexpected. However, illness due to SVDV has not been reported recently, not even from laboratories working with large amounts of live virus during large-scale screening. For this reason it is uncertain whether the reported human illness was actually caused by SVDV infection. Further, recent studies suggested that the virus has further adapted to swine and that SVDVs isolated after 1993 have lost the ability to infect human cells and, therefore, have likely lost any of their original zoonotic potential. Consequently, currently circulating strains and isolates obtained after 1993 are not considered zoonotic (Jimenez-Clavero et al. 2005).

**Epidemiology**

SVDV was isolated in 1971 in Hong Kong (Mowat et al. 1972). In 1972, SVD was diagnosed in Great Britain, Austria, Italy, Poland, and elsewhere (Table 42.2). More recently, Portugal (2007) and Italy (2009) have reported outbreaks. Italy is one of the few countries with a screening program for SVD antibodies and most of the recent outbreaks in Italy were detected by serological testing. It is possible that the virus is present in more countries than those that have reported the disease. The virus is probably present in some Asian countries, although the last reported outbreak was in Taiwan in 1999. North and South America are considered free.

Not only Euro-Asian pigs, but also American one-toed pigs are susceptible to SVDV (Wilder et al. 1974). Relatively high virus titers have been detected in the pharynx of sheep kept in close contact with SVDV-infected pigs (Burrows et al. 1974) and neutralizing antibodies were detected in some of these contact sheep. Similar experiments with cattle showed no signs of infection (Burrows et al. 1974). Thus, these studies suggest that the virus may have replicated in the sheep after close contact with infected swine, but there is nothing to indicate that sheep or other ruminants play a role in the epidemiology of SVD.

An epidemiological field study in Great Britain revealed that the primary method of spread was movement of pigs (48%), either transport of infected pigs (16%), use of SVDV-contaminated transport vehicles (21%), or through contacts at markets (11%). A second source of infection (15%) was feeding of contaminated waste food (Hedger and Mann 1989). The exceptionally high stability of the virus outside the host means that indirect routes of transmission, for example, virus-contaminated transport or waste food, play an essential role in the epidemiology of SVD. Contact with a contaminated environment can lead to viremia in one day and clinical signs within 2 days (Dekker et al. 1995a). Studies on SVDV transmission within an outbreak farm showed that spread between pens most likely occurred via a shared open drainage system or movement of pigs between pens. SVD, therefore, is considered a “pen disease” rather than a farm disease (Dekker et al. 2002; Hedger and Mann 1989).

Since clinically affected herds are immediately culled, it is not easy to study transmission in the field. Immunoglobulin M (IgM) and immunoglobulin G
(IgG) ELISAs have been developed in an attempt to estimate the time since the virus was introduced (Brocchi et al. 1995; Dekker et al. 2002). By approximately 50 days postinfection the antibody isotype profiles of most infected animals have stabilized. Thus, an estimation of the time of introduction beyond 50 days is not possible with serological tests (Dekker et al. 2002).

The duration of the shedding of SVD virus from vesicles is at least 7 days (Dekker et al. 1995a), but it has been recovered from feces for a longer period. There is one report of the recovery of the virus up to 126 days after infection (Lin et al. 1998) but it has been difficult to reproduce these findings (Lin et al. 2001).

The virus remains infectious for months in carcasses and processed meat, for example, salami or pepperoni sausages (Hedger and Mann 1989; Mebus et al. 1997). The survival of SVD in slurry is also long (Karpinski and Tereszczuk 1977).

SVDV is stable in a very wide pH range and consequently, acidic or alkaline disinfectants, which work well for FMDV, are not effective for SVDV. Like other picornaviruses, SVDV is resistant to detergents and organic solvents such as ether and chloroform. Only sodium hydroxide (1%) has been shown to adequately inactivate SVDV. If longer contact times between virus and disinfectant are possible, then formaldehyde (2%) was also effective after 18 minutes (Terpstra 1992).

Pathogenesis

It has been suggested that SVDV enters the pig through the skin or the mucous membrane of the digestive tract (Chu et al. 1979; Lai et al. 1979; Mann and Hutchings 1980). Experimental SVDV infection can lead to clinical signs within 2 days and SVDV has been isolated from a wide range of tissues (Chu et al. 1979; Dekker et al. 1995a; Lai et al. 1979). Viremia can develop within 1 day after pigs have contact with a SVDV-contaminated environment. This is the same as the interval observed when pigs are directly inoculated (Dekker et al. 1995a).

SVDV has a strong tropism for epithelial tissues, but virus titers in the myocardium and the brain may significantly exceed those in plasma. Therefore, epithelial tissues and sometimes myocardium and brain are probably the sites of virus replication (Chu et al. 1979; Lai et al. 1979). Lymph nodes may also contain high titers of SVDV after experimental infection. It is not known, however, whether virus in these tissues represents drainage of virus or virus replication (Dekker et al. 1995a). In vitro studies showed that infected porcine kidney cells could be detected by immunohistochemistry within 3.5 hours of inoculation. In situ hybridization, however, seems to be better when used for staining of SVDV-infected tissues (Mulder et al. 1997). More research is necessary to identify the cells that support SVDV replication. Identification of these cells may help to identify the mechanism behind the host tropism of the virus.

SVD may run a subclinical, mild, or severe course, but the latter is usually only seen when pigs are housed on a concrete floor in humid conditions (Hedger and Mann 1989; Kanno et al. 1996; Kodama et al. 1980). This suggests that the environment is an important factor in the observed virulence of the disease. Experiments to show differences in virulence between strains and chimeric viruses were performed, but reproducibility was problematic and the results, therefore, were not published. However, these experiments showed that lesions were often difficult to detect without careful examination, suggesting that field reports on difference in virulence in the field may be biased by observers.

Clinical Signs

Experimental infection by intradermal injection in the bulb of the heel can lead to clinical signs in 1–2 days. In experiments in which pigs were exposed to an infected environment, the first lesions were observed at 2 days after exposure (Dekker et al. 1995a), showing that the time to onset of clinical signs can be very short following natural exposure.

Mortality is not a feature of SVDV infection. Morbidity can be high, but depends on many factors, for example, virulence of the strain, type of farm, and most importantly, the time between infection and detection. Seroprevalence at the time of detection ranged from approximately 7% on a farm in which the disease was clinically detected to almost 90% in a collection center that presumably received infected pigs (Dekker et al. 2002). As previously given, the severity of the clinical signs can depend on the strain, but also on whether pigs are housed on straw or concrete.

Lesions

Clinical SVD is restricted to pigs. In pigs infected with SVDV, vesicles appear around the coronary bands (Figures 42.5 and 42.6), on the skin of the metacarpus and metatarsus, and to a lesser extent on the snout, tongue, and lips. Lesions are indistinguishable from those induced by FMDV infection. The clinical signs caused by SVD are, however, much milder than those caused by FMD. In experimental studies (Dekker et al. 1995a), fever was nearly always absent and lameness was almost never observed. Sudden death, due to heart degeneration, which is often observed in young piglets infected with FMDV, is not seen with SVDV.

In typical cases of SVD, lesions are first noticed at the junction of the heel and the coronary band (Figures 42.5 and 42.6). The whole of the coronary band may eventually be involved and the lesions may spread to the metatarsal and metacarpal regions. The horn and sole may be damaged so extensively that the claw(s) slough off. In lactating sows, lesions may be seen on...
stratum spinosum. In experimentally infected animals, nonsuppurative meningoencephalitis may occur, but this does not result in signs of impaired central nervous system (CNS) function (Chu et al. 1979).

**Diagnosis**

A farm with signs of a vesicular disease in pigs should be considered infected with FMDV until proven otherwise. Similar vesicular lesions are seen with SVD, vesicular stomatitis, and vesicular exanthema. FMD can be found in many parts of the world and is the most important differential diagnosis for SVDV. Vesicular stomatitis is endemic to large parts of the American Hemisphere and has only been exported four times to South Africa and twice to France. Vesicular exanthema is caused by a calicivirus and was last observed in the United States in 1959.

Lesions in pigs start with blanching of the coronary band. The location affected is often the posterior part of the interdigital area, which is most easily observed in a recumbent pig. The areas develop into large fluid-filled vesicles that rupture in 1–2 days, after which erosion is observed. True vesicular lesions are only caused by a limited number of diseases, but erosions seen after lesions have ruptured are not specific for SVD and have various causes, including trauma due to concrete floors.

Virus isolation on IB-RS-2 cells (De Castro 1964) is considered one of the most sensitive methods for laboratory diagnosis. SK6, PK-15, and primary or secondary porcine kidney cells are also susceptible to SVDV (Callens and De Clercq 1999; Nardelli et al. 1968). Several sensitive RT-PCR techniques have been developed to detect SVDV genomes, some of them designed to differentiate between various vesicular diseases (Fernandez et al. 2008; Lin et al. 1997; Niedbalski 2009; Reid et al. 2004). Virus isolation and RT-PCR are the tests of first choice for detection of SVDV in feces or organs. In vesicular material, however, the amount of...
virus is very high and ELISA can easily detect and identify antigen (Roeder and Le Blanc Smith 1987).

In the aftermath of an outbreak, screening for specific antibodies is essential to prove that no infected farms have been missed. SVDV infection produces high neutralizing antibody titers (Nardelli et al. 1968). VN tests are laborious, but ELISAs have been developed (Armstrong and Barnett 1989; Brocchi et al. 1995; Chenard et al. 1998; Dekker et al. 1995b; Hamblin and Crowther 1982). ELISA is easier to perform than VN test, but produces more false-positive results. However, the specificity of the ELISA can be increased by using monoclonal antibodies (Brocchi et al. 1995; Chenard et al. 1998; Dekker et al. 1995b; Hamblin and Crowther 1982). ELISA is easier to perform than VN test, but produces more false-positive results. However, the specificity of the ELISA can be increased by using monoclonal antibodies (Brocchi et al. 1995; Chenard et al. 1998). ELISA, the OIE standard test for SVDV, has been shown to be highly efficient in large-scale serosurveillance.

Immunity
The immune response to infection with SVDV is rapid. A detectable IgM response is present in 50% of the pigs 4 days after infection and IgG can be detected in 50% of the pigs by ~12 days (Brocchi et al. 1995; Dekker et al. 2002). Based on analysis of a large data set from various published experimental infection studies (Chenard et al. 1998), 50% of the pigs will be positive in the SVD ELISA at 7 days after infection and VN positive by day 8.

Experimental SVD vaccines have been developed to control the disease (Delagneau et al. 1974; Gourreau et al. 1975; McKercher and Graves 1976; Mowat et al. 1974). In addition to monovalent SVDV vaccines, combinations with FMDV (McKercher and Graves 1976; Mitev et al. 1978) and, more recently, an SVDV subunit vaccine have also been described, although the latter was not very efficacious (Jimenez-Clavero et al. 1998). Although the inactivated virus vaccines are effective for protecting against clinical signs, whether they reduce wild-type virus transmission has not been evaluated. No SVD vaccine is commercially available and, to date, vaccination of pigs has not been undertaken in the field.

SVDV is one single serotype, so prior infection or vaccination with any strain of SVDV should protect against any other strain of SVDV. Cross-neutralization with other PEVs or picornaviruses does not occur, and so no cross-protection is expected toward these other viruses.

Piglets born to SVDV-infected sows receive antibodies against SVDV from the sow. The half-life of maternal anti-SVDV antibodies in piglets is longer (30–50 days) (Bellini et al. 2010) than the half-life (7–21 days) of anti-FMDV maternal antibodies in piglets nursing FMDV-vaccinated sows (Francis and Black 1984). This means that maternally derived anti-SVDV antibodies can be found in pigs up to almost 6 months of age. This should be taken into account when interpreting results of serological tests on outbreak farms.

Prevention and Control
SVD is listed by the OIE, and the general policy in countries where SVD is detected is stamping out. This is primarily due to the fact that the clinical signs of SVD are indistinguishable from those of FMD. Current diagnostic tests can differentiate between these viral infections, but trading partners are not willing to accept SVDV-seropositive pigs, and therefore, the disease is still controlled by stamping out.

Although SVDV is very resistant to environmental factors and many commonly used disinfectants (Terpstra 1992), pigs transported in cleaned, disinfected vehicles do not become infected. Even on farms where SVDV is present in one part of the premises, the infection does not spread easily to other areas if strict hygiene measures are applied. Feeding of waste food has contributed significantly to transmission of SVD, but even when garbage feeding is not allowed, infection through contaminated feed can occur if pigs consume foodstuffs left by visitors or personnel, although the probability is much lower.

Stamping out whole farms followed by thorough disinfection and cleaning eliminates the virus. In The Netherlands in 1995, a finishing and breeding farm was detected with seropositive fattening pigs, indicating that virus had been introduced into these pigs. No virus could be isolated from any of the areas housing seropositive pigs. It was decided to slaughter the seropositive animals followed by disinfection and thorough cleaning of the areas that had housed the seropositive pigs. This procedure reduced the serological prevalence to zero. This might indicate that elimination of SVDV from a farm is possible, due to the fact that transmission is slow and limited, but is likely to fail if many pigs are clinically ill.

ENCEPHALOMYOCARDITIS VIRUS
Relevance
EMCV is a rodent-borne virus first described in 1940 (Jungeblut and Sanders 1940). Later, EMCV was isolated from a chimpanzee with myocarditis in Florida (Helwig and Schmidt 1945), and anti-EMCV antibodies or EMCV were subsequently detected in a variety of animal species (Tesh and Wallace 1978).

Infection of swine with EMCV is not uncommon, but clinical disease is infrequent. During an outbreak of acute disease in Panama (1958), Murnane et al. (1960) isolated EMCV from the lung and spleen of a pig that suddenly collapsed and died within a few minutes. This was the first time that EMCV had been recognized as a swine pathogen. In pigs, disease due to EMCV may take one of two main forms: an acute myocarditis, usually causing sudden death in young pigs, and/or reproductive failure in sows.

EMCV outbreaks with high mortality in pigs have been reported in Australia, South Africa, New Zealand, and/or reproductive
Cuba, and Canada (Acland and Littlejohns 1975; Dea et al. 1991; Seaman et al. 1986). In Europe, clinical disease caused by EMCV was first observed among domestic pigs in 1986 and has increased in frequency since. Outbreaks of acute myocarditis have been reported in Italy, Greece, Switzerland, Belgium, and Cyprus (Koenen et al. 1999). EMCV outbreaks are often clustered in so-called endemic areas (Maurice et al. 2007). In Belgium, EMCV was also frequently isolated in cases of reproductive failure (Koenen et al. 1999). In the endemic area of Italy, a seroprevalence of between 5% and 15%, and occasionally more than 60%, was found in farms with clinical EMC. In 50% of the farms without clinical EMC, a similar seroprevalence level was detected (Maurice et al. 2005). In the United Kingdom, antibodies against EMCV were detected in 28% of clinically normal slaughterhouse pigs (Sangar et al. 1977), but no attempt was made to isolate virus.

**Etiology**

EMCV belongs to the genus Cardiovirus of the family Picornaviridae. Several antigenically similar viruses, including Columbia-SK and Mengo, were isolated during the 1940s, and are considered to be in the same serotype as EMCV. Many properties of EMCV are common to other picornaviruses. It is ether resistant and stable over a wide range of pH. It is inactivated after 30 minutes at 60°C (140°F), but some strains have shown a marked thermal stability (Joo 1999).

Although EMCV is antigenically stable, the 1D (VP1 capsid protein) coding region of EMCV displays considerable genetic variability. A single mutation in the nucleotide sequence can be involved in attenuation or confer diabetogenicity to a particular EMCV strain (Nelsen-Salz et al. 1996). In particular, the Greek isolates appear to differ genetically from those isolated in Belgium, Italy, and Cyprus (Knowles et al. 1998).

EMCV replicates well in cell cultures originating from several animal species, including rodents, swine, and primates. BHK-21 and Vero cells are used most commonly. The virus also replicates in mice and chicken embryos and is pathogenic in many laboratory animals. Acute fatal disease is produced in mice and hamsters after inoculation by various routes. Neurological disease due to encephalitis has been reported, but myocarditis is more frequently seen at necropsy. Pathogenicity in rats, guinea pigs, rabbits, and monkeys appears to vary depending on the age of the animals and the virus strains used.

The virus demonstrates hemagglutinating activity with guinea pig, rat, horse, and sheep erythrocytes, but differences in this activity among EMCV strains have been reported (Kim et al. 1991). Serial passage of EMCV in cell culture can alter in vitro growth characteristics, reduce virulence, and affect hemagglutinating activity (Zimmerman 1994).

**Role in Public Health**

The impact of EMCV on public health is believed to be minimal. Despite the frequency of infection in swine, no association between infection and transmission of disease to humans has been recorded (Zimmerman 1994), even in persons at greatest risk (veterinarians, animal caretakers, laboratory staff). In the light of the ubiquitous presence of EMCV around the world in several animal species, including primates after a rodent plague (Canelli et al. 2010), secondary infections in immunocompromised persons can be expected to occur. In addition, the risk of human EMCV infection may become more important if pigs are used as donors for human xenografts. The experimental infection of a mouse through the transplantation of pig organs infected with EMCV validated this concern (Brewer et al. 2003). Recently, EMCV has been isolated from cases of febrile illness in humans in Peru (Oberste et al. 2009). Interestingly, the Peruvian isolates were most closely related to EMC viruses isolated from pigs in Europe.

**Epidemiology**

At one time considered a disease of subtropical or tropical areas, EMCVs are now noteworthy for their widespread geographical distribution and large number of susceptible hosts. EMCV infection has now been identified by virus isolation or the presence of antibodies throughout the world. In certain countries, a seasonal pattern of the outbreaks, with peaks in autumn, has been noted (Maurice et al. 2007).

EMCV is generally regarded as a rodent virus, although EMC viruses have been isolated from over 30 species of mammals and birds. In mammals, the host range includes monkeys, chimpanzees, elephants, lions, squirrels, mongoose, raccoons, and swine (both domestic and wild boar). An episode of lion deaths at a zoo was found to be due to feeding carcasses of African elephants that had died of EMCV infection (Simpson et al. 1977). Among all outbreaks in wildlife, those in elephants are best documented (Grobler et al. 1995; Hunter et al. 1998; Reddacliff et al. 1997). In rodents, the virus usually persists without causing disease (Acland 1989; Zimmerman 1994). Infected rodents show high levels of the virus in the heart, spleen, lung, pancreas, Peyer’s patches, and thymus, and excrete the virus in their feces and urine (Spyrou et al. 2004). Experimental infection of arthropods and arthropod cells with EMCV failed to show any virus replication, but in some cases, virus could be detected for up to 3 months (R. S. O’Hara et al., unpublished data).

Since infected pigs can excrete the virus, at least for a short period, direct pig-to-pig contact or contact with infected dead pigs are potential routes of virus spread at the farm level (Billinis et al. 1999; Maurice et al. 2002). Transplacental transmission may also occur.
(Christianson et al. 1992; Koenen and Vanderhallen 1997; Links et al. 1986). Although rigorous risk studies are scarce, some factors related to the introduction and/or spread of EMCV in pig farms include the presence of rodents, which may play a role as a virus reservoir and contribute to spread by means of either their feces or as infected carcasses (Acland 1989; Spyrou et al. 2004). Feed or water contaminated with EMCV by rodents or infected rodent carcasses are considered an important source of swine infection. These findings are confirmed by a matched case-control study (Maurice et al. 2007) in an area in Belgium where clinical EMCV outbreaks were regularly reported. The data indicated clusters of factors: (1) rodents, (2) general farm setup, and (3) general hygiene were associated with clinical clusters of factors: (1) rodents, (2) general farm setup, and (3) general hygiene were associated with clinical EMCV. However, the conclusion was that the presence of mice was the most significant risk factor for clinical EMCV infection.

Pathogenesis
Natural infection of swine is most likely to occur by oral exposure. Thereafter, the course of the infection in swine appears to be influenced by virus strain, exposure dose, passage history, and susceptibility of the individual animal. For example, some strains cause only reproductive failure or myocardial death, while others can give rise to both (Koenen and Vanderhallen 1997). Australian strains were shown to be more virulent than New Zealand strains (Horner and Hunter 1979; Littlejohns and Acland 1975) and certain isolates in Florida were found to cause only myocarditis without death (Gainer et al. 1968). Other critical factors, such as the route of infection and age of the pigs, were found to be important factors in the spread of the virus under experimental conditions (Billinis et al. 2004; Littlejohns and Acland 1975).

After experimental oral infections in young pigs, virus was demonstrated as early as 6 hours postinoculation in the intestinal tract. In heart and tonsils, focal positive reactions were found only in the cytoplasm of isolated macrophages and myocardial cells during the first 30 hours postinoculation. After 30 hours, some animals died with typical postmortem lesions and clear positive immunohistochemical reactions were observed in the tonsils and in the heart. Three days postinfection, the virus was also isolated from blood. The highest virus titers were recovered from heart muscle, both in experimental and natural infections. Myocardial lesions were predominant at necropsy. Gelmetti et al. (2006) concluded that virus replication in the heart, the target organ, is followed by myocarditis, with severe myocarditis resulting in sudden death in susceptible pigs. Proinflammatory cytokines such as IL-1β, tumor necrosis factor-α (TNF-α), and IL-6 have been implicated in the pathogenesis of myocarditis caused by EMCV infections (Robinson et al. 2009).

Clinical Signs
Many EMCV infections in a wide range of species are nonlethal and probably subclinical. Younger pigs are generally more susceptible to developing clinical disease, especially in the first weeks of life. In young pigs, the infection is most commonly characterized by acute disease with sudden death due to myocardial failure. Other clinical signs, such as anorexia, listlessness, trembling, staggering, paralysis, or dyspnea have also been observed. Experimentally infected swine (Craighead et al. 1963; Littlejohns and Acland 1975) have shown temperatures up to 41°C (106°F), and death between 2 and 11 days postinoculation (usually 3–5 days), or occasional recovery with chronic myocarditis. Extremely high mortality, approaching 100%, can occur in pigs of preweaning age (Joo 1999). Infections in pigs from postweaning age to adulthood are usually subclinical, although mortality may occasionally be observed, even in adult pigs. In experimental infections, Billinis et al. (2004) found a high fatality rate in 20- and 40-day-old pigs, but none of the challenged 105-day-old pigs died.

In breeding females, clinical signs may vary from inapparent infection to various forms of reproductive failure, including abortion and increased numbers of...
but not always present. In the tonsils, a positive immunohistochemical reaction is located in necrotic debris filling the crypts and in the cytoplasm of monocyte–macrophage lineage cells. This last finding was also noticed in lymph nodes (Gelmetti et al. 2006; Papaioannou et al. 2003; Psychas et al. 2001). Congestion with meningitis, perivascular infiltration with mononuclear cells, and some neural degeneration may be observed in the brain (Acland and Littlejohns 1975). Nonsuppurative encephalitis and myocarditis have also been described in swine fetuses with natural EMCV infection (Kim et al. 1989b).

**Lesions**

Pigs dying from the acute phase of cardiac failure may show only epicardial hemorrhage and no gross lesions. Hydropericardium, hydrothorax, and pulmonary edema are frequently observed at necropsy. The heart is usually enlarged, soft, and pale. The most striking lesions are found in the myocardium where multiple foci of various sizes are found, especially in the right ventricle, and may extend to varying depths within the myocardium. They are often ill defined, circular, and linear, and grayish/white in color (Figures 42.8 and 42.9). These lesions are observed more frequently in fattening pigs than in suckling piglets (Littlejohns and Acland 1975).

Infected fetuses are usually apparently normal, but can be hemorrhagic and edematous. With some virus strains, the fetuses can become mummified in various states of development, depending on the stage of infection. Macroscopic myocardial lesions are exceptional. Histopathologically, the most significant findings in young pigs are seen in the heart. A positive immunohistochemical reaction is chiefly localized to the cytoplasm of myocardial cells. Its intensity and distribution is in accordance with the severity of the lesions. Sometimes, the positive reaction is detected in the Purkinje fibers and in the endothelial cells next to these. Mineralization of necrotic heart muscle is common (Figure 42.10) but not always present. In the tonsils, a positive immunohistochemical reaction is located in necrotic debris filling the crypts and in the cytoplasm of monocyte–macrophage lineage cells. This last finding was also noticed in lymph nodes (Gelmetti et al. 2006; Papaioannou et al. 2003; Psychas et al. 2001). Congestion with meningitis, perivascular infiltration with mononuclear cells, and some neural degeneration may be observed in the brain (Acland and Littlejohns 1975). Nonsuppurative encephalitis and myocarditis have also been described in swine fetuses with natural EMCV infection (Kim et al. 1989b).

**Diagnosis**

In newborn and suckling piglets, the disease is often characterized by sudden death between 3 days and 5 weeks of age. In most cases the piglets are found dead without any clinical signs. In finishing pigs, sudden
death is also the most characteristic sign. All age categories can be affected, but mostly pigs of 60–70 kg (130–155 lb.) are involved. The disease is often restricted to one barn and deaths often occur in the late afternoon when the pigs are most active. In some pigs, squealing can be heard just before dying. In others, dyspnea can be noticed.

The clinical history of reproductive failure and preweaning mortality is a useful indication of EMCV infection (Joo 1999). EMCV-induced reproductive problems should be differentiated from other pathogens causing reproductive problems in sows. EMCV causes reproductive failure in sows of all parities, while porcine parvovirus infection is manifested by an increase in mummification, mainly in gilt litters, without neonatal mortality. Other infections such as FMD, porcine reproductive and respiratory syndrome (PRRS), pseudorabies, porcine circovirus, and leptospirosis should also be considered.

Histopathological lesions may play an important role in making a diagnosis. As described previously, a variable degree of nonsuppurative interstitial myocarditis or encephalitis (infiltration of lymphocytes, histiocytes, and plasma cells) is indicative of an EMCV infection.

A conclusive diagnosis of EMCV should be demonstrated by virus isolation in mice or cell culture. BHK-21 cells are the most sensitive, but HeLa or Vero cell lines are also commonly used. Infected cell monolayers show a rapid and complete cytopathic effect (CPE). Virus identification can then be made by cross-serum neutralization with a reference antiserum or by staining with an anti-EMCV fluorescent antibody conjugate. Molecular methods, such as nucleic acid probes or RT-PCR for the detection of EMCV, have been reported (Kassimi et al. 2002; Vanderhallen and Koenen 1997). These methods provide more sensitive and specific methods of diagnosis, especially when followed by sequencing.

Serological tests for the detection of serum antibodies against EMCV include hemagglutination inhibition (HI), ELISA, latex agglutination, immunofluorescent antibody assay (IFA), agar-gel immunodiffusion (AGID), and VN. The VN and ELISA are the most commonly used methods and have been shown to be specific. For VN, antibody titers of ≥1:16 appear to be significant (Joo 1999).

**Immunity**

Neutralizing antibodies can be detected as early as 5–7 days after inoculation and may persist for an extended period (from 6 months to 1 year). Maternal antibodies remain for at least 2 months. EMCV exists as a single serotype and there is little antigenic variation, therefore cross-protection between all EMCV strains is likely to occur. No cross-neutralization was found between EMCV and 62 human enterovirus serotypes or 11 porcine “enterovirus” serotypes (Zimmerman 1994). As discussed later in this chapter, most of these “enteroviruses” are now called teschoviruses.

**Prevention and Control**

There is no treatment for the disease but, in the acute phase, mortality may be minimized by avoiding stress or excitement of the pigs at risk. Rodents are often thought to play a role in the introduction and subsequent spread of the EMC virus in pig stables. Therefore, pig producers, especially in endemic areas, are advised to maintain rodent control to prevent clinical outbreaks of EMCV.

Pigs exposed to manure through slatted floors or to movement of manure between manure pits were found to be significantly protected against EMCV (Maurice et al. 2007), perhaps due to low level exposure leading to subclinical infections.

The virus can be inactivated in water containing 0.5 ppm residual chlorine. For disinfectants, iodine-based preparations or mercuric chloride can be used.

An inactivated EMCV vaccine is commercially available in the United States. The vaccine appears to be effective, since high humoral immunity is detected in vaccinated pigs and vaccinees were protected from clinical disease when challenged with virulent EMCV that killed 60% of unvaccinated controls. Also, protection against transplacental infection was demonstrated under experimental conditions.

**PORCINE TESCHOVIRUS**

**Relevance**

The first evidence of PTV infection was the occurrence of Teschen disease, a pig polioencephalomyelitis with high mortality, in Czechoslovakia over 75 years ago (Kouba 2009). PTVs are ubiquitous, and no conventional herd of pigs has been shown to be free of infection. While the majority of infections are subclinical, PTVs have been associated with a variety of clinical conditions, including polioencephalomyelitis, reproductive disease, enteric disease, and pneumonia. Strains that have not been shown to be pathogenic were previously referred to as enteric cytopathogenic swine orphan (ECSO) or enteric cytopathogenic porcine orphan (ECPO) viruses, but these terms are no longer in general use.

**Etiology**

PTVs were originally classified as PEVs. More recently, the genomic sequences of the prototype strains of all the PEV/PTV serotypes have been determined, as well as partial genomic data on a number of additional isolates (Doherty et al. 1999; Kaku et al. 1999, 2001; Zell et al. 2001). Comparative analyses of these data showed that the PEVs fell into three genetic groups: (1) PEV
types 1–7 and 11–13, (2) PEV type 8, and (3) PEV types 9 and 10. Prior to reclassification, PEVs were divided into three subgroups based on physicochemical properties, type of CPE produced in PK cells, and different cell culture host ranges (Knowles et al. 1979). The new classification scheme corresponded with the CPE groups defined earlier. The CPE group I viruses also activated in other cells of porcine origin such as the SST cell line or in primary swine testes cells (Knowles et al. 1979).

Thus, the 10 serotypes were renamed from PTV-1 to PTV-10 and reclassified as a single species, Porcine teschovirus, in a new genus, Teschovirus. An additional serotype, PTV-11, was also designated based on serological and molecular sequence data (Zell et al. 2001). The remaining three PEV serotypes are now classified in two species, Porcine sapelovirus (PEV-8, renamed PSV) and Porcine enterovirus B (PEV-9 and PEV-10), in the genera Sapelovirus and Enterovirus, respectively.

As with all picornaviruses, the virions of PTVs are spherical and nonenveloped, with a diameter of 25–30 nm. A single-stranded RNA genome is surrounded by an icosahedral capsid consisting of 60 copies of four polypeptides. A small basic virus-encoded protein (VPg) is linked to the 5′ end of the genome. No three-dimensional structure data are yet available.

The serotypic classification of PTVs is based upon the VN test (Dunne et al. 1971; Knowles et al. 1979). In the 1960s and 1970s there were numerous attempts to achieve a uniform classification of PEVs and these studies culminated in a classification of eight serotypes (Dunne et al. 1971). This was later extended to 13 serotypes (Auerbach et al. 1994; Knowles et al. 1979) (Table 42.1). A complement fixation test, suitable for rapid screening and typing of PTVs/PEVs, has also been described (Knowles and Buckley 1980). Subsequent findings (Knowles 1983) suggested that additional serotypes may exist. Honda et al. (1990b) compared the prototype strains found in Japan with 11 internationally recognized PTV serotypes by VN and suggested a further four candidate serotypes. Some limited cross-reactivity among the existing serotypes was evident, and Hazlett and Derbyshire (1978) showed that gastro-intestinal antibodies were more broadly specific than serum antibodies.

PTVs are stable when treated with lipid solvents and relatively stable to heat. However, heating in the presence of halide ions tends to destabilize the virus. PTVs are stable at pH 2–9. Hemagglutination has not been demonstrated.

PTVs are readily cultivated in the laboratory in cell cultures of porcine origin. They are normally grown in primary or secondary PK cell cultures or in established cell lines such as IB-RS-2, but they may also be cultivated in other cells of porcine origin such as the SST cell line or in primary swine testes cells (Knowles et al. 1979).

Role in Public Health

PTVs are not known to be infectious for humans.

Epidemiology

Tesch disease has continued to occur sporadically, mainly in Central Europe, but also in Africa. Milder forms of polioencephalomyelitis (Talfan disease, benign enzootic paresis), caused by serologically related but less virulent strains of PTV, have been reported in the last 50 years in western Europe, North America, and Australia.

The only known natural host for PTVs is the pig. The virulent PTV-1 strains associated with classical Teschen disease appear to be restricted to those areas in which the disease occurs and they have not been isolated in North America. Less virulent PTV-1 strains and representatives of the other PTV serotypes appear to be ubiquitous.

Transmission of PTV infection is most frequently by the fecal–oral route, and indirect transmission by fomites is likely to occur since the viruses are relatively resistant.

Endemic infection with several PTV serotypes can usually be demonstrated in conventional herds and is probably maintained in groups of weaned piglets. Singh and Bohl (1972) demonstrated waves of infection with six different serotypes over a period of 26 months in a long-term study of infection in a single herd. Piglets normally acquire infection shortly after weaning, when maternally derived antibodies are withdrawn and pigs from several litters are mixed, and it persists for at least several weeks. Adults rarely excrete virus but have high antibody levels. Pigs of any age are, however, fully susceptible to infection with a virus belonging to a serotype to which they have not previously been exposed.

PTVs are highly resistant to inactivation in the environment and may survive for long periods in liquid manure. Likewise, PTVs are relatively resistant to many disinfectants. Of 10 commonly used disinfectants tested by Derbyshire and Arkell (1971) against the Talfan virus, only sodium hypochlorite was effective.

Pathogenesis

Natural infection occurs by ingestion of the virus. It is well established (Long 1985) that initial replication occurs in the tonsil and intestinal tract. The large intestine and ileum are infected more frequently than the upper small intestine, and the former tissues contain higher titers of virus. It has not been clearly established which cells in the intestine support viral replication, but by analogy with experiments on poliovirus (Kanamitsu et al. 1967), it is probable that the reticuloendothelial tissue of the lamina propria is involved. Epithelial cell destruction is not a feature of these infections. Viremia follows regularly in infections with the virulent PTV-1 strains, but less regularly with the less
virulent strains, and leads to infection of the CNS (Holman et al. 1966).

It may be assumed that the pregnant uterus is also infected by viremic spread of the virus since embryonic or fetal infections were demonstrated in gilts following nasal or oral inoculation of PTV (Huang et al. 1980). Intranasal inoculation of the virus may lead experimentally to lung infection (Meyer et al. 1966), but the significance of the natural inhalation of viral aerosols is not known. It has also been clearly demonstrated that when piglets are inoculated parenterally with PTVs, the virus rapidly infects the intestine. Extraintestinal infections are relatively transient, whereas the virus persists in the large intestine for several weeks.

**Clinical Signs**

Although PTV infections are most frequently subclinical, various clinical syndromes have been associated with certain serotypes (Table 42.3) as outlined below.

**Polioencephalomyelitis.** The most severe form of polioencephalomyelitis is produced by the highly virulent PTV-1 strains that cause Teschen disease. This is a disease of high morbidity and high mortality, affecting all ages of swine and associated with major economic losses. The early signs of Teschen disease include fever, anorexia, and listlessness, rapidly followed by locomotor ataxia. In severe cases, there may be nystagmus, convulsions, opisthotonus, and coma. Paralysis ensues, and the animal may assume a dog-sitting posture or remain in lateral recumbency. Stimulation by sound or touch may elicit uncoordinated limb movements or opisthotonus. Death is common within 3–4 days of the onset of clinical signs. Since the appetite returns after the acute phase, some animals may be kept alive by careful nursing, but these cases show muscle wasting and residual paralysis.

The less virulent PTV-1 strains (Talfan disease, benign enzootic paresis) and strains belonging to other serotypes associated with polioencephalomyelitis produce a milder disease with relatively low morbidity and mortality. Mainly young pigs are affected, and the disease rarely progresses to complete paralysis. Recently, encephalomyelitis due to teschovirus infection has been reported in pigs in both the United States (Pogranichnyi et al. 2003) and Japan (Yamada et al. 2004).

**Reproductive Disorders.** The term SMEDI was introduced initially (Dunne et al. 1965) to designate a group of viruses, subsequently shown to be PTVs, that had been isolated in association with stillbirth (S), mummified fetuses (M), embryonic death (ED), and infertility (I). Subsequent studies by the same group of workers and by others (Pensaert et al. 1973; Pensaert and De Meurichy 1973) indicated that the SMEDI could be reproduced experimentally. However, it is now well established that parvovirus infection may also lead to ED and fetal mummification, and parvoviruses may be more frequently associated with these disorders of early and midgestation. Other findings (Cropper et al. 1976) substantiate a role for both PTV and parvoviruses in these disorders. Experimental (Bielaaski and Raeside 1977) and field (Kirkbride and McAdaragh 1978) data confirm an association between teschovirus infection and abortion in swine. These reproductive disorders are not usually accompanied by clinical signs in the sow or gilt. PTVs have also been isolated from the male genital tract (Phillips et al. 1972).

**Diarrhea.** The role of PTVs as enteric pathogens is uncertain. They have frequently been isolated from the feces of piglets with diarrhea, but since they can be readily isolated from normal piglets, particularly postweaning, and since diarrhea can be caused by a variety of other viral and bacterial agents, their presence may be coincidental. However, diarrhea has been produced experimentally by PTVs in piglets believed to be free of other pathogens. The diarrhea is mild and relatively transient, and it seems clear that PTVs are considerably less important enteric pathogens than rotaviruses or coronaviruses. When piglets were infected with PTVs together with rotaviruses, the disease was less severe than in piglets infected only with the rotavirus (Janke et al. 1988).

**Pneumonia, Pericarditis, and Myocarditis.** The role of PTVs as respiratory pathogens is also uncertain. It is probable that alone they rarely cause clinical signs of respiratory disease. Experimentally, two serotypes of

<table>
<thead>
<tr>
<th>Syndrome</th>
<th>PTV Serotypes</th>
<th>Other Picornaviruses</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polioencephalomyelitis</td>
<td>PTV-1, PTV-2, PTV-3, PTV-5</td>
<td>—</td>
</tr>
<tr>
<td>Reproductive disorders</td>
<td>PTV-1, PTV-3, PTV-6</td>
<td>PSV</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>PTV-1, PTV-2, PTV-3, PTV-5</td>
<td>PSV</td>
</tr>
<tr>
<td>Pneumonia</td>
<td>PTV-1, PTV-2, PTV-3</td>
<td>PSV</td>
</tr>
<tr>
<td>Pericarditis and myocarditis</td>
<td>PTV-2, PTV-3</td>
<td>—</td>
</tr>
<tr>
<td>Cutaneous lesions</td>
<td></td>
<td>PEV-9, PEV-10</td>
</tr>
</tbody>
</table>

Table 42.3. Natural or experimental clinical syndromes associated with porcine enteric picornavirus infection
PTV have been shown capable of producing pericarditis, and in one experiment myocardial involvement occurred (Long et al. 1969). These findings might lead to a suspicion of teschovirus infection in the case of sudden death in piglets, although EMCV might be a more likely candidate.

Possible Association with Cutaneous Lesions. During the course of investigations of SVD outbreaks in the United Kingdom, many adventitious agents were isolated from samples of epithelium and feces. Most of these isolates were identified as teschoviruses or entero-viruses (Knowles 1983).

Lesions
No specific changes have been associated with intestinal PTV infections. They do not appear to cause villous atrophy, which is characteristic of primary intestinal pathogens such as coronaviruses and rotaviruses. Other than muscle atrophy in chronic cases, no gross lesions are found in polioencephalomyelitis. The histological lesions associated with the latter are widely distributed in the CNS but are especially numerous in the ventral columns of the spinal cord, the cerebellar cortex, and the brain stem. The changes are more marked and extensive in Teschen disease than in milder encephalomyelitides, such as Talfan disease. The neurons show progressive diffuse chromatolysis (Koestner et al. 1966), and there are focal areas of gliosis and perivascular cuffing, particularly over the cerebellum, that may also occur.

The SMEDI syndrome is remarkable for the lack of specific lesions in stillborn or neonatal piglets, although mild focal gliosis and perivascular cuffing in the brain stem have been found occasionally. Placental changes are restricted to nonspecific degeneration.

Pneumonic lesions have been produced by several investigators. Smith et al. (1973) described areas of grayish-red consolidation in the ventral anterior lobes of lungs infected with a PTV-2 strain. Exudates were observed in the alveoli and bronchi, as well as slight perivascular and peribronchiolar cuffing, and some hyperplasia of the bronchiolar epithelium.

A PTV-3 strain consistently produced serofibrinous pericarditis experimentally and the more severely affected piglets showed focal myocardial necrosis (Long et al. 1969).

Diagnosis
Teschen disease (also known as teschovirus encephalomyelitis) is designated as a notifiable disease by the OIE and details of internationally accepted diagnostic methods are described (OIE 2008).

The occurrence of clinical signs associated with polioencephalomyelitis is suggestive of viral infection, but the differentiation of teschoviral infection from other neurotropic viruses requires isolation of the virus from the CNS, the demonstration of viral antigen by specific immunofluorescence, or the detection of viral RNA by RT-PCR. Similarly, with reproductive disorders, diarrhea, pneumonia, pericarditis, and myocarditis, there are no diagnostic clinical signs which would suggest PTV involvement and laboratory diagnosis is required.

Virus Detection
Virus isolation from the CNS requires the collection of tissues from a piglet showing early nervous signs. Animals that have been paralyzed for several days may no longer contain infectious virus in the CNS (Lynch et al. 1984). The virus may be isolated in PK cell cultures from suspensions of the spinal cord, brain stem, or cerebellum. The virus may subsequently be identified on the basis of its physicochemical characteristics or by immunostaining (Watanabe et al. 1971). Serological identification of the isolate is desirable. Isolation of a PTV from the gastrointestinal tract of a piglet with nervous signs does not establish the etiology of the disease, since the enteric infection may be coincidental.

In the SMEDI syndrome, mummified fetuses carried to term rarely contain live virus, but may contain viral antigen detectable by immunofluorescence. Virus isolation in PK cell culture may be attempted from tissues of aborted or stillborn fetuses. Lung tissue appears to be the most reliable source for the isolation of PTVs from fetuses (Huang et al. 1980). VN tests on the body fluids of such fetuses can be carried out against the SMEDI-associated PTV serotypes.

In the investigation of pneumonia or diarrhea, virus isolation from the respiratory or intestinal tract may be attempted but the virological findings should be interpreted cautiously, especially in relation to diarrhea, since enteric infections with PTVs are common in healthy piglets. In one study PTVs and PEVs were isolated from 57% of porcine fecal samples submitted for SVD diagnosis over a 7-year period (Knowles 1983).

Isolated viruses may be identified by VN (Knowles et al. 1979), complement fixation (Knowles and Buckley 1980), or immunofluorescence (Auerbach et al. 1994; Dauber 1999) if suitable reference reagents are available. Monoclonal antibodies capable of detecting PTVs have been described (Dauber 1999).

Now that genomic sequence data are available for all the PTVs, it is possible to use the RT-PCR to detect viral RNA in clinical samples or to identify viruses isolated in cell culture. Palmquist et al. (2002) described an RT-PCR using a single set of primers for the simultaneous detection and differentiation (based on amplicon size) of PTVs and PSV. Nested RT-PCR assays to specifically detect PTV-1 and to differentiate PTV, PSV and PEVs from each other using virus-specific primer sets have also been described (Zell et al. 2000). More recently, improvements to these assays resulted in the
development of a one-step real-time PCR (Krumbholz et al. 2003).

**Antibody Detection.** Serology is of little value for diagnosis, unless paired sera are available and the serotype known, in which case VN would be suitable. An ELISA suitable for mass screening has been described for the detection of antibodies against Teschen disease virus (Hubschle et al. 1983). However, as these viruses are ubiquitous serological surveys may not be very helpful.

**Immunity**

Infected pigs mount a classical humoral protective immune response (IgM and IgG). Mucosal immunity (IgA) may be produced and have a protective effect in the gastrointestinal tract. In an experimental study of PTV infection in piglets, it was shown that cell-mediated responses were weak, localized, and not associated with significant antiviral activity (Brundage et al. 1980).

Humoral antibody is thought to be important for protection. Immunosuppression of pigs infected with a PTV by cyclophosphamide treatment led to the lack of a serological response and a persistent infection of the intestinal tract (Derbyshire 1983). This resulted in severe diarrhea, and in one case, signs of encephalomyelitis. Presence of high levels of virus-neutralizing IgA antibody in the gastrointestinal tract may protect against oral challenge (Hazlett and Derbyshire 1977).

Since antibody is probably the most important factor in protection, at least in teschoviruses, the relatively large number of serotypes would suggest that cross-protection might not occur.

It has been reported for PTV-1 that maternal antibody has no effect on embryonic or fetal infections after the virus has reached the uterus (Huang et al. 1980). However, preinfection antibody in sows would probably limit infection and prevent the virus from reaching the uterus. Colostral antibody would be expected to protect piglets from infection.

**Prevention and Control**

As with most viral infections, control measures for PTV depend on prevention rather than treatment. Potential antiviral chemotherapeutics for porcine enteric picornaviruses have received little attention. Piglets with mild polioencephalomyelitis may recover if nursing care is provided during the period of transient paresis.

Vaccination has been practiced in the field only for the control of Teschen disease. The earlier Teschen disease vaccines, containing inactivated virus of pig tissue origin, have been superseded by attenuated or inactivated cell culture vaccines. Mayr and Correns (Mayr 1959) attenuated Teschen disease virus by cell culture passage and showed that live or formalin-inactivated vaccines prepared from this virus induced similar levels of protection in piglets. Success has been claimed for a Teschen disease eradication program involving ring vaccination and slaughter (Schaupp 1968). Restrictions on the import of swine and pork products from areas in which Teschen disease is endemic seem to be effective in limiting the spread of virulent PTV-1 strains. If such strains were introduced into North America, they would likely be controlled by a policy of quarantine and slaughter.

Vaccination has not been practiced against the milder forms of polioencephalomyelitis or against the other clinical manifestations of PTV infection in swine. Only the SMEDI syndrome is of sufficient economic importance to justify specific control measures in the field, but the multiplicity of serotypes that may be involved complicates the development of an effective vaccine.

The best current approach to the prevention of reproductive disorders associated with PTVs would appear to be the application of management practices that ensure that gilts are exposed to the infection with endemic viruses at least 1 month before breeding. This can be achieved naturally if the animals remain in a single building from birth to breeding, with thorough mixing of piglets from different litters at weaning. If breeding stock is segregated at an early age, they should be exposed to fecal material from recently weaned piglets. This can be readily accomplished by adding fresh feces to the feed of gilts or by dosing gilts with capsules of feces derived from a pooled sample collected from weaned piglets in several pens. The objective is to ensure exposure to the widest possible range of the viruses present in the herd. The operation of a closed-herd system reduces the risk of introducing extraneous viruses, but it is not possible to eliminate this risk, since the relatively resistant enteric picornaviruses can be transmitted by a variety of fomites. If the introduction of fresh stock is essential for breeding purposes, before the gilts or sows are bred they should be exposed (by fecal contamination as described above) to any virus that may be present or introduced.

Exclusion of PTVs by repopulation of herds with specific pathogen-free (SPF) stock seems to be difficult or impossible to achieve over a prolonged period, since these viruses have been isolated from commercial SPF herds and the accidental introduction of Talfan virus into SPF gilts maintained under strict isolation has been described (Parker et al. 1981). Since transplacental infection of fetuses may occur, even gnotobiotic pigs may be infected.

**SENECA VALLEY VIRUS**

SVV was initially found as a contaminant in cell cultures, but a number of closely related viruses have now been isolated from pigs (Hales et al. 2008). The complete genome sequence (Hales et al. 2008) and the three-dimensional structure of the virus capsid (Venkataraman et al. 2008) have been described. The pro-
teins of the virus are most closely related to the cardioviruses, but there are some significant differences in the genome organization and sequence. For these reasons, the virus is now classified as a new species (Seneca Valley virus) within a new genus (Senecavirus) of the Picornaviridae. A feature of SVV is its ability to replicate within tumor cells and there is interest in using it as an oncolytic virus against neuroendocrine cancers (Reddy et al. 2007).

There is no conclusive evidence that SVV is pathogenic for pigs, but there is one report of the RT-PCR-detectable SVV in a group of pigs from Manitoba, Canada with idiopathic vesicular disease (Pasma et al. 2008). Many of these pigs displayed lameness and had vesicles on the snout and feet, and some had fever. Investigations for FMDV, SVDV, and vesicular stomatitis virus were negative. However, initial attempts to reproduce the disease using SVV in experimentally inoculated animals have been unsuccessful (S. Alexandersen, unpublished results), although a serological response against the virus was detected.

**PORCINE KOBUVIRUS**

The Kobuvirus genus initially consisted of the human Aichi virus and the bovine kobuvirus. However, Reuter et al. (2009) reported the sequence of a PKV about 60% identical at the nucleotide sequence level to the Aichi virus and bovine kobuvirus. PKV was initially detected in an RT-PCR analysis for the presence of porcine sapovirus, a calcivirus, in fecal samples from healthy pigs in Hungary. A nonspecific PCR product was sequenced and found to be related to the known kobuviruses. Following the characterization of this virus, some 60% of the pigs at the farm were found to be carrying the virus, including about 90% of the animals less than 3 weeks of age. Subsequently, an extremely high (99%) incidence of PKV was found in piglets with diarrhea in Thailand (Khamrin et al. 2009), and in a study from China (Yu et al. 2009), about 30% of fecal samples from healthy pigs were positive by RT-PCR using specific primers for this virus. However, no experimental studies demonstrating a causal role in disease have yet been published.

**PORCINE SAPELOVIRUS**

Formerly called both PEV-8 and CPE type II group PEVs, PSV is a member of genus Sapelovirus, along with some simian and avian picornaviruses. PSVs are antigenically diverse (Dunne et al. 1971) and have been associated with the SMEDI syndrome (Dunne et al. 1965) and diarrhea (Honda et al. 1990a). Experimentally, pregnant pigs infected with PSV developed fetal infections (Huang et al. 1980). In addition to cells of porcine origin, PSV can be cultivated in monkey kidney (e.g., Vero) and BHK-21 cells (Knowles et al. 1979).

**REFERENCES**


OVERVIEW

Rotaviruses and reoviruses belong to the genera *Rotavirus* and *Reovirus*, respectively, in the family *Reoviridae*. Both rotaviruses and reoviruses are nonenveloped, icosahedral particles with a triple-layered capsid structure about 75 nm in diameter. The outer capsid layer can be removed by various chemical and enzymatic treatments, yielding noninfectious particles of about ~65 nm in diameter for rotaviruses and 50 nm in diameter for reoviruses. Viruses in the family *Reoviridae* have segmented double-stranded (ds) RNA. Rotaviruses and reoviruses have 11 and 10 segmented dsRNA, respectively. With some exceptions, each RNA encodes one structural or nonstructural protein (NSP). Due to the nature of the segmented genome, genomic reassortment among different strains occurs in rotaviruses and reoviruses, which may drive genetic diversity.

Both rotaviruses and reoviruses are ubiquitous. Rotaviruses are important causes of diarrheal disease in neonates and young of many species and a common cause of gastroenteritis in nursing and postweaning pigs. The role of reoviruses in the disease process is less clear. While reoviruses may be detected in pigs with respiratory, enteric, and reproductive diseases, they may also be recovered from healthy pigs.

PORCINE ROTAVIRUS

Relevance

Rotaviruses are a major cause of diarrhea in neonates and young humans and animals, including pigs. Rotaviruses were first discovered in calves (Mebus et al. 1969) and subsequently detected in humans (Bishop et al. 1973), pigs (Rodger et al. 1975) and other animals. Porcine rotaviruses are divided into four serogroups (A–C, E) based on the antigenicity of VP6. Group A rotaviruses are the most common cause of diarrhea in young pigs (both preweaning and postweaning) and account for up to 90% of all rotavirus diarrhea in commercial pig operations (Will et al. 1994).

Etiology

Rotaviruses are nonenveloped, icosahedral particles 65–75 nm in diameter (Figure 43.1). Rotavirus has 11 segments of dsRNA with a total genome size of ~18,522 base pairs (Estes and Cohen 1989). Each rotavirus genome segment encodes one of six viral structural proteins or one of six NSPs, except segment 11, which encodes both NSP5 and NSP6 (Estes and Kapikian 2007). The VP6 is the most abundant viral structural protein followed by glycoprotein VP7. The VP4 is a nonglycosylated protein and its proteolytic cleavage into VP5 and VP8 is important for viral infectivity.

Rotaviruses are triple-layered particles: the outer layer is comprised of VP7 and VP4; the inner layer of VP6; and the core of VP1, VP2, and VP3. The complete three-layered particles resemble a wheel (rota in Latin) when visualized by electron microscopy (EM). The outer capsid layer of VP7 and VP4 can be removed by various chemical and enzymatic treatments. The remaining double-layered particle is ~65 nm in diameter with rough edges. On EM of rotavirus samples, both double- and triple-layered particles are frequently observed. The double-layered rotavirus lacks the outer proteins that are involved in virus entry into susceptible cells, and only the triple-layered virus particles are infectious (Estes and Kapikian 2007).
Rotaviruses are divided into seven (A–G) morphologically indistinguishable, but antigenically distinct, serogroups based on the serological characteristics of VP6 (Estes and Kapikian 2007; Saif and Jiang 1994). Serogroup A rotaviruses are well characterized because they are the major cause of gastrointestinal disease in humans and animals. Groups B and C rotaviruses have been detected sporadically in animals and humans, but group C rotaviruses are becoming more common as a cause of diarrhea in suckling pigs under 1 week of age (Rossow et al. 2010). Group E rotaviruses have only been detected in pigs in the United Kingdom (Chasey et al. 1986), and groups D, F, and G have been detected in avian species.

The extracted rotavirus genome (segmented dsRNA) shows characteristic electrophoretic migration patterns (electropherotype) in a polyacrylamide gel for each distinct serogroup (Pedley et al. 1986; Saif and Jiang 1994). The rotavirus RNA segments cluster into four regions (I–IV). The cluster patterns corresponding to serogroups A, B, C, and D in regions I, II, III, and IV are 4:2:3:2, 4:2:2:3, 4:3:2:2 and 5:2:2:2, respectively. However, rotavirus groups and types need to be confirmed by serological and nucleic acid-based assays.

Rotaviruses in group A are further divided into G (VP7) serotypes and P (VP4) serotypes or genotypes (Table 43.1). Outer capsid proteins VP7 and VP4 elicit neutralizing antibodies (Abs) independently (Greenberg et al. 1983), and there are at least 15 G serotypes (VP7) and 26 P genotypes (VP4) among human and animal rotaviruses (Estes and Kapikian 2007).

Serotypes are defined by serological assays, for example, plaque reduction neutralization assays or fluorescent focus reduction assays, using polyclonal or monoclonal Abs (Bohl et al. 1984; Estes and Cohen 1989; Hoshino et al. 1984; Paul et al. 1988). Genotypes are defined by comparative sequence analysis and/or nucleic acid hybridization data. Strains that belong to the same genotype have higher than 89% amino acid sequence homology (Estes and Kapikian 2007; Gorziglia et al. 1990).

Because homotypic immunity is important for protection against rotavirus infection, determination of G and P types is important. Among porcine rotaviruses, at least 10 G serotypes (G1-6, G8-10, and 11) and seven P types (P1A[5], 2B[6], 7[5], 9[7], unidentified serotype[13], 12[19], and 14[23]) have been detected in association with diarrhea (Table 43.1). The predominant G types identified in pigs are G3 (CRW-like), G4 (Gottfried-like), G5 (OSU-like), and G11 (YM-like) (Table 43.2). However, human types G1, 2 and 9, and bovine types G6, 8, and 10, have also been detected in pigs. The most common P types in pigs are P2B[6] and P9[7], which are Gottfried-like and OSU-like types, respectively (Table 43.2). Other porcine P genotypes P[8] and P[6] (M37-like) and bovine P genotypes P[1], P[5] and P[11] have also been detected (Tables 43.1 and 43.2). In Japan, it was shown that G9 (with P2B[6] or unusual P types such as P[13] and P[23]) porcine rotaviruses were predominant in diarrhea outbreaks in young pigs in 2000–2002 (Teodoroff et al. 2005).
### Table 43.1. Serogroup, serotype, and genotype designations of selected porcine rotaviruses

<table>
<thead>
<tr>
<th>Serogroup</th>
<th>G (VP7) Serotype</th>
<th>Strain</th>
<th>P (VP4) Serotype [Genotype]</th>
<th>Strain</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>1</td>
<td>C60, C91, C95, CN117, C86, S7, S8A</td>
<td>1A[8]</td>
<td>S8A, S9B</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>OSU, EE, TFR-41, A34, MDR-13, A46, C134, CC117, S8B, 134/04-15</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>84/52E, 84/104E, 84/158F</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>Field sample</td>
<td>Not tested [13]</td>
<td>MDR-13, A46, Clon8</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>ISU-64, S5, A2, S8</td>
<td>12[19]</td>
<td>4F</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>P343</td>
<td>14[23]</td>
<td>A34</td>
</tr>
<tr>
<td></td>
<td>11</td>
<td>YM, A253</td>
<td>Not tested [26]</td>
<td>134/04-15</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>RU172</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>Ohio, NIAD-1, IA1146</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>Cowden, HF, IA850</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E</td>
<td>DC-9</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Dual G serotypes were reported.

Table is adapted and modified from Estes and Kapikian (2007) and Paul and Stevenson (1999).

### Laboratory Cultivation.

Porcine rotaviruses were first adapted to grow in roller tube cultures of porcine primary kidney cells by pretreatment of virions with trypsin or pancreatin (Theil et al. 1977). Viruses were later propagated in the African green monkey kidney cell line MA-104 (Bohl et al. 1984). The addition of proteolytic enzymes (trypsin or pancreatin) is essential for isolation of rotaviruses, since the proteolytic cleavage of VP4 is important for viral infectivity. This reflects rotavirus growth in the intestines of infected animals, where the proteolytic enzymes are abundant. For cultivation, viruses are pretreated with trypsin (10 µg/mL) or pancreatin (0.5–1 µg/mL), or trypsin or pancreatin is added to serum-free culture medium after viral adsorption.

The cytopathic effects produced by cell culture-adapted virus strains are characterized by rounding of cells followed by cell detachment from the surface. Cells are further lysed and the broken cell debris is present in the culture medium. Viral antigens can be demonstrated in the cytoplasm of virus-infected cells by immunofluorescence (Figure 43.2) or immunochromatographic methods.

Groups B and C porcine rotaviruses also have been propagated in roller cultures of primary porcine kidney cells in the presence of high concentrations of pancreatin (Sanekata et al. 1996; Terrett et al. 1987). The porcine group C rotavirus Cowden strain was adapted to grow in MA-104 cells (Saif et al. 1988). Group E
Table 43.2. Geographic and temporal variability in the dominant group A rotavirus G and P types in subclinical or diarrheic pigs in various countries

<table>
<thead>
<tr>
<th>Country</th>
<th>Report</th>
<th>Time Period</th>
<th>G Type</th>
<th>P Type</th>
<th>G/P Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Korea</td>
<td>Kim et al. 2010</td>
<td>2006–2007</td>
<td>G5 (70%)</td>
<td>P[7] (93%)</td>
<td>G5P[7] (64%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>n = 98 (38%)</td>
<td>G8 (17%)</td>
<td>P[23] (2%)</td>
<td>P [1] (1%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>n = 182/475</td>
<td>G9 (9%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>3–70 days</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>n = 18</td>
<td>G5 (28%)</td>
<td>P[23] (28%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Nursing weaned</td>
<td>G3 (17%)</td>
<td>P[7] (17%)</td>
<td>P[1]; P[13]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>G4, G1</td>
<td>G4 (55%)</td>
<td>P[6] (64%)</td>
<td>G4P[6] (45%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>n = 11 Ages?</td>
<td>G5(18%)</td>
<td>P[27] (18%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>n = 21/122 (17%)</td>
<td>G2,G9,G1 (9%)</td>
<td>P[13] (18%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>n = 31</td>
<td>G5 (29%)</td>
<td>P[6] (42%)</td>
<td>G4P[7]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ages?</td>
<td>G5 (15%)</td>
<td>P[6] (10%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>OH, CA, IA, KS, NE, SD</td>
<td>G3, G4 (9%)</td>
<td>P[6] (49%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>1–60 days</td>
<td>G9 (5%)</td>
<td>P[6] (50%)</td>
<td>G4P[6] (20%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>G7 (39%)</td>
<td>G4P[6] (20%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>n = 10</td>
<td>G9 (20%)</td>
<td>P[8] (10%)</td>
<td>G4P[6] (20%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>21–35 days</td>
<td>G1, G5</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>n = 57/216 26%</td>
<td>G4 (35%)</td>
<td>P[6] (10%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>OH, CA, IA, KS, NE, SD</td>
<td>G5 (15%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>n = 59</td>
<td>G3, G4 (9%)</td>
<td>P[6] (49%)</td>
<td>G5P[7] (10%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1–60 days</td>
<td>G9 (5%)</td>
<td>P[6] (50%)</td>
<td>G4P[6] (20%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>G7 (39%)</td>
<td>G4P[6] (20%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>n = 59</td>
<td>G3, G4 (9%)</td>
<td>P[6] (49%)</td>
<td>G5P[7] (10%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1–60 days</td>
<td>G9 (5%)</td>
<td>P[6] (50%)</td>
<td>G4P[6] (20%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>G7 (39%)</td>
<td>G4P[6] (20%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>n = 81/406 20%</td>
<td>G3 (27%)</td>
<td>P[13] (17%)</td>
<td>G3P[7] (14%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Nursing 13%</td>
<td>G4 (19%)</td>
<td>P[7] (16%)</td>
<td>G4P[6] (12%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Weaning 27%</td>
<td>G2,G11,G1 (9%)</td>
<td>NT (14%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Finisher 19%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>n = 88</td>
<td>G4 (16%)</td>
<td>P[6] (16%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ages?</td>
<td>G3 (9%)</td>
<td>P[6] (16%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>ND (45%)</td>
<td>ND (61%)</td>
<td></td>
</tr>
</tbody>
</table>

*Most fecal samples were from subclinically rotavirus-infected pigs (18% RV+). Of rotavirus-positive pigs, 76–87% shed subclinically with weaning (27%) > finisher (19%) > nursing (13%). Both bovine-like (G10, G6) and human-like (G1, G2, P[6]) rotavirus types were identified in pigs.

ND, not determined.

rotavirus, most group B rotaviruses, and some group A viruses still cannot be serially propagated in cell culture.

**Physicochemical and Biological Properties.** Rotaviruses are generally resistant to environmental conditions, for example, temperature, pH, chemicals, and disinfectants, including ether, chloroform, or detergents (deoxycholate) (Estes and Kapikian 2007). However, rotavirus infectivity is lost by repeated freeze–thaw cycles and treatment with ethylenediaminetetraacetic acid (EDTA) at 5 mM concentration, which removes the outer layer. Rotavirus is relatively stable at pH 3–9.

Triple- and double-layered rotavirus particles can be separated by centrifugation in sucrose or cesium chloride (CsCl) gradients. The density of triple-layered particles is 1.36 g/mL in CsCl and sediment coefficients are 520S–530S in sucrose. The double-layered particles have a density of 1.38 g/mL and sediment coefficients of 380S–400S (Tam et al. 1976). The density and sedimentation coefficient of single-layered core particles are 1.44 g/mL in CsCl and 280S, respectively (Bican et al. 1982).

**Role in Public Health**

Due to the frequent occurrence of genetic reassortment among rotaviruses, reassortants containing gene(s) from porcine or human rotaviruses (both group A and C rotaviruses) can be detected in human or porcine populations, respectively. However, the zoonotic potential of porcine rotavirus requires further investigation and continued surveillance.

**Epidemiology**

Although serological prevalence is related to the age of the pigs, group A rotaviruses are highly prevalent throughout the world. In some countries, up to 100% of adult pigs may be seropositive (Bridger and Brown 1985; Brown et al. 1987; Chassey et al. 1986; Hung et al. 1987; Nagesha et al. 1988; Terrett et al. 1987; Theil
and Saif 1985; Tsunemitsu et al. 1992). Seroprevalence to porcine groups B, C, and E is also high (Table 43.3).

Rotavirus serogroups A, B, C, E, and multiple serogroups and serotypes within serogroups A and C have been detected in pigs. The prevalence of group A rotaviruses in diarrheic samples varies from 10% to 70%, depending on the assay used, age of pigs, region, and so on (Table 43.4). Recent studies using sensitive methods, such as reverse transcription-polymerase chain reaction (RT-PCR), suggested that the prevalence of group A rotaviruses in diarrheic samples is high (up to 67%) (Table 43.4) (Halaihel et al. 2010; Katsuda et al. 2006; Kim et al. 2010; Lamhoujeb et al. 2010).

The primary route of transmission of rotaviruses is fecal–oral, although a recent study showed that gnotobiotic pigs orally or nasally inoculated with a virulent group A human rotavirus (Wa strain) shed rotaviruses at similar titers in nasal secretions as in feces for 3–4 days (Azevedo et al. 2005). Whether porcine rotavirus spreads among pigs via the respiratory route has not been confirmed.

Group A rotavirus is detected most frequently in pigs younger than 60 days of age and as early as 1 week of age. The highest prevalence occurs at 3–5 weeks of age (Bohl 1979). The duration of group A rotavirus shedding in feces is ~7.4 days and ranges from 1 to 14 days (Fu and Hampson 1987). Virus shedding is affected by the level of passive immunity and rotavirus serogroup. Adult animals do not generally shed rotavirus, but they may occasionally shed viruses and serve as sources of infection. In group B rotavirus infections, the duration and quantity of virus shedding is less than that of group A rotavirus (Bridger 1980; Theil and Saif 1985).

Previously, only a few outbreaks of group C or E rotaviruses were reported in the United States or United Kingdom (Chasey and Davies 1984; Kim et al. 1999), but recent data suggest group C rotaviruses are becoming dominant in suckling diarrheic pigs under 7 days of age (Rossow et al. 2010). In Brazil, genetically diverse porcine group C rotaviruses in diarrheic and normal fecal samples were reported in 2010 (Médić et al. 2010).

Generally, porcine rotaviruses circulate among pigs, their natural host. However, reassortment and interspecies transmission of rotavirus among pigs and cattle, pigs and horses, and pigs and humans has been reported (Alfieri et al. 1996; Cao et al. 2008; Das et al. 1993; Dunn et al. 1993; Ghosh et al. 2010; Laird et al. 2003; Matthijnssens et al. 2010; Nakagomi and Nakagomi 2002; Parra et al. 2008). Some of the reassortant viruses were identified in clinical samples from humans (Esona et al. 2009; Santos et al. 1999). For example, human rotavirus RMC321 strain from an outbreak of rotavirus diarrhea in India had characteristics of porcine rotavirus in most of the genes, including VP4, 6, and NSP1-5 (95–99% amino acid identity), indicating that porcine rotavirus can cross the species barrier and cause severe gastroenteritis in humans (Varghese et al. 2004).

In feces, rotaviruses are resistant to inactivation at 60°C (140°F) for 30 minutes and at 18–20°C (64–68°F) for at least 7–9 months (Woode 1978). Rotaviruses in organic material can be inactivated by 2% acid glutaraldehyde, 70% ethanol, 3.7% formaldehyde, 10% povidone-iodine, 67% chloramine T, and 0.5% trichloran (Sattar et al. 1983). Disinfectants that can inactivate rotaviruses include phenols, formalin, chlorine, and beta-propiolactone. Disinfectant spray containing ethanol (0.1% o-phenylphenol and 79% ethanol), bleach (6% sodium hypochlorite diluted to give 800 ppm free chlorine) and phenol-based products (14.7% phenol diluted 1.256 in tap water) effectively reduced the rotavirus titer by 95–99% after 10 minutes (Sattar et al. 1994). Ethanol (95%) acts as perhaps the most effective disinfectant by removing the outer capsid of rotavirus (Estes et al. 2001).

### Table 43.3. Prevalence of group A, B, and C rotaviruses in diarrheic pigs

<table>
<thead>
<tr>
<th>Country</th>
<th>Report/Assay</th>
<th>Pig Ages</th>
<th>Number/% Rotavirus Positive</th>
<th>% Group Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>United States</td>
<td>Janke et al. 1990/ polyacrylamide gel electrophoresis (PAGE)</td>
<td>All</td>
<td>90/NR</td>
<td>68 10 11 11</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Nursing</td>
<td>68/NR</td>
<td>76 7 7 9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Weaned</td>
<td>22/NR</td>
<td>41 18 23 18</td>
</tr>
<tr>
<td>Thailand</td>
<td>Pongsuwanna et al. 1996/PAGE</td>
<td>Piglets</td>
<td>26/5</td>
<td>89 4 8 0</td>
</tr>
<tr>
<td>South Africa</td>
<td>Geyer et al. 1996/ PAGE</td>
<td>Piglets (&lt;6 weeks)</td>
<td>NR/38</td>
<td>85 5 11 ND</td>
</tr>
<tr>
<td>Italy</td>
<td>Martella et al. 2007/ RT-PCR</td>
<td>Weaned (1–3 months)</td>
<td>102/98</td>
<td>72 ND 31(3) A + C</td>
</tr>
<tr>
<td>Korea</td>
<td>Jeong et al. 2007/ RT-PCR</td>
<td>Piglets (1–7 weeks)</td>
<td>137/26</td>
<td>ND ND 17(12) A + C</td>
</tr>
</tbody>
</table>

*In these studies, group C rotavirus alone occurred in 3% and 12% of samples, respectively.

NR, not reported.
Table 43.4. Rotavirus prevalence in various countries

<table>
<thead>
<tr>
<th>Country</th>
<th>Age</th>
<th>Rotavirus Positive/Total Number</th>
<th>% Positive</th>
<th>% Positive for Group A Rotavirus</th>
<th>Assay Used</th>
<th>Report</th>
</tr>
</thead>
<tbody>
<tr>
<td>United States</td>
<td>All ages</td>
<td>90/NR</td>
<td>NR</td>
<td>68</td>
<td>PAGE</td>
<td>Janke et al. (1990)</td>
</tr>
<tr>
<td></td>
<td>Suckling pigs</td>
<td>14/NR</td>
<td>NR</td>
<td>76.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Weaned pigs</td>
<td>29/NR</td>
<td>NR</td>
<td>40.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nigeria</td>
<td>All ages</td>
<td>43/96</td>
<td>44.8</td>
<td>100</td>
<td>ELISA</td>
<td>Attii et al. (1990)</td>
</tr>
<tr>
<td></td>
<td>1–3 weeks</td>
<td>29/52</td>
<td>34.2</td>
<td>100</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>4–6 weeks</td>
<td>14/41</td>
<td>55.8</td>
<td>100</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>&gt;6 weeks</td>
<td>0/3</td>
<td>0</td>
<td>100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>United States</td>
<td>All ages</td>
<td>96/1048</td>
<td>9</td>
<td>89</td>
<td>ELISA, PAGE</td>
<td>Will et al. (1994)</td>
</tr>
<tr>
<td>Thailand</td>
<td>Piglets</td>
<td>26/557</td>
<td>4.7</td>
<td>89</td>
<td>PAGE</td>
<td>Pongsuwanna et al. (1996)</td>
</tr>
<tr>
<td>South Africa</td>
<td>Piglets (&lt;6 weeks)</td>
<td>NR</td>
<td>37.8</td>
<td>84.6</td>
<td>PAGE</td>
<td>Geyer et al. (1996)</td>
</tr>
<tr>
<td>Poland</td>
<td>Piglets</td>
<td>169/531</td>
<td>32</td>
<td>100</td>
<td>ELISA</td>
<td>Markowska-Daniel et al. (1996)</td>
</tr>
<tr>
<td>Germany</td>
<td>All ages</td>
<td>5/149</td>
<td>3.4</td>
<td>NR</td>
<td>EM</td>
<td>Wieler et al. (2001)</td>
</tr>
<tr>
<td></td>
<td>1–7 days</td>
<td>0/33</td>
<td>0</td>
<td>NR</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>8–14 days</td>
<td>1/50</td>
<td>2</td>
<td>NR</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>15–21 days</td>
<td>1/19</td>
<td>5.3</td>
<td>NR</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>22–28 days</td>
<td>4/16</td>
<td>25</td>
<td>NR</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>36–42 days</td>
<td>0/31</td>
<td>0</td>
<td>NR</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brazil</td>
<td>All ages</td>
<td>53/99</td>
<td>53.5</td>
<td>100</td>
<td>PAGE</td>
<td>Barreiros et al. (2003)</td>
</tr>
<tr>
<td></td>
<td>&lt;7 days</td>
<td>10/19</td>
<td>53</td>
<td>100</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>8–21 days</td>
<td>12/20</td>
<td>60</td>
<td>100</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>&gt;21 days</td>
<td>31/60</td>
<td>52</td>
<td>100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Japan</td>
<td>All ages</td>
<td>179/269</td>
<td>66.5</td>
<td>NR</td>
<td>RT-PCR</td>
<td>Katsuda et al. (2006)</td>
</tr>
<tr>
<td></td>
<td>Suckling</td>
<td>103/153</td>
<td>67.3</td>
<td>NR</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Weaned</td>
<td>76/116</td>
<td>65.5</td>
<td>NR</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Korea</td>
<td>All ages</td>
<td>182/475</td>
<td>38.3</td>
<td>NR</td>
<td>RT-PCR</td>
<td>Kim et al. (2010)</td>
</tr>
<tr>
<td>Spain</td>
<td>All ages</td>
<td>37/221</td>
<td>16.7</td>
<td>NR</td>
<td>RT-PCR</td>
<td>Halaihel et al. (2010)</td>
</tr>
<tr>
<td></td>
<td>&lt;8 weeks</td>
<td>21/64</td>
<td>32.8</td>
<td>NR</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>&gt;8 weeks</td>
<td>16/157</td>
<td>10.2</td>
<td>NR</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Canada</td>
<td>All ages</td>
<td>21/122</td>
<td>17</td>
<td>NR</td>
<td>RT-PCR</td>
<td>Lamhoujeb et al. (2010)</td>
</tr>
</tbody>
</table>

*Diarrheic samples.
*bAmong rotavirus positive samples.
*cDiarrheic and nondiarrheic samples.
NR, not reported.

Pathogenesis

Rotaviruses replicate predominately in the cytoplasm of differentiated small intestinal villous epithelial cells (Buller and Moxley 1988) and in cecal or colonic epithelial cells (Collins et al. 1989; Theil et al. 1978; Ward et al. 1996b). The jejunum and ileum of the small intestine are most affected. Rotavirus antigens were observed in nearly all villous epithelial cells in the jejunum and ileum and in a few epithelial cells on villous tips in the duodenum at 12–48 hours postinoculation (Figure 43.3) (Collins et al. 1989; Saif 1999; Shaw et al. 1989; Stevenson 1990; Theil et al. 1978; Ward et al. 1996b).

Rotavirus replication in the villous enterocytes results in cell lysis and villous blunting and atrophy. The degree of villous atrophy and the distribution of atrophic villi in the small intestine depend on the age of the pig (Shaw et al. 1989), rotavirus strain (Collins et al. 1989), and serogroup (Saif 1999). Younger pigs have more severe and extensive villous atrophy, and porcine A and C rotaviruses tend to produce more severe villous atrophy and diarrhea than groups B and E rotaviruses in pigs (Saif 1999). Group B rotavirus produces scattered foci of infection in the villous tips of the distal small intestine and mild diarrhea (Saif and Jiang 1994).

The widely accepted mechanism of rotavirus-induced diarrhea in pigs and humans is rotavirus-induced villous atrophy with loss of intestinal absorptive cells. This causes malabsorption, which results in diarrhea (Kapikian et al. 2001). Piglets that developed rotavirus diarrhea following inoculation of porcine or human rotavirus demonstrated functional alterations in small intestinal villous epithelial cells, including impaired glucose-coupled sodium transport (Davidson et al. 1977; Rhoads et al. 1991), decreased disaccharidase activity (Graham and Estes 1991; Zijlstra et al. 1997), and increased thymidine kinase activity (Davidson
These pathophysiological changes all contribute to malabsorptive diarrhea (Saif 1999). The decreased level of disaccharidase activity results in the retention of disaccharides in the lumen of the small intestine, leading to hyperosmolarity of the intestinal contents, which causes an osmotic diarrhea. In addition, decreased Na\(^+\)K\(^-\)ATPase activity and glucose-coupled sodium absorption cause inadequate absorption at villous tips, disrupting the balance of the normal secretion of sodium and water from crypts of the villi.

In addition to villous atrophy, other mechanisms suggested for rotavirus-induced diarrhea include intestinal inflammatory responses (Zijlstra et al. 1999), activation of the enteric nervous system (Lundgren and Svensson 2001), and the enterotoxin function of rotavirus NSP4 (Ball et al. 1996; Estes et al. 2001).

**Clinical Signs**

The incubation period of the disease is 18–96 hours and is followed by depression, diarrhea, and sometimes fever. Naturally occurring rotavirus-associated diarrhea is most often reported in 1- to 41-day-old suckling pigs (Askaa et al. 1983; Bohl et al. 1978; Debouck and Pensaert 1983; Roberts et al. 1980; Svensmark et al. 1989; Yaeger et al. 2002) or pigs within 7 days following weaning (Bohl et al. 1978; Lecce and King 1978; Tzipori et al. 1980b; Woode et al. 1976). The age of onset is often consistent in a given herd.

In uncomplicated rotavirus diarrhea, where there is no concurrent infection with other enteric pathogens, diarrhea in suckling pigs is usually mild and limited to 2–3 days of duration. Feces are yellow or white, watery to creamy, and variably flocculant. Morbidity is variable, but is often less than 20%. Dehydration is usually mild and mortality due to dehydration is usually less than 15% of diarrheic pigs, with the highest mortality in young pigs. Suckling pigs are frequently infected with *Isospora suis* (Roberts et al. 1980) or enterotoxigenic *Escherichia coli* (ETEC) (Bohl et al. 1978) along with rotavirus. This combination results in more severe diarrhea, higher morbidity, and higher mortality (Lecce et al. 1982; Tzipori et al. 1980a).

Clinical signs in natural rotavirus infections are usually less severe than experimental inoculation studies involving pigs without maternal lactogenic immunity, for example, gnotobiotic pigs and colostrum-deprived pigs. Since rotavirus infection is endemic in almost all swine herds, the majority of dams have immunity against rotavirus and maternal immunity is passed on to piglets via colostrum and milk (Askaa et al. 1983; Ward et al. 1996a). Diarrhea occurs in pigs when the oral dose of rotavirus exceeds the protective level of passive immunity from dams (Saif 1985). In 1- to 5-day-old gnotobiotic pigs or conventional pigs deprived of colostrum, inoculation of rotavirus results in severe diarrhea with mortality up to 50–100% (McAdaragh et al. 1980; Pearson and McNulty 1977; Tzipori and Williams 1978; Ward et al. 1996b; Woode et al. 1976). Slightly older pigs (7–21 days of age) had less severe diarrhea and dehydration with lower mortality (Crouch and Woode 1978; Shaw et al. 1989; Theil et al. 1978), whereas diarrhea lasted only 1–1.5 days in 28-day-old pigs (Lecce et al. 1982; Tzipori et al. 1980c).

The importance of rotavirus in diarrhea of weaned pigs is less clear. Severe rotavirus diarrhea in weaned pigs has been associated with increased performance (Roberts et al. 1980; Svensmark et al. 1989; Yaeger et al. 2002).

**43.3.** Rotavirus antigen in the cytoplasm of villous epithelial cells as viewed by the indirect fluorescent antibody method (×90). (A) Ileum from 1-day-old gnotobiotic pig 16 hours postinoculation with porcine rotavirus. Nearly all villous epithelial cells contain viral antigen. (B) Midjejunum from a 27-day-old weaned conventional pig 3 days postinoculation. Viral antigen is in epithelial cells on villous tips.
pigs has been reported in combination with transmissible gastroenteritis virus (Bohl et al. 1978) or β-hemolytic ETEC (Lecce et al. 1982; Tzipori et al. 1980a). Inoculation of weaned pigs with rotavirus or β-hemolytic ETEC alone resulted in mild transient or no diarrhea, whereas inoculation with rotavirus followed by β-hemolytic ETEC resulted in enhanced colonization of β-hemolytic ETEC and severe protracted diarrhea. Reproduction of the clinical signs typically observed in natural outbreaks suggested that rotavirus plays an important role in postweaning diarrhea under certain conditions (Melin et al. 2004).

Mortality in pigs inoculated with rotaviruses may be associated with several interrelated factors that contribute to more severe diarrhea, for example, low environmental temperature (Steel and Torres-Medina 1984), malnutrition (Zijlstra et al. 1997, 1999), and high virus exposure dose (Shaw et al. 1989). Diarrhea causes dehydration and electrolyte imbalances, requiring treatment with oral electrolytes. Malnutrition increases the severity and duration of rotavirus diarrhea by hampering the regeneration of damaged intestinal villi, hence delaying the restoration of enzymatic and absorptive capacity (Zijlstra et al. 1997). Malabsorption results in malnutrition and may lead to energy deficiency and hypothermia. The higher mortality in neonatal pigs is likely related to more severe and extensive villous atrophy, combined with decreased extracellular fluid and energy reserves, compared to slightly older pigs.

Lesions
The typical rotavirus lesions in the small intestine result from viral replication leading to destruction of the villi and subsequent adaptive and regenerative responses. Gross lesions appear most severe in 1- to 14-day-old pigs (Collins et al. 1989; Janke et al. 1988; Pearson and McNulty 1977; Stevenson 1990; Theil et al. 1978). The stomach usually contains food and the distal one-half to two-thirds of the small intestine is thin-walled, flaccid, and dilated with watery, flocculent, yellow, or gray fluid. The lacteals in the distal two-thirds of the intestine contain no chyle and the associated mesenteric lymph nodes are small and tan. Gross lesions are less severe or are absent in pigs that are 21 days of age or older (Shaw et al. 1989; Stevenson 1990).

Light microscopic lesions (Paul and Stevenson 1999) and scanning electron microscopic lesions (Collins et al. 1989; McAdaragh et al. 1980; Stevenson 1990; Torres-Medina and Underdahl 1980) in suckling pigs have been extensively described. Degeneration of villous epithelial cells begins on the apical tip of villi by 16–18 hours postinoculation. The degenerated cells have swollen and rarefied cytoplasm, nuclear swelling, and irregular brush borders, and are partially detached from adjacent cells or from the basement membrane. Significant villous atrophy by sloughing of cells is observed by 16–24 hours postinoculation and becomes most severe by 24–72 hours postinoculation (Figure 43.4). The tips of atrophic villi are eroded or are covered by swollen or attenuated, nearly squamous epithelial cells (Figure 43.4). Beginning 48–72 hours postinoculation, significantly deeper crypts are seen due to crypt epithelial hyperplasia. The time required for complete regeneration of normal villi depends on the age of the pig.

43.4. Ileum from 3-day-old gnotobiotic pigs (hematoxylin and eosin [H&E]; ×35). (A) Normal villi in an uninoculated control pig. (B) Severe villous atrophy present 18 hours postinoculation.
Diagnosis

Since rotavirus infection is very common in neonatal pigs, rotavirus should be considered as a cause of diarrhea in pigs at 1–8 weeks of age. Differential diagnosis with other infectious agents should be considered, including *Escherichia coli*, transmissible gastroenteritis virus, *I. suis*, and *Clostridium perfringens*. The detection of rotavirus is conducted using fecal samples, intestinal contents, or tissue sections from acute phases of diarrhea. Since rotavirus shedding peaks in the first 24 hours after the onset of diarrhea, sample collection timing is important.

There are a number of methods available for detection of rotaviruses, including EM, immune EM, immunohistochemistry, immunofluorescence on frozen sections, or impression smears of the small intestine, enzyme-linked immunosorbent assay (ELISA), virus isolation, latex agglutination, dot-blot hybridization, RNA electropherotyping, and RT-PCR (Kapikian et al. 2001).

Commercial diagnostic ELISA kits are available for porcine group A rotavirus (Benfield et al. 1984; Goyal et al. 1987). Monoclonal antibody capture ELISAs have been developed for the detection of group B and C rotavirus but are not commercially available (Ojeh et al. 1992; Volken et al. 1988).

Immunohistochemistry is used for the detection of rotavirus antigen in formalin-fixed or paraffin-embedded sections of the small intestine using immunogold staining with protein A gold and specific antisera (Magar and Larochelle 1992).

Nucleic acid hybridization and RT-PCR are employed for the detection of viral RNA. Using specific radio- or nonradiolabeled probes, nucleic acid hybridization is a highly specific and sensitive method for detection and genotyping (Johnson et al. 1990; Koromyslov et al. 1990; Ojeh et al. 1993; Rosen et al. 1990; Zaberezhny et al. 1994). RT-PCR using specific primer sets is the most extensively used method for rotavirus detection, genogrouping, and genotyping (Barreiros et al. 2003; Ben Salem et al. 2010; Chun et al. 2010; Elschnier et al. 2002; Gouvea et al. 1994a,b; Halaihel et al. 2010; Katsuda et al. 2006; Kim et al. 2010; Martella et al. 2001; Pongsuwanna et al. 1996; Winiarczyk and Gradzki 1999; Winiarczyk et al. 2002).

Since the prevalence of rotavirus infection in pigs is high, the detection of Abs to rotaviruses has little value. However, the immune status of pigs can be evaluated by measuring immunoglobulin M (IgM) and immunoglobulin A (IgA) antibody levels. High IgM and IgA antibody levels indicate recent or active infection. Indirect immunofluorescence, complement fixation, immune adherence hemagglutination assay, ELISA, virus neutralization, hemagglutination inhibition (Eiguchi et al. 1987), inhibition of reverse passive hemagglutination, and immunocytochemical assays (Kapikian et al. 2001) are used to measure rotavirus antibody titers. Specific antibody responses (IgA, IgM, and immunoglobulin G [IgG]) against rotavirus may be detected by ELISA using isotype-specific monoclonal Abs (Azevedo et al. 2004; Parreno et al. 1999; Paul et al. 1989; To et al. 1998), and neutralizing Abs are detected by plaque reduction and fluorescent focus reduction neutralization assays (Hoshino et al. 1984; To et al. 1998).

Immunity

Rotavirus infection elicits both systemic and local immune responses. After rotavirus infection or oral vaccination, the predominant antibody responses were against VP6, followed by VP7, VP4, NSP2, and NSP4 (Chang et al. 2001; Iosef et al. 2002a; Yuan et al. 2004). Pigs were protected from homotypic (common P or G type) virus challenge, but not heterotypic viruses, following recovery from virulent rotavirus infection (Bohl et al. 1982; Hoshino et al. 1988; Saif et al. 1997). These results suggested the importance of the G- and P-type specific immune responses for the control of rotavirus infection. The outer capsid VP4 and VP7 induce virus-neutralizing Abs and independently confer protection against homotypic rotaviruses in pigs (Hoshino et al. 1988).

Although primary infection or vaccination confers minimal or no cross-protection among distinct rotavirus serogroups and serotypes, repeated vaccination or reinfection even with the same rotavirus strain broadens the range of protection against heterotypic rotaviruses (Chiba et al. 1993; Gorrell and Bishop 1999). However, virus-like particles containing only VP2 and VP6 did not induce active protective immunity in neonatal pigs (Azevedo et al. 2004; Gonzalez et al. 2004; Yuan et al. 2000, 2001) or passively protect neonatal pigs born to dams vaccinated with VP2/VP6 virus-like particles, indicating that Abs to VP6 are not sufficient to protect against rotavirus disease (Yuan and Saif 2002).

Immune responses to porcine and human rotavirus infection and vaccine efficacy have been studied in neonatal gnotobiotic pigs (Saif and Fernandez 1996; Saif et al. 1997; Yuan and Saif 2002). In gnotobiotic pigs orally inoculated with human rotaviruses, the level of intestinal IgA antibody-secreting cells and lymphocyte proliferation responses positively correlated with the level of protection induced, emphasizing the importance of local immune responses to rotaviruses (Saif et al. 1997; Ward et al. 1996c; Yuan and Saif 2002). The presence of neutralizing IgA Abs in the intestines and serum is most closely related to protection against rotavirus disease in pigs and humans (Azevedo et al. 2004; Coulson et al. 1992; To et al. 1998).

Pigs acquire maternal Abs from immune dams by ingesting colostrum during a short period of time (24–36 hours after birth) (Wagstrom et al. 2000). After that, maternal Abs only act locally to protect the gut (Saif
Therefore, pigs are most susceptible to rotavirus infection after the level of maternal Abs decreases. The predominant Abs in colostrum and milk are IgG and secretory IgA, respectively, with the latter being more effective in protection against rotavirus in the gut due to their resistance to cleavage by digestive enzymes and higher levels in milk (Saif and Fernandez 1996). Maternal IgG Abs passively transudate from serum back to the gut and are also transiently protective against rotavirus infection (Hodgins et al. 1999; Parreno et al. 2004, 1999; Ward et al. 1996a).

The roles of T and B cells in protective immunity to rotavirus infection have been extensively studied only in adult mice (reviewed by Franco and Greenberg 2000). Based on limited studies in pigs (Ward et al. 1996c), lambs (Bruce et al. 1995), calves (Oldham et al. 1993), and humans (Offit et al. 1993), it was suggested that CD8+ T cells play a major role in restricting and clearing primary rotavirus infection, and CD4+ T cells in generating mucosal antibody responses (Oldham et al. 1993).

**Prevention and Control**

There are no specific antivirals against rotaviruses. Therefore, treatment is limited to supportive therapy and prevention of secondary bacterial infection using antibiotics and fluid therapy (Bywater 1983; Paul and Stevenson 1999). To minimize dehydration and weight loss by rotavirus diarrhea, fluid electrolyte solutions containing glucose–glycine are administered (Bywater and Wood 1980), or L-glutamine is added to oral solutions to promote sodium and chloride absorption in the jejunum (Rhoads et al. 1991). The oral administration of transforming growth factor-α enhanced the recovery of the jejunum in rotavirus-infected piglets (Rhoads et al. 1995). Chicken egg powder containing Abs specific for rotavirus used in addition to sow’s milk reduced the prevalence of diarrhea in 2- to 3-day-old pigs (Hennig-Pauka et al. 2003).

Ambient temperature is important in reducing mortality from rotavirus diarrhea in nursing piglets, for example, an ambient temperature of 35°C (95°F) significantly reduced mortality (Steel and Torres-Medina 1984). A high-energy, weaned-pig diet on a scheduled feeding program also reduced rotavirus infection morbidity and mortality (Tzipori et al. 1980b).

Due to the ubiquitous and persistent nature of rotaviruses in the environment, eradication of rotavirus from a commercial swine herd is not practical. Thus, management practices that reduce the buildup of rotaviruses and exposure of susceptible pigs are crucial in controlling rotavirus infections. Adult sows can be subclinically infected and shed rotaviruses in the environment (Benfield et al. 1982). Therefore, sanitation is important to reduce virus buildup. Floors in farrowing and nursing facilities should be constructed for easy cleaning, fecal buildup should be minimized, and floors should be disinfected between groups.

Continuous-flow nurseries had significantly lower rates of rotavirus infection than all-in/all-out production, suggesting that rotaviruses in the environment induced active immunity in nursing pigs under the partial protection of maternal Abs (Dewey et al. 2003). Passive immunity is also important in the management of rotavirus infection in pigs. Replacement gilts should be exposed to feces from older sows to increase rotavirus antibody titers and enhance passive immunity. To ensure transfer of maternal immunity to suckling pigs, lactation diet, feed intake, comfort, and farrowing crate design should be optimized (Paul and Stevenson 1999).

Currently, an attenuated rotavirus vaccine containing two major serotypes is available for oral and intramuscular injection for nursing pigs (ProSystem® Rota, Merck Animal Health, Summit, NJ). The efficacy of inactivated rotavirus vaccines for sows or nursing pigs is uncertain (Saif and Fernandez 1996). In nursing pigs, maternal Abs can interfere with induction of active immunity against rotavirus disease and can often cause attenuated oral vaccine failure (Hodgins et al. 1999; Parreno et al. 1999). Maternal Abs may have less inhibitory effect on inactivated parenteral vaccines. However, inactivated rotavirus given intramuscularly to rotavirus-seronegative piglets did not induce secretory IgA Abs in the intestines or confer protection (Yuan et al. 1998).

**PORCINE REOVIRUS**

**Relevance**

Porcine reovirus was discovered in 1951 (Tyler 2001). “Reo” is an acronym for “respiratory and enteric orphan,” indicating that reoviruses were not causally associated with disease (Tyler 2001). Reovirus infection or Abs to reovirus have been detected in all animal species. Diseases associated with reovirus infection in animals have involved mainly the respiratory, gastrointestinal, and nervous systems (Fukutomi et al. 1996; Hirahara et al. 1988; Tyler 1998). Reovirus has been detected in both healthy pigs and pigs with clinical respiratory, enteric, and reproductive signs. At present, the role of reoviruses in diseases of pigs is not certain (Kasza 1970; Kirkbride and McAdaragh 1978; McFerran and Connor 1970).

**Etiology**

Reovirus is a nonenveloped icosahedral particle 75 nm in diameter with a rough outer rim (inner capsid 45–50 nm in diameter) (Figure 43.5) and a dsRNA genome with 10 segments. The density of a complete (mature) virion in cesium chloride is 1.36g/mL.

Porcine reoviruses are resistant to ether, chloroform, and trypsin, but susceptible to heat at 50°C (122°F) for 1 hour. Reoviruses are sensitive to 0.1% sodium deoxy-
cholate (Hirahara et al. 1988) and stable at acidic pH 3. Porcine reoviruses possess a hemagglutinin that agglutinates human group O and porcine erythrocytes at 4°C (39°F), 22°C (72°F) and 37°C (98.6°F).

Mammalian reoviruses have a common group antigen that can be detected by complement fixation, immunofluorescence, and immunodiffusion (Sabin 1959). Avian reoviruses have a group antigen that is shared among avian reoviruses, but distinct from mammalian reoviruses. All mammalian isolates can be serologically divided into three types (1, 2, and 3), which can be distinguished by serum neutralization and hemagglutination inhibition tests.

Reoviruses grow in a wide variety of cells from many species including porcine, bovine, feline, simian, human, and canine origin (Hirahara et al. 1988; Kasza 1970). Mouse L929 fibroblasts are the most commonly used cell line for viral cultivation, purification, and plaque assay (Tyler 2001). Reovirus replicates slowly and the majority (80%) of the nascent virus population remains cell associated. The cytopathic effects of reoviruses vary, depending on the cell line used. In general, cells become rounded, granular, and detached from the surface. Eosinophilic inclusion bodies are seen in the cell cytoplasm when stained with May–Greenwald–Giemsa stain (Paul and Stevenson 1999).

Epidemiology
Porcine reovirus infection is widespread in swine herds and Abs to all three types have been detected (Tyler 2001; Yang et al. 1976). The transmission of reoviruses is by fecal–oral and respiratory routes. Abs acquired from the dam persist for about 11 weeks, at which time pigs become susceptible to reovirus infection (Watt 1978).

Pathogenesis
Reoviruses mainly replicate in the respiratory and intestinal tracts. The virus can be detected in nasal secretions and feces as soon as 24 hours postinoculation and shedding may continue for 6–14 days. Hemagglutination-inhibiting Abs may be detected at 7 days postinfection and peak at 11–21 days postinfection.

Clinical Signs
Porcine reoviruses have been detected from healthy pigs, as well as pigs with respiratory or enteric diseases (Elazhary et al. 1978; Kasza 1970; McFerran and Connor 1970; Robl et al. 1971), and from aborted fetuses (Kirkbride and McAdaragh 1978). Experimental infection with reovirus has not consistently reproduced disease. In the majority of studies, conventional and gnotobiotic pigs 1–6 weeks of age inoculated with porcine or human reovirus type 1 via intranasal, intraperitoneal, or intracerebral routes showed a transient febrile reaction, but not clinical disease (Baskerville et al. 1971; Kasza 1970; McFerran and Connor 1970; McFerran et al. 1971; Watt 1978). Cesarean-derived, colostrum-deprived pigs and conventional pigs inoculated intranasally or exposed via aerosol to reovirus type 1 developed mild respiratory disease (pyrexia, sneezing, inappetence, and listlessness) (Hirahara et al. 1988). Seronegative sows between 40 and 85 days of gestation inoculated with reovirus via intravenous or intramuscular routes had term litters containing mummified, stillborn, weak live-born, and normal pigs. The virus was isolated from fecal tissues and the placenta of these sows (Paul and Stevenson 1999).

Lesions
The limited number of studies of pigs inoculated with reoviruses reported only mild microscopic lesions and few gross lesions. Although reproductive effects were seen in sows between 40 and 85 days of gestation inoculated via the intravenous or intramuscular routes (mummified, stillborn, weak live-born, and normal pigs), no specific gross or histopathological lesions were found. Oral inoculation of 1-week-old, colostrum-deprived pigs with reoviruses of enteric origin resulted in focal villous atrophy in the jejunum and ileum (Elazhary et al. 1978). Aerosol exposure of 4-week-old specific pathogen-free (SPF) pigs to type 1 reoviruses of porcine origin resulted in no gross lesions, but microscopic lung lesions characterized by lymphocytes and macrophages in alveoli and alveolar septae and mild peribronchiolar nodular lymphocytic hyperplasia were reported (Baskerville et al. 1971). Intranasal inoculation of 70kg SPF pigs with a porcine type 3 respiratory isolate resulted in lobular atelectasis, vesicular emphysema, and peribronchiolar nodular lymphocytic hyperplasia, with varying intensity between lobules (Paul and Stevenson 1999). Additional studies...
will be required to characterize the clinical disease and lesions in pigs caused by porcine reoviruses.

**Diagnosis**

Virus isolation and RT-PCR have commonly been used for detection of reoviruses. Reovirus typing can be done by virus neutralization and hemagglutination inhibition tests with reference antisera to three reovirus types (Paul and Stevenson 1999).

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RELEVANCE

Interest in retroviruses of pigs began in the 1970s after the discovery of retroviral particles in the supernatant of a pig kidney-derived cell line (Armstrong et al. 1971). Recent work has suggested an association between pig endogenous retroviruses (PERV) and mortality in commercial herds (Dieckhoff et al. 2007; Pal et al. 2011; Tucker and Scobie 2006). No association with disease was detected at the time of the initial discovery and interest in pig retroviruses waned.

In the early 1990s, xenotransplantation research on pigs and porcine tissues led to a renewed interest in PERV. Although pig-derived products, including insulin and Factor VIII, had been used in human medicine for years, the prospect of transplanting viable pig-derived tissues and cells into immunosuppressed humans led to careful consideration of the potential for the transmission of infectious agents from pig tissues to humans (Bloom 2001; Onions et al. 2000). The discovery that pig retroviruses were capable of infecting human cells in vitro (Patience et al. 1997) triggered research into the origins, biology, and means for prevention of xenograft-associated transmission of infectious agents (Scobie and Takeuchi 2009; Wilson 2008).

ETIOLOGY

PERV are members of the genus Gammaretrovirus in the family Retroviridae. They are enveloped, RNA viruses in Class VI of the Baltimore classification system. Class VI viruses replicate their genomes via a single-stranded, positive-sense RNA with a DNA intermediate. The genome is unique among viruses in that it is diploid and does not serve as mRNA, but as a template for reverse transcription (Weiss 2006).

Up to 8% of mammalian genomic DNA is believed to be retroviral in origin, reflecting the historical integration of viral nucleic acid after infection of host ancestors by ancient exogenous retroviruses (Kurth and Bannert 2009; Niebert and Tonjes 2005). Endogenous retroviruses are hypothesized to have positive evolutionary and physiological consequences for their host (Balada et al. 2009; Kurth and Bannert 2009). This evolutionary effect on host species diversification is proposed to be driven by proviral sequences enhancing recombination events (O’Neill et al. 2001), a feature also important in fostering the diversity of multigene families such as the Class II major histocompatibility complex (Doxiadis et al. 2008). It is also now clear that endogenous retroviral protein expression plays a key role in human placental syncytiotrophoblast development (Rote et al. 2004).

All retroviruses in pigs originate from one or more viral sequences encoded in the pig genome. Transmission occurs via the germ line, where they exist as proviruses. For this reason, they are termed PERV. Sequences vary between and within breeds in terms of location, copy number, completeness, and ability to be expressed as messenger RNA, viral proteins, and ultimately as replication-competent particles.

The pig genome contains hundreds of viral integration sites, but most are incomplete. Of the much smaller number of replication-competent PERV proviruses identified so far, all are C-type gammaretroviruses closely related to murine leukemia virus (MuLV), gibbon ape leukemia virus (GaLV), and koala retrovirus (KoRV) (Denner 2007). Typically, 6–15 replication-competent proviruses are present in a single pig (Akiyoshi et al. 1998; Dieckhoff et al. 2009; Herring et al. 2001; Niebert and Tonjes 2003). Closely related gammaretroviral sequences are found only in the Suidae family.
other retroviral remnants, including betaretroviruses, have been described in the pig genome, but these were highly incomplete and nonfunctional (Ericsson et al. 2001).

The full PERV genome contains the typical retroviral coding regions of gag (matrix and capsid proteins), pol (reverse transcriptase and integrase), and env (envelope proteins). Three subtypes of PERV (A, B, C) have been described based on env-mediated cell tropism (Patience et al. 1997; Wilson et al. 2000). Subtypes A and B are ubiquitous in pigs of all breeds and capable of infecting human cells in vitro, whereas PERV C is variably distributed between and within breeds, but only capable of infecting pig cells (Denner 2008a; Dieckhoff et al. 2009).

The presence of PERV DNA in the pig genome is ubiquitous across breeds, individuals, and tissues. There appears to be constitutive expression of some of these sequences since PERV mRNA was detected in most tissues in all animals tested, although tissue and pig age differences were found (Clemenceau et al. 1999; Moon et al. 2009). Variation in the presence and titer of viremia and viral release from activated peripheral blood mononuclear cells also occurs between breeds (Garkavenko et al. 2008), individuals (Oldmixon et al. 2002), and by age (Tucker and Scobie 2006). Although some of this variability is likely to be inherited, factors known to influence the expression of human endogenous retrovirus (HERV) RNA and release of viral particles include mitogens, cytokines, other microorganisms, steroid hormones, toxins, vitamins, and environmental signals, including radiation (Balada et al. 2009). The role of some of these factors, such as mitogens and cytokines, in stimulating PERV particle release has been confirmed in vitro (Cunningham et al. 2004).

Although initially only identified in the supernatant of some pig-derived cell lines, PERVs have since been isolated from pig plasma (Takefman et al. 2001), the supernatant of cultured primary pig endothelial cells, and peripheral blood mononuclear cells (Martin et al. 1998; Wilson et al. 1998). Recombinant PERV A/C was found after stimulation of peripheral blood mononuclear cells of miniature pigs (Wilson et al. 1998). These recombinants are believed to represent the copackaged products of separate PERV A and PERV C loci (Scobie et al. 2004). They replicate to high titers in human cells (Wood et al. 2004) and are found in the plasma of commercial pigs (Tucker and Scobie 2006).

Exogenous horizontally transmissible retroviruses have not been found in pigs. However, since the A/C recombinant is not present in proviral form within the host germ line, it is considered a candidate exogenous virus (Scobie et al. 2004; Wood et al. 2004). The presence of the full genomic A/C provirus in the somatic cells of some pigs indicates its potential for replication (Denner 2008b; Martin et al. 2006).

**ROLE IN PUBLIC HEALTH**

PERVs are a major infectious disease barrier to xenotransplantation. The recognition that human cells are permissive to PERV infection in vitro raised several concerns. First, that transplantation of pig tissues into immunosuppressed patients might result in PERV replication and neoplastic diseases, such as those associated with its close relatives, GalV, KoRV, and MuLV (Weiss et al. 2000). Second, that genetic modification of source pigs to enhance xenograft compatibility, either by including cell surface transgenes for human complement regulation or by removing galactose-alpha(1,3) galactose epitopes recognized by human natural antibodies, meant that PERV particles budding from these cells would be protected from components of the recipient’s innate immune system (Weiss 1998). Finally, it was hypothesized that recombination between PERV and HERVs or exogenous retroviruses (such as human immunodeficiency virus) in xenograft recipients could produce novel transmissible pathogens, although this idea has not been supported by in vitro work (Suling et al. 2003).

In recognition of these and other potential xenotransplantation hazards, the World Health Assembly issued resolution WHA57.18 (May 2004) requiring member countries to regulate and control source animal production and selection, patient surveillance, and sample archiving related to xenotransplantation. Many countries including the United States, Canada, the European Union, and Australasian member states have guidelines and regulations in place for oversight of activities related to xenotransplantation research.

**EPIDEMIOLOGY**

The epidemiology of PERV has been studied only in the context of xenotransplantation. Little is known regarding the potential for transmission of PERVs between pigs because, although viremia has been demonstrated, data are lacking on the shedding of infectious PERV in body fluids such as saliva, urine, and semen. Many questions remain concerning the activation of proviral transcription, factors that determine the titer of infectious particles in plasma or other body fluids, and the possibility of pig-to-human transmission, although recent literature would suggest that pig-to-pig transmission is not an issue (Kaulitz et al. 2011).

Despite the extensive use of porcine by-products in human medicine, PERV infection in humans and immunosuppressed nonhuman primates exposed to viable pig tissues has not been detected (Denner et al. 2009; Specke et al. 2009; Valdes-Gonzalez et al. 2010). Except for a report of short-term infection in fetal...
lambs and guinea pigs, all evidence for cross-species transmission is based on in vitro work, the results of which suggest that PERV does not pose as serious a risk for xenotransplantation as previously anticipated (Argaw et al. 2004; Popp et al. 2007; Scobie and Takeuchi 2009).

**PATHOGENESIS, CLINICAL SIGNS, AND LESIONS**

Gammaretroviruses in various species have been linked to neoplasia and immune dysfunction (Denner 1998). HERVs were linked to autoimmune and immunosuppressive diseases (Balada et al. 2009). Endogenous retroviruses in various animal species have been associated with a broad range of pathologies, including neoplasia, congenital defects, and autoimmune diseases (Balada et al. 2009; Kurth and Bannert 2009) through various mechanisms, including molecular mimicry, insertion-associated transcriptional activation, and immunosuppression.

A major complication in establishing causal, permissive, or even responsive roles for PERV in diseases of pigs is the fact that endogenous retrovirus expression can induce production of inflammatory markers, but these same inflammatory mediators also induce PERV expression. Nevertheless, limited evidence suggested that PERV plays a role in pig health. Specifically, PERV levels measured as viral RNA in plasma were higher in pigs housed in production units experiencing higher mortality and more endemic disease (Harrison et al. 2010; Tucker and Scobie 2006). In a miniature pig, PERV overexpression was found in melanoma tissue, but no correlation was made with the presence of infectious virions (Dieckhoff et al. 2007).

**DIAGNOSIS**

Detection methods may be used to determine the PERV status of source pigs and monitor for potential PERV infection in xenograft recipients. Laboratory methods to characterize source pig populations include: (1) infectivity cocultures of source pig primary cells with human and pig target cell lines; (2) measurement of reverse transcriptase activity in the supernatant of stimulated primary cells from source pigs; (3) measurement of viral load in source pig plasma; (4) determination of the PERV proviral copy number in the pig genome; and (5) determination of PERV class C prevalence in the herd (Garkavenko et al. 2008). To date, no anti-PERV antibodies have been reported in pigs.

Methods for monitoring for PERV infection in xenograft recipients include sensitive polymerase chain reaction (PCR) and reverse transcription-polymerase chain reaction (RT-PCR) methods for detection of proviral or transcript nucleic acids, detection of anti-PERV antibodies, and long-term archival of viable blood-derived cells (Denner et al. 2009). A key necessity is the inclusion of equally sensitive assays for the detection of porcine genomic DNA and RNA since microchimerism, the long-term existence of pig cells at disparate locations in the xenograft host, is a common feature of xenotransplantation that can explain the presence of low levels of PERV DNA and RNA in xenograft hosts (Paradis et al. 1999).

**PREVENTION AND CONTROL**

In terms of PERV-associated disease in pigs, the knowledge base is insufficient to determine the need or the strategy for control and prevention. However, genetic technologies such as high-density genotyping and marker-assisted selection could permit rapid identification of pigs free of deleterious proviruses if relevant loci are identified.

Efforts to reduce the probability of xenograft-associated PERV transmission initially focused on the identification of, and selective breeding against, proviruses considered important in cross-species transmission. For example, pigs carrying PERV C proviral sequences would be excluded as source animals due to the potential for recombinant formation of the high titer human tropic A/C form (Denner et al. 2009). However, the recognition that a residual risk would remain because of the sheer number of partial PERV proviruses and their potential to recombine to form replication-competent viruses in the future has led to additional strategies for prevention (Mattiuzzo et al. 2010; Scobie and Takeuchi 2009). No single optimum strategy for prevention of transmission has emerged and a multihurdle approach appears to be the most effective solution.

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OVERVIEW

Vesicular stomatitis viruses (VSVs) and rabies virus are members of the family Rhabdoviridae. VSVs belong to genus Vesiculovirus and rabies virus to genus Lyssavirus. These viruses have a large (65–185 nm), bullet-shaped virion composed of a host-derived plasma membrane, a phospholipid envelope, and an internal ribonucleoprotein core. After cell attachment, penetration, and uncoating, virus replication occurs in the cytoplasm of infected cells. The viral RNA-dependent RNA polymerase transcribes viral mRNAs from the viral genome. The viral genome codes for five major structural proteins: the nucleocapsid, phosphoprotein, matrix protein, glycoprotein, and the large polymerase (Wagner and Rose 1996).

VESICULAR STOMATITIS

Relevance

Vesicular stomatitis (VS) in cattle and pigs resembles foot-and-mouth disease (FMD) and, for that reason, is classified by the OIE World Organization for Animal Health as a multispecies notifiable disease. The presence of VS in livestock results in export restrictions on livestock from VS-affected to VS-free zones.

Etiology

Genetically related, but distinct, VSVs (Wunner et al. 1995) cause VS. Historical reports of VS date back to the 1800s (Hanson 1952), but VSV was first isolated in 1925 in New Jersey and in Indiana the following year (Cotton 1927). These isolates were found to be serologically distinct and classified as different serotypes: vesicular stomatitis New Jersey virus (VSNJV) and vesicular stomatitis Indiana virus (VSIV), respectively (Cartwright and Brown 1972). Both VSNJV and VSIV serotypes are pathogenic in domestic livestock, but only VSNJV has been associated with disease in swine. Two VSIV subtypes, Cocal virus and Alagoas virus, have caused disease in livestock in South America. VSV may be propagated in many cell culture systems (Swenson 2010).

Role in Public Health

Humans are susceptible to VSV and may become infected by contact or by aerosol. Clinical signs include influenza-like symptoms and blister-like lesions appearing 1–2 days after exposure. When working with live virus, handling diagnostic specimens potentially containing VSV, or handling animals suspected of having VS, appropriate measures should be in place to prevent exposure to VSV.

Epidemiology

VS affects cattle, horses, and swine and is the most common vesicular disease of livestock in the Americas. Antibodies to the VSVs have been found in a wide variety of wild animals, but naturally occurring clinical disease has not been reported in wildlife (Jenney et al. 1970; Tesh et al. 1970).

VS is not known to occur outside of the Americas (Swenson 2010). Within the Americas, VS, primarily caused by VSNJV and VSIV, occurs in endemic cycles in southern Mexico, Central America, and northern South America (Colombia, Venezuela, Ecuador, and Peru).

Both endemic and epidemic patterns of VS occur in the United States. In the southeastern United States, VSNJV was detected in domestic livestock almost yearly.
from the early 1900s to the mid- to late 1970s. Since then, VSNJV has only been detected in wildlife on Ossabaw Island, Georgia (Killmaster et al. 2011). In the western United States, epidemics of VSNJV (and VSIV to a lesser extent) occur in cattle and horses at 5- to 10-year intervals (Rodriguez 2002). Epidemics of VSNJV, sometimes spreading as far as Manitoba, Canada, accounted for approximately 80% of the epidemics in the United States (Hanson 1963). The remaining 20% of epidemics involved VSIV and a similar zone of spread (Hanson 1968). VSNJV epidemics generally affect a larger number of animals than VSIV epidemics. Naturally occurring VSNJV in domestic swine has not been reported in the United States since 1968 (Jenney and Brown 1972), and VSIV infection in swine has never been reported.

VSNJV can be transmitted via several routes, including direct animal-to-animal contact and biologically or mechanically by insect vectors. VSNJV was efficiently transmitted among domestic swine via contact transmission (Stallknecht et al. 2001). In experimental settings, the development of vesicles in inoculated animals facilitated contact transmission. Infection in contacts ranged from subclinical to clinical.

Radeleff (1949) proposed transmission of VSNJV by arthropod vectors, but the concept remained controversial because it was not clear how vectors could acquire the infection since vertebrate hosts do not develop a detectable VSV viremia. However, Mead et al. (2000, 2004) demonstrated that (1) hematophagous insects could become infected with VSNJV when they fed on or near virus-rich vesicular lesions; (2) VSNJV could be transmitted from VSNJV-infected to uninfected black flies (Simulium vitatum) during the process of cofeeding on the same vertebrate host; and (3) VSNJV-infected insects transmitted the virus to livestock.

In clinically affected animals, VSV is primarily localized to areas where lesions are present. Virus can be isolated in high concentrations from vesicular fluid, throat swabs, tonsils, saliva, and epithelium of clinically affected animals for up to 10 days postinfection (range 1–10 days), depending on the species.

VSVs are inactivated by exposure to 56°C (133°F) for 30 minutes (Watson 1981) or intense irradiation with UV light (Weck et al. 1979). Under natural conditions, VSNJV can remain viable in contaminated saliva on pails or food buckets for 3–4 days (Hanson 1952) and can be recovered from plant surfaces up to 24 hours after surface inoculation at room temperature (Drolet et al. 2009). VSVs are inactivated by a wide variety of lipid solvents, detergents, formalin, and many common disinfectants, such as household bleach.

**Pathogenesis**

VSV infection is localized to the site of inoculation with occasional spread to local draining lymph nodes. Virus is not found in other tissues. Primary replication seems to occur in keratinocytes (Scherer et al. 2007).

Virulence in swine is dependent on virus strain (VSNJV is more virulent than VSIV), route of inoculation, and dose. In domestic swine, experimental inoculation of the snout with $1 \times 10^{9.3}$ median tissue culture infectious dose per milliliter (TCID$_{50}$) of VSNJV resulted in seroconversion without clinical disease, whereas inoculation of the snout with doses $\geq 1 \times 10^{11.3}$ TCID$_{50}$ resulted in clinical disease followed by seroconversion. VSNJV produces large vesicular lesions at the inoculation site in 1–2 days and pigs shed high concentrations of virus for up to 7–8 days postinfection. Inoculation of the oral mucosa (including tongue), snout, and coronary bands results in the development of vesicular lesions in the majority of animals. In contrast, inoculation of haired skin results only in seroconversion and subclinical disease (Howerth et al. 2006; Scherer et al. 2007; Stallknecht et al. 1999).

Naturally occurring VS in domestic swine due to VSIV has not been reported, although swine are susceptible to VSIV and develop clinical signs when the inoculation dose is $\geq 1 \times 10^5$ TCID$_{50}$ (Stallknecht et al. 2004). Inoculation of VSIV at doses $\leq 1 \times 10^5$ TCID$_{50}$ did not result in clinical signs or a detectable antibody response. Compared to VSNJV, lesions are much smaller (if present) and virus is shed at low concentrations for 3–4 days.

The role of host factors, for example, age and breed, in clinical disease have not been investigated, but field studies suggest that age may be associated with the outcome of infection.

**Clinical Signs and Lesions**

VS is characterized by fever, if present, from 24 to 72 hours postinfection (HIPI) with noticeable vesicle formation in the oral mucosa, snout, teats, and coronary bands between 24 and 72 HPI. Multiple lesions on or near the same site, or on different sites, can occur on the same animal. Lesions typically begin at the site of infection as small, in some cases pinpoint, blanched areas with raised borders. These quickly develop into 2–4 cm grayish-red vesicles. If multiple lesions develop in the same area, they may coalesce and appear as a single vesicle. Vesicles usually rupture 1–2 days after formation, releasing straw-colored, virus-rich exudates (Howerth et al. 1997). There may be extensive epidermal erosion and ulceration followed by scabbing. In severe cases, the complete tongue epithelium may slough and, if lesions develop along a coronary band, the claw may separate. Lesions typically begin to re-epithelialize soon after eruption and, unless complicated by secondary infection, will completely heal in 1–2 weeks.

Excessive salivation due to lesions in the mouth is usually the first sign, and may be the only sign observed. Pain associated with oral lesions can lead to anorexia
and weight loss. Foot lesions and subsequent lameness are seen most frequently in swine, but occur in cattle and horses as well.

The VS incubation period in domestic livestock ranges from 1 to 3 days. Viremia has never been reported in naturally infected livestock and only once under experimental conditions. In that instance, Cotton (1927) inoculated the blood of horses and cows experimentally infected with VSNJV into guinea pigs. The subsequent development of vesicular lesions in the guinea pigs at the site of inoculation (foot pads) was interpreted as evidence of viremia in the horses and cows. This outcome has not been reproduced despite attempts by several investigators.

Mortality due to VSV infection is low, but morbidity rates during epidemics can be as high as 90%. VS is a self-limiting disease and animals typically recover in 2–3 weeks if there are no complications from secondary bacterial infections (Hanson 1952).

**Diagnosis**

Clinically, VS in swine is indistinguishable from FMD, swine vesicular disease (SVD), or vesicular exanthema of swine (VES). Therefore, it is imperative to collect and submit diagnostic samples for laboratory evaluation. Rule-outs for infectious causes of vesicular disease in swine include FMD, SVD, VES and other marine calicivirus infections, SVD, porcine parvovirus, enterovirus infection, and swinepox. Noninfectious causes include trauma, for example, burns (chemical or thermal) or course feed, toxins, plant awns, and photosensitization.

Clinical specimens for virus detection include vesicular fluid, tissue tags from ruptured vesicles, biopsies of affected areas, and swabs. To maintain virus viability, place diagnostic materials in a small volume of medium, chill to 4°C (39°F), and transport materials to the laboratory on ice packs. Do not freeze specimens, as this can adversely affect virus detection.

Virus isolation, complement fixation, antigen-capture enzyme-linked immunosorbent assay (ELISA), and reverse transcription-polymerase chain reaction are used for detection of virus or viral nucleic acid (Swenson 2010). Virus isolation can be done in continuous cell lines, embryonating chicken eggs, and mice. If present in high titers, VSV can be detected in <24 hours after inoculation and within 7 days of inoculation at lower titers.

Serum antibody can be detected by virus neutralization (VN), complement fixation (CF), and ELISA. VN-detectable serum antibody persisted for years in experimentally infected cattle (Sorensen et al. 1958) and in naturally infected horses by the VN test and a competitive ELISA. The antibody response detected by CF is usually of shorter duration, generally months. In experimentally infected calves and ponies, the competitive ELISA detected antibody before it was detectable by CF or VN (Katz et al. 1997). Because of the long duration of antibody, a fourfold change in antibody titer (CF or VN) in paired sera collected approximately 7 days apart is required to establish VSV infection.

**Immunity**

The immune response to VSV infection can vary with the virus serotype, the route of exposure, method of exposure, and the serological assay used to test samples (Katz et al. 1997; Stallknecht et al. 1999). Seroconversion can be detected as early as 5 days following exposure to VSV. The development of antibodies is associated with a decreased ability to detect viral shedding. In one study, virus was detected in 82 samples from experimentally infected pigs prior to seroconversion and in one sample following seroconversion (Stallknecht et al. 1999). In one study, animals re-exposed to homologous virus 49–77 days after initial exposure did not shed virus and did not show a change in antibody response. In contrast, animals re-exposed to a heterologous virus shed virus and responded serologically (Katz et al. 1997). In contrast, field studies suggested that animals were not protected when re-exposed to homologous virus, even in the presence of neutralizing antibodies (Rodriguez et al. 1990). Maternal antibodies have been detected in offspring born to infected dams. In an experimental study, maternal antibody was demonstrated at 3 months of age, but not at 7 months of age (Sorensen et al. 1958).

**Prevention and Control**

When vesicular disease is observed in swine, steps should be taken to stop the movement of animals and materials onto or off the premises until a diagnosis is made. The appropriate animal health authorities should be notified immediately.

As with most viral infections, treatment of VSV-infected animals is largely ineffective, except for palliative care such as feeding soft feed and providing padding for hard surfaces. Antibiotics may be useful to prevent or treat secondary bacterial infections. Treatment of vesicular lesions with topical antiseptics may promote faster healing and reduce the risk of secondary infections.

VSNJV is easily transmitted among swine by animal-to-animal contact, so affected animals and animals that may have had recent contact with them should be isolated. Minimize animal-to-animal contact to reduce transmission between animals. Disinfection of equipment, transport, and facilities occupied by infected animals is important.

Preventative measures include insect control, housing animals indoors during peak insect feeding times, and implementation of biosecurity procedures designed to avoid the introduction of VSV from affected premises via equipment, personnel, or animals. Insect repellants such as permethrin or other repellants...
approved for use on swine should also be considered. Vaccination with inactivated vaccines is practiced in some countries where VS is endemic.

RABIES VIRUS

Relevance
The potential for rabies in swine is present wherever contact with wildlife or canine reservoirs is possible. The predominate rabies reservoirs are foxes in Europe; dogs and foxes in Asia; dogs, jackals, and mongooses in Africa; dogs and vampire bats in South America; and foxes, raccoons, skunks, and bats in North America. Compared to cattle, the spillover of rabies into swine is low and probably reflects the separation between wildlife and swine that occurs with confinement operations. The United States averages about one case per year in domestic swine. In Europe, spillover to wild boars is an infrequent occurrence.

Role in Public Health
Rabies is an important zoonotic disease because of its high mortality rate in humans. However, there is little documented evidence of swine as a source of rabies for humans. Steele and Fernandez (1991) reported that of 521 people exposed to presumed rabid swine in the subcontinent of India, Pakistan, and Bangladesh from 1908 to 1972, none developed rabies. Notably, each exposed person received rabies prophylaxis.

Epidemiology
Rabies exists worldwide, except for certain islands and countries with strong geographical barriers, such as Norway and Sweden. Australia is free of rabies, but does have Australian bat lyssavirus. All warm-blooded mammals are susceptible to rabies, although some appear to be more susceptible than others. The susceptibility of animals to rabies is influenced by the quantity of the virus introduced, the site of the bite, the age of the animal, and the virus strain involved. Strain differences play an important part in species susceptibility.

Transmission occurs via the bite of an infected animal that has virus in its saliva. The amount and duration of virus in saliva varies significantly and is dependent on the amount of virus inoculated. In dogs, animals that received a high dose of virus died quickly with little virus in the saliva, while dogs that received lower doses of inoculums had longer incubation periods and more virus in the saliva (Fekadu 1991). The duration of virus shed was from 14 days before the onset of clinical signs to 4 days after onset. Studies have not been conducted to determine the amount and duration of virus shedding in swine, but it should be assumed that virus is present when determining treatment options following human exposure.

Rabies virus is susceptible to 1% sodium hypochlorite, 2% glutaraldehyde, 70% ethanol, and formaldehyde. It is inactivated by ultraviolet radiation, heat (1 hour at 50°C/122°F), and lipid solvents. The virus is rapidly inactivated by sunlight and does not retain infectivity for long out of the host.

Pathogenesis
Upon entering the body through a bite wound, the rabies virus quickly enters an eclipse phase where it remains undetectable by fluorescent antibody staining or virus isolation. Murphy et al. (1973) suggested that rabies virus replicates in muscle fibers prior to invading the nervous system. They postulated that replication of the virus in muscle fibers may be a necessary amplification step prior to infection of the peripheral nervous system and may account for the long and variable incubation period of the disease. Once the virus enters the axoplasm it travels to the dorsal root ganglia and then to the spinal cord and brain (Baer et al. 1965). As the virus spreads though the central nervous system (CNS), there is simultaneous centrifugal movement of the virus in peripheral nerves to non-nervous tissue, including the epidermis, cornea, epithelium of the mouth, nasal mucosa, intestine, lacrimal glands, pancreas, muscle fibers, myocardium, lungs, kidneys, adrenal medulla, and salivary glands.

Clinical Signs and Lesions
Rabies has classically been divided into furious and dumb forms. The clinical course of the disease is divided into the prodromal, excitement, and paralytic periods. During the prodromal period, there may be slight changes in temperament with a slight rise in temperature, dilation of pupils, and impaired corneal reflexes. The prominence of the excitement phase is what differentiates the furious form from the dumb form. Aggressive behavior, muscular tremors, incoordination, loss of balance, and increased salivation are common. The paralytic period, the final stage of the disease, is characterized by ascending paralysis, coma, and death.

Published reports on the clinical signs of rabies in swine are limited and, as in other species, not consistent. Sudden unexplained mortality with few clinical signs was reported in feeder pigs (Hazlett and Koller 1986). Morehouse et al. (1968) reported twitching, prostration, excessive salivation, and clonic muscular spasms. Other signs reported include uneasiness, incoordination, rapid chewing, fever, increased grunting, anorexia, marked thirst, and head and face rubbing (Dhillon and Dhingra 1973; DuVernoy et al. 2008; Merriman 1966; Morehouse et al. 1968; Yates et al. 1983).

As in other species, the incubation period in swine is variable, ranging from 17 days in a pig bitten by a skunk to 132 days in a potbellied pig bitten by a raccoon (DuVernoy et al. 2008). Baer and Olson (1972) reported...
the recovery of pigs from rabies. Specifically, four of six pigs developed clinical signs, including progressive paralysis, beginning 32–47 days after having been bitten by a rabid skunk. The signs subsided in 1–2 weeks with the pigs developing high antibody titers to rabies virus.

Gross lesions, other than those caused by self-mutilation, do not occur with rabies. Microscopic changes in the CNS in swine range from mild vasculitis and focal gliosis in the brain to extensive meningoencephalitis and neuronal degeneration in the brain and spinal cord (Morehouse et al. 1968).

Diagnosis
The fluorescent antibody technique for the detection of viral antigen in the brain is the preferred diagnostic test because of its speed and accuracy (Goldwasser and Kissling 1958). Antigen may also be detected in cornal impressions and in biopsies of tactile hairs in ante mortem samples (Blenden et al. 1983; Schneider 1969).

Immunity
Survival of pigs following exposure to rabies virus is common and is dependent on the site of the bite (bites around the face and neck are more likely to be fatal), the quantity of the virus introduced, the age of the animal (young animals are more susceptible), and the virus strain involved. Both humoral and cell-mediated immunity are necessary to prevent fatal infections. Once clinical signs develop, rabies is usually fatal.

Prevention and Control
Because of the expense of conducting duration-of-immunity efficacy tests and the limited market, there are no licensed rabies vaccines for use in swine. Off-label use of inactivated vaccines may be justified in expensive breeding stock in rabies-endemic areas, but vaccine efficacy has not been demonstrated. Overall, the best way to prevent rabies is to control the disease in wildlife reservoirs and use physical barriers to protect swine from wildlife.

REFERENCES
OVERVIEW

The family **Togaviridae** comprises the mostly arthropod-borne genus **Alphavirus** and genus **Rubivirus** with one member (rubella virus) pathogenic to humans. The virions are spherical, 70 nm in diameter, and consist of an envelope with fine peplomers surrounding an icosahedral nucleocapsid 40 nm in diameter. The peplomers are formed by 80 trimers of the envelope proteins E1-E2 heterodimer.

The genome is a linear positive-sense, single-stranded RNA 9.7–11.8 kilobases in size, arranged as 5′ M−G−[nsP1−nsP2−nsP3−nsP4]−[C−E3−E2−6K−E1]−(A)n 3′. Genomic RNA serves as mRNA to translate into a polyprotein, which is processed into mature nonstructural proteins (nsP). The structural proteins are translated from a subgenomic 26S mRNA and cotranslationally processed into mature proteins (Fauquet 2005). The E2 protein contains most neutralizing epitopes, while the E1 protein contains more conserved, cross-reactive epitopes.

Alphaviruses are stable at pH 7–8, but rapidly inactivated by very acidic pH. Virions have a half-life of 7 hours at 37°C (98.6°F) in culture medium and most are rapidly inactivated at 58°C (136°F) with a half-life of minutes. Generally, they are sensitive to organic solvents and detergents, which solubilize their lipoprotein envelopes.

Based on serological cross-reactivity, alphaviruses are grouped into eight antigenic complexes. Eastern equine encephalitis virus (EEEV) is in the EEEV complex, and Getah virus (GETV), Sagiyama virus (SAGV), and Ross River virus (RRV) are in the Semliki Forest complex.

EASTERN EQUINE ENCEPHALITIS VIRUS

EEEV is endemic in eastern Canada, the United States east of the Mississippi River, the Caribbean Islands, and Central and South America. Endemic cycles are maintained by ornithophilic mosquitoes, for example, *Culiseta melanura*. Epidemic cycles are mediated by arthropod vectors that feed on both birds and mammals, for example, *Aedes* spp., *Anopheles* spp., and in some settings the *Coquillettidia* spp.

EEEV is zoonotic and causes encephalitis, fever, drowsiness, and nuchal rigidity in humans. Symptoms may progress to confusion, paralysis, convulsion, and coma.

In pigs, response to EEEV infection is age dependent. Nursing pigs are most severely affected and infection in pigs older than 2 months is inapparent. Clinical signs include uncoordination, depression, seizure, vomiting, low fever, and mortality. Growth is usually retarded in survivors. Predisposing factors, such as environmental conditions and concurrent disease, may contribute to higher mortality.

In 1958, serological studies on pigs in the states of Georgia, Massachusetts, and Wisconsin showed that seroprevalence ranged from 17% to 26%. In Georgia, up to 20% of domestic herds and 16% of feral swine were seropositive, with virus neutralization (VN) titers of 1:4–1:128 (Elvinger et al. 1996). VN antibody titers in domestic swine were generally lower than feral swine, and pigs from sale barns and stockyards had seropositive rates of 7%. However, a natural EEEV outbreak was not reported until 1972 in 3-week-old pigs (Pursell et al. 1972), and in 1991, in pigs under 2 weeks...
of age, with 80% mortality (Elvinger et al. 1994). Thus, EEEV infection in pigs is not uncommon, but clinical episodes are rare.

The incubation period in pigs is unknown, since most infections are subclinical, but ranged from 1 to 3 days in experimental inoculations (Elvinger and Baldwin 2006). EEEV initially replicates in regional lymph nodes then invades extraneural tissues, resulting in high virus titers and secondary viremia. Viremia is key to the invasion of the central nervous system (CNS). Viremia lasts for 7 days postinoculation (DPI). EEEV is recoverable from oropharyngeal and rectal swabs for 4 DPI and from tonsils for 20 DPI. Potentially, infected young pigs could transmit EEEV to contacts and serve as a source of virus for vectors (Elvinger and Baldwin 2006).

No gross lesions are observed. Microscopic lesions include meningoencephalitis with necrosis, microabscesses, perivascular cuffing of neutrophils (early) and lymphocytes (later), and myocardial necrosis. Pigs that die in the acute phase may not have CNS lesions (Elvinger et al. 1994).

Diagnosis by viral isolation can be done using CNS tissues and/or the specimens specified above in Vero cells or other cell culture systems, followed by identification by immunofluorescence or polymerase chain reaction (PCR; Ostlund 2008).

There is no treatment for clinically affected pigs. Prevention is achieved by vaccination of animals at risk and/or control of the vector population. Vaccines are available for humans and horses. Vaccination may be economically justifiable in severe outbreaks or for the protection of valuable pigs. Vaccination of sows will provide protective maternal antibody to piglets.

GETAH VIRUS

GETV is present throughout Asia as far north as Russia and south to Sarawak in Malaysia. It is primarily a pathogen of horses, but the virus has been isolated from a variety of mosquito species in the vicinity of swine. Aedes vexans nipponii, Culex tritaeniorhynchus, Culex gelidus, and Culex fuscocephala are considered the main vectors, depending on the location.

GETV was first reported in swine in 1987; it was diagnosed in 8 of 12 newborn piglets exhibiting depression, tremors, yellowish-brown diarrhea, and 100% mortality (Yago et al. 1987). Subclinically infected piglets showed a transient depression in growth rate and sows were unaffected. GETV has also been recovered from late-term dead fetuses from a naturally infected sow (Shibata et al. 1991).

Experimental inoculation with GETV induced transient pyrexia and anorexia at 1–3 DPI, and in some piglets, mild depression and diarrhea. Viremia occurred at 1–2 DPI and the virus was recovered from spleen and various lymph nodes (Kumanomido et al. 1988). Sows inoculated before 26–28 days of gestation farrowed dead fetuses, with virus recoverable from the placenta, amniotic fluid, and fetal organs (Izumida et al. 1988).

In Japan, where GETV is endemic in some districts, the serum antibody rates were 3–19% in domestic pigs (Hohdatsu et al. 1990) and averaged 48% in wild boar, with higher seropositive rates in adult pigs (Sugiyama et al. 2009). In areas of Sri Lanka where there is abundant rainfall and small-scale pig herds are surrounded by rice fields, the seroprevalence in pigs was 41% (with no evidence of illness) compared to only 0.6% in humans (Peiris et al. 1994).

Diagnosis is done by isolation of GETV on RK-13 or Vero cells or by detection of viral RNA by PCR (Ogawa et al. 2009). Enzyme-linked immunosorbent assay (ELISA) or hemagglutination inhibition (HI) is available for use in paired sera. An inactivated vaccine has been used successfully in racehorses.

SAGIYAMA VIRUS INFECTION

SAGV was considered a variant of GETV, but can be differentiated from GETV by complement fixation due to the presence of the amino acid leucine in the SAGV capsid protein (vs. proline in GETV) (Wekesa et al. 2001). C. tritaeniorhynchus and Aedes vexans are believed to be the major vectors.

Natural pig infections without clinical signs were reported in the 1960s, when the VN antibody rate in pigs was 67% versus 18% in humans living in the vicinity of infected pigs (Scherer et al. 1962). The first SAGV outbreak was reported in 2006 in 9-week-old pigs exhibiting growth retardation, panting, abdominal breathing, and arthritis (Chang et al. 2006). The clinical picture may have been affected by concurrent infection with Japanese encephalitis virus (JEV) and porcine circovirus type 2 (PCV2), and subsequent intramuscular inoculation of nursery pigs produced no specific clinical signs.

A low level SAGV viremia occurs at 2–4 DPI, at which time the virus can be recovered from the spleen, tonsil, lymph nodes, and kidney, but not from oral and nasal swabs. The VN antibody can be detected by 4 DPI and viremia is resolved by 7 DPI. SAGV is considered non-pathogenic to pigs, but concurrent infections may interact synergistically to cause more severe clinical signs.

SAGV causes a cytopathic effect (CPE) in ST (swine testis; ATCC CRL-1746™, Rockville, MD), porcine kidney (PK-15), Vero, rabbit kidney (RK-13), and baby hamster kidney (BHK-21) cells. Virus isolation is definitive, but has little diagnostic value due to the transient nature of viremia. Antibody detection is viewed as an indicator of infection. VN antibody titers >1:48 or a seropositive rate >50% is suggestive of repeated exposure to SAGV.
There is no treatment for SAGV infection. Vaccine is not available and may not be cost-effective, leaving vector control as the most rational form of prevention and control.

ROSS RIVER VIRUS INFECTION

RRV is endemic to Australia, Papua New Guinea, and Irian Jaya, Indonesia. Several RRV epidemics occurred in South Pacific islands in 1979–1980, but it did not become permanently established there. The main vectors are *Aedes vigilax*, *Aedes camptorhynchus*, *Aedes polynesiensis*, and *Culex annulirostris*.

RRV is regularly transmitted via a human–mosquito–macropod marsupials–mosquito–human cycle. However, in epidemics in densely populated areas, a direct human–mosquito–human route is believed to occur because of the high levels of viremia reached in humans.

RRV is not known to cause disease in pigs. Experimental infection of juvenile domestic and feral pigs resulted in no detectable virus or only moderate viremia at 0–5 DPI (Harley et al. 2001). Seroprevalence in domestic pigs was 43% and 77%, by HI and VN, respectively, during epidemics (Rosen et al. 1981), and 15% in feral pigs by HI during interepidemic intervals (Gard et al. 1976).

In humans, RRV infection may cause symmetrical epidemic polyarthritis, predominantly involving peripheral joints, fever, skin rash, and constitutional effects like myalgia, fatigue, and malaise. Seroconversion (immunoglobulin G, IgG) in paired sera as demonstrated by ELISA is indicative of recent infection. Detection of immunoglobulin M (IgM) is diagnostically unreliable, particularly in endemic areas. PCR assays have been described for human and equine sera (Sellner et al. 1995; Studdert et al. 2003) and should be used in conjunction with serology. Nonsteroidal anti-inflammatory drugs (NSAID) provide the best and most effective symptomatic relief. No vaccine is available for human use.

REFERENCES


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Diseases caused by bacteria, including mycoplasmas and chlamydiae, are all considered in this section. While viral diseases such as porcine reproductive and respiratory syndrome virus (PRRSV) and porcine circovirus remain as some of the greatest challenges to worldwide pork production, bacterial diseases continue to have a significant impact on the industry. Concurrent bacterial infection with lung pathogens such as *Bordetella bronchiseptica* and *Mycoplasma hyopneumoniae* has been shown to increase the severity and duration of PRRSV-induced pneumonia (Brockmeier et al. 2000; Thacker et al. 1999). Bacterial respiratory and enteric infections are among the most common and economically significant diseases facing swine producers today. A major pneumonia complex is *M. hyopneumoniae*, with secondary bacterial bronchopneumonia (Straw et al. 1989). *Escherichia coli* is the most important cause of neonatal and postweaning diarrhea in pigs (Fairbrother and Gyles 2006).

Phenotypic classification of bacteria places them into groups based upon readily identifiable characteristics which include Gram stain reaction, microscopic cellular morphology, oxygen requirements, and ability to form endospores (Table 47.1).

The Gram stain reaction divides bacteria into gram-positive or gram-negative forms based upon differences in the composition and thickness of their cell wall. Although most of the swine bacterial pathogens are able to be Gram stained, there are notable exceptions. Even though mycoplasmas are of gram-positive lineage, their permanent lack of a cell wall precludes them from retaining the crystal violet stain. Treponemes and leptospires cannot be satisfactorily stained and observed microscopically by this method. Additionally, the high lipid and mycolic acid content in the cell walls of *Mycobacterium* species may render them difficult to Gram stain.

Bacterial cellular morphology comprises cocci (spheres), bacilli (rods), and spirochetes (curved rods or spiral forms). Within these three morphological groups there may be considerable variability in shape and size. Cocci may be found in clusters (*Staphylococcus* species) or in long or short chains (*Enterococcus* and *Streptococcus* species). While many bacilli are considered to be regular rods (members of the genera *Escherichia*, *Salmonella*, and *Listeria*), others may appear coccobacillary (*Pasteurella*), pleomorphic (*Aracanobacterium pyogenes* and *Actinobacillus* species), or filamentous in shape (*Haemophilus parasuis* and *Erysipelothrix rhusiopathiae*). Among the spirochetes there are those that are loosely or tightly coiled (*Brachyspira*, *Treponema*, and *Leptospira* species) or curved to a seagull shape (*Campylobacter* species and *Lawsonia intracellularis*). The size of the individual bacterial cell may vary depending upon its growth phase and the type of medium the organism was cultivated in or on. As a general rule most spirochetes, *Bacillus* species and clostridia are regarded as large. Medium-sized organisms include pseudomonads and members of the family *Enterobacteriaceae* (*salmonellae* and *E. coli*). *Brucella*, *Haemophilus*, and *Pasteurella* species are small in size, while mycoplasmas and chlamydiae are extremely small.

Bacteria may be further categorized by their ability to utilize or tolerate oxygen. Bacteria with an absolute requirement for oxygen are called obligate aerobes. There are no genera of swine bacterial pathogens that fall into this category. Facultatively anaerobic bacteria can survive in the presence or absence of oxygen and...
this category represents the majority of the swine bacterial pathogens. Microaerophilic bacteria require trace amounts of oxygen for growth but may be killed by normal atmospheric concentrations. Members of the genus *Campylobacter* best represent the microaerophiles. Obligate anaerobes are killed by even trace amounts of oxygen. Many clostridia are obligately anaerobic.

Another classification of bacteria, primarily gram-positive bacilli, is based on their ability to produce spores. Spore-forming bacteria produce a unique resting cell called the endospore when vegetative cells are deprived of a necessary growth factor or requirement. Spores are extremely resistant to harsh environmental conditions and disinfectants. The two genera of spore formers with veterinary medical importance are *Bacillus*, the members of which are aerobic spore formers, and *Clostridium*, whose species are anaerobic spore formers. Notable non-spore-forming bacilli that represent swine pathogens include *Actinobacillus suis*, *E. rhusiopathiae*, and *A. pyogenes*.

Knowledge of the characteristics of a given bacterium is useful to the practitioner. The Gram stain reaction of a suspected pathogen can provide information regarding which antimicrobial agent to prescribe for empiric therapy while the veterinarian is awaiting the results of culture and susceptibility testing. When carrying out primary observations on stained clinical materials, it is important to know the Gram stain and cellular morphology of the common pathogens that might be present in the specimen. Knowledge of a bacterium’s oxygen requirements is an important consideration when collecting and transporting diagnostic specimens to the laboratory. Organisms that produce spores, such as *Clostridium difficile* and *Clostridium perfringens*, may be extremely difficult to eliminate from the farrowing house environment because the spores are resistant to most disinfectants, heat, and ultraviolet light. This is an important consideration for practitioners faced with the challenge of managing these infections in neonates.

The majority of swine bacterial pathogens are able to be grown on artificial culture media which provide necessary nutrients. Of these, many may form discrete colonies on plated media within 24–72 hours of inoculation. Other bacteria like *Leptospira* and *Mycobacterium* species may require several months for growth to occur in culture media. Some obligate intracellular parasites, such as chlamydiae and *L. intracellularis*, can only be cultivated in vivo or in cell culture systems because they are unable to produce energy in amounts required to sustain their metabolism outside a living host.

Swine bacteria may be considered as normal flora or commensals, opportunists, or frank pathogens. Commensals live on or in the host harmlessly. Most of the aerobic and anaerobic bacteria in the porcine alimentary tract are commensals. Opportunistic pathogens cause disease when the host’s innate or acquired immunity is compromised in some way. *A. pyogenes* represents one of the most common opportunists. Frank pathogens are consistently able to infect a percentage of healthy hosts by evading various defense mechanisms to cause disease. *Bacillus anthracis* is an example of a frank pathogen.

### Table 47.1. Classification of the principal bacterial pathogens of swine

<table>
<thead>
<tr>
<th>Classification</th>
<th>Genus/Genera</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gram-positive aerobic to facultatively anaerobic cocci</td>
<td><em>Enterococcus</em>, <em>Staphylococcus</em>, <em>Streptococcus</em></td>
</tr>
<tr>
<td>Gram-positive aerobic to microaerophilic non-spore-forming bacilli</td>
<td><em>Arcanobacterium</em>, <em>Erysipelothrix</em>, <em>Listeria</em>, <em>Mycobacterium</em>, <em>Rhodococcus</em></td>
</tr>
<tr>
<td>Gram-positive anaerobic spore-forming bacilli</td>
<td><em>Bacillus</em></td>
</tr>
<tr>
<td>Gram-positive anaerobic spore-forming bacilli</td>
<td><em>Clostridium</em></td>
</tr>
<tr>
<td>Gram-positive anaerobic non-spore-forming bacilli</td>
<td><em>Actinobaculum</em></td>
</tr>
<tr>
<td>Gram-negative aerobic to facultatively anaerobic bacilli</td>
<td><em>Actinobacillus</em>, <em>Bordetella</em>, <em>Brucella</em>, <em>Burkholderia</em>, <em>Escherichia</em>, <em>Haemophilus</em>, <em>Pasteurella</em>, <em>Salmonella</em>, <em>Yersinia</em></td>
</tr>
<tr>
<td>Gram-negative microaerophilic to anaerobic curved to spiral-shaped bacilli</td>
<td><em>Brachyspira</em>, <em>Campylobacter</em>, <em>Lawsonia</em>, <em>Leptospira</em>, <em>Treponema</em></td>
</tr>
<tr>
<td>Bacteria without cell walls and obligately intracellular bacteria</td>
<td><em>Mycoplasma</em>, <em>Chlamydia phila</em></td>
</tr>
</tbody>
</table>

Bacterial pathogenicity is a multifactorial process. Bacteria initially must establish an infection. This process involves bacterial attachment or other means of gaining entry into the host, evading host defenses, multiplying to significant numbers, causing damage to the host either directly or indirectly, and concluding with transmission of the agent to another susceptible host (Gyles and Prescott 2004). Host immune factors, the numbers of organisms present in the initial exposure, and the virulence attributes of the bacterium all play a role in the development of disease.

As a general rule, bacteria cause disease by two primary mechanisms: tissue invasion and toxin production (Songer and Post 2005). To invade host tissues, bacteria employ methods for adhering to and/or penetrating cells, producing extracellular substances to facilitate the process of invasion, and overcoming host defenses. Adhesins are surface proteins that cause adhesion of organisms to host cells. Many strains of pathogenic *E. coli* possess surface adhesive organelles called pili that mediate cellular attachment. Invasion may
follow, which affords bacteria the ability to evade the humoral immune response and multiply in a protected site. *Listeria monocytogenes* and *Yersinia* species are examples of facultatively intracellular bacteria that employ invasins to gain entry into host cells (Niemann et al. 2004). Certain bacteria produce extracellular enzymes like coagulase produced by *Staphylococcus hyicus* and streptokinase produced by beta-hemolytic streptococci that enable these agents to spread widely within the host tissue. For those bacteria that cause disease through toxin production, exotoxin and endotoxin are the major types. Exotoxins are proteins released primarily by gram-positive bacteria into their extracellular environment. Exotoxins vary greatly in their potency, ranging from the highly toxic botulinum toxin to the weakly toxic product released by *A. pyogenes*. Other swine pathogens that may produce exotoxins are *C. perfringens*, enteropathogenic strains of *E. coli*, *Pasteurella multocida*, and *S. hyicus*. Endotoxins are the lipopolysaccharides found in the cell wall of gram-negative bacteria. These may be released from both actively growing bacterial cells and those that have been lysed as a result of the effect of certain antibiotics or through successful host defense mechanisms like lysozyme production. The release of endotoxins is a very important component of the toxicity of gram-negative bacteria and is directly responsible for many of the clinical signs produced by these pathogens including fever, shock, and disseminated intravascular coagulopathy.

Many swine bacterial diseases may be recognized by their specific clinical signs, gross postmortem lesions, or epidemiological features (Tables 47.2 and 47.3). Direct contact, close contact with infected droplets or feces, or mechanical transfer by fomites or vectors are the ways in which bacteria are commonly spread. Succeeding chapters provide in-depth coverage of bacterial diseases including relevance, potential public health significance, epidemiology, pathogenesis, clinical signs, lesions, diagnosis, immunity, and prevention and control.
REFERENCES

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Marcelo Gottschalk

ACTINOBACILLUS PLEUROPNEUMONIAE

Relevance
Actinobacillus pleuropneumoniae (App) is the etiologic agent of pleuropneumonia in pigs. Named originally as Haemophilus pleuropneumoniae (Kilian et al. 1978; Shope et al. 1964), it was later designated as App based on DNA homology studies relating it to Actinobacillus lignieresii (Pohl et al. 1983). An organism also isolated from swine pleuropneumonia, originally known as “Pasteurella haemolytica-like,” was later determined to be a nicotinamide adenine dinucleotide (NAD)-independent biotype of App (Pohl et al. 1983) and is known as App biotype II (see below).

App is among the most important bacterial pulmonary pathogens in pigs and is found worldwide. In its most virulent form, App induces severe rapidly fatal fibrinohemorrhagic and necrotizing pleuropneumonia in naïve swine of all ages. Survivors often have devitalized bacteria-laden sequestra in their lungs that are poorly penetrated by antibiotics and that serve as sources of the organism for later outbreaks. The economic importance of App is principally due to the mortality, production, and medical costs in acute outbreaks as well as antimicrobial and/or immunoprophylaxis. In chronically infected herds, results from studies investigating impact on average daily gain have been controversial (Andreasen et al. 2001; Hartley et al. 1988; Hunneman 1986).

Virulence of App strains varies remarkably; some strains produce high mortality, others are avirulent and yet others are intermediate in virulence. High mortality outbreaks are relatively infrequent in the United States and Canada, but remain a problem in Latin American, Asian, and European countries (Gottschalk et al. 2003a).

Certain App can also manifest as insidious endemic infections in herds, functioning as secondary pathogens where they contribute to increased mortality in conjunction with other viral and bacterial pathogens as a component of the porcine respiratory disease complex (PRDC).

App can be frustrating for swine producers and veterinary clinicians alike. Many herds are infected with multiple strains that reside in the tonsils, nasal cavities, and chronic pneumonic lesions. Herds can carry high virulence strains for extended periods of time without episodes of disease or suggestive lesions at the abattoir. Individual carriers of App can be hard to identify. Outbreaks may suddenly appear in the presence of concomitant diseases, as a consequence of changes in management, or from other significant stressors. There are not readily available tests to definitively identify and differentiate highly virulent strains of App from strains of lesser virulence.

Both the organism and the disease have been studied extensively. Knowledge gained has allowed for better diagnostic tests and strategies, new vaccines, and relatively effective eradication strategies. However, App remains a significant cause of economic loss to the swine industry and there remains opportunity for improvement in control and eradication of this agent.

Etiology
App is a small, gram-negative, encapsulated rod with typical cocccobacillar morphology. App isolates are classified on the basis of the NAD requirement for growth into biotype I (NAD dependent) and biotype II (NAD independent) (Pohl et al. 1983). Biotype I strains do not grow on blood agar unless it contains NAD or...
unless NAD is supplied by a staphylococcal nurse streak where colonies of App grow in immediate proximity as “satellites.” App forms colonies 0.5–1 mm after 24 hours of incubation and is beta-hemolytic, particularly when sheep red blood cells are used. In fact, App produces an increased zone of hemolysis within the zone of partial lysis surrounding a beta-toxinogenic Staphylococcus aureus (the CAMP [Christie, Atkins, and Muench-Petersen] phenomenon) (Kilian 1976; Nicotet 1970). This CAMP phenomenon is related to the possession by App strains of combinations of the three cytolyisins ApXL, ApXI, and ApXIII (Frey et al. 1994; Jansen et al. 1995). Additional detailed morphological and biochemical characteristics may be found in original reports by Shope (1964) and Kilian et al. (1978). Biotype I strains should be differentiated from other actinobacilli normally present in the upper respiratory tract of swine (Gottschalk et al. 2003b; Kielstein et al. 2001). Biotype II strains grow easily on blood agar plates without the presence of NAD; colonies resemble and must be differentiated from those of Actinobacillus suis using a panel of biochemical tests. This is especially important because A. suis may induce pleuropneumonia similar to App (MacInnes and Desrosiers 1999; MacInnes et al. 2008; Yaeger 1995), but has different implications for control.

App biotype I has been divided into 13 serotypes (1–12, 15) and biotype II into 2 serotypes (13–14) for a total of 15 serotypes (Blackall et al. 2002; Kamp et al. 1987; Kilian et al. 1978; Nielsen 1985a,b, 1986b; Nielsen et al. 1997; Nielsen and O’Connor 1984; Rosendal and Boyd 1982). Serotype 5 is subdivided into subtypes 5a and 5b (Nielsen 1986a); however, this subdivision has neither epidemiological nor pathological significance and most laboratories do not perform the subtyping. The above association of serotypes and biotypes are predominant but not exclusive. Biotype II strains belonging to serotypes 2, 4, 7, 9, and 11 (normally found among biotype I strains) have been reported (Beck et al. 1994; Maldonado et al. 2009). Additionally, two biotype I serotype 13 strains have been isolated from the United States and Canada that are antigenically different from the reference serotype 13 biotype II strain (Gottschalk et al. 2010a). An encapsulated biotype II strain nontypeable with sera raised against the 15 known serotypes has been recovered from pigs with pleuropneumonia in a closed herd in the United States (Gottschalk et al. 2003a). However, more than 1000 sera from other swine herds representing the same and several other states in the United States were serologically negative when tested using a lipopolysaccharides (LPS) antigen prepared from the nontypeable strain, indicating that the nontypeable strain is not widely distributed.

Serologic specificity is conferred by the capsular polysaccharides (CPS) and the cell wall LPS of App. However, some capsular serotype groups have cell wall similarities and share identical LPS O chains, thus explaining the cross-reactions observed between serotypes 1, 9, and 11; serotypes 3, 6, and 8; and serotypes 4 and 7 (Dubreuil et al. 2000; Perry et al. 1990). More recently, antigenic cross-reactions between serotypes 3, 8, and 15 can also be explained by structural similarities (Gottschalk et al. 2010b; Perry et al. 2005). Combinations of a given serotype at the CPS and a different serotype at the LPS levels have been reported. For example, strains of CPS/LPS serotype 1/7 and 2/7 have been reported in North America and Europe, respectively (Gottschalk et al. 2000; Nielsen et al. 1996). The biotype I serotype 13 strains recovered in North America are antigenically different from the biotype II serotype 13 reference strain (isolated in Europe) in that they have an LPS that clearly cross-reacts with LPS from App serotype 10 (Gottschalk et al. 2010a). These strains can cause false-positive serotype 10 serologic reactions in LPS-based serologic tests (see below). Because cross-reactions among serotypes are common, at least two different techniques should be used to confirm the serotype (Mittal et al. 1992). It has been suggested that serotypes of App should be more rigorously defined by specifying both capsular (K) and LPS (O) antigens (Perry et al. 1990), but this nomenclature has not been widely adopted (Dubreuil et al. 2000).

**Public Health**

App does not infect humans and poses no public health risk.

**Epidemiology**

**Distribution of App.** App is widely distributed and only infects pigs. The primary reservoir is domestic swine, but a study from Slovenia showed that more than 50% of wild boars (Sus scrofa) were seropositive to App, suggesting them as a possible reservoir (Vengust et al. 2006). Outbreaks of pleuropneumonia have been reported in domestic swine from practically all European countries and from different parts of the United States, Mexico, South America, Japan, Korea, Taiwan, and Australia as well as in many other countries. However, the distribution of serotypes involved in outbreaks in different regions of the world is radically different. Moreover, strains of a certain serotype may be typically highly virulent in one region and yet strains of the same serotype may be typically of low virulence in another region. Therefore, it is critical when testing imported pigs for App prior to introduction into a closed herd to select diagnostic tests for the most important virulent serotypes present in their region of origin.

Distribution of serotypes of App in different countries has been summarized (Clota et al. 1996; Kucerova et al. 2005; Mittal et al. 1992). In some regions one or more serotypes predominate that are virulent and cause
most outbreaks: serotypes 1 and 5 in North America, serotype 2 in most of Europe, and serotype 15 in Australia, to name a few. Serotype 2 strains are highly virulent in European countries owing to secretion of two cytotoxins, ApxII and ApxIII, but serotype 2 strains in North America secrete only ApxII and are of low virulence (Gottschalk et al. 2003a). Serotype 4 is one of the most frequent virulent serotypes in Spain, but is uncommon in most other countries (Maldonado et al. 2009). This serotype was isolated from asymptomatic animals in Canada (Lebrun et al. 1999), but there are no other reports of serotype 4 strains in North America. Serotype 15, originally described and the predominant serotype in Australia (Blackall et al. 2002), has also been reported in North and South America (Gottschalk et al. 2003a) as well as in Japan (Koyama et al. 2007).

Biotype II strains are more commonly isolated in Europe than in North America, where only one report is available (Frank et al. 1992). Traditionally biotype II strains have been considered of low virulence; however, there are reports of their isolation in several cases of fatal pleuropneumonia (Gambade and Morvan 2001). As mentioned above, a nontypeable biotype II strain was isolated from pigs with fatal pleuropneumonia in a closed commercial herd in the United States, but it has not been found elsewhere in North America (Gottschalk et al. 2003a). More recently, Maldonado et al. (2009) reported that 25% of isolates recovered from diseased pigs in Spain belonged to biotype II of which serotypes 7, 2, 4, and 11 were the most common.

Most conventional herds are infected with one or more serotypes of App, but often these strains are of low virulence (Gottschalk et al. 2003a). In a recent study in Canada, 78% of herds were App positive based on polymerase chain reaction (PCR) detection in the upper respiratory tract of piglets (MacInnes et al. 2008). In the same study, 70% of the herds were seropositive by lipopolysaccharide-based enzyme-linked immunosorbent assay (LPS-ELISA) with distribution of serotypes as follows: 26% were positive for serotype 7 (4), 17% for 12, 15% for 3 (6, 8, 15), 6% for 5, 4% for 2, and 2% for 1 (9, 11). These data reflect a shift from traditionally predominant and virulent serotype 1 and 5 strains to relatively low virulence strains of other serotypes with a commiserate decrease in disease outbreaks. This shift is likely due to intentional changes in management and control strategies, including lower weaning ages, implementation of all-in/all-out management in nurseries and grower barns, limiting of commingling of animals from multiple sources, improved biosecurity, increased testing and monitoring for virulent serotypes, and implementation of a regional program for control of serotypes 1 and 5 in breeding herds in some Canadian provinces (MacInnes et al. 2008). In contrast, there has been a simultaneous increase in clinical App in recent years in many European countries as reflected in increased chronic pleuritic adhesions in abattoirs (Hoeltig et al. 2009; Sjölund and Wallgren 2010). This is likely due to older weaning ages mandated by new welfare laws, since App is known to be a late colonizer in piglets and earlier weaning dramatically reduces carriage rates in weaned pigs.

The somewhat paradoxical trend of high seroprevalence combined with decreasing disease prevalence in the aforementioned Canadian data highlights a concept important in the epidemiology of App. Low virulence strains tend to be widely distributed in herds, thus having high seroprevalence, whereas highly virulent strains that cause most disease in a herd are carried by a much lower proportion of animals with a corresponding lower seroprevalence. Hence, most prevalent serotypes (detected by serology) will very often be different from serotypes recovered from diseased pigs.

Strains belonging to the same serotype have been genotypically compared. In general, and using different techniques, most studies have shown a relative homogeneity within a particular serotype with one or few clones represented (Chatellier et al. 1999; Fussing 1998; Kokotovic and Angen 2007; Möller et al. 1992). Various molecular genetics techniques have been used in epidemiological studies in attempts to differentiate serotypes and strains. For example, a multiplex PCR for the identification and genotyping of App was developed by Rayamajhi et al. (2005). This technique, based on five different primer sets that amplify the four potential Apx genes from App in a single-step reaction, was successful in differentiating 11 of 15 reference serotypes. These results highlight the difficulty of molecular epidemiological studies and are typical in that a particular genotyping method often differentiates some, but not all App serotypes due to the existence of divergent clones within some serotypes. Further rapid subtyping tests are needed and a combination of different tests will have to be used to reach conclusions about epidemiological relationships (Kokotovic and Angen 2007).

**Routes and Duration of Shedding, Transmission.** App infects only the respiratory tract of pigs. In peracute and acute pulmonary infections, it can be found not only in pneumatic lung, but also in large numbers in nasal discharges. Survivors may remain carriers for several months (Desrosiers 2004), mainly in chronic lung lesions and tonsils. Some exposed animals remain healthy and become subclinical carriers, mainly in tonsils. Less frequently, carrier animals may harbor the pathogen in the nasal cavity (Chiens et al. 2010; Kume et al. 1984). Subclinical carriage of App in tonsils occurs for not only low virulence strains, but also for high virulence strains that can sometimes be present in well-managed herds for long periods without clinical disease. Environmental stressors or concurrent pulmonary pathogens can result in sudden herd outbreaks.
Transmission between herds occurs through the introduction of carrier animals to naïve populations. The main route of spread is by direct nose-to-nose contact or by droplets within short distances. Moving and mixing pigs increases the risk of spread. In acute outbreaks infection may not occur in every pen, suggesting the possible role of aerosols and air movement in the transmission of the disease over longer distances within buildings or the indirect transmission of contaminated exudates from acutely infected pigs by farm personnel. Studies confirm that App may be transmitted by aerosol over short distances (Desrosiers 2004), and Kristensen et al. (2004b) reported that airborne transmission between closely located pig units is possible, but rare. In contrast, Zhuang et al. (2007) suggested that local spread of App serotype 2 from neighboring herds was a predominant factor in the contamination of Danish specific pathogen-free (SPF) genetic herds. Introduction of the disease by artificial insemination or embryo transfer is improbable, since the genital tract is not a common site of infection, and antimicrobials in the diluent would prevent survival of the contaminating organism. Birds and small rodents are unlikely to transfer App and are not considered significant sources of the infection.

Infection is maintained in endemically infected herds by vertical transmission from infected sows to their offspring via close contact. The frequency of transmission is likely related to the amount of bacteria shed in sow nasal secretions and level of maternal immunity in piglets. The persistence of colostral antibody in piglets ranges from 2 weeks to 2 months of age, depending on the initial level of acquired colostral antibodies (M. Gottschalk, unpublished observations; Vigre et al. 2003). Usually only a few piglets are infected from their dams during the late suckling period when maternal immunity has declined, then lateral spread occurs after weaning as decay of maternal immunity renders more pigs susceptible (Vigre et al. 2002) or if mixed with pigs from naïve sources.

Survival of the organism in the environment is of short duration, especially in warm, dry conditions. However, when protected with mucus or other organic matter, it can survive for several days (even weeks) and it can survive in clean water for periods of up to 30 days at 39°F (4°C). Common disinfectants are effective against App when organic matter is first removed by thorough washing (Gutiérrez et al. 1995).

Pathogenesis

**Virulence Factors and Pathogenesis of the Infection.**

The incubation period can be quite variable. Inoculation of pigs with large numbers of virulent App can lead to fatal pleuropneumonia in as few as 3 hours. Following exposure by oronasal contact or inhalation, App first colonizes the squamous epithelial cells on the surface and later in the crypts of the palatine tonsil (Chiers et al. 1999). Colonized epithelial cells vacuolate and desquamate and, along with transmigrating neutrophils, distend tonsillar crypts. In contrast, App does not bind well to the ciliated epithelium of the trachea or bronchi (Bossé et al. 2002); an exception may be the trachea of newborns (Auger et al. 2009). When able to reach the lower respiratory tract, App is able to adhere to pneumocytes that line alveoli (Bossé et al. 2002; Van Overbeke et al. 2002).

Colonization is dependent upon bacterial-to-cell adhesion that seems to be mediated by polysaccharides and proteins (Van Overbeke et al. 2002). Evidence from other pathogen–host cell interactions suggests that adherence to host cells is a complex and multifactorial process (Bossé et al. 2002). The oligosaccharide core of LPS seems to play an important role in adherence of App to swine cells (Chiers et al. 2010). Knocking out the rfaE gene, which is involved in biosynthesis of LPS, resulted in a mutant strain that is no longer able to adhere (Provost et al. 2003). The presence of proteinaceous fimbriae and fimbrial subunits on the surface of App has been well confirmed (Zhang et al. 2000). However, their role in adhesion remains to be elucidated.

Once in the respiratory tract environment, certain bacterial nutrients, particularly iron, are scarce. App expresses a number of factors that are involved in the acquisition and uptake of iron (Chiers et al. 2010; Jacques 2004). Among other mechanisms, App is capable of utilizing porcine transferrin and haem compounds including free haem, haemin, haematin, and hemoglobin as well as a siderophore (Bossé et al. 2002; Chiers et al. 2010; Jacques 2004).

In pigs that are tonsillar carriers of virulent strains of App, the mucociliary apparatus normally clears any stray bacterial cells that are inhaled. This prevents App access to and subsequent replication in alveoli, the essential step to development of pleuropneumonia. Factors that overwhelm or reduce the function of the mucociliary apparatus are necessary to deliver sufficient App to alveoli for subsequent disease development. High inhaled doses of App in finely atomized particles can result in App reaching alveoli. This is most likely during acute outbreaks when sick pigs are shedding large amounts of App. Alternatively, cilia can be damaged by colonizing *Mycoplasma hyopneumoniae* or by replication of pseudorabies (Aujeszky’s) virus or influenza virus in tracheal and bronchial epithelial cells. Marois et al. (2009) showed that experimental inoculation of 10-week-old pigs with a serotype 9 strain of App produced disease only in pigs previously exposed to *M. hyopneumoniae* and not in nonexposed controls. Other factors may also impair ciliary function, like chilling or high environmental ammonia levels.
Once in the alveoli, the outcome of a battle between host immunity and virulence factors of App determines whether App is killed or causes pleuropneumonia. Initially LPS on the surface of App acts as a potent attractor of macrophages and neutrophils as well as stimulates host alveolar macrophages to secrete inflammatory cytokines. Cho et al. (2005) showed that locally released cytokines play an important role in the pathogenesis of swine pleuropneumonia. These cytokines activate macrophages and increase vascular permeability, flooding alveoli with important antibacterial serum proteins including complement and anti-App immunoglobulin G (IgG) antibodies (maternally derived, vaccinated, or previously exposed animals). App has several strategies to resist these host responses. The polysaccharide bacterial capsule of App inhibits engulfment by phagocytes as has been demonstrated by the characterization of nonvirulent, isogenic noncapsulated mutants (Bosse and Matyunas 1999; Rioux et al. 2000). Both macrophages and neutrophils can phagocytose App only in the presence of convalescent pig serum owing to the opsonic activity of anti-App IgG (Bosse et al. 2002; Cruysen et al. 1992). App is also resistant to the action of complement (Rioux et al. 1999, 2000; Ward and Inzana 1994).

The most important factors involved in impairment of the phagocytic function of both macrophages and neutrophils are the elaborated protein RTX (repeats in the structural toxin) toxins ApxI, ApxII, and ApxIII (Frey 2003; Haesebrouck et al. 1997). ApxI is strongly hemolytic and cytotoxic, ApxII is weakly hemolytic and moderately cytotoxic, and ApxIII is nonhemolytic but strongly cytotoxic (Frey 2003). Apx I toxin has recently been shown to induce apoptosis in porcine alveolar macrophages (Chien et al. 2009), whereas ApxIII is highly toxic for peripheral blood mononuclear cells. In general, strains of serotypes 1, 5, 9, and 11 produce ApxI and ApxII; strains of serotypes 2, 3, 4, 6, 8, and 15 produce ApxII and ApxIII; strains of serotypes 7, 12, and 13 produce only ApxII; and strains of serotypes 10 and 14 produce only ApxI (Gottschalk et al. 2003a). It seems that strains of serotype 3 secrete low levels of ApxII. A fourth toxin (ApxIV) is produced (in vivo only) by all serotypes (Schaller et al. 1999). The possible role of this toxin on phagocyte damage remains to be elucidated (Frey 2003). Recently, Liu et al. (2009), using an ApxIV knockout mutant, showed that ApxIV is essential for expression of full virulence of App.

Finally, other virulence factors have also been suggested to play important roles in the pathogenesis of the infection, such as outer membrane proteins, factors involved in biofilm formation, and proteases, as well as many products coded by genes that are clearly upregulated during infection, although in some cases their exact function is not yet known. For a complete review see Chiers et al. (2010). Complete genome sequencing and preliminary analysis of at least two different serotypes of App (serotypes 5 and 3), as well as analysis of the functions of the encoded proteins, will extend current knowledge on the metabolic and virulence characteristics of this pathogen (Foote et al. 2008; Gouré et al. 2009; Xu et al. 2008).

Tissue damage in the lung is extensive and due principally to the combined effects of the App Apx cytoxins on a variety of lung cells (Frey 2003; Frey et al. 1993; Haesebrouck et al. 1997) and the App LPS-stimulated host inflammatory response. Host phagocytes are attracted by LPS- and Apx-induced chemokines. Macrophages are activated and secrete toxic oxygen metabolites, and macrophages as well as neutrophils are killed by Apx toxins and release lysosomal enzymes that together further damage lung cells. Damage to endothelial cells results in activation of the coagulation pathway, microthrombi formation, and localized ischemic necrosis (Bosse et al. 2002). In most fatal cases of peracute pleuropneumonia, death is caused by endotoxic shock arising from absorption of copious amounts of App-derived LPS.

Factors Affecting Severity of Disease. Differences in virulence between serotypes or even within the same serotype have often been observed. In the field, strains of serotypes 1 and 5 in North America and 2, 9 and 11 in Europe are generally found to be more virulent than those of other serotypes. It is suggested that such differences are due to their combination of Apx toxins, capsular structure (Jacques et al. 1988), LPS composition (Jensen and Bertram 1986), and type of hemolysin (Frey 2003). In addition to production of ApxIV by all strains, virulent strains seem to produce two (not one) of either Apx I, II, or III. Interestingly, atypical strains that are either more or less virulent than others in their respective serotype have often been shown to have either added (more virulent) or lost (less virulent) an Apx toxin (Beck et al. 1994; Gottschalk et al. 2003a; Maldonado et al. 2009). However, not all differences in virulence are explained by capsule, LPS, and Apx toxin profiles as exemplified by some low virulence serotype 1 strains that have no atypical CPS, LPS, or toxin profiles (Gottschalk et al. 2003a). Attempts have been made using molecular genetic techniques directed toward Apx toxin and other gene targets to differentiate degrees of virulence in strains of App (Chatellier et al. 1999; Möller et al. 1992); however, there are currently no methods to definitively differentiate virulence levels of App strains apart from controlled animal inoculation experiments.

The outcome of infection by App and severity of outbreaks as gauged by increased morbidity and mortality can be impacted by several factors. As already mentioned, strain virulence and presence of other
pathogens like *M. hyopneumoniae*, pseudorabies virus and, likely, swine influenza virus significantly impact disease. Interestingly, serotypes considered as low pathogenic (such as serotype 3 and 12, at least in North America) can sometimes induce necrotizing bronchopneumonia or pleuropneumonia, especially in the presence of these other pathogens. In contrast, experimental studies showed that infection with porcine reproductive and respiratory syndrome virus (PRRSV) may not necessarily result in more severe App-induced disease (Pol et al. 1997). This is in agreement with field observations in geographical areas of endemic PRRSV infection, where the incidence of App pleuropneumonia does not increase significantly. Factors such as crowding and adverse climatic conditions such as rapid changes in temperature and high relative humidity coupled with insufficient ventilation encourage the development and spread of the disease and, consequently, also affect morbidity and mortality. It is therefore not surprising that the highest incidence of outbreaks is observed in growing and finishing pigs, mainly in seasons with adverse weather conditions. As a rule, large herds which mix pigs frequently are more at risk than small herds or herds with separate units.

Immune status of the animals relative to the infecting serotype of App is also important, whether passive, convalescent, or vaccinal. Conventional animals that might have been in contact with low virulent serotypes of App or with *A. suis* may be more resistant to the infection from a specific strain than SPF animals that are negative to all serotypes of App (M. Gottschalk, unpublished observations). However, this may depend on the ability of the previous colonizing serotypes to induce antibodies against the toxins elaborated by the newly infecting strain.

**Immunity**

Experimental or natural infections stimulate an immune response, and circulating antibodies can be detected approximately 10–14 days postinfection. These antibodies reach a maximum level within 4–6 weeks postinfection and may persist for many months (Desrosiers 2004). In some cases, subclinically infected animals may present low levels or absence of antibodies against toxins (Chiers et al. 2010). However, more studies are needed to confirm this fact. Immune sows confer passive immunity on their offspring. Such colostral antibodies may persist for about 5–12 weeks (Vigre et al. 2003), but this may depend on the sensitivity of the test used to detect the antibodies and on the initial level of acquired colostral antibodies. Protection may only last for as little as 3 weeks in some cases (Nielsen 1975), but these data may be due to the use of a very low sensitivity test such as the complement fixation test (CFT). The antibodies are directed against a wide range of bacterial structures and products, including capsule, LPS antigens, toxins (which can be neutralized), outer membrane proteins, superoxide dismutase, and iron-binding proteins. Both local immunoglobulin A (IgA) antibodies and serum IgG antibodies are produced.

**Clinical Signs**

Clinical signs vary with the age of the animals, their state of immunity, the environmental conditions, and the degree of exposure to the infectious agent. The clinical course can be peracute, acute, or chronic (Nicolet et al. 1969; Shope 1964; Shope et al. 1964). All stages of disease, from intermediate to fatal, subacute, or chronic, may develop within an affected group.

In the peracute form, one or more weaned pigs in the same or different pens suddenly become very sick with fever to 106.7°F (41.5°C), apathy, and anorexia. There is a short period of slight diarrhea and vomiting. The affected animals lie on the floor without distinct respiratory signs, heart rate is increased, and cardiovascular function fails. The skin on the nose, ears, legs, and later the whole body becomes cyanotic. In the terminal phase, there is a severe dyspnea with mouth breathing, animals remain in a sitting posture, and rectal temperatures drop. Shortly before death, there is usually a copious, foamy, blood-tinged discharge through the mouth and nostrils. In peracute forms it is common to find one or more animals dead without any premonitory signs and with typical foamy blood-tinged nasal discharge (Figure 48.1). Experimental studies have shown that the course of the disease may be as little as 3 hours from infection to death.

![48.1. Animal that died from peracute pleuropneumonia caused by Actinobacillus pleuropneumoniae presenting with typical foamy blood-tinged nasal discharge (courtesy of Dr. Enric Marco).](image-url)
In the acute form, many pigs in the same or different pens are affected. Body temperature rises to 105–106°F (40.5–41°C), the skin may be reddened, and the animals are depressed, are reluctant to rise, refuse food, and are reluctant to drink (Pijpers et al. 1990). Severe respiratory clinical signs with dyspnea, cough, and sometimes mouth breathing are evident. Cardiovascular failure is usually present, with congestion of the extremities. There is a marked loss of condition, which is apparent within 24 hours of the onset of the disease. The course of the disease differs from animal to animal, depending on the extent of the lung lesions and the time of initiation of therapy.

The chronic form develops after the disappearance of acute signs. There is little or no fever, and a spontaneous or intermittent cough of varying intensity develops. Appetite may be reduced, and this may contribute to decreased rate of gain. Affected animals can be identified by their intolerance of exercise. When moved, they lag behind the group and struggle only feebly when restrained. In chronically infected herds there are often many subclinically diseased animals. The clinical signs may be exacerbated by other respiratory infections (mycoplasmal, bacterial, or viral). Atypical mild respiratory signs with low mortality, resembling influenza, have also been reported (Tobias et al. 2009).

Less common manifestations may be seen in some outbreaks. In neonatal pigs fatal septicemia may be observed. In primary epidemics involving pregnant females, abortions may be observed (Wilson and Kierstead 1976), especially in SPF herds. Head tilt and drooping of one or both ears may be observed owing to middle ear infection associated with App infection (Duff et al. 1996).

Lesions

The gross pathological lesions are located mainly in the lungs (Nicolet and König 1966) and vary according to clinical course of disease. Pneumonia can be unilateral or bilateral, diffuse or multifocal, and well-demarcated in the affected lung(s) and tends to most often involve the cardiac and apical lobes, as well as at least part of the diaphragmatic lobes (Figure 48.2).

In peracute cases the trachea and bronchi are filled with a foamy, blood-tinged, mucus exudate and few gross changes may be obvious. In slightly later peracute cases, the pneumonic areas appear dark red-purple and slight to moderate firm yet resilient with little or no fibrinous pleurisy. On cut surface there is diffuse hemorrhage and areas of necrosis are friable.

In acute cases, layers of fibrin are obvious on the pleural surface and infrequently on the epicardium and pericardium (Figure 48.3) in animals that live for at least 24 hours after infection. The thoracic cavity usually contains a blood-tinged fluid. Affected areas of the lung are firm, rubbery, and mottled dark red-purple to lighter white in areas that contain abundant fibrin. On the cut surface, the parenchyma is heterogeneous (Figure 48.4). There are areas of hemorrhage and other areas that are necrotic and friable. White lines of fibrin are observed surrounding necrotic areas and distending interlobular septa. In some areas, interlobular septa may be instead distended with hemorrhagic red fluid.

In chronic cases, fibrosis of the previously fibrinous pleuritis results in firm adhesions between visceral and parental pleura. These areas often result in tearing of
Diagnosis

Confirmation of App Pleuropneumonia and Typing of Strains. App pleuropneumonia should be suspected when typical clinical signs and gross lesions are observed. Differentials in peracute cases where lungs are dark red and edematous should include other diseases that may produce similar lesions such as classical swine fever (Chapter 38), influenza (Chapter 40), pseudorabies (Chapter 28), septicemic salmonellosis (Chapter 60), and erysipelas (Chapter 54). In acute cases with typical fibrinohemorrhagic pleuropneumonia, A. suis (see below) (MacInnes and Desrosiers 1999; MacInnes et al. 2008; Yaeger 1995) and pleuritic strains of Pasteurella multocida (Chapter 58) should also be considered. Diagnosis is confirmed by culture, identification, and often typing of App. Lung samples for culture should come from lesioned areas of lungs collected from peracutely or acutely affected untreated animals. In chronic cases, where sequestra are in lungs and/or fibrous pleuritis is observed at necropsy or in the abattoir, App culture is typically negative. Surveillance of the herd using serology can be used to determine herd status. It is relatively easy to demonstrate App in pneumonic lesions from freshly dead animals. Primary isolation of App from tissues and secretions may be carried out on 5% sheep blood agar with a cross-streak of Staphylococcus epidermidis or S. aureus. After aerobic incubation overnight (in the presence of 5% CO₂), small colonies appear in the neighborhood of the streak (NAD requirement) surrounded by a clear zone of complete hemolysis. This allows a rapid presumptive bacteriologic diagnosis. For some serotypes (such as serotypes 7 and 12), the zone of hemolysis is usually less intense. Altered blood agars (“chocolate agar”) or PPLO agar supplemented with NAD allow the growth of the organism, but it is less distinctive on these media. Presumptive biochemical identification can be carried out by demonstrating the CAMP phenomenon and urease activity. Usually, serotyping will confirm the identity of App. Most isolates recovered from acutely infected animals are typical App and its identification is relatively straightforward.

When biochemically atypical isolates are recovered (e.g., urease negative isolates) or when the isolates are untypeable, it is recommended to confirm identity by PCR (see below). Biotype II (NAD-independent) isolates have been recovered more frequently in recent years (Gambade and Morvan 2001; Gottschalk et al. 2003a; Maldonado et al. 2009). These isolates might be misidentified as A. suis. In these cases, complete biochemical identification must be done before sending the isolate for serotyping. In fact, A. suis isolates present strong cross-reactions with sera against serotypes 3-6-8 of App and without a correct identification, these isolates can be serotyped and reported as App (M.
The detection of App identity of biotype II isolates of App is strongly recommended to perform a PCR test to confirm the identity of biotype II isolates of App. Serotyping allows rapid confirmation of identity of typical App isolates, is essential for choice of bacterins when they are used in a preventive strategy, and allows assessment of the herd and area epidemiology of various serotypes of App to be understood and monitored. Only biochemically (typical) or genetically (atypical) confirmed isolates should be serotyped. Cross-reactions are a problem when serotyping and it is recommended that two to three types of tests should be done to accurately identify the serotype. The slide agglutination test should not be used, since many isolates present cross and/or nonspecific reactions. The coagglutination test (Mittal et al. 1987) is routinely used, but many cross-reactions between serotypes occur due to common epitopes and a confirmatory test, such as agar gel diffusion and indirect hemagglutination, must be used (Mittal et al. 1987). In some particular cases, cross-reactions are so strong that some isolates will be classified as “serotype 6/8” (M. Gottschalk, unpublished observations). The use of monoclonal antibodies for serotyping has also been reported (Lacouture et al. 1997; Lebrun et al. 1999; Rodriguez-Barbosa et al. 1995). Monoclonal antibodies, especially those directed against the capsular epitopes, can easily differentiate, for example, serotype 1 from serotypes 9 and 11, and serotype 7 from serotype 4. However, monoclonal antibodies against the LPS will cross-react between serotypes owing to the sharing of common LPS between some serotypes (see above; Lacouture et al. 1997; Lebrun et al. 1999). Some of these monoclonal antibodies even showed that LPS epitopes are common between App serotype 7 and A. lignieresii (Lebrun et al. 1999).

PCR tests have been developed to help serotype isolates and to solve serological cross-reaction problems, especially those mentioned for serotypes 3, 6 and 8 (Zhou et al. 2008). In addition to App serotype specific PCR, multiplex PCR tests for different serotypes have been developed, such as a test for serotypes 1, 7, and 12 (Angen et al. 2008a); serotypes 1, 5, and 7 (Ito 2010); and serotypes 1, 2, and 8 (Schuchert et al. 2004). A PCR test based on the Apx toxin genes has been suggested to differentiate most serotypes (Rayamjahi et al. 2005). However, some serotypes cannot be differentiated and its use showed poor correlation with serological serotyping when field strains are tested (M. Gottschalk, unpublished observations). On the other hand, toxin typing using a well-described PCR (Frey et al. 1995) can be used to determine which Apx toxin genes are carried by a certain isolate, which may help to anticipate its virulence. For example, a nonvirulent serotype 1 strain recovered from healthy animals from a herd free of App-related disease was shown to produce ApxI only (Gottschalk et al. 2003b). Similarly, low virulent serotype 2 strains isolated in North America produce ApxII only, whereas highly virulent European strains produce both ApxII and ApxIII (Gottschalk et al. 2003a). Finally, a DNA microarray-based identification and typing system for App has also been reported (Xiao et al. 2009), although its use so far has been limited.

Bacteriological diagnosis of chronic disease is more complex, because it is difficult to culture App from such lungs. In lungs that contain sequestra, direct detection of App antigens in lung tissues can be performed by extracting antigens and testing by ring precipitation, coagglutination, latex agglutination, enzyme-linked immunosorbent assay (ELISA) and/or counterimmunoelectrophoresis (Bunka et al. 1989; Dubreuil et al. 2000; Mittal et al. 1983). However, results should be interpreted with care, since most of these tests have not been validated in the field. Bacterial presence can also be observed in tissues by using either a fluorescent or an immunoperoxidase antibody test (Bunka et al. 1989; Gutierrez et al. 1993). Nucleic acid from bacteria may also be detected by labeled DNA probes in tissue and PCR (Cho and Chae 2003). Direct confirmation by PCR of the presence of App in lung tissue is not yet routine. In cases where only fibrous pleuritic lesions are detected in the abattoir, App antigen may not be detected in lung. Therefore, serology (see below), along with disease history in the source herd, may be the easiest method to determine herd status.

**Detection of Tonsillar Carriers.** The detection of App from clinically healthy carrier animals is even more complex. This may be required in cases of seedstock introduction into negative herds or in eradication programs where the results of serology tests are equivocal. Bacteria are usually localized in tonsils and, less frequently, in the nasal cavities where they must be cultured from a milieu of other commensal bacteria, including several other NAD-dependent bacterial species (Kielstein et al. 2001). In fact a new species that is biochemically very similar to App, *Actinobacillus porcintonsillarum*, has been reported that may share common CPS and/or LPS epitopes with serotypes 1 and 9 of App (Gottschalk et al. 2003b). This species was shown to produce and secrete ApxII toxin by a complete ApxII operon (which does not exist in App strains) (Kuhnert et al. 2005). Selective media have been described (Jacobsen and Nielsen 1995), although their sensitivity is very low owing to typical heavy contamination of plates. To overcome the presence of a highly contaminating flora, an immunomagnetic separation technique for the selective isolation of a given serotype of App from tonsils has been developed and applied to serotype 1 (in Canada) (Gagné et al. 1998) and serotype 2 (in Denmark) (Angen et al. 2001).

Molecular techniques can also be used for the detection of App from tonsils. Several PCR techniques that...
Serology. Serology is the preferred and most cost-effective method for App surveillance. Serological testing has been used widely for the diagnosis, management, and eradication of virulent serotypes of App. In fact, serology is the most effective tool used to detect subclinical App infections (Broes and Gottschalk 2007). Some countries, such as Canada and Denmark, use serology for epidemiological surveillance of breeding herds on a routine basis. Different assays designed to detect antibodies against either the toxins or somatic and/or capsular antigens have been developed (for more details, see Dubreuil et al. 2000). Most tests detecting antibodies against ApxI, ApxII, and ApxIII toxins have low specificity, since other microorganisms such as A. suis can also produce similar toxins (Dubreuil et al. 2000; Nielsen et al. 2000). Some commercial kits lack specificity because they are based on ApxII as antigen and ApxII is produced by most serotypes.

An ELISA test detecting antibodies against the ApxIV toxin has been reported (Dreyfus et al. 2004) and commercialized. Although the manufacturer suggests high specificity and sensitivity for the assay, limited information has been published (Eamens et al. 2008). This test should be highly specific for App as it is the only bacterial species known to produce ApxIV, but it cannot differentiate among serotypes. Thus, most conventional herds would have high levels of antibodies owing to infection by several low virulence serotypes and its use as a diagnostic tool for high virulence serotype(s) would be limited. Although some unexplained positive reactions in App negative herds have been described (Broes et al. 2007), acceptable results may be obtained when this test is used for routine surveillance of high health status herds free of all serotypes of App (M. Gottschalk, unpublished observations). Finally, interpretation of this test must take into account that subclinically infected herds with animals harboring bacteria in only tonsils may not induce high levels of antibodies against App toxins (Chiers et al. 2002). In addition, some strains possess insertion sequences in their genome that abolish ApxIV production. Animals infected with such strains do not produce antibodies against this toxin (Tegetmeyer et al. 2008).

Although some ELISA tests based on CPS have been reported, some cross-reactions (probably due to antigen contamination during the purification process) were observed (Dubreuil et al. 2000). Common surface proteins have also been tested as antigens, but their use is limited due to the presence of cross-reactions with other microorganisms commonly present in pigs (Eamens et al. 2008).

The tests most commonly used are ELISA tests using O-chain LPS as antigens (Dubreuil et al. 2000; Gottschalk et al. 1994; Grøndahl-Hansen et al. 2003; Klausen et al. 2007). A kit based on these antigens is also commercially available. These ELISA tests can identify groups of serotypes as follows: 1, 9 and 11; 2; 3, 6, and 8; 4 and 7; 10; and 12. An ELISA test, using mixed O-chain LPS antigens from different serotypes, has also been reported (Grøndahl-Hansen et al. 2003). The O-antigens of serotype 15, 3, and 8 are chemically identical (Perry et al. 2005), and animals infected with App serotype 15 presented clear and strong serological reactions by ELISA using a serotype 3 O-chain LPS antigen (Gottschalk et al. 2010b). In addition, serotype 15 strains also present cross-reaction with serotypes 3 and 6 in serotyping. An atypical strain of App serotype 1 strain that did not express O-chain LPS antigen has also been described (Jacques et al. 2005). The relationship between chemical composition and antigenic behavior is not always clear. For example, the O-chain LPS of serotype 13 reference strain is highly similar to that from serotype 7 (MacLean et al. 2004); however, cross-reactions between these two serotypes have never been
observed in the ELISA test using LPS as antigen (M. Gottschalk, unpublished observations).

O-chain LPS antigens are also used in blocking ELISA tests using polyclonal antibodies (Andersen et al. 2002). Serological tests such as the CFT and the 2-mercaptoethanol are no longer used by most laboratories due to problems of sensitivity and specificity, respectively (Dubreuil et al. 2000). Interestingly, some countries (such as China and Russia) still require a negative App CFT test for imported swine, even though the low sensitivity and propensity for false-positives of the CFT are widely known (Gottschalk et al. 1994; Klausen et al. 2007).

Even though serological tests are valuable to identify subclinically infected herds and animals, serological testing occasionally generates ambiguous results, and genetic detection and/or bacterial isolation from tonsils should be carried out. In fact, tonsil colonization without induction of antibody response has been demonstrated (Chiers et al. 2010). App is a very dynamic pathogen and sometimes diagnosticians and practitioners must face atypical situations. Broes et al. (2007) presents such cases as well as diagnostic approaches.

**Treatment**

App has traditionally been susceptible in vitro to ampicillin, cephalosporin, chloramphenicol, colistin, sulfonamide, cotrimoxazole (trimethoprim + sulfamethoxazole), and gentamicin, to which it exhibits low minimum inhibitory concentrations (MIC). Sporadic data from the United States and other countries reflect a relatively high number of resistant App isolates to the betalactam family of antimicrobials (penicillin, ampicillin, amoxycillin) (Nadeau et al. 1988). Response of pigs with clinical App to penicillin treatment may be inconsistent (Sjölund et al. 2009). High MIC values indicating relative resistance are found for streptomycin, kanamycin, spectinomycin, spiramycin, and lincomycin (Gilbride and Rosendal 1984; Nadeau et al. 1988; Yoshimura et al. 2002). Resistance to tetracyclines, and to a lesser extent, trimethoprim–sulfonamide, seems to have increased in the last few years (Gutiérrez-Martín et al. 2006; Hendriksen et al. 2008; Morioka et al. 2008). Although it is accepted that there is no clear correlation between the distribution of the antibiotic resistances and the serotypes of App (Matter et al. 2007), higher levels of resistance were described in some studies for serotypes 1, 3, 5, and 7 (Gilbride and Rosendal 1984; Vaillancourt et al. 1988). In a recent study in Switzerland, all strains tested were susceptible against cephalothin, ceftriaxone, amoxicillin–clavulanic acid, and florfenicol (Matter et al. 2007), which is in agreement with reports from other countries (Gutiérrez-Martín et al. 2006; Morioka et al. 2008). A relatively high susceptibility against tiamulin, enrofloxacin, and tilmicosin was also found (Matter et al. 2007).

In theory, the antimicrobial of first choice should be the one with the lowest MIC. However, the pharmacokinetic properties should also be taken into consideration. Sjölund et al. (2009) showed that antibiotics with minimal MIC in their categories significantly varied in their capacity to control acute infection. Enrofloxacin and ceftiofur have been shown to be particularly effective after experimental challenge (Kobisch et al. 1990; Stephano et al. 1990). Satisfactory results in the field have been reported with tiamulin (Anderson and Williams 1990) and a combination of lincomycin and spectinomycin (Hsu 1990). Tilmicosin has also been reported as effective (Paradis et al. 2004). One study indicates that, using experimentally infected animals, tulathromycin administered as a single dose at either 2.5 mg/kg or 5 mg/kg body weight was at least as effective as three daily doses of ceftiofur regarding percentage of lung lesions, daily weight gain, days with clinical disease, and rectal temperature (Hart et al. 2006).

Antibiotic therapy is effective in clinically affected animals only in the initial phase of the disease, when it can reduce mortality. Interestingly, the success rate of an antibiotic treatment may influence the immune response of animals. In fact, highly effective antibiotics may prevent a good antibody response, leaving animals susceptible for a later reinfection (Sjölund et al. 2009). On the other hand, the nature of the lesions means that delay in treatment can result in a degree of infarction and chronic damage which will leave the animal as a respiratory cripple even if it recovers. Antibiotics should be given parenterally (subcutaneously or intramuscularly) and in high dosage, as affected animals may not eat or drink (Pijpers et al. 1990). To ensure effective and durable blood concentrations, repeated injections may be required, depending on the pharmacokinetic properties of the antibiotic used. The success of therapy depends mainly on early detection of clinical signs and on rapid therapeutic intervention. Water treatment may be used to treat members of the affected group which are still able to drink. Feed medicated with any of the above antimicrobials may be used successfully if all pigs have a normal food and water intake. Feed and water medication can be used as prophylactic antimicrobial therapy to prevent acute outbreaks in highly infected herds. Continuous medication or pulse dosing may be practiced, but neither should be used for long, and the antimicrobial sensitivity of the organism should be monitored continuously. Strategic medication should be targeted at periods of risk, which can be identified by routine postmortem examinations, clinical examinations, and herd antibody profiles. A combination of parenteral and peroral medication in a recent outbreak often yields the best results. In spite of apparent clinical success, it must be remembered that antibiotic therapy does not eliminate infection in a
herd. Chronic infections in lung abscesses or on the tonsils of carriers persist to form an important source of infection for other animals. Severely affected animals may not recover even after treatment and nursing and should be killed. Results obtained so far indicate that bacteria from carrier animals cannot be eliminated under antibiotic treatment (Angen et al. 2008b; Fittipaldi et al. 2005).

Prevention and Control
When herds are free of App, strict biosecurity should be practiced to prevent infection. The greatest risk is presented by introduction of potentially infected swine, usually seedstock. Seedstock should be purchased from herds demonstrated negative for App by a history of freedom from disease and negative serology for App. Incoming animals should be quarantined and serologically tested as negative before introduction. At times herds may be positive for certain serotypes known to be low virulence and have little or no history of disease. In these cases, similar precautions to introduction of serotypes known to be generally more virulent should be practiced. In herds that are already infected by virulent serotypes, App-negative seedstock should be vaccinated with products appropriate for the infecting virulent serotypes according to label directions and time should allowed for development of immunity before introduction.

During outbreaks of pleuropneumonia on App-infected farms, the first priority must be to control mortality by the treatment of affected individuals, usually including all contact animals in the affected pen and surrounding pens using the methods and antimicrobials outlined above. Disease may be treated at an early stage by treating groups of animals and moving them into clean airspaces where they are maintained in isolation until slaughter. Generally, good environmental management minimizes outbreaks in infected herds and includes maintaining appropriate environmental temperature with minimal fluctuations, seasonally appropriate ventilation, use of solid partitions between pens, all-in/all-out movement of pigs, appropriate stocking densities, and younger weaning ages (<21 days).

A wide range of vaccines have been developed for this disease (for a complete review, see Ramjeet et al. 2008). Commercial vaccines fall into two main groups: killed organisms (bacterins) and subunit toxin-based vaccines. Bacterins still represent 90% of commercially available vaccines. Vaccination with bacterins is serotype specific (Nielsen 1984), with possible cross-immunity with cross-reacting serotypes (Nielsen 1984, 1985c). The protection afforded has been extended by including all the serotypes present in an area. Animals vaccinated with bacterins will produce antibodies that will cross-react with ELISA tests that use polysaccharides as antigens. The type of adjuvant used may affect efficacy, and care may also be necessary before using certain adjuvants in pigs intended for human consumption, as some vaccines can produce undesirable granulomatous lesions at the site of injection (Straw et al. 1985).

Subunit vaccines, composed of the three major RTX exotoxins (ApxI, ApxII, and ApxIII) with or without a 42kDa outer membrane protein of App, have been developed and shown to give high protection against all 12 major serotypes (serotypes 1–12) under experimental conditions as well as in field trials (van den Bosch and Frey 2003). Toxin–bacterin combined vaccines have very recently been commercialized, and field results will be available in the following years. The ApxIV toxin, produced in vivo, seems also to confer immunity and protection against at least two different serotypes of App (Wang et al. 2009), although it has not yet been incorporated in any commercial vaccine. Liu et al. (2009) suggested that ApxIV protein is not required for efficient protection of pigs against App infection. Keeping in mind the very complex pathogenesis of porcine pleuropneumonia, inclusion of other bacterial virulence factors in vaccines might also be of value (Haesebrouck et al. 2004). A wide range of antigens administered either by parenteral, aerosol, or oral routes have been found to be experimentally protective, but none of them have been used in the field. Finally, live vaccines using laboratory-obtained non-virulent mutants have also been developed, and showed to protect against homologous and heterologous serotypes (Bei et al. 2007; Lin et al. 2007; Park et al. 2009). Some of these live vaccines even use the differentiate infected from vaccinated animals (DIVA) concept (Liu et al. 2009; Maas et al. 2006). One of these live vaccines is a nonencapsulated serotype 5 App mutant, containing a kanamycin resistance (KnR) gene within the capsule locus of the genome. Although some concern has been raised concerning potential transmission of antibiotic resistance to normal flora, further studies indicated that this would be unlikely (Inzana et al. 2004).

Vaccines may provide high levels of protection against morbidity in experiments, reduce mortality, reduce the number of treatments required, increase daily live weight gain, and may improve feed conversion efficiency. The quality of the carcass is also improved, with fewer condemnations for pneumonia and lower slaughtering costs through reductions in pleurisy and pericarditis. The decision to vaccinate should be carefully evaluated; the costs of mortality alone should not be the sole consideration, because the other effects on productivity listed above contribute to the benefits of vaccination. In some cases, individual and intensified medical treatments of affected pigs may be needed to reduce the impact of App (Sjölund and Wallgren 2010). Vaccination of piglets is usually advised; animals should not
receive the first dose during the first week of age to avoid interference with maternal antibodies. Sows can also be vaccinated without adverse effects (Kristensen et al. 2004a) and replacement animals can be vaccinated before their introduction in an infected herd.

**Eradication and Regional Control.** Control of pleuropneumonia in a region or breeding pyramid involves health schemes aimed at pleuropneumonia-free breeding and multiplying herds, serological monitoring, monitoring at slaughter and postmortem examination of casualties, control of management, and controlled pig traffic (serological testing, quarantine). For herds infected with App intending to join such a scheme, an eradication program is the method of choice but requires careful evaluation of the economic consequences. Depopulation and restocking with pigs originating from certified pleuropneumonia-free herds can be used; however, this method is expensive and may lead to the loss of important bloodlines. Other methods which have succeeded in the past include an eradication program in the existing herd area but weaning at another farm, at the same time supported by a program of vaccination, medication, and culling and repopulation with disease-free gilts (Larsen et al. 1990). Age of weaning and level of maternal antibodies may have an important influence on the colonization of piglets by App (Vigre et al. 2002). There are several herds that successfully eradicated App (depending on the serotype) using a good medicated early weaning program (M. Gottschalk, unpublished observations). Some serotypes, such as serotypes 12 and 3, are highly infective (even in the absence of clinical signs), and transmission of the infection from sows to piglets occurs relatively fast, preventing in some cases the success of good medicated early weaning programs. Small one-site breeding herds (up to 400 sows) with a relatively low percentage of seropositive animals (up to 30%) have used the “test and removal” of seropositive sows under medication (Nielsen et al. 1976). Certain reports suggest that only partial success (Lariviere et al. 1990) or even failure (Hunneman 1986) has resulted from the application of such eradication programs. In addition, the successful outcome of this method is principally based on the serological test used: a test with low sensitivity will not eliminate all carrier sows, and a test with low specificity will eliminate healthy noncarrier animals, which considerably increases the cost of the program. A successful elimination of certain serotypes of App with partial depopulation and antibiotic treatment has been suggested (Andersen and Gram 2004). However, it has also demonstrated that antibiotic treatment cannot completely eliminate the pathogen from carrier animals (Angen et al. 2008b; Fittipaldi et al. 2005). So far, there is no solid proof that partial depopulation can eradicate all serotypes of App. Before an eradication program is carried out, all aspects of biosecurity and the characteristics of the farm should be taken into consideration to avoid risks of recontamination (Zhuang et al. 2007).

**ACTINOBACILLUS SUIS**

The gram-negative bacterium *A. suis* is a ubiquitous opportunistic pathogen that colonizes the upper respiratory tract of pigs (MacInnes and Desrosiers 1999). In a recent survey, MacInnes et al. (2008) showed that as many as 94% of the tested herds were suspected to be infected by *A. suis*, although no clinical cases were observed. Although originally reported as causing septicemia and death in only suckling and recently weaned pigs, disease can be observed in suckling, weaning, fattening pigs, and even adult animals, especially in high health status herds (Yaeger 1995, 1996). Prevalence of disease may be greater in relatively new herds, before animals develop immunity (Wilson and McOrist 2000).

*A. suis* produces septicemia and localized infections and has been associated with a variety of clinical signs including sudden death, dyspnea, cough, lameness, fever, weakness, wasting, abscesses, neurological signs, abortion, cyanosis, and diffuse hyperemia. One of the most suggestive gross lesions is the presence of petechial to ecchymotic hemorrhages in the lungs, kidneys, heart, liver, spleen, skin, or intestines and petechial hemorrhages on the ears, abdomen, and skin, and cutaneous erysipelas-like lesions (MacInnes and Desrosiers 1999). Yaeger (1995) reported three common forms of *A. suis* disease.

First is an acute fulminant septicemic form occurring mainly in suckling and recently weaned pigs. Very often, pigs are simply found dead. Consistent gross lesions include petechial to ecchymotic hemorrhages in multiple organs and serous to serofibrinous exudates in the thoracic and abdominal cavities. Pleuritis, pericarditis, arthritis, and miliary abscesses in a variety of organs may be observed. Histologic lesions consist of foci of necrosis in multiple organs associated with bacterial thromboemboli. Differential diagnosis should include other agents of septicemia.

A second form is respiratory disease mainly affecting grow–finish pigs, most commonly in high health status herds. Pigs may exhibit a cough and fever and, as with young pigs, sudden death may also be the sole presenting sign. However, in these cases, the most remarkable gross lesion is generally a multifocal or diffuse hemorrhagic and necrotizing pneumonia or pleuropneumonia, petechial hemorrhages on the serosal surfaces of abdominal and thoracic viscera and, in some cases, a fibrinous peritoneal exudate. Differential diagnosis should include mainly App (Yaeger 1995, 1996).
The third form of *A. suis* disease is acute septicemia in adult animals, also most commonly observed in high health status herds. Animals may exhibit lethargy, anorexia, fever, and red, rhomboid skin lesions resembling erysipelas. Abortions may occur and animals may also die due to septicemia. Gross lesions in these cases consist of multifocal petechial hemorrhages, serofibrinous exudates in the thoracic and abdominal cavi ties, and occasionally small foci of hepatic necrosis. This form, particularly when skin lesions (as described above) are present, may be confused with erysipelas.

*Actinobacillus suis* isolates from both healthy and diseased pigs are very similar based on biochemical analysis, restriction endonuclease fingerprinting, slide agglutination, and toxin typing (MacInnes and Desrosiers 1999). The organism has genes that encode toxins that are very similar to ApxI and ApxII of App. These toxins likely contribute to the virulence of this organism. However, *A. suis* produces lower levels of Apx toxins than does App, which may explain why *A. suis* is generally less virulent (MacInnes and Desrosiers 1999). On the other hand, *A. suis* is able to resist bile and serum, suggesting that it has additional, but as yet unidentified, virulence factors when compared to App. It has been shown that different serologically distinct groups of cell surface antigens exist: O1/K1, O1/K2, and O2/K2, presenting some variation in virulence (Slavic et al. 2000). However, a clear classification of serotypes and their association to virulence is not used routinely. Critical virulence factors of *A. suis* are unknown; however, an outer membrane protein was shown to be an important adhesin for swine cells (Ojha et al. 2010).

Preliminary diagnosis is based on typical clinical signs and gross lesions. Diagnosis is confirmed by culture and identification of *A. suis* from tissues with typical microscopic lesions. A strain-specific ELISA test has been shown to be useful to follow up maternal and active antibodies in an infected herd (Lapointe et al. 2001).

Antibiotic treatment should be applied early in the appearance of signs; ceftiofur, gentamicin, and trimethoprim/sulfadiazine seem to be the antibiotics of choice, followed by ampicillin, sulfadimethoxine, and tiamulin (MacInnes and Desrosiers 1999). There are no reports in the literature suggesting resistance of a large number of *A. suis* strains to different antibiotics. Vaccination has been implemented in some herds using autogenous vaccines, with variable results. In one of these herds, gilts and sows were vaccinated, although these animals already presented high titers of antibodies before vaccination (Lapointe et al. 2001).

**OTHER ACTINOBACILLUS SPECIES**

*Actinobacillus equuli*, a primary infectious agent of horses, has been suggested as being involved in several swine infections in Europe many years ago (Ramos-Vara et al. 2008). However, most of these infections were probably caused by *A. suis*, since both pathogens are phenotypically and genetically very similar. A revised definition of actinobacilli recovered from animals (from a diagnostic point of view) has been published (Christensen and Bisgaard 2004). On the other hand, swine infection by real and confirmed *A. equuli* has been recently reported in the United States and Canada (M. Gottschalk, unpublished observations; Ramos-Vara et al. 2008) with, in some cases, high morbidity and mortality that required the use of autogenous vaccines. The source of the infection in most herds remains unknown, but diagnostic laboratories must be aware of the potential of *A. equuli* in pigs and clearly differentiate from *A. suis* infections (Christensen and Bisgaard 2004).

Many species of *Actinobacillus* other than App can be found in swine tonsils, such as *Actinobacillus minor*, *Actinobacillus porcinus*, *Actinobacillus rossii*, Bisgaard Taxon 10, and *A. porcintonsillarum* (Lowe et al. 2010). As mentioned in the “*Actinobacillus pleuropneumoniae*” section, the latter species may be misidentified with the etiological agent of pleuropneumonia. Although considered as nonvirulent, recent reports indicate that *A. porcintonsillarum* can be isolated from diseased animals presenting a variety of pathologies (Martínez and Maldonado 2006; Ohba et al. 2007) and it seems to present a higher antibiotic resistance than App (Matter et al. 2007). The virulence potential of other actinobacilli has not been demonstrated.

**REFERENCES**


Bordetellosis

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RELEVANCE

*Bordetella bronchiseptica* (Bb) was first isolated and associated with respiratory disease in dogs in 1910 (Ferry 1910). Soon after, it was found to cause respiratory disease in other mammals (Ferry 1912). By the 1940s it was isolated in association with swine pneumonia, and it was being investigated as a cause of atrophic rhinitis of swine by the 1950s (Phillips 1943; Switzer 1956). In swine, Bb is widespread and plays multiple roles in respiratory disease. It is the primary etiological agent of nonprogressive atrophic rhinitis (NPAR), a mild to moderately severe reversible condition. Nasal colonization by Bb also promotes colonization by toxigenic strains of *Pasteurella multocida*, which leads to severe, progressive atrophic rhinitis (PAR; see Chapter 58) (Cross 1962; de Jong and Nielsen 1990; Duncan et al. 1966b; Pedersen and Barford 1981; Rutter 1983). In young pigs, Bb is a primary cause of bronchopneumonia and in older pigs contributes to the porcine respiratory disease complex (PRDC) (Duncan et al. 1966a; Dunne et al. 1961; Palzer et al. 2008). Its presence also enhances colonization with secondary bacteria other than *P. multocida* and increases the severity of respiratory disease associated with additional primary and secondary pathogens, including porcine reproductive and respiratory syndrome virus (PRRSV) (Brockmeier 2004; Brockmeier et al. 2008, 2000, 2001; Loving et al. 2010; Vecht et al. 1989, 1992).

ETIOLOGY

*Bordetella* is a genus of *Betaproteobacteria* consisting of eight formally recognized species. A ninth species has been described in the literature but has so far not been accorded standing in nomenclature. Bb is the only species of importance in swine. The so-called Bb cluster consists of *Bordetella pertussis*, *Bordetella parapertussis*, and Bb, all of which cause respiratory disease. Bb infects a broad range of mammals, occasionally causing acute disease, but more often resulting in chronic or inapparent infection of the upper respiratory tract (Goodnow 1980; Mattoo and Cherry 2005; Staveley et al. 2003). A variety of techniques indicate that species of the Bb cluster are highly clonal (Gerlach et al. 2001; Musser et al. 1986; Parkhill et al. 2003). It has been theorized that *B. pertussis* and *B. parapertussis* independently emerged from a Bb-like progenitor through rearrangement and large-scale genome decay. It has been suggested that they be reclassified as subspecies.

Bb is an aerobic, motile, gram-negative rod or cocccobacillus approximately 1.0 × 0.3 µm in size. The bacterium grows slowly, but readily, on blood agar or other nonselective media as well as on MacConkey agar. Convex colonies roughly 1–2 mm in diameter, usually hemolytic on blood agar, develop after 36–48 hours at 98.6°F (37°C). Bb is nonfermentative but positive for oxidase, catalase, urease, and citrate. Pure cultures used for additional characterization of isolates are often propagated on Bordet–Gengou (BG) agar containing 10% defibrinated sheep’s blood, a medium initially developed for growth of the fastidious species *B. pertussis*. Stainer–Scholte (SS) broth (Stainer and Scholte 1970) supplemented with 40 µg/mL streptomycin may be used for liquid cultures, but the resulting material should be recultured on a blood-containing agar medium prior to subsequent use to ensure that the hemolytic phenotype originally observed has been maintained.

No serotyping methodology useful for discriminating among strains or evaluating population diversity is


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currently available for Bb. Nearly all Bb strains express one of two antigenically distinct O-antigen serotypes, O1 or O2, that are not cross-reactive (Buboltz et al. 2009a). However, these antigens are not suitable for typing purposes since they are encoded by separate loci that may recombine (Buboltz et al. 2009a).

Researchers have turned to molecular typing methods to identify and characterize genetic relationships among strains. These include ribotyping, random amplified polymorphic DNA (RAPD) fingerprinting, pulsed-field gel electrophoresis (PFGE), and multilocus sequence typing (MLST) (Gueirard et al. 1995; Khattak and Matthews 1993; Musser et al. 1986, 1987; Register et al. 1997). While the population structure of the classical bordetellae appears to be clonal, phylogenetics and comparative genomic analyses indicate that Bb strains are more diverse than *B. pertussis* or *B. parapertussis* strains (Cummings et al. 2004; Diavatopoulos et al. 2005; Musser et al. 1986, 1987; van der Zee et al. 1997). In fact, some strains of Bb are more distantly related to each other than they are to *B. pertussis* or *B. parapertussis* (Diavatopoulos et al. 2005).

It is well known that the consequence of colonization by Bb can vary from asymptomatic infection to lethal pneumonia (Goodnow 1980; Mattoo and Cherry 2005). Using inbred and specific pathogen-free mice, the 50% lethal dose (LD₅₀) can differ by up to 100,000-fold between bacterial strains, suggesting that substantial differences in virulence may be due to strain variation alone (Buboltz et al. 2008; Gueirard and Guiso 1993; Gueirard et al. 1995). Recently published reports have demonstrated that phylogenetic lineages can differ in virulence factor expression and virulence (Buboltz et al. 2008, 2009b). These reports support the overarching idea that the diversity of Bb-related disease and broad host range may be due, in part, to the distinct sets of virulence factors used by strains of different phylogenetic lineages (Cummings et al. 2006; Giardina et al. 1995).

**PUBLIC HEALTH**

Human illness resulting from Bb infection is rare but on the rise (Berkelman 2003; Llombart et al. 2006; Mattoo and Cherry 2005; Tamion et al. 1996; Woolfrey and Moody 1991). Most at risk are infants and immunocompromised individuals with exposure to carrier animals; however, disease in immunocompetent adults also occurs. A variety of clinical presentations have been documented, including tracheobronchitis, whooping cough, pneumonia, sinusitis, septicemia, meningitis, and peritonitis, occasionally with a fatal outcome. In the majority of cases exposure to domesticated pets, particularly dogs, cats, and rabbits, is the proven or presumed source of infection. No cases obviously related to transmission from swine have so far been reported. However, one of the few animal isolates among a genetically related group of Bb strains strongly associated with human infections is of swine origin (Diavatopoulos et al. 2005). It is currently unknown whether healthy adults may act as asymptomatic carriers following exposure to naturally infected or recently vaccinated swine or other animals.

**EPIDEMIOLOGY**

Bb has a worldwide distribution and is known to infect poultry and a broad range of wild and domesticated mammalian species (Farrington and Jorgenson 1976; Goodnow 1980; Hammond et al. 2009; Heje et al. 1991; Lacasse and Gamble 2006; Ngom et al. 2006). It is highly prevalent among swine and frequently isolated from pigs with pneumonia or atrophic rhinitis as well as those that are apparently healthy (Backstrom et al. 1988; Giles et al. 1980; Hensel et al. 1994; Palzer et al. 2008; Rutter et al. 1984; Straw and Duran 1999).

Transmission of Bb occurs primarily by aerosol droplets. It is facilitated by close contact, but airborne transmission over short distances, including within a barn or production unit, is probable (Brockmeier and Lager 2002; Stehmann et al. 1992). Infectious aerosols generated by sneezing or coughing in pigs with active disease further promote spread of the agent. Pigs of all ages are susceptible to infection, but many litters are colonized by Bb at an early age, most likely due to exposure from nursing sows. Bb persists in the nasal cavity for at least several months (Backstrom et al. 1988; Riising et al. 2002; Rutter 1981) and perhaps indefinitely, and the introduction of carrier pigs is a source of infection for older animals in systems where an all-in/all-out approach is not practiced. Newly purchased breeding stock may also be a source of infection. Spread within a herd is rapid, particularly in immunologically naïve animals (Smith et al. 1982).

Antibody passively acquired by piglets from the colostrum of infected or vaccinated sows protects against turbinate lesions and pneumonia but not against infection (Kobisch and Pennings 1989; Magyar et al. 2002; Riising et al. 2002; Rutter 1981). However, vaccination of sows may delay infection for up to several weeks (Rutter et al. 1984), at which time lesions typically fail to develop or are significantly reduced in severity (de Jong and Akkermans 1986; Duncan et al. 1966b; Giles et al. 1980).

Bb has been isolated from rodents, birds, raccoons, opossums, and other animals trapped in close proximity to swine (Farrington and Jorgenson 1976; Le Moine et al. 1987). Although so far undocumented, cross-species transmission to pigs following exposure to infected wild animals or domesticated pets, such as dogs and cats, may occur. Insect vectors may also be capable of introducing the organism into a herd or production unit (Beatson 1972). It has been suggested that nonporcine strains are of low virulence in pigs.
(Ross et al. 1967), but it is unclear whether the cultures used were propagated so as to retain their original phenotypes. This conclusion should be reevaluated in light of the current understanding of virulence factor regulation in Bb and the importance of maintaining a Bvg+ phenotype during culture (see “Pathogenesis” section). A subsequent study reported no differences in the bacteriological properties of swine isolates as compared to isolates obtained from several other hosts (Bemis et al. 1977).

Transmission via contaminated fomites has not been evaluated but should not be excluded, given the ability of Bb to survive ex vivo. The bacterium remains viable for up to 45 days in soil (Mitscherlich and Marth 1984) and for at least several weeks in lake water or non-nutritive liquid media at temperatures ranging from 50 to 98.6°F (10–37°C) (Porter et al. 1991; Porter and Wardlaw 1993). The half-life of aerosolized organisms at ambient temperature and ~75% relative humidity is 1–2 hours (Stehmann et al. 1992).

Bb can be inactivated by sonication (Harris and Switzer 1972), heating to 140°F (60°C) (Bemis and Kennedy 1981; Lendvai et al. 1992), or treatment with formaldehyde (Jenkins 1978). It is sensitive to several chemical disinfectants suitable for farm use (Thomson et al. 2007).

**PATHOGENESIS**

The pathogenesis of Bb is dependent on the sequential, coordinated synthesis of an array of virulence factors, including adhesins, toxins, and other bacterial products that may alter host functions, facilitate immune evasion, or otherwise assist in transmission or survival. Expression of most virulence genes requires coexpression of the BvgAS (Bordetella virulence genes) system (Beier and Gross 2008). At temperatures of ~77°F (25°C) or lower, bvgAS genes are not expressed, and the resultant Bvg state is nonpermissive for the synthesis of toxins, adhesins, and other known or suspected virulence proteins. Environmental cues, including a rise in growth temperature that occurs as the organism moves from an external environment into the tissues of the respiratory tract, trigger transcription of bvgAS and subsequent expression of the BvgAS-inducible genes (Bvg+ state). This process, known as phenotypic modulation, is fully reversible and is an important adaptive response of the organism to changes in the environment.

The bvgAS genes are also subject to phase variation, in which a small proportion of growing cells spontaneously acquire deletions or frameshift mutations that irreversibly abolish expression of all BvgAS-activated genes, regardless of the growth conditions. When subculturing Bb it is important to pick for passage only single, well-isolated Bvg+ colonies (small, domed, and hemolytic on blood agar) in order to avoid gradual transition of the culture to an irreversible Bvg+ state (colonies are larger, flat, and nonhemolytic on blood agar).

So-called early BvgAS-inducible genes, including many whose products are involved in attachment, are among the first to be activated during phenotypic modulation from Bvg+ to Bvg-. Expression of “late” genes, including several toxins, commences only after accumulation of sufficient levels of the bvgAS gene products. The existence of the BvgAS system suggests that precise control of the temporal expression of virulence factors in response to a changing environment is important for optimizing the growth and survival of Bb as it cycles through transmission, colonization, growth and spread, immune evasion, and shedding.

In the initial stage of infection Bb attaches to epithelial cells lining the nasal mucosa. Organisms display preferential adherence to ciliated cells (Duncan et al. 1966b; Yokomizo and Shimizu 1979), but attachment to nonciliated epithelia may also occur and may be important in establishing microcolonies or biofilms (Irie et al. 2004). Several known or suspected Bb adhesins have been characterized and these likely function in a redundant or cooperative manner. Filamentous hemagglutinin (FHA), which is both secreted and associated with the cell surface, is a highly immunogenic protein required for optimal colonization of the upper respiratory tract (Cotter et al. 1998; Edwards et al. 2005; Hibrand-Saint Oyant et al. 2005; Irie and Yuk 2007; Nicholson et al. 2009). At least four different domains with distinct binding specificities have been identified, some of which exert immunomodulatory effects (Hannah et al. 1994; Ishibashi et al. 1994; Prasad et al. 1993; Relman et al. 1990). Fimbrial proteins, which form a complex of hair-like strands extending from the cell surface, similarly possess multiple binding specificities, one of which enhances FHA-mediated attachment (Geuijen et al. 1997; Hazenbos et al. 1995). Fimbriae are important for colonization and persistence in the trachea and also influence the humoral immune response to infection (Edwards et al. 2005; Funnell and Robinson 1993; Geuijen et al. 1997; Mattoo et al. 2000). Studies suggest the outer membrane protein pertactin may also contribute to colonization, perhaps as an accessory adhesin, but its precise function remains unclear (Hibrand-Saint Oyant et al. 2005; Nicholson et al. 2009). In contrast, its importance as a protective immunogen is well established (Kobisch and Novotny 1990; Montaraz et al. 1985; Novotny et al. 1985). Sequence heterogeneity in a region comprising an immunodominant protective epitope (Boursaux-Eude and Guiso 2000; Register 2001, 2004) may alter the specificity of host immune responses, providing a potential mechanism for immune evasion (Hijnen et al. 2007).

Once Bb is established within the respiratory tract, expression of toxins contributes to the progression of disease. Of central importance is a dermonecrotic toxin...
(DNT) that exerts pleiotropic effects, including impairment of bone formation (Horiguchi et al. 1995). Following reports demonstrating that strains with reduced demonercotic activity failed to induce turbinate and lung lesions (Magyar et al. 1988; Roop et al. 1987), additional investigations comparing isogenic mutants deficient solely in DNT production unequivocally established that the toxin is essential for development of pneumonia and turbinate atrophy in both mice and pigs (Brockmeier et al. 2002; Magyar et al. 2000). A bifunctional toxin that has both adenylate cyclase and pore-forming activities, referred to as adenylate cyclase toxin (ACT), also contributes to virulence by disrupting innate immunoprotective functions. Phagocytic cells appear to be a primary target of the Bb ACT (Harvill et al. 1999). The toxin also modulates cytokine production in dendritic cells (Skinner et al. 2004) and alters serum and secretory antibody responses in pigs (Hibrand-Saint Oyant et al. 2005). On the basis of studies using ACT from the closely related human pathogen B. pertussis, Bb ACT is likely to exert additional immunomodulatory effects (Vojtova et al. 2006). Since ACT, like most Bordetella toxins, is expressed only by Bvg+ bacteria, its hemolytic activity is a convenient visual marker for the Bvg status of isolates grown on blood-containing media. Tracheal cytotoxin (TCT) is a peptidoglycan breakdown product arising from normal cell wall remodeling during growth. Unlike most other gram-negative bacteria, Bb lacks the capability to recycle TCT (Cookson and Goldman 1987), and it is instead released extracellularly, where it interacts synergistically with lipopolysaccharide to cause ciliostasis and extrusion of ciliated cells from the mucosal epithelial lining (Flak et al. 2000). In contrast to the Bb protein toxins, TCT is not controlled by the Bvg system and is produced by both Bvg+ and Bvg− bacteria undergoing replication. TCT is likely responsible for the impairment of mucociliary clearance that occurs early in the course of infection.

Age and immune status play roles in the pathogenesis of disease with Bb as well. In the nonimmune pig, younger animals typically develop more severe disease both in regards to bronchopneumonia and atrophic rhinitis. Pigs with passive or acquired immunity from vaccination or natural infection develop less severe disease, although they may still be colonized. Coinfection with Bb and other pathogens in the respiratory tract also affects disease severity. The ability of Bb to predispose to colonization of the upper respiratory tract with P. multocida leading to PAR has been well established (de Jong and Nielsen 1990; Pedersen and Barfod 1981; Rutter 1983). However, Bb has also been shown to predispose to disease with Streptococcus suis (Vecht et al. 1989, 1992). Preinoculation of pigs with Bb prior to S. suis resulted in increased clinical signs and fever, increased isolation of S. suis, increased pneumonia and disseminated lesions due to S. suis, and increased mortality (Vecht et al. 1989, 1992). Bb has been shown to enhance colonization of the nasal cavity with Haemophilus parasuis as well (Brockmeier 2004).

The means by which Bb exacerbates or predisposes to secondary bacterial infections is not known but damage inflicted to innate protective mechanisms during colonization likely plays a major role. TCT-induced destruction of the epithelial lining of the upper respiratory tract significantly impairs the function of the mucociliary clearance escalator, leaving the host susceptible to both upper and lower respiratory tract infection upon subsequent exposure to other primary or opportunistic pathogens. Bb is cytotoxic for swine alveolar macrophages (Brockmeier and Register 2000; Forde et al. 1999), which may result in decreased phagocytosis and clearance of bacteria able to gain access to the lung. Alteration or ablation of the normal flora in the nasal cavity resulting from damage to the turbinates by DNT may also leave the host vulnerable to infection by other agents. DNT additionally causes pneumatic lesions characterized by necrosis, hemorrhage, neutrophil accumulation, and eventually fibrosis (Brockmeier et al. 2002). Increased accumulation of mucus, exposure of submucosal areas to which other bacteria may adhere, and increased nutrient availability could all play a role in nonspecifically predisposing to colonization by other bacteria. However, a DNT mutant of Bb was still capable of predisposing to infection with toxigenic P. multocida and the subsequent development of atrophic rhinitis appeared unaffected (Brockmeier et al. 2002). Consequently other factors must also contribute to secondary infection and disease associated with that agent. One plausible specific interaction is suggested by the composition of the capsule produced by type D strains of P. multocida, which are those most frequently associated with PAR. The type D capsular polysaccharide is largely composed of a heparin-like substance (Rimler 1994) that could potentially interact with a heparin-binding domain of the secreted Bb adhesin FHA (Menozzi et al. 1994). Thus P. multocida may appropriate FHA synthesized and secreted by Bb, using it as a bridge to mediate binding to cells of the respiratory tract. B. pertussis, a close relative of Bb, has also been shown to enhance adhesion of secondary pathogens. Streptococcus pneumoniae and Haemophilus influenzae acquired the ability to adhere to cilia which were pretreated with FHA (Tuomanen 1986).

Additionally, the pathogenicity of both Bb and swine viruses commonly infecting the respiratory tract can be influenced by coinfection. PRRSV predisposes to bronchopneumonia with Bb (Brockmeier et al. 2000). Although PRRSV alone has not been shown to enhance infection with P. multocida, infection with both Bb and PRRSV leads to increased pulmonary infection with P. multocida (Brockmeier et al. 2001). By facilitating colonization with other prevalent bacterial pathogens, Bb,
in combination with PRRSV and its immunosuppressive properties, may leave pigs more susceptible to pulmonary or systemic infection with opportunistic bacteria. Coinfection with Bb and swine influenza virus (SIV) or porcine respiratory coronavirus (PRCV) leads to increased severity of pneumonia with an earlier onset and longer resolution (Brockmeier et al. 2008; Loving et al. 2010). Coinfected pigs show a greater and more sustained production of proinflammatory cytokines that may contribute to enhancement of pulmonary lesions (Brockmeier et al. 2008; Loving et al. 2010).

**CLINICAL SIGNS**

Clinical signs vary greatly among pigs infected with Bb depending on age, immune status, and coinfection with other pathogens. In uncomplicated disease, clinical signs typically appear around 2–3 days after infection and are associated with rhinitis and bronchitis, including sneezing, nasal discharge, ocular discharge, and a dry, repeated cough. More severe signs can occur in neonatal pigs when bronchopneumonia develops, with associated dyspnea and lethargy occurring as well. Clinical signs may abate after several weeks but the respiratory tract remains colonized for months.

The clinical presentation may expand to include PAR, bronchopneumonia, or systemic disease when coinfection with *P. multocida*, *H. parasuis*, and *S. suis* or other bacterial or viral pathogens occurs (Brockmeier 2004; Brockmeier et al. 2001; de Jong and Nielsen 1990; Pedersen and Barfod 1981; Rutter 1983; Vecht et al. 1989, 1992). Coinfection with toxigenic strains of *P. multocida* can lead to PAR characterized by more severe upper respiratory signs, including epistaxis and either brachygnathia or deformation of the snout laterally (Pedersen and Barfod 1981; Rutter 1983). Pneumonia caused by Bb can also occur secondarily to infection with common viral pathogens such as PRCV, SIV, or PRRSV (Brockmeier et al. 2008, 2000; Loving et al. 2010). Pigs with passively acquired maternal or adaptive immunity may become colonized in the absence of clinically apparent disease, but these pigs are more vulnerable to secondary infections with other bacterial pathogens. Bb is highly infectious and transmits rapidly and easily via direct contact or aerosol transmission, resulting in high morbidity but generally low mortality except in very young pigs or complicated coinfections (Brockmeier and Lager 2002).

**LESIONS**

Bb may colonize and cause injury throughout the entirety of the respiratory tract, but lesions are especially associated with the nasal cavity, consisting of atrophic rhinitis, and the lung, consisting of suppurative bronchopneumonia (Duncan et al. 1966a,b). Macroscopic lesions of the nasal cavity include nasal exudate and mild-to-moderate turbinate atrophy, which are often referred to as NPAR (Figures 49.1 and 49.2). Distortions of the nasal septum, curvature of the snout, and/or brachygnathia are usually not seen with uncomplicated Bb infection, but may occur in mixed infections with toxigenic strains of *P. multocida* that
develops and may take months to completely resolve. Coinfection with other bacteria and/or viruses may change the character of the lesions, but suppurative bronchopneumonia is nearly always present.

**DIAGNOSIS**

Many pathogens other than Bb often cause pneumonia in pigs. When acting as a primary pathogen, Bb alone may be isolated from pneumonic lungs. However, this organism most commonly occurs in mixed infections and is frequently found together with one or more other respiratory disease agents. In such cases, the actual contribution of Bb to clinical disease may be difficult to determine. Lung lesions, especially acutely, can appear very similar to those caused by SIV, PRCV, *P. multocida*, *Actinobacillus suis*, *Actinobacillus pleuropneumoniae*, *Mycoplasma hyopneumoniae*, *Salmonella choleraesuis*, *S. suis*, and *H. parasuis*. When investigating the cause of rhinitis in young pigs, porcine cytomegalovirus and *P. multocida* should be considered as a differential diagnosis. Various grading systems for the quantitative assessment of turbinate atrophy have been used to evaluate treatment of PAR. These systems are based on transversely sectioning the snout at the level of the first or second premolar tooth and assigning a score estimating the amount of atrophy of each of the four scrolls of the ventral turbinates.

Detection of infection with Bb is typically based on isolation and biochemical testing of suspect colonies cultured from nasal swabs or postmortem lung washings or biopsy specimens. Mini-tipped swabs are available that facilitate nasal sampling in young pigs. Swabs should be placed in a non-nutritive transport medium, such as phosphate buffered saline (PBS), and kept at 39°F (4°C) for delivery to a diagnostic laboratory, preferably within 24 hours.

Bb grows readily on blood agar; however, use of a selective medium is desirable to prevent overgrowth by other faster-growing and typically more numerous commensal and pathogenic bacteria that may be present. Many laboratories use a modified MacConkey agar with 1% glucose and 20 µg/mL furaltadone (Farriington and Switzer 1977), but a peptone agar formulation (Smith and Baskerville 1979) may be superior when the number of Bb is low. In a direct comparison, neither medium performed as well as blood agar with cephalaxin for isolation of Bb from nasal swabs (Lariviere et al. 1993). Blood agar plates containing 20 µg/mL penicillin, 10 µg/mL amphotericin B, 10 µg/mL streptomycin, and 10 µg/mL spectinomycin also perform well for isolation from highly contaminated sites such as the nasal cavity (Brockmeier 1999). As noted above, a blood-containing medium should be utilized for subsequent passage of primary isolates so that it is possible to evaluate and maintain the original Bvg phenotype. Although the required reagents are widely available,

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**49.3. Macroscopic lung lesions in a pig infected with B. bronchiseptica showing fibrotic lesions in the right cranial lobe (arrows) 4 weeks after infection.**
conventional identification methods are time consuming and suffer from poor sensitivity. A DNA probe hybridization assay using colony lifts of primary isolation plates that is highly specific and more sensitive than traditional isolation and biochemical testing has been described (Register et al. 1995). More recently a PCR target originally evaluated for use with human specimens (Hozbor et al. 1999) was found to be 100% sensitive and specific for Bb when tested with bacterial genera and species commonly found in the swine respiratory tract (Register and DeJong 2006).

Early methods employed for the serological diagnosis of Bb infection were based on the detection of agglutinating serum antibody in assays using either tube (Jenkins 1978) or plate (Shashidhar et al. 1983) formats. Enzyme-linked immunosorbent assays (ELISAs) for detection of antibody in serum (Kono et al. 1994; Venier et al. 1984) or nasal secretions (Kono et al. 1994) were subsequently described. Because these assays depend solely on antibody binding and provide objective end points, they provide greater sensitivity and reproducibility than agglutination-based methods. Serology may be useful for monitoring the status of a herd but is rarely used routinely for diagnostic purposes.

**IMMUNITY**

Studies characterizing the mechanisms of protection against Bordetellosis have been carried out primarily in mice and provide useful information for understanding immunity to infection/vaccination. Studies have shown that immunoglobulin A (IgA) is important for clearance of organisms from the upper respiratory tract, whereas immunoglobulin G (IgG) is critical for clearance from the lungs; thus, vaccines that elicit strong IgA responses are critical for complete respiratory tract clearance (Kirimanjeswara et al. 2003; Wolfe et al. 2007). Infection-induced immunity, as opposed to vaccine-induced immunity, provides significant protection in the upper and lower respiratory tract. Although antibody titers are higher following vaccination, as opposed to infection, the antibody generated following vaccination is less effective at providing protection (Gopinathan et al. 2007). Together, this information suggests that immune responses elicited at the site of natural infection are likely to be the most efficacious and live, attenuated vaccines delivered intranasally are an obvious approach.

Vaccines that elicit a response to pertactin, a 68kDa protein on the bacterial surface, have been shown to be highly protective in reducing disease severity (Kobisch and Novotny 1990; Li et al. 1992). Vaccines that induce large amounts of antibody to lipopolysaccharide appear to provide the least protection from disease (Novotny et al. 1985). Pertactin gene heterogeneity has been described (Register 2004) and is an important consideration for creating a vaccine that is efficacious against field strains. Although pertactin appears to be a primary protective antigen, reactivity to additional outer membrane proteins is important for complete protection evidenced by superior protection provided by whole cell vaccines (Kobisch and Novotny 1990; Novotny et al. 1985). Current commercial vaccines typically contain Bb bacterin as well as a P. multocida toxoid for optimal protection against PAR. Vaccination does not provide sterilizing immunity but does significantly limit or even abolish clinical disease.

Piglets typically become naturally infected with Bb within the first few weeks of life, thus sow vaccination is useful for preventing piglet infection. Although sow vaccination can decrease disease severity and increase piglet performance, it does not eliminate colonization of pathogenic organisms from piglets (Rising et al. 2002). In addition, passive antibody from the sow to piglet can interfere with parenteral vaccination of the piglet (Smith et al. 1982). Vaccination of nonimmune piglets can provide protection from turbinate atrophy as piglets vaccinated with bacterin at 1 and 4 weeks of age develop circulating antibody lasting beyond 12 weeks of age (Farrington and Switzer 1979). However, until the piglet mounts a response to the vaccine, it remains susceptible to natural infection.

**PREVENTION AND CONTROL**

Bb is largely susceptible to chlortetracycline, oxytetracycline, and enrofloxacin; all are antibiotics approved for the treatment of swine respiratory disease caused by pathogens that are often associated with Bb, such as P. multocida, H. parasuis, and S. suis (Kadlec et al. 2004). Tulathromycin specifically lists swine respiratory disease caused by Bb as an indicated use. *Bordetella* are largely resistant to ceftiofur, which is approved for treatment of swine respiratory disease of mixed bacterial etiology. Accordingly, ceftiofur is not the optimal choice when treating mixed infections that include Bb as a component (Kadlec et al. 2004). Antibiotics may alleviate pneumonia and reduce clinical signs but total clearance of *Bordetella* from the upper respiratory tract is difficult. Normally, the need for treatment of atrophic rhinitis arises when there are signs of PAR in the herd, which almost always involves coinfecition with toxigenic strains of *P. multocida*. Use of antibiotics to control cases of atrophic rhinitis includes administration by feeding or parenteral route to sows and piglets around the time of farrowing/weaning in an effort to limit the extent of colonization in young pigs. Trimethoprim-sulfonamide preparations have historically been successfully used for this purpose, but there is some evidence of resistance to this combination of antibiotics (Rutter 1981). Oxytetracycline has also been used successfully in this manner to reduce the incidence of PAR in herds (de Jong and Oosterwoud 1977).
Treatment of older pigs probably has limited efficacy on the disease progression of atrophic rhinitis. Vaccines for Bb largely consist of bacterins often in combination with \textit{P. multocida} bacterins or toxoids, but there are a few attenuated intranasal vaccines available as well. When using vaccination to prevent PAR, the \textit{P. multocida} toxin (PMT) is an important component of the vaccine, and those with added PMT toxoid offer superior protection (Foged et al. 1989; Hsuan et al. 2009; Nielsen et al. 1991; To et al. 2005; Voets et al. 1992). Vaccination of sows around 6 weeks and again around 2 weeks prior to farrowing appears to work well to protect piglets and minimize transmission (Rissing et al. 2002). Protection of piglets, whether through antibiotic treatment, passive antibody through sow vaccination, or a combination of both, during the first few weeks of life appears to be an important factor in the control of atrophic rhinitis (Pejsak et al. 1994). Vaccination of piglets around 2 and 4 weeks of age has had mixed results; when less favorable results occur it is probably due to the occurrence of maternal antibody interference and/or prior colonization of young piglets before an immune response has developed (Farrington and Switzer 1979; Smith et al. 1982). Attenuated vaccines are usually given intranasally within a few days of birth in an attempt to prevent colonization with virulent strains of Bb by competitive exclusion and to induce a mucosal immune response. Results with these vaccines have been mixed, possibly because of maternal antibody interference or due to the nature of the attenuated strain (de Jong 1987; Pejsak et al. 1994). Bb attenuated via mutation of the \textit{bvg} locus is avirulent but poorly colonizes the respiratory tract and would not be expected to elicit a robust immune response. Strains defective only in DNT production have been engineered and are greatly attenuated, but are not desirable for vaccine use since, like wild type, they predispose to colonization with \textit{P. multocida} (Brockmeier and Register 2007). Most vaccine studies have examined efficacy in terms of prevention of atrophic rhinitis (Pejsak et al. 1994). Vaccination of piglets around 2 and 4 weeks of age has had mixed results; when less favorable results occur it is probably due to the occurrence of maternal antibody interference or due to the nature of the attenuated strain (de Jong 1987; Pejsak et al. 1994). Bb attenuated via mutation of the \textit{bvg} locus is avirulent but poorly colonizes the respiratory tract and would not be expected to elicit a robust immune response. Strains defective only in DNT production have been engineered and are greatly attenuated, but are not desirable for vaccine use since, like wild type, they predispose to colonization with \textit{P. multocida} (Brockmeier and Register 2007). Most vaccine studies have examined efficacy in terms of prevention of atrophic rhinitis, but since Bb predisposes to infection with other respiratory pathogens, vaccination or elimination from a herd may have broader effects on respiratory health. Although vaccines may prevent clinical outbreaks and decrease the \textit{Bordetella} burden, they are highly unlikely to prevent colonization.

Bb is a ubiquitous pathogen that is highly infectious. By itself it often causes mild clinical signs that go unreported, resulting in subclinical chronic carriers that make it difficult to prevent introduction to or elimination from a herd. Its contribution to respiratory disease is often overlooked because more severe clinical signs are caused by the secondary bacteria that colonize or invade. Measures to control or eliminate PAR from a herd include combinations of improved husbandry, such as improved air quality/ventilation and pig flow (all-in/all-out management with proper cleaning and disinfection between groups), mediated early weaning, and vaccination of the breeding herd and/or pigs. Intensive use of these methods has been shown to reduce the bacterial burden of both Bb and \textit{P. multocida} and, in combination with nasal swab testing and elimination of positive animals, has even been able to eliminate toxigenic \textit{P. multocida} from herds. Whether or not Bb can be totally eliminated by these methods remains unclear.

**REFERENCES**


OVERVIEW OF BRACHYSPIRA SPECIES

The genus *Brachyspira* contains seven officially named and several unofficially proposed species of spirochetes, some of which were previously assigned to the genus *Serpulina*. Properties of members of the genus have been reviewed (Stanton 2006). These gram-negative anaerobic bacteria are genetically distinct from other spirochetes, and have adapted to occupy specialized niches in the large intestines of various birds and animals, including swine. Six *Brachyspira* species colonize swine: the two main pathogenic ones are *Brachyspira hyodysenteriae*, the agent of swine dysentery (SD), and *Brachyspira pilosicoli*, the cause of porcine intestinal (or colonic) spirochetosis (PIS/PCS). Of the others, the proposed species “*Brachyspira suanatina*” and some strains of *Brachyspira murdochii* and *Brachyspira intermedia* also occasionally may cause colitis in swine, while *Brachyspira innocens* is usually regarded as being a nonpathogenic commensal. Relationships between the officially named species based on their 16S rRNA gene sequences are shown in Figure 50.1.

All the *Brachyspira* species resemble short slender snakes with several loose coils (Figure 50.2). Those that colonize swine vary from 5 to 11μm in length and from 0.2 to 0.4μm in width. Like other spirochetes, the *Brachyspira* species have two sets of periplasmic flagella, with each set anchored at opposite ends of the spirochete cell. Each set winds around the cell body beneath the outer envelope, and the free ends overlap the other set halfway along the cell. These flagella confer a pronounced corkscrew-like motility on the spirochetes, and this helps them to penetrate and move through the viscous digesta and mucus overlying the colonic epithelium.

The DNA of *Brachyspira* species has a low guanine and cytosine ratio, in the range 24.6–28%. Genome sizes for the species vary from ∼2.5 to 3.2 million base pairs (Mbp), each with >2300 protein coding sequences. Most of the species share close similarities in their 16S rRNA gene sequences, implying that species separation occurred relatively recently. The genome sequences of *B. hyodysenteriae* strain WA1, *B. pilosicoli* strain 95/1000, and *B. murdochii* strain 56–1501 have been published (Bellgard et al. 2009; Pati et al. 2010; Wanchanthuek et al. 2010). *Brachyspira hyodysenteriae* contains a prophage-like gene transfer agent (GTA) that randomly packages ∼7.5kB fragments of host DNA and transfers it to other *B. hyodysenteriae* strains (Humphrey et al. 1997; Matson et al. 2005). Other *Brachyspira* species harbor similar GTA sequences (Motro et al. 2009; Stanton et al. 2003), although it is not known whether they are functional. The GTAs could contribute to the extensive gene rearrangements that occur within and between *Brachyspira* species (Zuerner et al. 2004).

The *Brachyspira* species are anaerobic, but can tolerate brief exposure to oxygen. All species grow slowly, and can easily be overgrown by other anaerobic members of the enteric microbiota unless the isolation medium is made selective by incorporating appropriate antimicrobials. Typically the species form a low flat film of growth after 3–5 days at 98.6–108°F (37–42°C), without forming colonies. Growth on plates containing 5% defibrinated ovine or bovine blood is surrounded by a zone of beta-hemolysis: this is strong for *B. hyodysenteriae* and “*B. suanatina*” and weak for all the other *Brachyspira* species (Figure 50.3).

Descriptions of the metabolic activities of *B. hyodysenteriae* and other intestinal spirochetes have been published (Stanton 2006). Metabolic pathways in the
three sequenced species have been reconstructed (Bellgard et al. 2009; Wanchanthuek et al. 2010), and only minor differences appear to exist between them.

Useful features that can help distinguish between the species include their strength of beta-hemolysis, their ability to produce indole, and their enzymic profile in the commercial API-ZYM kit (bioMérieux, Marcy l’Etoile, France; Fellström et al. 1997). Some comparative features of the porcine spirochetes are presented in Table 50.1. None can be completely relied upon for identification as strains with unusual phenotypes are occasionally found (Thomson et al. 2001).

**BRACHYSPIRA HYODYSENTERIAE: SWINE DYSENTERY**

**Relevance**

SD was recognized in the 1920s, but the spirochetal etiology was only demonstrated in the early 1970s (Glock and Harris 1972; Taylor and Alexander 1971). The causal agent originally was named *Treponema hyodysentia* (Harris et al. 1972), but this species and the weakly beta-hemolytic *Treponema innocens* (Kinyon and Harris 1979) were later transferred to the genus *Serpula*, then to *Serpulina* (Stanton 1992), and are now classified in the genus *Brachyspira* (Ochiai et al. 1997)—together with the five other official species.

In infected herds SD causes considerable financial loss due to mortality, slow growth, poor feed conversion, and cost of treatment. Costs also arise from the need to implement preventative measures in herds that do not have SD, and from the disruption to the supply and movement of pigs when the disease becomes introduced into breeding company herds.

**Etiology**

*Brachyspira hyodysentia* is a discrete species within the genus *Brachyspira*. Analysis of the population structure using multilocus enzyme electrophoresis (MLEE) has shown that the species is diverse and contains numerous genetically distinct strains (Lee et al. 1993a). Subsequent analysis of MLEE data was used to deduce that the species is recombinant, but has an epidemic population structure with widespread epidemic clones (Trott et al. 1997b). Recent studies using multilocus sequence typing (MLST) (La et al. 2009b; Råsbäck et al. 2007b) and multiple-locus variable-number tandem-repeat analysis (MLVA) (Hidalgo et al. 2010a) have confirmed this diversity; however, based on the index of association values, *B. hyodysentia* appears to be a nonrecombinant clonal species. The existence of numerous distinct strains also can be demonstrated using techniques such as pulsed-field gel
Table 50.1. Differentiation of six Brachyspira species that infect swine by their hemolys pattern on Trypticase Soy blood agar, biochemical reactions, and utilization of sugars

<table>
<thead>
<tr>
<th>Species</th>
<th>Hemolysis</th>
<th>Indole</th>
<th>Hippurate</th>
<th>API-ZYM&lt;sup&gt;4&lt;/sup&gt;</th>
<th>D-ribose</th>
</tr>
</thead>
<tbody>
<tr>
<td>B. hyodysenteriae</td>
<td>Strong</td>
<td>+&lt;sup&gt;a&lt;/sup&gt;</td>
<td>–</td>
<td>1</td>
<td>–</td>
</tr>
<tr>
<td>B. intermedia</td>
<td>Weak</td>
<td>+</td>
<td>–</td>
<td>1</td>
<td>–</td>
</tr>
<tr>
<td>B. innocens</td>
<td>Weak</td>
<td>–</td>
<td>–</td>
<td>2</td>
<td>–</td>
</tr>
<tr>
<td>B. murectii</td>
<td>Weak</td>
<td>–</td>
<td>–</td>
<td>3</td>
<td>–</td>
</tr>
<tr>
<td>B. pilosicoli</td>
<td>Weak</td>
<td>+&lt;sup&gt;a&lt;/sup&gt;</td>
<td>+&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4</td>
<td>+</td>
</tr>
<tr>
<td>“B. suanatina”</td>
<td>Strong</td>
<td>+&lt;sup&gt;b&lt;/sup&gt;</td>
<td>–</td>
<td>5</td>
<td>–</td>
</tr>
</tbody>
</table>

<sup>a</sup>Indole-negative strains of B. hyodysenteriae and indole-positive strains of B. pilosicoli have been recorded.

<sup>b</sup>Weakly positive indole

<sup>c</sup>Hippurate-negative strains of B. pilosicoli have been recorded.

<sup>d</sup>Reactions in the commercial API-ZYM test:
1. alpha-glucosidase positive, alpha-galactosidase negative.
2. alpha-glucosidase positive or negative, alpha-galactosidase positive.
3. alpha-glucosidase negative, alpha-galactosidase negative.
4. variable reactions, including positive reactions for both enzymes, beta-glucosidase negative.
5. alpha-galactosidase negative, beta-glucosidase positive.

+, positive reaction; –, negative reaction.

electrophoresis (PFGE) (Atyeo et al. 1999a) and/or random amplified polymorphic DNA analysis (Hidalgo et al. 2010b). Isolates also can be differentiated into serogroups and serovars, which are defined on the basis of lipooligosaccharides (LOS) extracted from the cell envelope (Hampson et al. 1997).

Molecular analysis of isolates has shown that new variants of B. hyodysenteriae may emerge on farms (Atyeo et al. 1999a; Hidalgo et al. 2010b). Other than random mutational and recombination events, GTAs may contribute to this “microevolution” of strains through transduction of new sequences from other Brachyspira species or strains. Newly emerged strains could have altered phenotypic properties, potentially including altered antimicrobial susceptibility, colonization potential, or virulence. Drifts in antigenicity of surface LOS among isolates recovered from the same farm over a number of years have been recorded (Combs et al. 1992).

Brachyspira hyodysenteriae is 6–8.5 µm long, 0.32–0.38 µm wide and has 7–14 periplasmic flagella inserted at each cell end. The cell is covered by a loose outer membrane. Brachyspira hyodysenteriae strain WA1 has a circular chromosome of ~3.0 Mbp and a ~36 kilobase pairs (kbp) circular plasmid (Bellgard et al. 2009). Many B. hyodysenteriae proteins associated with transport and metabolism have greater similarity to those in Escherichia coli and Clostridium species than to those of other spirochetes, suggesting that the genes encoding them gradually have been acquired from other enteric species by horizontal gene transfer.

A number of outer membrane proteins and lipoproteins of B. hyodysenteriae have been described and given various names; however, they are best described according to their molecular size (Hampson et al. 2006). For example, a 29.7 KDa lipoprotein has been called BmpB or BlpA, but is now named Bhlp29.7. The gene sequence encoding Bhlp29.7 is part of a locus encoding four tandem paralogous genes for lipoproteins of approximately 30 kDa (Cullen et al. 2003). A 39 kDa variable surface protein (Bhp39) encoded by linked gene copies, which is possibly involved in immune avoidance due to differential gene expression, has also been described (McCaman et al. 2003; Witchell et al. 2006).

The B. hyodysenteriae outer envelope contains LOS, a semirough form of lipopolysaccharide (Halter and Joens 1988). The rfbBADC genes that encode enzymes involved in rhamnose biosynthesis, and that are predicted to be associated with O-antigen assimilation in LOS, are located on the plasmid in strain WA1 (Bellgard et al. 2009). Lipopolysaccharide from B. hyodysenteriae has some of the same biological properties as LOS from other gram-negative bacteria, and the toxic effects may act locally to disrupt the colonic epithelial barrier (Greer and Wannemuehler 1989; Nibbelink et al. 1997; Nuessen et al. 1983).

Brachyspira hyodysenteriae has many genes involved in motility and chemotaxis (Bellgard et al. 2009), and shows both chemotaxis and viscostaxis toward mucin (Milner and Sellwood 1994; Naresh and Hampson 2010). This vital activity allows B. hyodysenteriae to associate with the gut mucosa (Kennedy et al. 1988). The flaB genes that encode flagella have resulted in both reduced motility and colonization ability (Kennedy et al. 1997; Rosey et al. 1996).

The NADH oxidase activity of B. hyodysenteriae may enhance its ability to colonize the colonic mucosa by protecting it from oxygen toxicity. Consistent with this, strains with an inactivated NADH oxidase (nox) gene show a reduced ability to colonize swine and cause disease (Stanton et al. 1999).
The hemolytic activity of \( B. \) \textit{hyodysenteriae} is probably an essential virulence factor. Three genes (\( tlyA \), \( tlyB \), and \( tlyC \)) encoding putative hemolysins of \( B. \) \textit{hyodysenteriae} were originally described based on their ability to induce a hemolytic phenotype in \( E. \) \textit{coli} (ter Huurne et al. 1994). Another gene (\( hlyA \)) has been characterized that encodes an 8.93KDa acyl carrier protein with hemolytic activity (Hsu et al. 2001). The \( tly \) genes may be regulatory elements rather than encoding hemolysins; nevertheless, inactivation of \( tlyA \) has been shown to reduce both the hemolytic activity and the virulence of \( B. \) \textit{hyodysenteriae} (Hyatt et al. 1994). Strongly hemolytic avirulent isolates of \( B. \) \textit{hyodysenteriae} have been described (Lysons et al. 1982; Thomson et al. 2001), and a study of these may help to define other \( B. \) \textit{hyodysenteriae} virulence factors.

\textit{Brachyspira hyodysenteriae} grows in an anaerobic environment at 98.6–108°F (37–42°C) on Trypticase Soy agar and similar agar containing 5–10% defibrinated blood. After 3–5 days there is a low flat haze of growth surrounded by a zone of strong beta-hemolysis, which is enhanced by cutting the agar during inoculation (Olson 1996). Growth of \( 10^8 \)–\( 10^9 \) cells per milliliter of \( B. \) \textit{hyodysenteriae} can be obtained within 2–3 days in prereduced anaerobic Trypticase Soy broth medium (Kunkle et al. 1986), and in brain heart infusion broth containing 10% (v/v) fetal bovine serum (Stanton and Lebo 1988). Adding 1% \( O_2 \) to the atmosphere enhances growth.

**Public Health**

\textit{Brachyspira hyodysenteriae} does not infect human beings.

**Epidemiology**

SD has a worldwide distribution. The incidence varies in different countries and regions, and changes with time. SD remains a relatively common and important endemic problem in many countries in the European Union, South America and Southeast Asia. In the last 20 years a decline in SD incidence has occurred in the United States following establishment of new high health status herds in nontraditional swine-rearing states, and with introduction of larger units, multisite production, and early weaning systems. Routine medication with carbadox may have also suppressed the disease, as the incidence has increased again in states where carbadox has been withdrawn from use.

\textit{Brachyspira hyodysenteriae} naturally infects pigs (including feral pigs) and occasionally some species of birds (rheas, chickens, ducks, and geese). On infected farms it has been isolated from mice, rats, dogs, and feral birds, including seagulls.

On endemically infected swine farms transmission mainly occurs by ingestion of infected feces. This is especially likely in single-site, farrow-to-finish herds with continuous flow and poor farm biosecurity. \textit{Brachyspira hyodysenteriae} may be spread in feces by animal caretakers who do not change their clothing or footwear. Transmission between pens may occur in housing systems where there are open channels between pens. Lagoon water containing effluent can be a source of infection (Glock et al. 1975), and should not be recycled. Feral and other animals on farms are potential reservoirs that may transmit infection.

New outbreaks of SD usually occur following introduction of asymptomatic carrier pigs that are not quarantined and/or treated prophylactically. Outbreaks also occur in herds following introduction by contaminated feed or animal trucks, or by visitors who have had contact with infected pigs. When investigating risk factors for SD, Robertson et al. (1992) found that allowing visitors onto farms and the presence of rodents were both associated with disease. On the other hand, provision of boots and protective clothing for visitors, the presence of security fencing, the use of home-mixed feed, and obtaining replacement breeders from the same source were protective.

\textit{Brachyspira hyodysenteriae} is shed in feces for variable periods. Transmission has occurred following exposure of susceptible pigs to previously infected animals that had shown no clinical signs for 70 days (Sonner and Harris 1978).

\textit{Brachyspira hyodysenteriae} is relatively resistant in moist feces. It survives in feces diluted in water for 48 days from 32 to 50°F (0–10°C), for 7 days at 77°F (25°C), and less than 24 hours at 98.6°F (37°C) (Chia and Taylor 1978). In another study, it survived for 10 days in soil at 50°F (10°C), for 78 days in soil in the presence of 10% pig feces, and for 112 days in pure pig feces (Boye et al. 2001). Drying of dysenteric feces rapidly eliminates \( B. \) \textit{hyodysenteriae} (Chia and Taylor 1978). Phenolics and sodium hypochlorite are the most effective disinfectants.

**Pathogenesis**

Following ingestion in feces, \( B. \) \textit{hyodysenteriae} survives the acidic environment of the stomach and eventually reaches the large intestine. Experimentally, an inoculum of \( 10^5 \) colony-forming units (CFU) may produce SD (Kinyon et al. 1977), although it may be necessary to use higher doses (e.g., \( 10^{10} \)CFU). As previously indicated, spirochetal proliferation and mucosal colonization require specialized features, including the ability to utilize available substrate, to penetrate and move through viscous mucus down a chemotactic gradient into the crypts, and to avoid potential oxygen toxicity at the surface of the colonic mucosa. Clinical signs and lesions start to develop as cell numbers reach \( 10^6/cm^2 \) of mucosa (Hughes et al. 1977; Whipp et al. 1979). Spirochetes appear in the feces 1–4 days before diarrhea starts (Kinyon et al. 1977), concurrent with a shift in colonic bacteria from predominantly gram-positive to mainly gram-negative species (Robinson et al. 1984).
Brachyspira hyodysenteriae strains vary in their virulence (Achacha et al. 1996), but the basis of this is poorly understood. The presence of spirochetes close to epithelial cells in the lumen and crypts of the cecum and colon stimulates an outpouring of mucus (Wilcock and Olander 1979a,b). They attach to epithelial cells in the crypt, but the significance of this is unclear since attachment to animal cell cultures does not cause cellular damage or invasion (Bowden et al. 1989; Knoop et al. 1979).

The mechanisms of tissue destruction in SD have not been fully elucidated. The hemolysin(s) and LOS play a role by acting locally to disrupt the epithelial barrier in the colon, resulting in epithelial sloughing. Subsequent submucosal invasion by secondary bacteria and the protozoan Balantidium coli may contribute to lesion formation.

Diarrhea appears to result from colonic malabsorption due to a failure of epithelial transport mechanisms to actively transport sodium and chloride ions from lumen to blood, and not from the activity of enterotoxins and/or prostaglandins released from the inflamed mucosa (Argenzio et al. 1980; Argenzio 1981; Schmall et al. 1983). Sterile filtrates of *B. hyodysenteriae* broths do not cause fluid accumulation in ligated colonic loops in pigs or in sucking mice and sterile filtrates do not produce changes in Y-1 adrenal cells (Whipp et al. 1978). Inactivated whole cells and sonicates of *B. hyodysenteriae* also do not cause lesions or fluid accumulation in ligated colonic loops. Occasional peracute deaths may arise from endotoxin release.

Diet has a major influence on SD expression. Colonization by *B. hyodysenteriae* can be inhibited by feeding diets that are either highly digestible (Pluske et al. 1996) or rich in inulin (Hansen et al. 2010; Thomsen et al. 2007). The protective mechanism may involve changes in the colonic microbiota, with increases in species that inhibit the spirochete (Klose et al. 2010; Leser et al. 2000; Mølbak et al. 2007). On the other hand, some other anaerobic bacteria that form part of the microbiota can facilitate *B. hyodysenteriae* colonization and augment inflammation and lesions (Joens et al. 1981; Whipp et al. 1979).

**Clinical Signs**

SD occurs mainly in grower and finisher pigs, and less frequently in weaners. It is often seen a few weeks after animals are moved from the nursery, coinciding with a dietary change and removal of antimicrobials used to control respiratory and enteric diseases. Older piglets from gilts that have not been exposed to *B. hyodysenteriae* and piglets in newly infected herds are occasionally affected.

The first evidence of SD is usually soft, yellow to gray feces. Partial anorexia and increased rectal temperature of 104–105°F (40–40.5°C) may occur. A few hours to days after infection, large amounts of mucus and often flecks of blood are found in the feces. This progresses to watery stools containing blood, mucus, and shreds of white mucofibrinous exudate, with concurrent staining of the perineum. Most pigs recover over several weeks, but their growth rate remains depressed. Prolonged diarrhea leads to dehydration and the animals become weak and emaciated.

The incubation period for SD is variable, ranging from 2 days to 3 months, but disease usually occurs within 10–14 days in naturally exposed pigs. Disease usually spreads gradually, with new animals becoming affected daily. The course varies between individual animals within and between herds. Occasionally pigs are peracutely affected and die within a few hours.

In outbreaks of SD, morbidity in weaner pigs may approach 90% and mortality may be 30% if effective treatment is delayed. Experimentally, mortality in untreated pigs may reach 50%. In chronically affected herds, particularly if they are being medicated, the disease may not be clinically evident.

In experimentally induced SD the occurrence and severity of disease is dependent upon the amount of stress on the pig, the quantity of infectious inoculum, the growth phase of the culture (with active log-phase inoculum being most infectious), the diet, the group size, and the weight of the pig (Jacobson et al. 2004).

On endemically infected swine farms clinical signs often recur cyclically at 3- to 4-week intervals in individual pigs and in large groups. Reappearance may occur after removal of antimicrobials from the water or feed. Pigs may also develop diarrhea after moving to new pens, mixing with different animals, weighing, or a change in feed. Stresses such as overcrowding and exposure to extreme changes in temperatures may also precipitate disease. Where antibiotic medication is routine, any cause of loss of appetite (such as pneumonia) stops the intake of drug and the animal may then succumb to SD.

**Lesions**

Typical changes in acute SD include hyperemia and edema of the large intestinal walls and mesentery. Mesenteric lymph nodes may be swollen with small amounts of clear ascitic fluid present. There may be white, slightly raised foci on the serosa caused by submucosal aggregates of mononuclear cells. The mucosa is usually swollen, with loss of the typical rugose appearance, and is covered by mucus and fibrin, with flecks of blood. The colonic contents are soft to watery and contain exudate.

As the condition progresses the edema in the colon wall may decrease. Mucosal lesions become more severe, with increased fibrin exudation and formation of thick, mucofibrinous pseudomembranes containing blood. As lesions become chronic the mucosal surface usually becomes covered by a thin, dense, fibrinous exudate, resembling superficial necrosis. Lesions can be
found in clinically healthy pigs and appear as discrete reddened areas of the mucosa, usually covered with mucus, but with normal colonic contents.

The distribution of lesions within the large intestine varies. Sometimes the entire organ may be involved, while at other times only certain segments may be affected. Lesions tend to become more diffuse in the later stages of the disease. Hepatic congestion and hyperemia or congestion of the gastric fundus may occur; however, such lesions are not specific for SD.

Significant microscopic lesions are only found in the cecum, colon, and rectum. Typical acute lesions include a thickened mucosa and submucosa due to vascular congestion and extravasation of fluids and leukocytes. Goblet cell hyperplasia is present and the epithelial cells at the base of the crypts may be elongated and hyperchromic. There may be spirochetes in goblet cells in the colonic crypts and penetrating intercellular gaps in the epithelium. A loss of cohesion between colonic enterocytes occurs, with necrosis and shedding of the epithelium. Spirochetes may be found attached to the luminal surface and inside disrupted epithelial cells. There may be increased numbers of leukocytes in the lamina propria, with accumulation of neutrophils in and around capillaries near the lumen. Some spirochetes may be seen in the lamina propria, particularly around blood vessels. Bleeding may occur from small vessels under areas of eroded epithelium, and this may be invaded by the colonic microbiota.

Later changes include accumulation of fibrin, mucus, and cellular debris in mucosal crypts and on the luminal surface of the large intestine. Superficial necrosis of the mucosa may be extensive, but deep ulceration is not typical. Increased numbers of neutrophils may be seen throughout the lamina propria. Spirochetes are most numerous in the lumen and within crypts in the acute phase of the disease (Figure 50.4). Chronic changes are not very specific, with less hyperemia and edema being present. There is often more advanced superficial necrosis of the mucosa, which usually has a thick, fibrinous pseudomembrane.

Early ultrastructural changes include the presence of large numbers of spirochetes at the luminal surface and within crypts. Adjacent epithelial cells show destruction of microvilli, swelling of the mitochondria and endoplasmic reticulum, loss of other organelles, and decreased density. *Brachyspira hyodysenteriae* may also be seen inside epithelial cells, goblet cells, and in the lamina propria (Glock et al. 1974; Taylor and Blakemore 1971).

Hematological changes include an inconsistent increase in total leukocyte counts, usually with a marked left shift. Acute phase proteins may increase (Jonasson et al. 2006). There may be early transient increases in erythrocyte sedimentation rates and fibrinogen levels, and total plasma protein may be elevated with altered blood concentrations of some amino acids, but not glucose or lactate (Jonasson et al. 2007). Levels of sodium, chloride, and bicarbonate in the blood decrease. A marked metabolic acidosis and a terminal hyperkalemia may occur.

### Diagnosis

A number of enteric diseases may be confused with SD, and it often occurs concurrently with other enteric infections (Møller et al. 1998; Thomson et al. 1998). Proliferative enteropathy (PE) caused by *Lawsonia intracellularis* (Chapter 59) may clinically resemble SD, but SD does not affect the small intestine. Salmonellosis (Chapter 60) can have similar clinical signs and lesions; however, with salmonellosis there may be hemorrhage or necrosis in parenchymatous organs and lymph nodes, and mucosal lesions in the small intestine. Deep ulcerative enteric lesions are also much more typical of salmonellosis. Trichuriasis (Chapter 66) may be differentiated from SD by the presence of numerous *Trichuris suis* in the large intestine. Gastric ulcers and other hemorrhagic conditions may result in blood in the feces, but this tends to be “tarry” due to digestion of the blood. PIS/PCS represents the most difficult differential diagnosis as it can closely resemble mild cases of SD.

Pigs that have died from SD are often emaciated, dehydrated, and have a rough hair coat with fecal staining of the perineum. Characteristically there is diffuse mucohemorrhagic enteritis confined to the large intestine, the lesions of which have been described earlier. To help identify and localize the spirochete, nucleic acid probes can be used for fluorescent in situ hybridization (FISH) on fixed colonic tissue (Boye et al. 1998).

Samples for microbiology are best taken from several acutely affected animals, as they have large numbers...
(10⁸–10⁹/g) of *B. hyodysenteriae* in their colonic mucosa and feces. Asymptomatic pigs are not suitable as they may only periodically shed the organism at detectable levels (>10³ cells/mL contents). Medication may reduce the number of organisms to below detectable levels. Colonic contents are optimal samples, but feces can be used. If disease is mild or subclinical it may be necessary to examine large numbers of samples to detect a positive animal. Fellström et al. (2001) recommended pooling batches of five rectal swabs to increase detection rates.

Spirochetes can be seen in smears from the colonic mucosa or feces, but this does not distinguish between the different *Brachyspira* species. In the United Kingdom an indirect fluorescent antibody test has been used to detect *B. hyodysenteriae* in smears (Hunter and Saunders 1977), although false positive reactions can occur. Monoclonal antibodies (MAbs) should improve specificity (Lee and Hampson 1996), but when one of these was bound to magnetic beads to extract *B. hyodysenteriae* from feces, this did not increase the sensitivity of detection (Corona-Barrera et al. 2004b).

A definitive diagnosis of SD requires the demonstration of *B. hyodysenteriae* in the colonic mucosa or feces. Traditionally, this has been done by selective anaerobic culture and analysis of phenotypic properties of the isolated organisms. Optimal culture media and conditions are as described earlier, but the agar should be made selective with the addition of 400 μg/mL spectinomycin and 25 μg/mL each of colistin and vancomycin to make CVS (colistin, vancomycin, and spectinomycin) medium (Jenkinson and Wingar 1981). Alternatively, the more selective B medium with lower concentrations of the three antimicrobials, but with 25 μg/mL spiramycin and 12.5 μg/mL rifampin added, can be used (Kunkle and Kinyon 1988).

On blood agar, *B. hyodysenteriae* produces zones of strong beta-hemolysis around a film of growth in which colonies are hard to distinguish. Plates without hemolysis should be reincubated and regularly checked for up to 10 days. False negative results can occur due to inappropriate handling or storage of samples, such as exposure to extreme temperatures, drying, or delays during transport.

Mixed spirochete species can be present on a plate, and individual isolates must be cloned to purity before phenotypic characterization (Table 50.1). Antigen-based methods, including a fluorescent antibody test, a growth-inhibition test, and a rapid slide agglutination test have been described for identification of *B. hyodysenteriae*, but these have largely been superseded by polymerase chain reaction (PCR) testing.

PCR amplification of specific sequences is widely used for detection and identification of *B. hyodysenteriae*. The most usual targets are portions of the 23S rRNA gene (Leser et al. 1997), the nox gene (Atyeo et al. 1999b) and the tlyA gene (Fellström et al. 2001). PCR is usually conducted on growth from the primary isolation plate. Results are obtained in 3–5 days and the isolates are available for antimicrobial sensitivity testing and/or strain typing.

Another PCR-based methodology involves amplification of portions of specific genes, followed by restriction enzyme digestion of the products to give species-specific banding patterns after gel electrophoresis (restriction fragment length polymorphism [RFLP] analysis). Targets include the genes for 16S rRNA (Stanton et al. 1997), 23S rRNA (Barcellos et al. 2000), and the nox gene (Rohde et al. 2002). Unfortunately, isolates of *B. hyodysenteriae* that fail to amplify in the 23S rDNA PCR have been identified (Thomson et al. 2001).

The original PCR tests have been extended; for example, as a duplex reaction identifying *B. hyodysenteriae* and *B. pilosicoli* in DNA extracted directly from feces (La et al. 2003). Recently, multiplexed real-time PCRs have been described for *Brachyspira* species and *L. intracellularis* (Song and Hampson 2009; Willems and Reiner 2010), and these have the added advantage that they allow quantitation of the bacterial load in clinical samples.

Several serological tests have been reported for identifying herds with SD (reviewed by La and Hampson 2001), but they are not currently available as commercial kits, and few laboratories conduct them. Generally these tests have not been based on species-specific antigens and so they have had low specificity and/or sensitivity. An enzyme-linked immunosorbent assay (ELISA) using LOS as a plate-coating antigen has proved helpful for identifying infected herds, but not for detecting individual pigs with SD (Joens et al. 1982). Lipopolysaccharide-based ELISA systems require knowledge of the serotypes of organisms present in the herds to be tested so that the appropriate LOS can be used as a plate-coating antigen. An ELISA using recombinant Bhp29.7 as a plate-coating antigen has recently been described (La et al. 2009a), and other recombinant antigens are being evaluated.

**Immunity**
Changes occur in antibody levels and in cell-mediated reactivity in pigs with SD, but their importance are unclear. Serum immunoglobulin G (IgG) levels to *B. hyodysenteriae* correlate with the duration of clinical signs, while immunoglobulin A (IgA) levels in the colon reflect recent exposure (Rees et al. 1989b). Neither is strongly correlated with protection from SD (Joens et al. 1982; Rees et al. 1989b).

Pigs recovered from SD may be protected against subsequent challenge with *B. hyodysenteriae* for up to 17 weeks (Joens et al. 1979; Olson 1974), although some animals (7–43%) remain susceptible (Jenkins 1978; Joens et al. 1979; Rees et al. 1989a) and ~10% may only become protected after two bouts of disease.
Prevention and Control

Methods to Treat Pigs or Control Outbreaks. Only a few effective antimicrobials are still available for treatment of SD, and resistance to important drugs such as the pleuromutilins threatens the pig industry. Such drugs should only be used for specific therapy where other control measures are not effective, and for eradication programs. It is important to use either agar or broth dilution methods to determine the minimum inhibitory concentration (MIC) of the agents.

Severely affected animals may require intramuscular antimicrobials for at least 3 days; however, in most cases water medication for 5–7 days is preferable. If this is not possible then in-feed medication for 7–10 days can be used, although affected animals may have a low feed intake. Pigs should have free access to drinking water. Oral glucose–electrolyte solutions can be given to severely affected pigs. Treatment of acute SD may be followed by in-feed medication at subtherapeutic levels for 2–4 weeks to prevent reinfection.

The drugs most commonly used to treat SD are the pleuromutilins tiamulin and valnemulin, as well as tylosin and lincomycin. Their dose rates and potential side effects are listed in Table 50.2. Based on pharmacokinetic properties and in vitro susceptibility data the pleuromutilins appear to be the most suitable antimicrobials; however, decreased susceptibility to tiamulin among \textit{B. hyodysenteriae} isolates has been reported in several countries. Tiamulin usage selects for clones of \textit{B. hyodysenteriae} with decreased tiamulin susceptibility.

Table 50.2. Dosage level, duration of administration, and side effects for the four drugs most commonly used for the treatment of swine dysentery

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dosage and Duration</th>
<th>Side Effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tiamulin</td>
<td>10 mg/kg BW; im for 1–3 days</td>
<td>Rare: erythema. Local reactions at the injection site. Lethal side effects may occur in combination with ionophores.</td>
</tr>
<tr>
<td></td>
<td>8 mg/kg BW; po for 5–7 days in drinking water</td>
<td>Adverse effects including lethargy, depression, erythema, edema, pyrexia, ataxia, anorexia and deaths have been reported. Lethal side effects may occur in combination with ionophores.</td>
</tr>
<tr>
<td></td>
<td>Or: in-feed medication with 100 ppm for 7–10 days, followed by 30–40 ppm for 2–4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>weeks</td>
<td></td>
</tr>
<tr>
<td>Valnemulin</td>
<td>In-feed medication 3–4 mg/kg BW for 1–4 weeks.</td>
<td></td>
</tr>
<tr>
<td>Tylosin</td>
<td>10 mg/kg BW; im twice daily for 3–5 days.</td>
<td>Diarrhea, pruritus, erythema, rectal edema and prolapse have been reported.</td>
</tr>
<tr>
<td></td>
<td>Or: 5–10 mg/kg BW; po in drinking water for 5–7 days, followed by in-feed</td>
<td></td>
</tr>
<tr>
<td></td>
<td>medication with 100 g/t feed for 3 weeks, followed by in-feed medication 40 g/t feed.</td>
<td></td>
</tr>
<tr>
<td>Lincomycin</td>
<td>8 mg/kg BW; po in drinking water. Not to be used for more than 10 days and not for use in swine weighing more than 250 lb.</td>
<td>Rare</td>
</tr>
<tr>
<td></td>
<td>In-feed medication 100 g/t feed for 3 weeks or until signs of disease disappear,</td>
<td></td>
</tr>
<tr>
<td></td>
<td>followed by 40 g/t. Not for use in swine weighing more than 250 pounds.</td>
<td></td>
</tr>
</tbody>
</table>

The information in this table is an abbreviated summary of product labeling. For information regarding withdrawal times (which vary extensively between countries), review national regulations and product labels.

BW, body weight; im, intramuscular; po, per os (oral).
(Karlsson et al. 2004), so to reduce the risk of emerging resistance to pleuromutins, and if herd records or MIC determinations indicate that other drugs are effective, these should be used. Resistance to both tylosin and lincomycin frequently occur in Brachyspira species (Hommez et al. 1998; Karlsson et al. 2003). Macrolide and lincosamide resistance is caused by a single point mutation in the 23S rRNA gene, and tylosin resistance develops within 2 weeks in vitro (Karlsson et al. 1999). Multidrug-resistant strains of B. hyodysenteriae are increasingly being reported (Duinhof et al. 2008).

Acetylisovaleryltylosin (aivlosin) is a modification of an old drug, and may be useful both to prevent and treat SD when used in feed. Other antimicrobials including bacitracin, spiramycin, gentamicin, dimetridazole, ronidazole, virginiamycin, olaquindox, and carbadox have been used for treatment and prevention of SD. Unfortunately, resistance to several of these has been reported, and the availability of others is now much reduced internationally. These drugs usually have low MICs against B. hyodysenteriae, but their pharmacokinetic properties result in low concentrations in the gastrointestinal tract—which makes them only suitable as a prophylactic (de Graaf et al. 1988). Carbadox and metronidazole have both been shown to induce expression of the B. hyodysenteriae GTA, which in turn may increase transfer of resistance genes between strains (Stanton et al. 2008). Ionophore growth promoters such as salinomycin and monensin may prevent losses; however, toxicity may occur if they are used with pleuromutins or drugs that interfere with hepatic metabolism.

All-in/all-out management with cleaning and disinfection between batches reduces the risk of reinfection of medicated pigs, and limits spread of infection. Ideally, affected batches of pigs should be moved to clean buildings after medication to break the cycle of infection. Careful disposal of infected bedding, the use of boot scrubbers and disinfectant footbaths, cleaning and disinfection of equipment used in infected areas, and changing of protective clothing are all vital measures. As outbreaks of SD are often associated with stressful conditions such as pig handling, crowding, transportation, severe weather conditions, or dietary changes, it is important to minimize these. Attention should also be paid to the form and composition of the diet.

Mice and rats can act as reservoirs of B. hyodysenteriae in pig herds, so implementing efficient rodent control is very important. Unfortunately, it is virtually impossible to prevent mechanical transmission of infectious material by birds and other possible wildlife vectors in outdoor units.

Bacterin vaccines for SD are available commercially in some countries, and may provide a degree of protection (Diego et al. 1995). Unfortunately they tend to be LOS serogroup specific, which then requires the use of autogenous or multivalent preparations. Furthermore, they are relatively difficult and costly to produce on a large scale because of the fastidious growth requirements of the spirochete. One publication reported that immunization with a B. hyodysenteriae bacterin actually exacerbated dysentery (Olson et al. 1994). A commercial proteinase-digested bacterin may offer a better level of protection than conventional bacterins (Waters et al. 2000b).

Naturally avirulent or low-virulence strains have been used experimentally as vaccines (Hudson et al. 1976), sometimes in combination with bacterins (Lysons et al. 1986). Modified live strains have been produced by inducing mutations in genes affecting motility (Rosey et al. 1996), hemolysis (Hyatt et al. 1994) and protection from oxygen toxicity (Stanton et al. 1999); however, such strains may have a reduced ability to colonize pigs and they produce limited protection.

The use of a recombinant B. hyodysenteriae 38kDa flagellar protein as a vaccine failed to prevent colonization in pigs (Gabe et al. 1995), but the immunogenic Bhlp29.7 outer membrane lipoprotein of B. hyodysenteriae did provide a 50% reduction in incidence of disease following experimental infection (La et al. 2004). Other recombinant proteins have given similar levels of protection under experimental conditions (Song et al. 2009), and this demonstrates the general validity of this approach.

Methods to Prevent Introduction or Avoid Outbreaks of Brachyspira hyodysenteriae. Herds that are closed or in a closed pyramid should remain free of SD if they are geographically isolated and precautions are taken to prevent contamination. Introduction of new stock is the greatest risk, so a reliable history of the source herd is essential. Purchased animals should be quarantined for at least 3 weeks, and treated to eliminate B. hyodysenteriae. Infectious materials may also be carried into a herd by fomites such as workers’ boots, farm implements, and feed or animal trucks—and measures must be implemented to prevent this from occurring.

Methods to Eliminate Brachyspira hyodysenteriae from Herds. Depending on the herd structure, the production system, and on economical considerations, SD eradication can be performed in several ways. These vary from intensive medication of all pigs for a short period, to introduction of medicated early weaning and multisite production, through to an ongoing program performed by emptying and disinfection of each herd unit in a cycle, and introducing medicated animals to cleaned and disinfected units. Considerable effort is needed to plan and organize the eradication, and to achieve the full understanding and cooperation of all personnel involved. Generally, eradication becomes
more difficult as herd size increases, and as the company operation becomes larger and more complex. Wood and Lysons (1988) suggested that the chances of an eradication program succeeding were around 80–90% in carefully selected herds. The cost for eliminating SD may be recouped in 6–12 months through improved production and reduced drug usage (Windsor and Simmons 1981; Wood and Lysons 1988).

Total depopulation, cleanup, disinfection, and repopulation with SD-free stock is sometimes the only method available to eliminate \textit{B. hyodysenteriae}; however, it should only be instituted after accurate financial calculations (Wood and Lysons 1988). In this process the general guidelines described below for elimination without depopulation should be carefully followed. Evaluations have shown that elimination without depopulation is financially more attractive than depopulation/repopulation (Polson et al. 1992), but the probability of successful eradication should influence the method chosen.

Attempts to eliminate SD without total depopulation should only be undertaken if an effective antimicrobial agent against \textit{B. hyodysenteriae} isolates from the unit is available. It is also important to establish a source of replacement breeding stock that is free from SD, or to isolate and medicate all replacement stock before allowing them to enter the herd.

General guidelines for an SD eradication program include the following. Diagnosis of SD should be confirmed by laboratory testing. Several isolates should be obtained and tested for their MIC to available antibiotics. Isolates should also be typed to determine whether more than one strain is present. Herds with a continuous production system should try to change to batch production before the eradication. The program should be performed during a warm season when environmental survival of \textit{B. hyodysenteriae} is diminished. The number of animals in the herd should be reduced. Ideally, all weaners, growers, and finishers should be removed. Stock replacement should cease during the program. A rodent and insect control program should be implemented, and measures taken to stop wild birds from entering buildings. Dogs and cats should not be allowed within the herd area. Environmental contamination of all areas where pigs are housed, as well as the watering and feeding equipment, should be removed by high-pressure washing with hot water and disinfection. Slats should be lifted, and slurry tanks emptied, cleaned, and disinfected. In outdoor production units shelters should be cleaned, disinfected, and relocated to fresh sites. Remaining organic material should be burned, and the ground ploughed and reseeded, or left empty for several months. All sows, gilts, and boars should be medicated via drinking water or feed for at least 14 days, then moved into cleaned and disinfected buildings that have been empty for at least 2 weeks. Piglets born during the medication period should be weaned, treated parenterally with the same antimicrobial, and finished off-site. Piglets born after the sows have finished their medication can be weaned and reared on-site.

\textbf{BRACHYSPIRA PILOSICOLI: PORCINE INTESTINAL/COLONIC SPIROCHETOSIS}

\textbf{Relevance}

Taylor et al. (1980) first described porcine intestinal spirochetosis (PIS/PCS). In this study pigs that were orally challenging with weakly beta-hemolytic intestinal spirochete strain P43/6/78 developed mucoid diarrhea containing flecks of blood and had lesions of colitis. This isolate is now the type strain of the species \textit{B. pilosicoli}, although previously it has been referred to as "\textit{Anguillina coli}" (Lee et al. 1993b), "\textit{Serpulina coli}" (Duhamel et al. 1993), a group IV weakly hemolytic intestinal spirochete (Fellström and Gunnarsson 1995), and \textit{Serpulina pilosicoli} (Trott et al. 1996a).

Pigs with PIS/PCS show a variable loss of condition. This leads to increased time to reach market weight, and disrupts efficient production flow in infected herds (Duhamel 1998).

\textbf{Etiology}

Studies using MLEE have demonstrated that \textit{B. pilosicoli} forms a distinct species, that strains show extensive diversity, and that the population is recombinant (Lee and Hampson 1994; Trott et al. 1998). Recombination and genomic rearrangements may be caused in part by the activity of GTAs (Zuerner et al. 2004). Currently PFGE is the most commonly used technique for typing strains of \textit{B. pilosicoli} (Atyeo et al. 1996; Fossi et al. 2003).

\textit{Brachyspira pilosicoli} is 6–10\(\mu\)m long, 0.25–0.30\(\mu\)m wide,characteristically has four to seven periplasmic flagella attached at each cell end, and pointed ends. The \textit{B. pilosicoli} strain 95/1000 has a \(\sim 2.59\) Mbp circular chromosome, genes for a GTA, and an integrated prophage, but does not possess plasmids (Wanchan-thonk et al. 2010). The \textit{B. pilosicoli} outer envelope contains LOS, and this is serologically heterogeneous among different strains (Lee and Hampson 1999). \textit{Brachyspira pilosicoli} lacks the \textit{rfbBADC} gene cluster found on the \textit{B. hyodysenteriae} plasmid, and hence the species are predicted to have a different LOS structure. A number of outer membrane proteins and lipoproteins of \textit{B. pilosicoli} have been described (reviewed by Trott et al. 2001). More work is needed to define their potential role in disease, including whether they may be involved in attachment and/or in generating protective immunity.

\textit{Brachyspira pilosicoli} is predicted to be more tolerant of oxidative stress than \textit{B. hyodysenteriae}. Unlike \textit{B. hyodysenteriae}, it has genes for a glycine reductase complex that allows use of glycine while protecting from
oxidative stress, and for aconitase and related enzymes in the incomplete tricarboxylic acid (TCA) cycle that allows glutamate synthesis, and function of the cycle during oxidative stress (Wanchanthuek et al. 2010). *Brachyspira pilosicoli* has substantially fewer methyl-accepting chemotaxis genes than *B. hyodysenteriae*, and hence these species are likely to have different chemotactic responses that may help to explain their different host range and colonization sites.

*Brachyspira pilosicoli* is cultured under the same anaerobic conditions as *B. hyodysenteriae*. After 3–5 days on Trypticase Soy blood agar *B. pilosicoli* forms a thin spreading surface haze surrounded by a zone of weak beta-hemolysis. Slicing the agar prior to inoculation can improve the recovery of *B. pilosicoli*, but a zone of enhanced hemolysis is not usually seen. Once isolated, the spirochete grows readily in various anaerobic liquid media, as described for *B. hyodysenteriae*.

**Public Health**

*Brachyspira pilosicoli* colonizes human beings who are usually either immunocompromised or live in developing communities where hygiene is poor and fecal contamination of water supplies may occur (Margawani et al. 2004). Infection may be associated with chronic diarrhea and/or failure to thrive. Strains of *B. pilosicoli* from humans can cause disease when inoculated into pigs and chickens (Duhamel et al. 1995; Muniappa et al. 1997; Trott et al. 1995, 1996b). The potential for transmission of *B. pilosicoli* from animals to humans cannot be dismissed, although the risk of healthy pig industry workers developing disease from contact with pigs is slight.

**Epidemiology**

PIS/PCS has been reported in most pig-producing countries. Increased recognition of the condition has resulted from improved diagnostic methods, the withdrawal of routine antimicrobial growth promoters, and the fact that other major intestinal diseases are now better controlled in many countries.

Investigations in different regions have found that a variable but often high proportion of farms having pigs with persistent diarrhea problems are infected with *B. pilosicoli*, whereas little or no infection occurs in farms without diarrhea.

A wide range of species may be naturally infected with *B. pilosicoli*, and typical clinical signs and lesions have been recorded in all host species (reviewed by Duhamel 2001). Isolates from pigs, dogs, birds, and humans can be closely related genetically (Lee and Hampson 1994; Trott et al. 1998).

Transmission occurs by the fecal/oral route and infection may be introduced into naïve herds by carrier pigs. *Brachyspira pilosicoli* can persist in the environment, and the disease can recur between batches of pigs if the premises are not adequately cleaned and disinfected. Feral animals and birds may be a source of infection. On one pig farm, *B. pilosicoli* was detected in chickens, effluent pond water, and wild ducks on the effluent pond (Oxberry and Hampson 2003). An isolate from the pond belonged to the same genetic type as one from a pig, a finding consistent with a previous observation suggesting that feral water birds may contaminate water supplies and so represent a potential source of *B. pilosicoli* infection for pigs (Oxberry et al. 1998). Rodents appear unlikely to serve as an important long-term biological reservoir of *B. pilosicoli*.

The on-farm epidemiology of *B. pilosicoli* can be highly variable (Oxberry and Hampson 2003). Sometimes the incidence is low and largely confined to one age group, while in other herds it may be widespread and associated with numerous different strains. The presence of multiple *B. pilosicoli* strains within certain farms might explain why PIS/PCS commonly recurs in convalescent animals, or in those treated with antimicrobials. In such cases, reinfection may be with a different strain, possibly having different antigenic determinants, antimicrobial susceptibilities, or potential to colonize and cause disease.

In Finland, most farms had their own distinct genotypes of *B. pilosicoli*, while it was rare to find the same type on different farms (Fossi et al. 2003). The within-farm genotypes appeared fairly stable, as the same genotypes were found on three farms that were reexamined after 3 years.

*Brachyspira pilosicoli* is shed via the feces. Shedding may be intermittent, and continue over many weeks in some pigs. It is relatively resistant in the environment. It survives in lake water at 39°F (4°C) for 66 days (Oxberry et al. 1998) and remains viable at 50°F (10°C) for 119 days in soil, and for 210 days in soil with 10% pig feces and in feces (Boye et al. 2001). *Brachyspira pilosicoli* is susceptible to many commonly used disinfectants, although the efficacy of some of these is reduced by organic matter such as feces (Corona-Barrera et al. 2004a).

**Pathogenesis**

Cells of *B. pilosicoli* are motile, and strains vary in their attraction to mucin (Naresh and Hampson 2010). Once in the large intestine, the spirochete is able to penetrate the mucus overlying the colonic mucosa. In the initial stage of infection *B. pilosicoli* cells can adhere in large numbers to the luminal surface of cecal and colonic epithelial cells. Attachment occurs in mature apical enterocytes between crypt units, but not in immature cells deeper in the crypts (Trott et al. 1996b).

Despite the availability of a genome sequence, few *B. pilosicoli* attributes that might contribute to disease have been identified. Progress in this area remains hampered by a lack of means for genetic manipulation. *Brachyspira pilosicoli* proteases have been identified...
(Dassanayake et al. 2004); however, their potential role in disease has not been determined. Cellular attachment by B. pilosicoli strains has been achieved in vitro using intestinal epithelial cell lines, but putative adhesins or host cell receptors have not been identified (Muniappa et al. 1998; Naresh et al. 2009). In infected Caco-2 cell monolayers the cell junctions were the initial target sites for attachment (Naresh et al. 2009). Colonized monolayers demonstrated a time-dependent series of changes, including accumulation of actin at the cell junctions, a loss of tight junction integrity, and evidence of apoptosis—although the mechanisms causing these changes were not identified. The colonized monolayers demonstrated a significant increase in interleukin-1β (IL-1β) and IL-8 expression. If the chemokine IL-8 is also released in vivo, it may be responsible for attracting neutrophils to the infected colon.

As with SD, B. pilosicoli colonization and/or disease expression can be influenced by diet. An analysis of risk factors on farms revealed that reduced prevalence results from using home-mixed and/or nonpelleted diets (Stege et al. 2001). Adding carboxymethyl cellulose to an experimental pig diet increased the viscosity of the intestinal contents and enhanced colonization with B. pilosicoli (Hopwood et al. 2002). High levels of soluble nonstarch polysaccharide (“soluble fiber”) in grains like barley and rye may also increase viscosity, and therefore enhance B. pilosicoli colonization. Consistent with this, pigs fed diets based on cooked white rice (highly digestible and low in soluble fiber) have shown reduced colonization with B. pilosicoli compared to pigs fed conventional diets (Hampson et al. 2000; Lindecrona et al. 2004). Feeding a pelleted diet rather than a meal increased the risk of colonization, but fermented liquid feed or lactic acid had no influence on colonization (Lindecrona et al. 2004).

**Clinical Signs**

PIS/PCS most commonly occurs soon after weaning or in recently mixed growers placed on a new diet, but it can occur in finishers and occasionally in pregnant sows and recently introduced breeding stock. PIS/PCS may affect groups of pigs in a unit or be present in pigs of mixed ages. Various manifestations may be seen in weaners, growers, and finishers on the same farm. Not all infected animals develop diarrhea; however, subclinical infections may still depress growth rates.

The first clinical signs are the hollowing of the flanks and the passage of loose, sometimes sticky feces. Fecal consistency changes to that of wet cement or porridge, and may glisten. These may be the only signs in finishers, but weaners and growers usually develop watery to mucoid diarrhea which is green or brown and occasionally contains thick tags of mucus, and sometimes flecks of blood. Diarrhea is usually self-limiting and lasts 2–14 days, although some animals may relapse.

Affected pigs appear ill thrifty, have fecal staining of the perineum, a tucked-up appearance, and sometimes are febrile, but usually continue to eat. Pigs that develop loose feces may show significant loss of condition, decreased feed conversion, and delays in reaching market weight (Thomson et al. 1997, 1998).

Following experimental inoculation, B. pilosicoli may be shed in the feces within 2–7 days, although the incubation period may extend to 20 days. In experimental infections 17–100% of pigs may become infected, with 17–67% developing diarrhea and 8–100% having colitis (Duhamel 2001). Mortality is rare in the field.

Pigs with PIS/PCS may have concurrent sickness that exacerbates disease, particularly intestinal diseases such as SD, salmonellosis, and PE, or infection with porcine circovirus type 2 (PCV2) (Duhamel et al. 1995; Girard et al. 1995; Möller et al. 1998; Stege et al. 2000; Thomson et al. 1998, 2001).

**Lesions**

Gross lesions are limited to the cecum and colon and may be subtle, particularly in the early stages. Soon after the onset of clinical signs, the cecum and colon may be flaccid and fluid-filled with an edematous serosal surface and enlarged mesenteric and colonic lymph nodes. The large intestinal contents are usually abundant, watery, green or yellow, and frothy. Mild congestion and hyperemia of the mucosal surface may be present, with some erosions and necrotic foci. Inflammation in the later stages may result in multifocal erosive, ulcerative, or mucohemorrhagic colitis. The mucosa becomes thickened and local ecchymotic or petechial hemorrhages may be seen on the surface. In chronic cases and in resolving lesions the hemorrhages may be covered by small tags of adherent fibrin, necrotic material, and digesta that appear as conical scales adherent to the mucosa.

Lesions are generally confined to the mucosa and submucosa, but may extend into the muscularis. The mucosa is usually thickened, edematous, and occasionally hyperemic, and characterized by dilated, elongated crypts filled with mucus, cellular debris, and degenerate inflammatory cells. The presence of B. pilosicoli within crypts and the lamina propria may be associated with neutrophilic exocytosis (crypt abscesses) and a mixed infiltrate of neutrophils and lymphocytes in the lamina propria (Figure 50.5). In chronic infections the lamina propria is usually infiltrated with large numbers of monocytes, lymphocytes, and plasma cells (Duhamel 2001). The crypt cell mitotic rate can be increased, and immature, cuboidal, or squamous epithelium may be present on the surface of the mucosa between crypt units. Columnar epithelium on the surface of the colon may be covered by a dark fringe of spirochetes attached by one cell end forming a characteristic “false brush border” (Girard et al. 1995; Taylor et al. 1980) (Figure
By transmission electron microscopy, polar-attached spirochetes with four to seven periplasmic flagella may be seen invaginated into the terminal web cytoplasm, effacing microvilli and disrupting microfilaments without penetrating the host cell plasmalemma (Figure 50.7). Scanning electron microscopy may reveal the adherent spirochetes forming a patchy fringe on the surface of colonic epithelial cells. Spirochetes may also be observed invading between epithelial cells in the extrusion zone between adjacent crypt units (Duhamel 1998).

Epithelial damage followed by local invasion and subsequent colitis combine to cause an increase in the water content of the cecal and colonic contents, together with excess mucus production. Erosion of the epithelium leads to replacement by immature cells and reduction of the surface area of the colon for absorption of water, electrolytes, and volatile fatty acids. This then leads to reduced feed conversion efficiency and diminished weight gain (Duhamel 1998; Thomson et al. 1997).

**Diagnosis**

Clinical signs of PIS/PCS can be very similar to those of PE. PIS/PCS may occur together with PE (Chapter 59), salmonellosis (Chapter 60), postweaning colibacillosis (Chapter 53), SD, yersiniosis (Chapter 64), PCV2 enteritis (Chapter 26), trichuriasis (Chapter 66), and/or “nonspecific colitis,” a diet-responsive colitis (Chapter 15; Smith and Nelson 1987; Wood 1991). All these conditions should be considered in the differential diagnosis of porcine colitis.

Postmortem examination of several affected pigs should be undertaken. The body weights (BWs) of animals from a production batch may be uneven. Localized areas of mild or occasional severe colitis are

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50.5. Photomicrograph of a section of porcine colon infected with a Brachyspira pilosicoli strain. Neutrophilic exocytosis (crypt abscess material) is present in dilated intestinal crypts (arrows). The lamina propria is diffusely infiltrated with inflammatory cells. Marker bar = 37µm.

50.6. Light photomicrograph of the colonic epithelium from a pig with PIS/PCS. Note the dense fringe of spirochetes attached by one cell end to the colonic epithelium to form a “false brush border” (arrows). Bar = 16µm.

50.7. Transmission electron photomicrograph of the colonic epithelium from a pig with PIS/PCS. Note the large numbers of spirochetes attached by one cell end to the apical membrane of enterocytes, causing effacement of the microvilli and disruption of terminal web microfilaments (arrow). Bar = 2µm.
usually observed, and macroscopic and microscopic lesions are as described earlier. Immunohistochemical staining with specific antibodies or FISH with specific oligonucleotide probes (Boye et al. 1998; Jensen et al. 2000) can be used to confirm the presence of *B. pilosicoli* attached to the surface of colonic enterocytes, within diluted intestinal crypts, and occasionally within the lamina propria.

A definitive diagnosis of PIS/PCS requires the demonstration of *B. pilosicoli*, but its isolation from feces does not always correlate with diarrhea or epithelial attachment, and the significance needs to be interpreted in the context of a complete diagnostic investigation (Thomson et al. 1998). Fecal samples for culture and/or PCR should be obtained from a cross-section of affected pigs. Swabs taken from the colonic wall can be used to prepare wet smears for viewing by phase contrast microscopy, or they can be fixed and Gram stained.

CVS medium is preferred for isolating *B. pilosicoli*, while BJ medium may inhibit *B. pilosicoli* growth—and consequently is not recommended (Duhamel and Joens 1994; Trott et al. 1996c). Pure cultures of spirochetes isolated from pigs can be differentiated using the simple biochemical tests outlined in Table 50.1. Generally, *B. pilosicoli* can be identified using strength of beta-hemolysis, hippurate hydrolysis, metabolism of ribose, and lack of beta-glucosidase activity (Fellström and Gunnarsson 1995; Trott et al. 1996c).

Diagnosis is usually supported by the use of specific PCR tests targeting the genes for 16S rRNA (Fellström et al. 1997), 23S rRNA (Leser et al. 1997), or the nox gene (Atyeo et al. 1999b). Duplex and multiplex PCRs, sometimes also detecting other enteric pathogens, have been described. Real-time PCR assays that allow spirochete quantitation are also becoming available (see the section on SD).

As with *B. hyodysenteriae*, RFLP analysis can be used to identify *B. pilosicoli* isolates. Indirect fluorescent antibody tests using MAbS raised against specific outer membrane proteins of *B. pilosicoli* have the potential for diagnostic use on feces (Lee and Hampson 1995), but a MAb-based immunomagnetic separation of *B. pilosicoli* from feces did not improve the sensitivity of detection compared to culture/PCR (Corona-Barrera et al. 2004b).

To date, no routine commercial tests are available to measure species-specific serum antibody to *B. pilosicoli*.

**Immunity**

The host immune mechanisms directed against *B. pilosicoli* are poorly understood. In the original study, agglutinating serum antibodies were recorded in pigs recovered from experimental infection (Taylor et al. 1980). In another study no significant antibody levels against whole cell preparations of *B. pilosicoli* were found after 18 days in experimentally infected pigs with mild colitis (Hampson et al. 2000). In other experiments, pigs developed low levels of serum IgG against *B. pilosicoli* whole cell extracts and membrane preparations 2–7 weeks after challenge (Zhang and Duhamel 2002; Zhang et al. 1999). Little is known about protective immunity in PIS/PCS. The existence of long-term colonization suggests that the spirochete may be able to evade immune mechanisms. Cross-protection is unlikely as *B. pilosicoli* strains show considerable variability in their LOS (Lee and Hampson 1999). Nothing is known about maternal immunity, although natural infection has not been recorded in unweaned piglets.

**Prevention and Control**

**Methods to Treat Pigs or Control Outbreaks.** Treatment and control of PIS/PCS are largely modeled on procedures developed for SD, although modifications can be made because of the milder economic impact of PIS/PCS. Antimicrobial therapy can be used to reduce *B. pilosicoli* infection and maintain productivity while improving welfare; also, it may be required to prevent sudden increases in morbidity due to recent introduction of naïve pigs, change of diet, or other stressors. Affected pigs should be treated by water or feed medication at similar levels and durations as recommended for SD. Parenteral treatment may be necessary for severely ill pigs. Although information on the in vitro antimicrobial susceptibility of *B. pilosicoli* is limited, a number of antimicrobials that are effective against *B. hyodysenteriae*, including tiamulin, valnemulin, carbadox, dimetridazole, and, to a lesser extent, lincomycin, have been shown to have low MIC values when tested against porcine *B. pilosicoli* isolates (Fossi et al. 2000; Hommez et al. 1998; Kinyon et al. 2002; Trott et al. 1996c). Fewer isolates have been found to be susceptible to tylosin, and resistance to several antimicrobials, including tiamulin, has been recorded. Olaquindox may be a useful prophylactic as it has MIC values <1.0 μg/mL against *B. pilosicoli*, and the spirochete could not be isolated from herds previously receiving 100 ppm olaquindox in feed (Fellström et al. 1996).

Management strategies that limit access of pigs to contaminated environments can reduce the impact of PIS/PCS. Replacing continuous-flow systems with all-in/all-out systems reduces the risk of infection (Stege et al. 2001). Modification to the diet composition and/or physical form, or adding zinc oxide in the feed at 3 kg/t, may be helpful (Love 1996).

**Methods to Prevent Introduction or Avoid Outbreaks of *Brachyspira pilosicoli*.** It is difficult to avoid the introduction of *B. pilosicoli* into herds because of the presence of reservoir hosts such as feral birds, although similar strategies to those described for SD can be followed. Unfortunately, no effective vaccines are available for *B. pilosicoli*. An autogenous bacterin induced systemic antibody titers, but the pigs still became
colonized and developed diarrhea after challenge (Hampson et al. 2000).

**Methods to Eliminate Brachyspira pilosicoli from Herds.** The methods described for the elimination of SD may also be effective for PIS/PCS, but the economic impact generally does not warrant such costly procedures. Fossi et al. (2001) reported eradicating Brachyspira pilosicoli from a 60-sow herd by tiamulin treatment followed by relocation of the breeding herd, thorough cleaning and disinfection of the premises, and then returning the adult animals to the original location. This protocol would be more difficult to follow in larger herds, and the existence of reservoir hosts presents an ongoing threat of reintroduction.

**OTHER BRACHYSPIRA SPECIES THAT MAY CAUSE COLITIS**

"Brachyspira suanatina" is a proposed strongly beta-hemolytic species that was recently identified in Scandinavia, and that has been reported to cause colitis in pigs (Råsbäck et al. 2007a). Isolates of the species resemble those found in feral mallards, suggesting that this may have been their origin. Currently there is uncertainty both about the distribution of "B. suanatina" and its overall significance to pig health.

Occasionally the other weakly hemolytic *Brachyspira* species have been thought to be capable of causing colitis and contributing to chronic diarrhea in pigs. Some strains of *B. intermedia* have been suspected of having pathogenic potential, and this is not surprising given the extreme diversity of this group (Phillips et al. 2010). *Brachyspira murdochii* appears to be the species most frequently associated with lesions of mild colitis (Komarek et al. 2009; Weissenböck et al. 2005), and a recent experimental infection of pigs with a *B. murdochii* strain confirmed that it was mildly pathogenic (Jensen et al. 2010). Infections or colonizations with one or more of the nonpathogenic or mildly pathogenic *Brachyspira* species are probably quite common: arguably their greatest significance is that the presence of these spirochetes may complicate diagnosis of SD and/or PIS/PCS—both of which are much more economically important diseases, and require implementation of rigorous control measures.

**ACKNOWLEDGMENTS**

The author wishes to acknowledge the input of the authors of the chapters on SD and PIS/PCS in previous editions of this book. Much of the current chapter is based on these influential works.

**REFERENCES**


Brucellosis

Brucellosis is an infectious disease caused by bacteria of the genus *Brucella*, characterized by abortion and infertility in numerous mammalian species, and is also one of the most important zoonotic diseases worldwide (Cutler et al. 2005). Brucellosis in swine is primarily caused by *Brucella suis*. The organism was first isolated by Traum (1914) from aborted porcine fetuses in Indiana (Frye 1983) and was recognized as a separate *Brucella* species in 1929 (Huddleston). *Brucella suis* causes infertility and abortion in infected herds, but is most important as a zoonotic agent.

Beginning in the 1920s, increasing awareness and knowledge of brucellosis by the human medical community in the United States led to increasing diagnosis and confirmation of human infection. Initially, many human *B. suis* isolates were misidentified as *Brucella abortus* due to limitations in microbiological techniques. Likewise, the lack of phage testing and oxidative metabolic tests prior to the 1960s likely led to the misidentification of swine isolates of *B. suis* biovar 3 as *Brucella melitensis* biovar 2 (Alton 1990). Nevertheless, *B. suis* was recognized as the most frequent cause of human brucellosis in the United States between 1959 and 1974 (Frye 1983).

Between 1956 and 1960, serological testing of greater than 88,000 herds across the United States demonstrated a herd infection rate of approximately 6.15%. Programmatic eradication began in the United States in 1959. Control or eradication programs in livestock reservoirs are justified by data confirming them as the most efficient and economical approach for reducing human brucellosis (Jelastopulu et al. 2008; Roth et al. 2003; Zinsstag et al. 2007).

At this time, there are eight recognized species of *Brucella* (*B. abortus*, *B. melitensis*, *B. suis*, *Brucella canis*, *Brucella ovis*, *Brucella neotomae*, *Brucella ceti*, and *Brucella pinnipedialis*) that differ in their host preference (Table 51.1; Alton et al. 1988) and in some microbiological and genetic markers (Alton et al. 1988; OIE 2008). Recently, new bacterial species with characteristics of *Brucella* have been isolated from the common vole (Scholz et al. 2008) and from humans in a breast implant infection (Scholz et al. 2010) and chronic destructive pneumonia (Tiller et al. 2010). They have been proposed as new *Brucella* species: *Brucella microti* from voles and *Brucella inopinata* from humans. However, *B. microti* may be a component of the *B. suis* biovar 5 cluster (Audic et al. 2009). Using a combination of microbiological, serological, and molecular tests, *B. melitensis*, *B. abortus*, and *B. suis* have been further divided into 3, 8, and 5 biovars, respectively (Table 51.2; Alton et al. 1988).

Brucellosis in swine is predominantly caused by *B. suis*, a species that for many years was considered as a highly pathogenic *B. abortus* variant (Alton 1990; Huddleston 1929). While domestic swine (*Sus scrofa domesticus*) are primarily infected by *B. suis*, brucellosis in pigs may also be due to *B. abortus* and *B. melitensis* in areas where brucellosis is endemic in cattle and small ruminants, respectively.

*Brucella suis* organisms are gram-negative coccobacilli measuring 0.6–1.5µm in length and 0.5–0.7µm in width. In nature, *B. suis* invariably occurs in the smooth (S) form and colonies of *B. suis* cannot be differentiated by visual observation from isolates of other smooth *Brucella* species. Of the five recognized biovars of *B.
**Table 51.1.** Differential microbiological characteristics of species of the genus *Brucella* (Source: Chapter 2.4.3 (pp. 626 and 627), Bovine brucellosis. In 2008 OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals, Sixth Edition (http://web.oie.int/boutique/index.php?page=ficprod&id_produit=124&lang=en; http://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/2.08.05__PORCINE_BRUC.pdf))

<table>
<thead>
<tr>
<th>Species</th>
<th>Colony Morphology</th>
<th>Serum Requirement</th>
<th>RTD 10^4</th>
<th>RTD 10^4</th>
<th>Oxidase</th>
<th>Urease</th>
<th>Preferred Hosts</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>B. abortus</em></td>
<td>S</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>Cattle and other Bovidae</td>
</tr>
<tr>
<td><em>B. suis</em></td>
<td>S</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>4f</td>
<td>+</td>
<td>Swine, wild boar, European hare,</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>reindeer, and wild rodents</td>
</tr>
<tr>
<td><em>B. melitensis</em></td>
<td>S</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>Sheep and goats</td>
</tr>
<tr>
<td><em>B. neotomae</em></td>
<td>S</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>Desert wood ratk</td>
</tr>
<tr>
<td><em>B. canis</em></td>
<td>R</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>Rams</td>
</tr>
<tr>
<td><em>B. ceti</em></td>
<td>S</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>Dogs</td>
</tr>
<tr>
<td><em>B. pinnipedia</em></td>
<td>S</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>Pinnipeds</td>
</tr>
</tbody>
</table>

*Phages: Tbilisi (Tb), Weybridge (Wb), and Izatnagar1 (Iz1).*

bNormally occurring phase: S: smooth, R: rough.

c* B. abortus* biovar 2 generally requires serum for growth on primary isolation.

dSome African isolates of biovar 3 are negative.

eIntermediate rate, except reference strain 544 and some field strains.

fSome isolates of *B. suis* biovar 2 are not or only partially lysed by phage Wb or Iz1.

gRapid activity.

hSome isolates are lysed by phage Wb.

iSlow rate, except some strains that are rapid.

jMinute plaques.

k* Neotoma lepida.*

lBut some isolates are lysed by Tb.

mMost isolates are lysed by Wb and Iz1.

RTD, routine test dilution.

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**Table 51.2.** Differential characteristics of the recognized *Brucella* biovars (Source: Chapter 2.4.3 (pp. 626 and 627), Bovine brucellosis. In 2008 OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals, Sixth Edition (http://web.oie.int/boutique/index.php?page=ficprod&id_produit=124&lang=en; http://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/2.08.05__PORCINE_BRUC.pdf))

<table>
<thead>
<tr>
<th>Species</th>
<th>Biovar</th>
<th>Preferred Hosts</th>
<th><strong>CO₂</strong> Requirement</th>
<th><strong>H₂S</strong> Production</th>
<th>Thionin</th>
<th>Basic Fuchsin</th>
<th>Agglutination with Monospecific Sera</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>B. melitensis</em></td>
<td>1</td>
<td>Sheep and goats</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>A</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td><em>B. abortus</em></td>
<td>1</td>
<td>Cattle</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>+</td>
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<tr>
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<td>3</td>
<td>+</td>
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<td>−</td>
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<td>+</td>
<td>−</td>
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<tr>
<td></td>
<td>5</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>−</td>
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<td></td>
<td>6</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>−</td>
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<td></td>
<td>9</td>
<td>+/−</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td><em>B. suis</em></td>
<td>1</td>
<td>Swine</td>
<td>−</td>
<td>+</td>
<td>−^c</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>Swine, wild boar</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>European hare</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>Reindeer</td>
<td>−</td>
<td>−</td>
<td>−^d</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>Wild rodents</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>−</td>
</tr>
</tbody>
</table>

^aDye concentration in serum dextrose medium: 20µg/mL.

^bMost strains are basic fuchsin resistant. Sensitive strains have been isolated.

^cMost strains are basic fuchsin sensitive. Resistant strains have been isolated (in South America particularly).

^dNegative for most strains.
Brucella suis, biovars 1, 2, and 3 are mainly responsible for brucellosis in swine. Biovar 4 has been exclusively isolated from reindeer or caribou (Rangifer tarandus and its various species), moose (Alces alces), American bison (Bison bison), arctic foxes (Alopex lagopus), and wolves (Canis lupus) in subarctic areas, and biovar 5 has been exclusively isolated from wild rodents in the former USSR (OIE 2008).

Brucella suis biovars 1, 2, and 3 can be isolated in adequate culture media (see the section “Diagnosis” below) and may be presumptively identified by slide agglutination with monospecific A antiserum and some additional bacteriological tests (Alton et al. 1988). As biovars 1 and 3 are virulent human pathogens, appropriate precautions should be taken when handling and disposing of potentially infective material. Under laboratory conditions, manipulation of the cultures or contaminated material from infected animals must be done under strict biosafety conditions. Biolevel 3 containment is recommended for safely working with pathogenic strains of B. suis.

Confirmatory identification of species and biovars should be performed in a specialized brucellosis reference laboratory. The precise identification of biovars depends on phage tests, production of H2S (only biovar 1 produces H2S), and growth in the presence of dyes (Table 51.2). Some strains of B. suis biovar 1 are atypical in that they grow on media containing 20 µg/mL of basic fuchsin (Lucero et al. 2008). Most strains of B. suis are inhibited by O safranin at a concentration of 1/10,000 and B. suis usually reacts more rapidly in the urease test than either B. abortus or B. melitensis. Differentiation of biovars 1, 2, and 3, is not straightforward and may require additional tests beyond the above described.

Complete genomes of eight strains belonging to five Brucella species have been fully sequenced, confirming a high degree of similarity. The 3.3 Mb complete genomic sequence of B. suis biovar 1 (strain 1330) has been determined, being very similar in chromosome structure, organization, and gene content to available genomes of B. melitensis and B. abortus (Chain et al. 2005). Molecular techniques based on the polymerase chain reaction (PCR) are currently available for differentiating B. suis from the remaining Brucella species in a single step (Garin-Bastuji 2008; López-Gofri et al. 2008; Mayer-Scholl et al. 2010). However, direct PCR tests have not so far been able to fully differentiate the 5 biovars within B. suis (Ferraio-Beck et al. 2006).

Other PCR-based methods have been used to improve typing of the main B. suis biovars. The most widely used have been the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analyses of genes omp2a and omp2b, which can differentiate between the reference biovars 1, 2, and 3 (Cloeckaert et al. 1995), and of gene omp31, which was able to differentiate biovars 1 and 3 from biovar 2 (Vizcaíno et al. 1997). Recently, fingerprinting based on a PCR method for multiple-locus variable-number tandem-repeat analysis (MLVA) has been developed for the molecular typing of B. suis (García-Yoldi et al. 2007). This is useful for comparing relative differences between strains in different biovars, within each biovar and in epidemiological investigations. (García-Yoldi et al. 2007). It is expected that on the basis of these results, new multiplex PCR assays will be developed in the near future for the differentiation of B. suis biovars in a single test.

PUBLIC HEALTH

Brucella melitensis, followed by B. abortus and B. suis, are the main species involved in the infection of human beings, thus being the main target of eradication campaigns. Human brucellosis is the world’s leading bacterial zoonosis, with most cases being derived from contaminated unpasteurized milk and milk products (Pappas et al. 2006). Brucella suis biovars 1, 3, and 4 are pathogenic in humans, whereas current data suggest that biovar 2 is a very rare human pathogen reported only in immune-compromised hosts, particularly when exposed to infected wildlife (Garin-Bastuji et al. 2006; Lagier et al. 2005; Paton et al. 2001). Within the Brucella genus, only B. melitensis is generally considered to be as pathogenic (or more) in humans as biovars 1 and 3 of B. suis (Corbel 2006).

Occurrence of human B. suis infections is generally limited to areas of the world where biovars 1 and 3 are endemic in domestic or feral swine. For example, B. suis is a significant cause of brucellosis in swine and people in some Latin American countries (Alton 1990; Boletín Epidemiológico Periódico 2004; Lucero et al. 2008). In those countries a majority of human isolates have been biotype 1, but atypical isolates that grow not only on thionin, but also on basic fuchsin, safranin O, thionin blue, malachite green, and penicillin (Corbel et al. 1984) have been described (García-Carrillo 1990) and now represent nearly half of all human isolates in Latin America.

Transmission from pigs to people is relatively efficient owing to the long bacteremia in pigs, the presence of B. suis in many tissues during bacteremia, a long period of shedding from pigs, and a relatively low infective dose in humans. At increased risk are those whose occupations facilitate direct exposure to infected pigs such as abattoir workers (Hendricks et al. 1962; Trout et al. 1995) and farmers (Alton 1990). Direct transmission has also been documented in hunters of feral swine or wild boar (Garin-Bastuji et al. 2006; Irwin et al. 2009; Lagier et al. 2005; MMWR 2009; Robson et al. 1993).

Although indirect transmission through consumption of contaminated dairy products is common for B. abortus and B. melitensis, indirect transmission of B. suis...
is uncommon. Like *B. abortus*, when cattle are infected with *B. suis* the organism localizes to the mammary gland and is shed in milk (Ewalt et al. 1997). Several reports have documented zoonotic transmission of *B. suis* through unpasteurized bovine milk (Beattie and Rice 1934; Borts et al. 1943).

In humans, brucellosis is a febrile disease that is often chronic and debilitating as a result of granulomatous inflammation in a variety of organs. Onset may be abrupt following incubation of 2–3 weeks or, in approximately half of patients, can be insidious with symptoms developing over a period of weeks to months after exposure (Corbel 2006). Symptoms include fever and nonspecific flu-like signs such as headache, malaise, back pain, myalgia, and generalized aches. Drenching sweats can occur, particularly at night. In the absence of specific treatment (Ariza et al. 2007), infection may persist for weeks or months. Relapse of infection within 6 months after treatment is not uncommon (Wallach 1998), but is not usually associated with emergence of antibiotic-resistant strains (Corbel 2006). The duration of the human illness and its long convalescence means that brucellosis is not only a medical problem, but also economically important due to time lost from normal work activities (Corbel 2006).

**EPIDEMIOLOGY**

*Brucella suis* has worldwide distribution. Both biovars 1 and 3 of *B. suis* have been reported in domestic swine in the United States, but are essentially eradicated. In Central and South America, data suggest a wide distribution of *B. suis* in domestic swine with infections predominantly caused by biovar 1 (Luna-Martínez and Mejía-Terán 2002; Poester et al. 2002; Samartino 2002). In China, which has a large population of swine, *B. suis* is of economic importance, with both biovars 1 and 3 reported. Although current data are limited, swine brucellosis also appears to be endemic in parts of Southeast Asia, including Indonesia, the Philippines, Taiwan, and other islands in the Pacific (Alton 1990). Porcine brucellosis is also believed to be widespread across sub-Saharan Africa, although data are limited and populations of swine are relatively small (McDermott and Arimi 2002).

Due to success of regulatory programs, increased use of confinement operations and changes in other management practices for production of swine, feral swine, or wild boar have become the predominant reservoir host for *B. suis* in a number of countries or regions (Australia, United States, Western and Central Europe, and others). Available evidence suggests that prevalence of *B. suis* biovar 2 infection in the Eurasian wild boar (*Sus scrofa scrofa*) is very high with estimates ranging from 8% to 32% throughout continental Europe (Al Dahouk et al. 2005; Cvetnić et al. 2003; Garin-Bastuji and Delcueillerie 2001; Hubálek et al. 2002; Koppel et al. 2007; Muñoz et al. 2010). Feral swine pose a risk for domestic swine when contact occurs. In continental Europe, excluding Finland, Norway, and Sweden, the spillover of *B. suis* biovar 2 from wild boars (*Sus scrofa scrofa*) or wild hares (*Lepus europaeus*) to swine raised in outside pens remains a problem (Cvetnić et al. 2009; EFSA 2009; Godfroid et al. 2005; Godfroid and Käsbohrer 2002). Transmission from wild boars to pigs is thought to be through the venereal route, as crossed piglets (striped) have been reported, at least in France and Portugal, although other routes might also be possible. Specific control measures are not in place in most of these countries because *B. suis* biovar 2 is not an important cause of human brucellosis. France is an exception where hare- and wild boar-proof fences have been made compulsory for outdoor-ranged pig farms and where infected herds are systematically depopulated. *Brucella suis* biovar 2 has not been reported in other parts of the world.

The European hare (*L. europaeus*) is also a possible source of *B. suis* biovar 2 for domestic pigs through swill feeding with offal from hunted infected hares, or via natural ingestion of dead hares in pastures. Interestingly, the unique *B. suis* biovar 2 strain isolated from European hares in the north of Spain (Lavin et al. 2006) is genetically different from haplotypes identified in wild boar (Muñoz et al. 2010). This particular hare strain demonstrates different molecular patterns from those of the Thomsen biovar 2 reference strain, and *B. suis* biovar 2 isolates obtained from hares in Northern European countries (B. Garin-Bastuji and J. M, Blasco, unpublished results). This suggests that at least in some regions, the *B. suis* biovar 2 strains infecting European brown hares and wild boar may be different. However, this observation needs to be confirmed in further studies using larger numbers of animals.

Swine may also maintain infections with other *Brucella* spp. In addition to recovery of *B. suis*, *B. abortus* biovar 1 field strains and the strain 19 vaccine strain were isolated from feral swine in South Carolina (Stofregen et al. 2007). As this feral swine population was isolated by development and topography from domestic livestock for approximately 40 years, the report suggests that *B. abortus* can be transmitted and maintained in a swine population for long periods of time. *Brucella melitensis* has also been reported as a cause of brucellosis in domestic pigs (B. Garin-Bastuji, personal results; Lucero et al. 2008).

It should be noted that indoor, environmentally controlled swine farms can also be severely affected by *B. suis*, where spread within a farm is rapid. Likewise, in large vertically integrated systems with livestock flow from a few nucleus seed stock farms to large numbers of multiplication and commercial farms, infection can spread rapidly between farms encompassing large geographic regions, especially if the nucleus
seed stock farm or boar stud that supplies semen to the system are infected.

Environmental persistence of *Brucella* is considered of low epidemiological importance. Maintenance of *B. suis* in swine populations generally requires continued infection of susceptible hosts by direct or close contact (Olsen et al. 2010). *Brucella suis* bacteria are not found free-living in the environment and are not considered to exist as commensal bacteria. However, *Brucella* can survive in the environment for several months with cold temperatures and moisture (Walker 1999). *Brucella* can withstand drying and freezing, and also survive for considerable periods in cold climates in aborted fetuses, manure, hay, dust, equipment, and clothes (CFSPH-ISU 2007). Direct sunlight reduces survival time and *Brucella* is destroyed by pasteurization or cooking, and by most common disinfectants. Limited data suggest that *B. suis* biovar 2 may not survive outside the host as long as other brucellae (B. Garin-Bastuji, unpublished results).

The main risks associated with the introduction of porcine brucellosis in pig herds are the introduction of infected live animals, contact with infected wildlife reservoirs, and artificial insemination with semen from infected boars. Indirect transmission through mechanical vectors such as dogs, cats, wild carnivores, and migrating wild birds has been suspected but their precise involvement has never been properly assessed (EFSA 2009). *Brucella suis* has also been shown to persist in some arthropods such as ticks, but this method seems unlikely to be a common means of introduction (Alton 1990).

When *B. suis* first enters an uninfected herd, abortions often lead to tremendous contamination of feed, water, and premises. Within a few months *B. suis* can spread from a single infected animal to over 50% of the animals within the herd. Infection rates of up to 70–80% are not uncommon in the early stages of outbreaks (Bathke 1980; B. Garin-Bastuji and J. M. Blasco, unpublished results; Szulowski 1999). As the infection becomes endemic, the prevalence of infection may fall.

Like other brucellae, lateral transmission of *B. suis* can be through mucosal exposure from direct contact, aerosol, or ingestion of infected materials such as aborted fetuses or placental membranes (Alton 1990). Oral exposure is probably the main mode of entry, but conjunctival infection and penetration through breaks in the skin also occur. Spread of infection within the herd occurs primarily by consumption of the products of abortion and contamination of foodstuffs and surroundings by infected discharges, usually of uterine origin (Alton 1990). At the time of abortion, fetuses, placentas, and vaginal discharge contain myriad *B. suis* bacteria. Uterine infection and vaginal shedding usually ends by 40 days after abortion; however, in a proportion of sows may continue for 30–36 months (Manthei 1974; Manthei and Deyoe 1970).

*Brucella suis* differs from most other brucellae by its propensity for venereal transmission that also accounts for significant spread within and between herds (Alton 1990). Shedding in semen of boars is sporadic and of long duration as a consequence of *B. suis* containing granulomas in male reproductive organs. In one study, infection persisted at least 3–4 years in male reproductive organs (Manthei 1964).

*Brucella suis* can also be transmitted vertically. Piglets can be infected in utero, resulting in infected live-born pigs that can be clinically normal, or weak and subject to increased preweaning mortality. A majority of congenitally infected pigs clear the infection by 6 months of age; however, in one study, 8% of 230 pigs were bacteremic beyond 3 months and 2.5% were tissue positive at 2 years of age (Manthei et al. 1952). Congenitally infected pigs may become latent carriers that can transmit the disease in the absence of clinical and immunological evidence of infection (Acha and Szefres 2003; J. M. Blasco, unpublished results). Vertical transmission also occurs in suckling pigs through consumption of contaminated maternal milk. Shedding in milk of sows is likely sporadic and, like semen shedding in boars, can be of long duration owing to *B. suis* containing granulomas in mammary glands.

**PATHOGENESIS**

*Brucella suis* causes a long-term, usually nonfatal infection characterized by granulomatous inflammation in a variety of organs. Localization in male and female reproductive organs is responsible for the predominant clinical signs of abortion and infertility as well as the main routes of shedding. The capacity of *Brucella* spp. to invade, survive, and proliferate in cells, especially macrophages and placental trophoblasts, is the basis for pathogenicity.

The minimum infectious dose of *B. suis* is not known, but doses of $10^{4.6}$ are adequate to infect most experimentally inoculated pigs (Alton 1990). *Brucella suis* typically enters by penetration of mucous membranes or, less commonly, through breaks in the epithelium. Location is determined by route of exposure, usually oropharyngeal or intestinal mucosa with oral exposure or vaginal-uterine mucosa with venereal exposure. Studies with *B. abortus* suggest that penetration of mucosa occurs preferentially through follicle-associated epithelial cells such as M cells and/or by uptake by intraepithelial phagocytes that may also aid in transport to submucosal lymphoid patches or regional lymph nodes (Ackermann et al. 1988). Initial localization and replication is in lymphoreticular tissues draining the site of infection.

Bacteremia soon follows, due either to free bacteria or *B. suis*-laden blood-borne phagocytes. Bacteremia lasts an average of 5 weeks, but can extend up to 34 months in sows (Deyoe 1967, 1972a,b). Bacteremia
results in secondary infection of lymphoid tissues and other organs throughout the body. Lymph nodes commonly involved include mandibular, gastrohepatic, internal iliac, suprathyroid, and supramammary (Deyoe and Manthei 1967). Sites of particular predilection include mammary glands, placenta, and synovial tissues, but seminal vesicles, prostate, epididymides, testes, uterus, oviducts, liver, spleen, bones, tendons, bursae, and brain may also be affected (Alton 1990; Rosenbusch 1951). Localization of *B. suis* appears to be reduced in reproductive tissues of sexually immature gilts as compared to adults (Deyoe 1967). Compared to brucellosis in ruminants, lesions of *B. suis* in swine tend to be more widespread and more frequently involve bones and joints (Enright 1990).

In sites of localization, *B. suis* stimulates release of cytokines, chemokines, and other inflammatory mediators that induce a granulomatous inflammatory response evidenced by accumulation of lymphocytes, plasma cells, macrophages, and multinucleate giant cells (Foster and Ladds 2007; Schlaf and Miller 2007). Enlarging granulomas undergo caseous necrosis centrally and eventually are encapsulated by connective tissue.

The mechanisms whereby *Brucella* spp. avoid elimination in the extracellular environment, enter macrophages (and other cells) and avoid destruction in phagolysosomes, and successfully replicate in a protected intracellular niche, are only partially understood. The O-side chain on the lipopolysaccharide (O-LPS) of virulent smooth strains is important for extracellular survival in that it has low immunogenicity and is relatively resistant to complement-mediated bacterial lysis and to bactericidal cationic peptides (Allen et al. 1998; Lapaque et al. 2005). The O-LPS has also been implicated in helping *Brucella*-infected cells inhibit apoptosis (Gross et al. 2000) and evade the host immune system (Lapaque et al. 2005). Specifics of immune modulation by *Brucella* spp. are discussed under the section “Immunology” below.

Whereas opsonized *Brucella* cells are internalized by Fc receptors and rapidly degraded in macrophages (Gorvel and Moreno 2002), nonopsonized bacteria enter by a different mechanism that involves lipid rafts in the host cell membrane (Naroe and Porte 2002). Internalized *Brucella* initially localize in phagosomes. Early acidification of the phagosomes (a normal prelude to fusion with lysosomes) induces a type IV secretion system (VirB) in *Brucella* that inhibits phagosome maturation, prevents lysosomal fusion, and is vital for modification and trafficking of the phagosome into a specialized endoplasmic reticulum-like vacuole for bacterial replication, the so-called brucellasome (Celli 2006). Most brucellae (approximately 70–85%) are eliminated by phagolysosome fusion, but creation of the brucellasome allows intracellular survival of some bacteria that ultimately replicate (Pappas et al. 2005). Survival of *Brucella* in brucellasomes is also enhanced by their use of stationary-phase physiology and siderophores to scavenge iron in the nutrient-poor environment (Roop et al. 2003) and by their ability to resist oxidative killing utilizing multiple molecular mechanisms to detoxify free radicals, including superoxide dismutase, alkyl hydroperoxide reductase C, and others.

Severity of disease depends on the virulence and dose of the infecting strain as well as the resistance or susceptibility of the host as determined by innate and acquired immunity mechanisms (Enright 1990). The virulence of *B. suis* biovars 1 and 3 are thought to be similar in swine (Alton 1990). It is also believed that virulence of biovar 2 is similar to 1 and 3 in swine, but there is no experimental proof. Both genders and all ages are believed to be equally susceptible to infection by *B. suis* (Alton 1990). In contrast, limited evidence suggests that Duroc and Jersey Red crosses may be less susceptible to disease when challenged with *B. suis* (Cameron et al. 1942).

**CLINICAL SIGNS**

When *B. suis* first enters an uninfected herd, abortions, increased perinatal mortality, and infertility may have significant economic impacts. In endemically infected herds, *B. suis* usually causes only mild-to-moderate clinical signs and often goes undetected. Reproductive failure characterized by abortion, stillbirth, and infertility in sows, congenitally infected pigs with reduced viability, and infertility in boars are the main clinical features of epidemic *B. suis* infection. However, most infected pigs do not exhibit clinical sickness. Pyrexia or anorexia is usually absent and leukograms typically remain normal in acute and chronic *B. suis* infections.

The main clinical signs of brucellosis are not pathognomonic and other causes of reproductive failure should be considered in a differential diagnosis. The more significant include porcine reproductive and respiratory syndrome virus (Chapter 31), pseudorabies virus (Chapter 28), porcine circovirus (Chapter 26), porcine parovirus (Chapter 29), classical swine fever virus (Chapter 38), swine influenza virus (Chapter 40), porcine enteroviruses (Chapter 42), *Leptospira* spp. (Chapter 56), and *Erysipelothrix* spp. (Chapter 54) (Dial et al. 1992; Kirkbride 1990; Manthei 1974).

Abortion may be only a minor component of the disease presentation under field conditions (Johnson and Huddleson 1931), and may occur at any stage of gestation. Infection of females at breeding through natural service by infected boars or by artificial insemination using contaminated semen causes placental infection that impairs oxygen and nutrient delivery and results in embryonic death and abortion as early as 21–27 days of gestation. The small size of the abortus may go
unnoticed, and the first evidence of fetal loss may be irregular returns to estrus beginning 40–45 days after breeding. In one study where naïve females were exposed by artificial insemination with *B. suis*-positive semen, abortions began as early as 22 days after insemination and irregular returns to estrus were observed from 30 to 45 days after infection (Manthei and Deyoe 1970).

Experimental oral or parenteral inoculation of pregnant females after day 40 of gestation results in fetal infection, with subsequent abortions observed mid-to late gestation. Abortions occurring under field conditions are generally associated with oral exposure between 50 and 100 days of gestation. Occasionally, sows expel stillborn or weak fetuses from 100 to 110 days of gestation (Manthei 1974). Metritis with vaginal discharges and placental retention may be observed in a portion of sows that abort. Infertility may be observed and is related to duration of infection and severity of lesions in the uterus (Manthei and Deyoe 1970; Thomsen 1934). Persistence of uterine infection is usually 30–40 days after abortion, but may be as long as 4–36 months (Manthei 1974).

Pigs born congenitally infected with *B. suis* suffer increased neonatal mortality (Hutchings et al. 1946a,b). In congenitally infected pigs that survive and in those that are infected any time prior to the onset of sexual maturity, clinical signs are rare and generally limited to swollen joints and lameness. After sexual maturity, the course of infection appears to be longer, with greater chronicity associated with infections of males as compared to females. In mature boars, *B. suis* was recovered from tissue samples of 66.7% at 6 months after experimental infection with 50% remaining culture positive at 42 months. In comparison, approximately 25% of experimentally infected females were culture positive between 6 and 42 months after experimental infection (Deyoe 1972a). Data from one study of feral swine suggest a similar sex difference with *Brucella* recovered from 93% of males as compared to 61% of females (Stoffregen et al. 2007).

In intact boars, although *B. suis* often localizes in accessory sexual organs, testicles, and epididymides, clinical evidence is only rarely observed as enlarged or atrophic nodular testicles and/or epididymides. Use of infected boars results in lower conception rates and fewer live-born pigs. However, depending on whether lesions are unilateral or bilateral in reproductive organs, infected males may or may not demonstrate reduced fertility or libido even when high numbers of *B. suis* are present in the semen (Hutchings and Andrews 1946; Manthei and Deyoe 1970).

Swollen joints and tendon sheaths, accompanied by lameness and incoordination, may be observed. Less common signs include posterior paralysis, spondylitis, and abscess formation in various organs. (Enright 1990; Schlafer and Miller 2007).

**LESIONS**

*Brucella suis* incites granulomas that may appear as abscesses when less chronic, but given more time well-encapsulated granulomas with caseous centers form. Generally, granulomas appear as isolated or coalescing cream to yellow nodules in affected tissues. As already mentioned, sites of particular predilection include mammary glands, placentas, and synovial tissues, but seminal vesicles, prostate, epididymides, testes, uterus, oviducts, liver, spleen, bones, tendons, bursae, brain, and lymph nodes may also be affected (Alton 1990; Rosenbusch 1951). Microscopically granulomas are composed of central amorphous caseous necrotic debris that is surrounded by a variable rim composed of mixed aggregates of macrophages, epithelioid macrophages, multinucleate giant cells, lymphocytes, and plasma cells. At the periphery, circumferential fibroblasts and collagen form a capsule.

In the uterus and uterine tubes, multifocal, miliary, 2–3 mm yellow nodules may be observed in the mucosa from which caseous exudate can be expressed when incised. Nodules may coalesce to form plaques that thicken the mucosa. In the oviducts, nodules may lead to obstruction and pyosalpinx. The endometrium is typically expanded by lymphoplasmacytic infiltrates, including hyperplastic lymphocytic nodules. Suppurative infiltrates are present in superficial endometrial glands and in the uterine lumen. Partial desquamation or squamous metaplasia, including rete pegs and intercellular bridges, may be observed in the epithelium of the uterus or superficial glands. Uterine ligaments may also contain small and irregular granulomas on their surface (Schlafer and Miller 2007).

In the pregnant uterus, miliary lesions develop into a superimposed diffuse, catarrhal endometritis with hemorrhage and edema, and a catarrhal exudate that contains large numbers of bacteria. Lesions in aborted fetuses and placenta are uncommon (Manthei and Deyoe 1970). In the fetus, there can be blood-tinged subcutaneous edema, particularly around the umbilicus, and infusions within body cavities (Schlafer and Miller 2007).

In intact males, orchitis and epididymitis are characterized by multiple abscesses and/or granulomas in parenchyma, occasionally accompanied by fibrinopurulent or hemorrhagic periorchitis. External appearance of testes is usually normal, but granulomas may be apparent on cut surfaces. Occasionally testes may be enlarged or, when very chronic, testicular atrophy and a variable degree of enlargement of the epididymides are characteristic. Infection of accessory glands can be associated with vesicular gland hypertrophy, and microabscesses in vesicular glands, prostate, or bulbourethral glands (Foster and Ladds 2007). Mineralized foci may also be in the testes and accessory sexual glands and organs, particularly in the epididymides.
and seminal vesicles. Single or multiple spermatoceles may be present in connective tissues that appear as abscesses filled with creamy or caseous exudate. Hemorrhage and suppurative inflammation in the tunica vaginalis is also a frequent finding resulting from rupture of the spermaticulo.

Articular lesions are characterized by purulent or fibrinopurulent synovitis affecting the compound and large joints of the limbs. Osteomyelitis is typically observed in the lumbar vertebra and is commonly associated with destruction of the intervertebral cartilages. Compression of the spinal cord or pathological fractures with damage to the cord may result in paraparesis or paralysis. Bone lesions are usually granulomatous with dry caseous necrosis but can become suppurrative leading to abscessation (Schlafer and Miller 2007).

**DIAGNOSIS**

**Direct Diagnosis**

The presumptive diagnosis of brucellosis in pigs can be made by microscopic examination of Stamp’s stained smears from vaginal swabs, placentas, and/or aborted fetuses. However, this test lacks sensitivity and specificity.

Bacterial culture is the definitive method for identifying brucellosis in swine. Attempting bacteriological isolation on a small sample of targeted lymph nodes is likely to detect as many positives as serological diagnosis (Alton 1990; Rogers et al. 1989). Isolation of *Brucella* spp. from swine samples or tissues not only demonstrates an in vivo infection, it eliminates concerns regarding cross-reactive serological responses and provides material for species- and biovar-specific testing.

For culture, the most practical samples taken from living animals include vaginal secretions (swabs), milk, semen, fetal membranes, and samples from aborted fetuses (stomach contents, spleen, and lung). Preferred samples from dead pigs are spleen and lymph nodes from the head, mammary, and genital tract. *Brucella suis* can also be isolated from testes, epididymides, vesicular glands, prostate, and bulbourethral glands of boars.

*Brucella suis* grows well at 98.6°F (37°C) in air on general multipurpose culture media (i.e., blood agar) without the addition of blood or serum. However, selective media is recommended and plates should be incubated at 98.6°F (37°C) with 5–10% CO₂, because contaminants are frequent and brucellosis in swine can also be caused by other, more fastidious bacterial species. *Brucella suis* biovar 2 is more sensitive than biovars 1 and 3 to antibiotics contained in common *Brucella*-selective medias. Therefore, the simultaneous use of both Farrell’s and modified Thayer-Martin’s medium (Marin et al. 1996) is recommended for this biovar.

Colonies generally appear after 3–4 days, but cultures should not be discarded as negative until 8 days of incubation. *Brucella suis* colonies are morphologically indistinguishable from other smooth brucellae, and can be presumptively identified by agglutination with monospecific antisera since the three most relevant biovars (1, 2, and 3) always agglutinate with the A but not the M monospecific antiserum (Alton et al. 1988; OIE 2008). It is recommended that species and biovar identification be performed in a *Brucella* reference laboratory.

The direct diagnosis of *B. suis* from samples has been also attempted using several PCR-based protocols (Bouaada et al. 2009; OIE 2008). However, the sensitivity of these methods is currently significantly lower than that of culture.

**Indirect Diagnosis**

There is little information available on the diagnostic value of serological tests for brucellosis in swine. None of the conventional serological tests used for the diagnosis of brucellosis in domestic ruminants are fully reliable for diagnosing swine brucellosis when used on individual pigs. It is recommended that these serological tests be interpreted on a herd or collective basis. In a herd with positive serological responses, other diagnostic procedures, such as bacteriological isolation or molecular assays which detect *Brucella* DNA, should be utilized to confirm serological data.

The major antigen involved in the serological response against infection caused by smooth brucellae in all animal species is the smooth lipopolysaccharide (S-LPS). Most standard brucellosis serological tests were initially developed for detection of *B. abortus* infections in cattle and use the polysaccharide O side-chain of LPS (O-PS) from *B. abortus* as an antigen. Because the structural component of the O-PS is similar to epitopes on *Yersinia enterocolitica* O:9, *Escherichia coli* O:157, and others (Bundle et al. 1989), cross-reactions occur. *Yersinia enterocolitica* O:9 infection in pigs is quite widespread in many countries and, accordingly, a major source of false-positive serological reactions (EFSA 2009). This is the most likely explanation for reports of positive responses by swine on brucellosis tests in the absence of epidemiological or bacteriological evidence of infection.

Sensitivity and specificity estimates of serology tests should be related to the “gold standard” of bacterial isolation, yet recovery of *B. suis* from infected animals is not 100% and may be significantly lower in chronic infections. This may explain the great variation in estimates of sensitivity and specificity for these tests in swine as reported in scientific literature. Ranges for estimates of sensitivity of standard tests are: standard tube (SAT; 51.1–100%), mercaptoethanol (38.5–100), rivanol (23.1–100), complement fixation test (CFT; 49.1–100), card test (Rose-Bengal test [RBT]; 20–100), buffered plate antigen (61–77.1%), and fluorescent polarization assay (FPA; 63–98.9%) (Ferris et al. 1995;

The SAT should not be used for diagnosis of brucellosis in pigs because of false-positives resulting from nonspecific antibodies, thought to be immunoglobulin M (IgM), in swine serum. The sensitivity of the CFT is low because swine complement interacts with guinea-pig complement to produce anticomplementary activity. In fact, the diagnostic sensitivity of the CFT is significantly lower than that of the RBT (J. M. Blasco and B. Garin-Bastuji, unpublished results; Ferris et al. 1995; Rogers et al. 1989). However, despite relative insensitivity, the CFT has high specificity for identifying true infected herds when interpreted on a collective basis (L. Dieste et al., unpublished results).

An expert panel of the European Food Safety Authority recently conducted meta-analytical estimates of diagnostic sensitivity and specificity of serological tests for porcine brucellosis based on the data mined from a systematic literature review in order to recommend best tests. Conclusions suggest that indirect (iELISA) and competitive (cELISA) enzyme-linked immunosorbent assays (ELISAs) are suitable candidates based on their high sensitivity and specificity. However, ELISAs have not been fully standardized for use in pigs. Primary reference standards are currently being developed by the World Organization for Animal Health (OIE). Formal procedures such as those implemented by the OIE should be considered for accreditation of candidate tests for the purpose of control of B. suis in pigs (EFSA 2009).

The delayed-type hypersensitivity reaction (allergic skin test) based on the use of S-LPS-free Brucella cytoplasmic proteins (widely known as Brucellin), extracted from rough B. melitensis strain B115 (Bhongbhibhat et al. 1970), is of great diagnostic interest since these proteins are not present in Y. enterocolitica O:9. In addition to bacterial culture, the skin test is the only other confirmatory test suitable to fully discriminate between true brucellosis infections and the infections caused by Y. enterocolitica O:9 or other cross-reacting bacteria. Moreover, Brucellin does not stimulate the formation of antibodies that would be reactive in RBT, CFT, or ELISAs. The allergic skin test was developed for use in ruminants but it has been proven also very effective for confirming the disease at the herd level in pigs (EFSA 2009).

**IMMUNITY**

Although most smooth strains induce a vigorous immune response after infection, the chronic nature of Brucella infections indicates that these immune responses are insufficient to eliminate Brucella from their intracellular niche (Olsen et al. 2010). The capacity of Brucella to inhibit or avoid components of the host immune responses appears to be a critical determinant in pathogenesis. The lipid A of the LPS of Brucella strains stimulates a greatly reduced inflammatory response in mammalian hosts than does the endotoxin of other gram-negative bacteria (Barquero-Calvo et al. 2007). The unusual chemical composition of the O-chain of the LPS of smooth strains also plays a direct role in inhibiting host immune responses. Specifically, macrophages have great difficulty in degrading the perosamine O-chain of the Brucella LPS, and undigested O-chain directly inhibits the ability of infected macrophages to present antigens to T-cells via the major histocompatibility complex (MHC) class II pathway (Forestier et al. 2000).

Cellular immunity associated with Th1 responses (interleukin [IL]-2, IL-12, IL-18, interferon-γ [IFN-γ], tumor necrosis growth-α [TNF-α], and tumor necrosis growth-β [TNF-β] cytokines) and cytolytic activity by some T-cell subsets are believed important for immunity against intracellular pathogens (Tizard 2009). A critical component of immunity against B. suis is the production of IFN-γ, considered the crucial effector for activating macrophages for efficient killing and inhibition of intracellular replication. In human brucellosis, chronicity of infection has been associated with reductions in production of IFN-γ and increase in cells expressing IL-13, a Th2 cytokine (Rafiei et al. 2006). Pretreatment of monocytes with IFN-γ yields anti-Brucella activity, not only by increased bacterial killing but also by preventing intracellular replication (Jiang et al. 1993). Recent discoveries in monocyte biology revealed that IFN-γ stimulates phagosome maturation, phagosome/lysosome fusion, and increases rates of autophagy, which degrades intracellular vesicles and organelles.

The lipopolysaccharide and other antigens of B. suis are highly immunogenic in eliciting humoral responses in infected swine. The lipopolysaccharide of Brucella is a prototypical T-cell-independent antigen because it can directly activate B cells to produce antibody without the aid of helper T cells. Antibodies have been considered to be beneficial against Brucella through their opsonic properties and their complement-mediated killing abilities, mediating antibody-dependent cellular cytotoxicity, and by binding to bacterial receptors to prevent adherence of bacteria to host tissues. Opsonization is considered as the principal mechanism involved in protection by specific antibodies because it enhances phagocytic uptake of brucellae and intracellular killing. The pattern of antibody production in pigs infected with B. suis has not been properly established. However, it should be similar to that induced by other Brucella infections, with IgM antibodies predominating in the
first 2 weeks after infection, and immunoglobulin G (IgG) isotypes increasing slowly during the first 3 weeks of infection. The relative contribution of IgG versus IgM antibody isotypes in brucelcidal functions of macrophages has not been evaluated. The role of antibodies with regard to complement-mediated killing mechanisms is questionable because some Brucella spp. strains are not susceptible to complement (Baldwin and Goenka 2006; Moreno and Gorvel 2004).

**PREVENTION AND CONTROL**

At the present time, there are not sufficient data to recommend any vaccine as being efficacious for preventing brucellosis in swine. Although China once reportedly used their B. suis strain 2 vaccine orally in swine (Xin 1986), data on its safety and efficacy are not available, and recent reports suggest it is not currently being used in swine in China (Deqi et al. 2002). Previous reports suggesting that oral vaccination with $10^{11}$ colony-forming units (CFU) of B. abortus strain RB51 was efficacious in swine (Edmonds et al. 2001) differ from experimental and field data which found that parental vaccination of RB51 did not protect pigs from brucellosis (Olsen and Stoffregen 2005).

Due to the prevalence of B. suis biovar 2 in wild pigs and hares in most European countries, swine reared in open-air breeding systems or free-ranging systems are the most frequently infected (EFSA 2009). Risk of infection depends on the prevalence of infection in wildlife in the area and on the level of farm biosecurity to prevent contact with infected wildlife reservoirs. Currently, with the exception of increasing hunting pressure to reduce population densities, no measures are available to reduce or eliminate the infection in wildlife reservoirs. Therefore, the only means to minimize the risk of transmission to pigs in outdoor systems is to implement biosecurity measures that prevent contacts with wildlife such as double hare- or wild boar-proof fencing (EFSA 2009). Current research in swine evaluating immune-contraception and orally deliverable vaccines may eventually provide the needed tools to help in managing the disease in wildlife reservoirs.

Owing to the present lack of effective tools to manage the disease, whole-herd depopulation appears to be the only viable option to eradicate brucellosis from domestic swine herds. As serological surveillance is best performed in swine on a herd basis, regulatory efforts to control or eradicate B. suis in swine should be directed toward herds, rather than individual animals (Olsen and Stoffregen 2005). Biosecurity of B. suis-free herds is paramount to prevent introduction or reintroduction from wildlife or other infected farms (Acha and Szyfres 2003).

In the absence of total depopulation, antibiotic treatment, alone or in combination with test-and-slaughter, is the only suitable alternative to minimize the clinical and economical impacts of the disease. However, as no published reference is available on the efficacy of antibiotics for treating brucellosis in pigs, publications treating brucellosis in other animal species and humans are the only standard for devising porcine therapies (Ariza et al. 2007; Marin et al. 1989; Nicoletti et al. 1985). Treatment using oxytetracycline (20 mg/kg per body weight [BW] for 4–6 weeks) combined with aminoglycosides (streptomycin $-20$ mg/kg BW daily for 2–3 weeks or gentamicin $-0.5$ mg/kg daily for 2 weeks) is considered the best regimen for treating brucellosis in animals (Grilló et al. 2006; Marin et al. 1989; Nicoletti et al. 1985). However, the parenteral administration of aminoglycosides may be problematic in pigs. Recent data suggest that prolonged oral treatment with oxytetracycline (20 mg/kg BW for at least 90 days) is cost-effective and minimizes the clinical impact of B. suis biovar 2 while maintaining productivity on infected farms (J. M. Blasco, unpublished results). In addition to antibiotic treatments, increased culling of sows that abort or are infertile as well as slaughter of skin-test-positive pigs could significantly reduce prevalence of disease. However, this method of control is not validated by published experimental data.

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INTRODUCTION

Clostridia are strict to oxygen-tolerant anaerobic gram-positive, spore-forming rods. *Clostridium perfringens* types A and C, *Clostridium difficile*, *Clostridium tetani*, *Clostridium novyi*, *Clostridium botulinum*, *Clostridium septicum*, and, rarely, *Clostridium chauvoei*, are frank pathogens of swine that may also invade wounds or lesions (Table 52.1). Many reside in the intestinal tract.

ENTERIC INFECTIONS

Enteric infections are caused by *C. perfringens* and *C. difficile*. *Clostridium perfringens* is divided into five toxigenotypes (A–E) based on production of major toxins that include alpha (*C. perfringens* alpha toxin [CPA]), beta (*C. perfringens* beta toxin [CPB]), epsilon (*C. perfringens* epsilon toxin [ETX]), and iota (*C. perfringens* iota toxin [ITX]) (Table 52.2). Type A strains are part of the intestinal microbiota of warm-blooded animals and are thus common in the environment. Other types are less commonly found in normal animals.

*Clostridium perfringens* Type C Enteritis

Infections with *C. perfringens* type C occur worldwide (Azuma et al. 1983; Barnes and Moon 1964; Field and Gibson 1955; Hogh 1965; Matthias et al. 1968; Morin et al. 1983; Plaisier 1971; Szent-Ivanyi and Szabo 1955). Type C causes fatal hemorrhagic necrotic enteritis in young piglets. Hallmark lesions are profound, usually segmental, transmural necrosis and emphysema in the small intestine, sometimes extending into the cecum and proximal colon, but toxin effects are also likely systemic.

**Etiology and Epidemiology.** Type C is a primary pathogen, but can colonize lesions of other diseases, such as transmissible gastroenteritis (TGE). Types B (Bakhtin 1956) and D (Harbola and Khera 1990) have been reported from type C-like syndromes, but the vagaries of the in vivo typing system suggest the need for prudence in accepting these as even rare causes of severe enteritis in neonatal pigs.

Infection may be transmitted from piglet to piglet, but the ultimate source is likely sow feces. Preventing introduction of disease by screening of replacement stock is likely not a viable option. Type C is a minor component of the sow’s intestinal microbiota, but piglets act as enrichment vessels, in which small numbers of type C outcompete other bacteria and multiply to large numbers, with resulting disease production.

The organism persists in the environment mainly as spores, which are resistant to heat, disinfectants, and ultraviolet light.

Disease may appear as early as 12 hours after birth, but is most common in 3-day-old piglets. Onset is rare in pigs older than 1 week (Bergeland et al. 1966; Matthias et al. 1968; Meszaros and Pesti 1965). Type C enteritis occurs epidemically in nonvaccinated populations (Bergeland et al. 1966) and can reach a prevalence of 100% of litters. The case fatality rate varies with the form of the disease, but 100% mortality in litters of nonimmune gilts is not unusual, and total herd mortality may be as high as 50–60% (Bergeland et al. 1966; Hogh 1967b). As herd immunity rises due to exposure of sows to infected piglets, disease may become endemic. Milder cases may then occur over a period of months, but acute disease continues to occur due to...
mucosal lesions are intensely hemorrhagic, with gas bubbles in the intestinal wall. Mesenteric lymph nodes may be reddened. Microscopic examination reveals necrotic jejunal villi and a surface covered by a carpet of large gram-positive bacilli. Crypt epithelium may be necrotic, and there is profuse hemorrhage throughout the mucosa and submucosa.

Acutely affected piglets may survive for 1–2 days after onset of clinical signs. They have reddish-brown diarrhea containing gray shreds of tissue debris, and may be dehydrated. There may be perineal scalding, with adherent reddish feces. Nursing is minimal, and these piglets rapidly become gaunt and weak. Gross lesions (Figure 52.1) are usually localized, and emphysema may be observed in sharply demarcated jejunal segments. Acute fibrinous peritonitis may occur, with loose adherence among adjacent segments of jejunum (Figure 52.2). The intestinal wall is usually thickened and yellow or grayish, with blood and necrotic debris. Deposition of urate crystals in kidney is common. Microscopic examination reveals widespread villous necrosis. The exposed submucosa is carpeted by bacteria, shed epithelial cells, fibrin, and degenerating inflammatory cells. Submucosal vessels are necrotic and many contain thrombi. Emphysema may be evident in submucosa, tunica muscularis, and under the serosa. Large gram-positive bacteria may be present in deeper layers of intestinal wall.

Diarrhea is nonhemorrhagic in pigs with subacute disease. These active, alert, and appetent animals become progressively emaciated; they may be thin and overall shortfalls in herd immunity (as with repeated introduction of naïve gilts or sows).

**Clinical Signs and Lesions.** Clinical signs vary with immune status and age of affected piglets. *Peracute* affected piglets develop hemorrhagic diarrhea, with perineal staining. Affected piglets are weak, reluctant to move, and become rapidly moribund, risking crushing by the sow. Rectal temperature falls to 95°F (35°C) or below, and abdominal skin may blacken before death. Many piglets are found dead within 12–36 hours of birth. Death occurs in some animals without diarrhea. The most immediate and striking findings at necropsy are intensely hemorrhagic small intestines (Figure 52.1) and bloodstained fluid in the abdominal cavity. Lesions are typically in jejunum and ileum, but may extend anterior to within a few centimeters of the pylorus and posterior to the proximal colon. Alternatively, only a few centimeters of jejunum may be affected. Gross mucosal lesions are intensely hemorrhagic, with gas bubbles in the intestinal wall. Mesenteric lymph nodes may be reddened. Microscopic examination reveals necrotic jejunal villi and a surface covered by a carpet of large gram-positive bacilli. Crypt epithelium may be necrotic, and there is profuse hemorrhage throughout the mucosa and submucosa.

*Acutely affected piglets* may survive for 1–2 days after onset of clinical signs. They have reddish-brown diarrhea containing gray shreds of tissue debris, and may be dehydrated. There may be perineal scalding, with adherent reddish feces. Nursing is minimal, and these piglets rapidly become gaunt and weak. Gross lesions (Figure 52.1) are usually localized, and emphysema may be observed in sharply demarcated jejunal segments. Acute fibrinous peritonitis may occur, with loose adherence among adjacent segments of jejunum (Figure 52.2). The intestinal wall is usually thickened and yellow or grayish, with blood and necrotic debris. Deposition of urate crystals in kidney is common. Microscopic examination reveals widespread villous necrosis. The exposed submucosa is carpeted by bacteria, shed epithelial cells, fibrin, and degenerating inflammatory cells. Submucosal vessels are necrotic and many contain thrombi. Emphysema may be evident in submucosa, tunica muscularis, and under the serosa. Large gram-positive bacteria may be present in deeper layers of intestinal wall.

Diarrhea is nonhemorrhagic in pigs with subacute disease. These active, alert, and appetent animals become progressively emaciated; they may be thin and

**Table 52.1.** Major clostridia and associated syndromes affecting swine

<table>
<thead>
<tr>
<th>Clostridium Species</th>
<th>Syndrome</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. perfringens</em> type C</td>
<td>Neonatal hemorrhagic and necrotic enteritis</td>
</tr>
<tr>
<td><em>C. perfringens</em> type A</td>
<td>Neonatal necrotizing enteritis, gas gangrene</td>
</tr>
<tr>
<td><em>C. difficile</em></td>
<td>Neonatal colitis</td>
</tr>
<tr>
<td><em>C. septicum</em></td>
<td>Malignant colitis</td>
</tr>
<tr>
<td><em>C. chauvoei</em></td>
<td>Blackleg</td>
</tr>
<tr>
<td><em>C. novyi</em></td>
<td>Sudden death in sows</td>
</tr>
<tr>
<td><em>C. tetani</em></td>
<td>Tetanus</td>
</tr>
<tr>
<td><em>C. Botulinum</em></td>
<td>Botulism</td>
</tr>
</tbody>
</table>

**Table 52.2.** Production of so-called major toxins by types of *Clostridium perfringens* and associated diseases

<table>
<thead>
<tr>
<th>Toxin Type</th>
<th>Major Diseases</th>
<th>Major Toxins</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Food poisoning, poultry necrotic enteritis, lamb enterotoxemia, porcine neonatal necrotizing enterocolitis, bovine neonatal hemorrhagic enteritis</td>
<td>Alpha</td>
</tr>
<tr>
<td>B</td>
<td>Lamb dysentery, ovine chronic enteritis, bovine/equine hemorrhagic enteritis</td>
<td>Alpha, beta, epsilon</td>
</tr>
<tr>
<td>C</td>
<td>Fowl necrotic enteritis, hemorrhagic or necrotic enterotoxemia in piglets, lambs, calves, goats, foals, acute enterotoxemia (“struck”) in adult sheep</td>
<td>Alpha, beta</td>
</tr>
<tr>
<td>D</td>
<td>Ovine enterotoxemia, caprine enterocolitis, bovine enterotoxemia (calves, possibly adults)</td>
<td>Alpha, epsilon</td>
</tr>
<tr>
<td>E</td>
<td>Bovine (possibly ovine) neonatal enterotoxemia</td>
<td>Alpha, iota</td>
</tr>
</tbody>
</table>
Pathogenesis. *Clostridium perfringens* has a very short generation time, allowing type C organisms to multiply to numbers approaching $10^8$–$10^9$ per gram of contents in only a few hours (Ohnuna et al. 1992). Attachment to jejunal epithelial cells at villous apices (Arbuckle 1972; Walker et al. 1980) is followed by desquamation of these cells and proliferation of the organism along the basement membrane. Necrosis of the villous lamina propria is extensive, and hemorrhage accompanies necrosis. The necrotic zone advances to involve crypts, muscularis mucosa, and submucosa, and occasionally the muscular layers. Perforation of intestinal wall leads to peritonitis as well as emphysema (sometimes with thrombosis) in muscle layers, beneath the peritoneum, and in mesenteric lymph nodes. Bacteria remain adhered to necrotic villi or are shed into intestinal lumen with cell debris and blood, and sporulation may be observed (Kubo and Watase 1985).

The lethal and necrotizing beta toxin (CPB) is the key factor in pathogenesis of type C infections (Hogh 1967a; Warrack 1963). A recently developed mouse model has opened new opportunities for a specific study of type C enterotoxemias (Uzial et al. 2009). Toxoid vaccines protect against infection, but it is worth noting that these are crude, containing many clostridial antigens other than beta toxoid. Experimental evidence for the primacy of CPB in pathogenesis is equivocal. Disease was reproduced by oral administration of toxin, but not in type C-free pigs (Field and Goodwin 1959). Typical lesions appeared in gut loops inoculated with broth cultures of type C (Bergeland 1972), but necrosis was not produced by inoculation with CPB alone. Numerous potentially confounding factors are found in much of the published literature, including use of older pigs as experimental animals. Trypsin secretion deficiencies and colostral protease inhibitors probably account for susceptibility of piglets less than 4 days old.

CPB has been detected in both intestinal contents and peritoneal fluid of affected pigs, suggesting that death is primarily due to effects of intestinal damage and toxemia. Toxin-containing preparations administered intravenously (IV) in high doses cause sudden death, and lower doses cause polioencephalomalacia,
adrenal cortical necrosis, nephrosis, and pulmonary edema. Hypoglycemia and secondary bacteremia may raise the mortality rate (Field and Goodwin 1959; Hogh 1967b).

*Clostridium perfringens* beta2 toxin (CPB2), produced by most or all porcine type C isolates, may also play a role in pathogenesis (see below, under the section “*Clostridium perfringens Type A Enteritis*”).

**Diagnosis.** Compatible clinical signs, pattern of mortality, and nature of gross and microscopic lesions are sufficient basis for a presumptive diagnosis of type C enteritis. Diagnosis of chronic disease may depend upon a more detailed history of herd infection, elimination of other causes of necrotic enteritis, and detection of type C organisms in lesions. Coccioidosis (infection with *Isospora suis*) and other causes of villous atrophy (e.g., rotavirus infection, TGE, and porcine epidemic diarrhea) may induce lesions which become colonized by *C. perfringens* type C. It is especially important in subacute and chronic cases to distinguish type C infections from those caused by *C. perfringens* type A, and this can be done only by bacteriological culture and toxin detection or genotyping.

Laboratory aspects of diagnosis include bacteriological culture and examination of smears of intestinal contents and mucosal lesions and histological sections of intestine for large, gram-positive rods. *Clostridium perfringens* is not an efficient sporulator, but ovoid to eccentric spores are sometimes observed. Colonies after 24 hours’ incubation on horse or bovine blood agar are usually 3–5 mm in diameter, grayish, and circular. The organism usually produces an inner, complete zone of hemolysis, caused by theta toxin (perfringolysin O, PFO), and a less complete outer zone caused by CPA. An anaerobic organism producing a double zone of hemolysis and being in morphology a large gram-positive rod, is *C. perfringens*. Genotyping is a vital part of isolate characterization.

Microscopic lesions are almost pathognomonic. Diagnosis can be confirmed by demonstration of CPB in eluates of hemorrhagic intestinal contents or in peritoneal fluid. Mouse protection tests are seldom used today, having been replaced by enzyme immunoassays (Havard et al. 1992). Polymerase chain reaction (PCR) methods to detect genes for the major toxins are an acceptable substitute for toxin detection in typing of isolates, and can provide useful supportive findings in diagnosis (Buogo et al. 1995; Meer and Songer 1997; Songer and Meer 1996).

In the absence of methods to detect toxins in pathological specimens, bacterial isolation and genotyping are useful aids in establishing a diagnosis. In most cases, type C is isolated in large numbers and in pure culture from scrapings of the intestinal mucosa. Results may be misleading in rare situations, in that type C can be found as a secondary agent, colonizing lesions of TGE and other viral diseases. Cultures may be negative in chronic cases, and when positive, usually yield a mixture of type C and type A organisms. Thus, it is important to determine genotype or phenotype of multiple primary isolates. Diagnosis must be based, in some cases, upon findings in the herd as a whole, rather than from examination of individual diseased animals.

**Treatment and Prevention.** Treatment is of little use in animals with clinical signs (Hogh 1967b; Szabo and Szent-Ivanyi 1957), and prophylaxis is the preferred approach. Passive immunization with equine antitoxin can be useful for the protection of litters of nonimmune sows in an outbreak, and protection may last as long as 3 weeks (Ripley and Gush 1983). Antitoxin should be injected parenterally as soon after birth as possible. Oral antimicrobials, such as ampicillin or amoxicillin, may also be given prophylactically, beginning immediately after birth and continuing daily for 3 days. There are reports of antimicrobial resistance in *C. perfringens*, and tetracycline resistance plasmids have been identified (Rood et al. 1985). However, the organism remains uniformly susceptible to penicillins. Ceftiofur may be an alternative for treatment of piglets, and bacitracin methylene disalicylate can be administered to sows before and after farrowing to prevent infection of piglets.

Prevention is best achieved by vaccination of sows with type C toxoid, at breeding or midgestation and 2–3 weeks before farrowing (Kennedy et al. 1977). Commercial toxoid vaccines are quite effective, and vaccination usually eliminates the disease within one farrowing cycle. Tenfold reductions in mortality are common (Ripley and Gush 1983), assuming normal responses of the sow to vaccination (Matishek and McGrinley 1986) and ingestion by piglets of adequate amounts of colostrum. Booster injections should be given about 3 weeks before subsequent farrowings. Toxoid may also be of value in protecting weaned pigs (Meszaros and Pesti 1965).

**Clostridium perfringens Type A Enteritis**

*Clostridium perfringens* type A is included in the microbiota of the swine intestine (Mansson and Smith 1962), but strains properly equipped to do so also cause enteric disease. Neonatal pigs and, rarely, weaned pigs (Jestin et al. 1985) are affected worldwide (Amtsberg et al. 1976; Collins et al. 1989; Nabuurs et al. 1983; Ramisse et al. 1979; Secasiti 1984). The association of type A with hemorrhagic bowel syndrome is not yet adequately supported by experimental evidence.

**Etiology and Epidemiology.** *Clostridium perfringens* type A resembles type C in culture, but it produces CPA as its sole major toxin (Table 52.2). Recent information (Bueschel et al. 2003; Waters et al. 2003) suggests a role for beta2 toxin (CPB2) in porcine clostridial enteritis,
and nearly all type A strains from this condition produce this protein (Bueschel et al. 2003; Waters et al. 2003; see below). Clinical signs have been reproduced experimentally (Johannsen et al. 1993a; M. A. Anderson and J. G. Songer, unpublished results).

Human clostridial food poisoning usually occurs when *C. perfringens* enterotoxin (CPE)-producing strains grow in slowly cooling meats following cooking. Involvement of CPE-producing strains in animal disease is apparently uncommon (Collins et al. 1989; Estrada Correa and Taylor 1989; Miwa et al. 1997; van Damme-Jongsten et al. 1990). CPE-positive food poisoning strains are most commonly associated with poultry, but porcine strains can also be fully enterotoxigenic. Strains with the gene for CPE (denoted cpe) located chromosomally are most commonly found in human food-borne disease, while those with plasmid-borne cpe occur more frequently in animals and cause antibiotic-associated diarrhea in humans.

Piglet infections with nonenterotoxigenic type A occur usually during the first week of life, and sows are the likely source of infection. Antibody is widespread in finishing pigs and in sows (Estrada Correa and Taylor 1989). Some strains of type A cause piglet disease, while others do not (Table 52.3). However, currently available in vitro methods do not allow differentiation between “normal flora” type A strains and those that cause disease, with the possible exception of CPB2 positivity via PCR. Type A is ubiquitous in gut contents and in soil, although most of these strains are CPB2 negative and not disease producing. Thus, discussion of the epidemiology of type A enteric infections amounts to little (or nothing) more than speculation.

As noted previously, *C. perfringens* is an inefficient sporulator, but spores are nonetheless likely to be important in maintaining the organism in the environment. It can also be isolated from pig feed.

**Clinical Signs and Lesions.** Piglets develop creamy or pasty diarrhea within 48 hours of birth, displaying rough hair coat and perineal fecal staining (Johannsen et al. 1993a). Diarrhea lasts for up to 5 days, and feces become mucoid and sometimes pink. Most piglets recover, but may lag behind their unaffected peers through growing and finishing. Disease has been reproduced in gnotobiotic colostrum-deprived and conventional pigs (Johannsen et al. 1993d; M. A. Anderson and J. G. Songer, unpublished results).

At necropsy, small intestine is flaccid, thin walled, and gas filled, with watery contents and no blood. Inflammation of the mucosa is mild, occasionally with adherent necrotic material. Large intestine may be distended, with whitish, pasty contents, but without lesions. Microscopic lesions in piglets may include superficial villous tip necrosis and accumulation of fibrin, but villi may also be completely normal in appearance (Figure 52.4). Jejunal and ileal lesions may be heavily colonized with *C. perfringens* (Nabuurs et al. 1983), although it is common to find masses of organisms in the lumen (Johannsen et al. 1993c). Capillaries may be dilated, but there is no hemorrhage. There are no stomach lesions, but stomach contents may be a rich source of virulent organisms.

Superficial mucosal necrosis and villous atrophy are common in infections by enterotoxigenic *C. perfringens* (Collins et al. 1989; Estrada Correa and Taylor 1989; Nabuurs et al. 1983). Signs after experimental inoculation range from creamy diarrhea and emaciation with low mortality to profuse, bloodstained diarrhea, enteritis, and death (Olubunmi and Taylor 1985). Small intestinal villi and enterocytes are morphologically normal, and colon and cecum remain normal; numerous sporulating, gram-positive rods are found in lumen or layering epithelial cells at the tips of villi.

CPE-producing strains have been linked to diarrheal disease in growing pigs. Onset and severity of diarrhea

<table>
<thead>
<tr>
<th>Strain</th>
<th>Source</th>
<th>Clinical Signs/Lesions</th>
</tr>
</thead>
<tbody>
<tr>
<td>JGS1882</td>
<td>Porcine neonatal enteritis</td>
<td>++/+++</td>
</tr>
<tr>
<td>JGS4024</td>
<td>Porcine neonatal enteritis</td>
<td>++++/++</td>
</tr>
<tr>
<td>Various</td>
<td>Sow normal feces</td>
<td>None</td>
</tr>
<tr>
<td>Various</td>
<td>Piglet normal feces</td>
<td>None</td>
</tr>
<tr>
<td>JGS1936</td>
<td>Bovine neonatal enteritis</td>
<td>None</td>
</tr>
<tr>
<td>JGS4142</td>
<td>Bovine jejunal hemorrhage</td>
<td>None</td>
</tr>
<tr>
<td>JGS4151</td>
<td>Strain 13 (human gas gangrene)</td>
<td>None</td>
</tr>
<tr>
<td>JGS4104</td>
<td>Turkey necrotic enteritis</td>
<td>None</td>
</tr>
<tr>
<td>JGS1235</td>
<td>Chicken necrotic enteritis, cholangiohepatitis</td>
<td>None</td>
</tr>
</tbody>
</table>

**Table 52.3.** Virulence of *Clostridium perfringens* type A strains for neonatal pigs

*Figure 52.4.* Association of *C. perfringens*-like organisms with villus tips and accumulation of large numbers of organisms in jejunal lumen in *C. perfringens* type A infection in a 3-day-old piglet (courtesy of Jane Christopher-Hennings).
correlate with presence of CPE in feces. Affected pigs develop serum antibodies to CPE (Jestin et al. 1985).

**Pathogenesis.** Pathogenesis is poorly understood, but is likely multifactorial. Numbers of CPA- and CPB2-producing organisms in ileum and jejunum reach $10^8$–$10^9$ per gram of contents. Attachment and invasion do not occur in experimental infections (Johannsen et al. 1993c; M. A. Anderson and J. G. Songer, unpublished results). Intestinal epithelial necrosis occurs in experimental infections, and in a much less pronounced form in natural cases. Minimal gross and microscopic lesions suggest that type A enteritis is mainly a secretory diarrhea.

No direct information is available on the role of specific toxins in pathogenesis. No consistent changes are found in gut loops inoculated with purified CPA, but slight villous edema occurs when 6-hour-old piglets are given 80–800 mouse lethal doses (Johannsen et al. 1993b). The specific role of cpb2 in pathogenesis is unknown, but its strong association with enteric disease in pigs suggests that it is at least a marker of virulence (Bueschel et al. 2003; Gibert et al. 1997; Herholz et al. 1999). Few isolates from normal pigs contain CPB2, but >90% from porcine neonatal enteritis are positive, and the gene is rarely silent (Bueschel et al. 2003). In the rare cases in which it is involved, CPE causes villous necrosis and fluid secretion into intestinal lumen. Anti-CPE antibody is present in colostrum, and disease associated with CPE commonly occurs in weaned pigs after maternal antibody disappears (Estrada Correa and Taylor 1989).

**Diagnosis.** Diagnosis of type A enteritis is often equivocal. The most useful findings are compatible clinical signs and isolation of large numbers of *C. perfringens* from affected jejunum or ileum. The stomach is often a rich source of virulent organisms. Genotyping almost invariably reveals type A organisms with CPB2. Microscopic examination may reveal organisms intimately associated with the mucosa, or they may be massed in the lumen. Detection of CPA in gut contents is also supportive, but tests are not widely available. Commercial assays for CPE are prone to false positives, but diarrhea has been associated with fecal CPE titers >1:32 (Popoff and Jestin 1985). Failure to demonstrate other agents is also supportive.

**Treatment and Prevention.** Treatment with antimicrobials is more likely to be successful than in type C infections. Vaccination against type A is possible via custom biologics, and products available in some countries for immunization of other species are used off label in pigs. Commercial vaccines do not include CPE toxoid. Growth promoters, such as avoparcin (Taylor and Estrada Correa 1988) and salinomycin (Kyriakis et al. 1995), have been used in feed, and bacitracin methylene disalicylate can be used prophylactically in sows or for treatment of piglets (Madsen 1995).

**Clostridium difficile Infection**

**Etiology and Epidemiology.** Human *Clostridium difficile* infection (CDI) accounts for most cases of antibiotic-associated diarrhea (Bartlett 1992; Bartlett et al. 1978; Borriello and Wilcox 1998; Johnson et al. 1999). Spores germinate in ileum, cecum, and colon (Kelly et al. 1984); vegetative cells fill niches emptied by antimicrobial therapy and produce toxins. Disease presents as diarrhea, colitis, pseudomembranous colitis, or fulminant colitis (Kelly et al. 1984). The last can be accompanied by ileus, toxic megacolon, and bowel perforation.

CDI also occurs in antibiotic-treated hamsters (Libby et al. 1982), guinea pigs, and neonatal foals (Jones et al. 1988), and has emerged as a type of enteritis in neonatal pigs. Nearly two-thirds of piglets with enteritis in major swine-producing areas of the United States have CDI (Songer et al. 2000). Prevalence in individual herds can be 100%, and may be as high as 50% on a per-litter basis in swine production systems (J. G. Songer and K. W. Post, unpublished observations).

**Clinical Signs, Lesions, and Pathogenesis.** Typical CDI affects piglets 1–7 days of age born to gilts or multiparous sows. They present with a history of early-onset scours, rarely with respiratory distress (with hydrothorax and/or ascites). Gross lesions usually include mesocolonic edema (Figure 52.5), and large mesocolonic edema in a 4-day-old piglet with C. difficile infection (courtesy of J. Glenn Songer).
intestines may be filled with pasty to watery yellowish feces. Extensive sampling in CDI-affected herds has revealed that two-thirds of litters and more than one-third of individual pigs are toxin positive in an infected barn. Piglets without enteric signs may be toxin positive (Bakker et al. 2010; Hunter et al. 2010; J. G. Songer, unpublished results; Waters et al. 1998; Weese et al. 2010; Yaeger et al. 2002).

Focal suppuration in colonic lamina propria is the hallmark lesion, and colonic serosal and mesenteric edema is common. Infiltration of mononuclear inflammatory cells and neutrophils into edematous tissues is seen frequently. Segmental erosion of colonic mucosal epithelium and volcano lesions (exudation of neutrophils and fibrin into the lumen) may occur (Songer et al. 2000). Pathogenesis of C. difficile-associated disease (CDAD) in domestic animals is likely mediated by monomeric C. difficile toxin A (TcdA, 308 kDa, an enterotoxin). Clostridium difficile toxin B (TcdB, 270 kDa, a cytotoxin and enterotoxin) apparently does not bind to any tissue of neonatal pigs (Keel and Songer 2007, 2011), nor does it produce lesions in porcine intestinal explants (Keel and Songer 2007). Thus, it seems very likely that natural disease in piglets is mediated by TcdA alone.

**Diagnosis.** The gold standard for diagnosis of porcine CDAD is detection of TcdA and TcdB in feces or colonic contents. The reference method is measurement of neutralizable cytotoxicity in monolayers of Chinese hamster ovary or other cells, but most laboratories now use commercially available enzyme immunoassays. Culture for C. difficile can be somewhat challenging, in that this organism is more strictly anaerobic than some clostridia (Post et al. 2002; Songer et al. 2000). Most isolates are fully toxigenic, but some produce only TcdB or no toxins at all. Gross lesions are minimal, but careful microscopic examination of colon and cecum reveals suppurative foci, as described above. Colitis due to other causes in suckling pigs is relatively rare.

**Treatment and Prevention.** Immunoprophylaxis of CDI in domestic animals has not been studied, but precedent in other species suggests that immunity will be.antitoxic. Antibodies against TcdA (Allo et al. 1979) and TcdB (Kink and Williams 1998; Viscidi et al. 1983) prevent toxin binding in mouse and hamster models, eliminating secretion, inflammation, and clinical disease. Results of in vitro antimicrobial susceptibility testing suggest that tylosin may be effective in treatment of piglets.

**CELLULITIS AND GAS GANGRENE**

Clostridial wound infections are comprised of acute inflammation, edema, and extensive tissue emphysema and local tissue necrosis. Inflammation spreads rapidly from the primary site, often with terminal generalized sepsis. Clostridium septicum, C. perfringens type A, C. novyi, and C. chauvoei are most likely to cause porcine clostridial cellulitis and gas gangrene.

**Clostridium septicum Infection (Malignant Edema)**

**Etiology and Epidemiology.** Clostridium septicum is apparently the most common etiological agent of clostridial cellulitis and gas gangrene of swine. It is an anaerobic gram-positive rod that forms oval subterminal spores, is found in soil and feces (Finegold et al. 1983; Kahn 1924; Princewill 1985; Princewill and Oakley 1976), and is a frequent postmortem invader (MacLennan 1962). Incidence of malignant edema is particularly high on certain premises that have had large populations of livestock for many years, suggesting that there is a buildup of spore numbers in the environment of these farms.

**Clinical Signs and Lesions.** Clostridium septicum causes malignant edema, which has an acute course and is often fatal in less than 24 hours. Hemorrhage, edema, and necrosis develop as the infection spreads along muscular fascial planes. Common sites include the inguinal and ventral abdominal region, the head and ventral cervical area, and the shoulder. The affected animal is reluctant to bear weight on affected limbs, and skin overlying the swollen area has a blotty reddish-purple discoloration. Tissues that are initially painful and warm, with pitting edema, become rapidly crepitant and cold. In the terminal stage, affected swine lie in lateral recumbency and commonly groan during forced expiration.

Swelling at the primary infection site overlies subcutaneous edema that is colorless with focal hemorrhages or uniformly sanguineous fluid. Adjacent skeletal muscle may be edematous, with essentially normal color, or may be black, dry, and crepitant. The affected muscle may have a butyric odor comparable to that in ruminant blackleg (C. chauvoei infection). Regional lymph nodes are enlarged and hemorrhagic, and may be emphysematous. Acute fibrinohemorrhagic peritonitis is common, the spleen is slightly enlarged, and there is moderate pulmonary edema and congestion. Varying amounts of blood-tinged fluid and fibrin may be found in the pleural cavity and pericardial sac.

Postmortem accumulation of subcutaneous gas progresses until the subcutis of the entire carcass is emphysematous. Focal postmortem liver damage is evident within several hours after death. These foci become confluent, giving the liver a uniform tan color with numerous gas bubbles. Microscopically, the edematous subcutis contains large numbers of degenerating acute inflammatory cells and bacteria. Septic thrombi in subcutaneous veins and lymphatics are commonly found (Figure 52.6). Affected skeletal muscle fibers undergo
SECTION IV  BACTERIAL DISEASES

may be considered on premises where the disease recurs, but is rarely practiced in swine. Antibodies to somatic and toxin antigens provide lifelong immunity (Green et al. 1987). Treatment with antimicrobials may be successful if applied early (Zeller 1956). Experimental prophylactic use of tetracyclines, penicillin, or chloramphenicol prevents disease in mice (Taylor and Novak 1952).

**Clostridium perfringens Type A Infection (Gas Gangrene)**

**Etiology and Epidemiology.** Most cases of *C. perfringens*-associated myonecrosis are apparently caused by type A strains. The source of infection is usually endogenous, and most cases are induced by trauma. High intraherd incidence in young piglets is often a complication of injection of iron-containing preparations for prevention of nutritional anemia. Anecdotal evidence supports a view that such injections create a tissue microenvironment that favors growth of *C. perfringens* (Jaartsveld et al. 1962; Taylor and Bergeland 1992) in pure or mixed culture. The case fatality rate approaches 50%.

**Clinical Signs and Lesions.** Affected animals have marked swelling of the entire affected limb, and in piglets with iatrogenic infections, the swelling extends cranially to the umbilical area. The skin overlying the swollen area has a dark reddish-brown discoloration. There is extensive edema and large amounts of gas may be found in muscle and subcutis. The inflammatory exudate is stained by the injected iron preparation and the lesion usually has a putrid odor. Postmortem decomposition is rapid, and livers of pigs dead for more than a few hours may have gray, lytic foci that surround minute gas bubbles. Acute thrombophlebitis may be evident microscopically, and muscle fibers undergo fragmentation and liquefaction necrosis. Uterine gangrene and decomposition of its contents may follow dystocia and bungled attempts to assist in delivery. Foul-smelling, reddish, watery vulval discharge may be seen, and death ensues in 12–24 hours. The uterus is usually dark green or black, malodorous, and contains gas bubbles. There may be foul-smelling reddish fluid in the peritoneal cavity. Decomposition of the remainder of the carcass is rapid, and lesions are rarely identified in other sites.

**Pathogenesis.** Spores germinate, vegetative cells multiply in ischemic tissue, and infection spreads to healthy muscle. CPA and PFO play local and systemic synergistic roles in myonecrosis (Awad et al. 2000), although the sum of our knowledge in this area comes from studies in mice. The protective effect of CPA-containing toxoids against gas gangrene has long been known, and antibodies against native CPA and its C-terminus protect mice against challenge with toxin or multiple

coagulation necrosis with fragmentation and lysis, and bacteria are readily found between degenerating muscle fibers.

**Pathogenesis.** Most cases originate from wounds, and local tissue damage favors establishment of the infection. Lesions are largely the result of the necrotizing effect of alpha toxin. Hyaluronidase may cause disappearance of the endomysium (Aikat and Dible 1960), which may aid spread of the infection through muscle. Toxemia is probably the ultimate cause of death. Alpha toxin is a pore former (Ballard et al. 1993), and IV infusion specifically affects coronary and pulmonary circulation and causes pulmonary edema (Kellaway et al. 1941).

**Diagnosis.** Presumptive diagnosis is based upon gross lesions. Laboratory confirmation is based on pathological findings, exclusion of other diseases, and identification of the organism. Bacteria are seen in direct smears of affected subcutis or muscle, and fluorescent-labeled antibody staining is a rapid and accurate method to positively identify *C. septicum* (Batty and Walker 1963). Bacteriological culture is an alternative method, but is time consuming and often less reliable than immuno-fluorescence (Martig 1966). Swarming of *C. septicum* may cause small numbers of the organism to appear predominant, resulting in a false-positive diagnosis.

**Treatment and Prevention.** Prevention is preferred to treatment, given the fulminant clinical course. It is important to practice good sanitation and prevent injuries. Adequate sanitary procedures should be followed when making injections or performing surgery, because infection is not infrequently iatrogenic. Immunization may be considered on premises where the disease recurs, but is rarely practiced in swine. Antibodies to somatic and toxin antigens provide lifelong immunity (Green et al. 1987). Treatment with antimicrobials may be successful if applied early (Zeller 1956). Experimental prophylactic use of tetracyclines, penicillin, or chloramphenicol prevents disease in mice (Taylor and Novak 1952).
median lethal doses (LD_{50s}) of spores (Titball et al. 1993; Williamson and Titball 1993).

**Diagnosis.** Diagnosis is based upon clinical and pathological findings, together with isolation and identification of *C. perfringens*. Gram-stained smears of the lesion are helpful in estimating the relative numbers of bacteria. Isolation of the organism is easily accomplished by anaerobic incubation for 18–24 hours on blood or egg yolk agar.

**Prevention and Treatment.** Prevention of gas gangrene requires prevention of deep, contaminated wounds and prompt treatment of any such wounds with systemic penicillins. Treatment may be successful if instituted early in the course of the disease. Infection of mice was prevented by administration of penicillin coincident with *C. perfringens*, but if the antimicrobial was delayed by as little as 3 hours, the survival rate was appreciably lowered (Hac and Habert 1943). Clinically ill pigs may recover following penicillin injection (Jaartsveld et al. 1962).

Specific immunization of domestic animals against infection by type A in North America is focused at present upon anti-CPA immunity, and both toxoids and bacterin-toxoids are widely available elsewhere in the world.

*Clostridium chauvoei* Infection (Blackleg)

**Etiology and Epidemiology.** *Clostridium chauvoei* is a pleomorphic, anaerobic, gram-positive rod that readily forms central to subterminal spores. It causes blackleg (Burke and Opeskin 1999; Kuhnert et al. 1996), an emphysematous, necrotizing myositis that resembles malignant edema in ruminants and other domestic species (Table 52.1).

**Clinical Signs and Lesions.** There have been very few substantiated reports of blackleg in swine. The disease has occurred in swine kept under poor hygienic conditions on premises with previous losses of cattle from blackleg (Gualandi 1955; Sterne and Edwards 1955), although *C. septicum* may also be involved in these cases. Disease may follow consumption by swine of meat from blackleg-affected calves (Eggleston 1950), and in these cases, swelling of the face and throat is prominent. Lesions are perhaps more common in limbs (Mavenyengwa and Matope 1995). Signs include high fever, anorexia, depression, and lameness, with crepitant lesions and sudden death. Lesions are often dry and emphysematous at the center, but edematous, hemorrhagic, and necrotic at the periphery, with little leukocytic infiltration.

**Pathogenesis.** Pathogenesis of *C. chauvoei* infection in pigs has been little explored. Infection may be by the oral route, rather than beginning as a wound infection. The organisms may lie dormant in various tissues until a microenvironment favorable for their growth is generated. The roles of alpha toxin, which is necrotizing, hemolytic, and lethal, as well as of beta toxin, a DNase (Ramachandran 1969), remain undefined. Flagellar expression is associated with virulence, and phase variation occurs in motility and flagellation (Tamura et al. 1995). Flagella are apparently immunogenic (Kojima et al. 2000; Verpoort et al. 1966).

**Diagnosis.** A diagnosis of blackleg can be made only by bacterial identification, due to similarities in the clinical presentation and pathology of *C. septicum* and *C. chauvoei* infections. The fluorescent antibody test, applied to direct impression smears of infected tissue, is a rapid and practical method of identification (Batty and Walker 1963). Bacterial isolation may be difficult in decomposing specimens, since *C. chauvoei* is easily overgrown by other bacteria, including *C. septicum*.

**Treatment and Prevention.** Prevention of *C. chauvoei* infection requires minimizing exposure. *Clostridium chauvoei* is not known to be a common soil organism, but anecdotal evidence suggests that keeping swine on known contaminated premises or allowing them to eat carcasses of ruminants dead of blackleg are risk factors.

*Clostridium novyi* Infection (Sudden Death)

**Etiology and Epidemiology.** *Clostridium novyi* is an anaerobic, spore-forming, gram-positive rod. Toxigenic types A and B are involved in swine infections (Duran and Walton 1997; Itoh et al. 1987).

**Clinical Signs and Lesions.** *Clostridium novyi* has been associated with sudden death in swine (Batty et al. 1964), with unusually rapid postmortem decomposition. Necropsy findings include rapid postmortem tympany, submandibular swellings, bloodstained fluid in pleural, pericardial, and peritoneal cavities, serosal hemorrhages, and splenic enlargement. The hallmark is marked hepatic degeneration and emphysema, often referred to as “aerochocolate liver.” A bronze color and the presence of large numbers of small gas bubbles are common (Duran and Walton 1997). The organism can be demonstrated in various tissues, including liver and heart blood.

The disease affects large finishing pigs and breeding stock, principally sows, and appears to occur more frequently in older, periparturient sows of parity greater than 4 in moderate to good condition (Duran and Walton 1997).

**Pathogenesis.** Livers of normal sows often yield *C. novyi* when subjected to bacteriological culture, although the route by which the organism reaches the liver has not been documented. The conditions under which dormant spores germinate are also unknown.
Pathogenesis is likely mediated by the lethal, necrotizing alpha toxin. Alpha toxin is a so-called large clostridial cytotoxin (Busch et al. 2001; Selzer et al. 1996), and is produced by strains of types A and B. Beta toxin, a phospholipase related to CPA of \textit{C. perfringens}, is produced in small quantities by type B strains. Dissemination of alpha toxin causes edema, serosal effusion, hepatic necrosis, and peracute or acute death (Cotran 1979; Elder and Miles 1957; Rutter and Collee 1967).

**Diagnosis.** Diagnosis of \textit{C. novyi} infection in swine is difficult, since suspect cases are usually found dead, and the interval between death and necropsy introduces the possibility of postmortem invasion and proliferation of \textit{C. novyi}. Other possible causes of death should be excluded, but disease should be suspected when there is a history of sudden death and typical necropsy findings in the herd. Subcutaneous edema is particularly notable in cervical and inguinal regions. Pulmonary edema and tracheal froth, serofibrinous or serosanguinous exudates in pericardial and pleural cavities, and unusually rapid decomposition with accumulation of gas in the liver are all common. Gas bubbles in the liver in an otherwise fresh carcass are particularly significant (Duran and Walton 1997).

The organism is rapidly identified by fluorescent antibody staining of direct smears of infected tissue. It is the most fastidious of the clostridia commonly encountered in swine (Duran and Walton 1997; Itoh et al. 1987; J. G. Songer and K. W. Post, unpublished observations).

**Treatment and Prevention.** Given that the definition of the syndrome is sudden death, treatment is not a factor in management of the disease. It may be possible to prevent disease by feeding bacitracin methylene disalicylate in the periparturient period. Prevention may also be achievable by use of bacterin-toxoids or toxoids, and second-generation vaccines may be based upon native or recombinant alpha (Amimoto et al. 1998) or beta toxoids.

**NEUROTOXIGENIC CLOSTRIDIA**

\textit{Clostridium tetani} (Tetanus)

\textit{Clostridium tetani} causes tetanus, characterized by toxin-mediated, uncontrollable spasms of voluntary muscles. Swine of all ages may be affected, but most cases involve young pigs and originate with castration wounds or umbilical infection.

**Etiology and Epidemiology.** \textit{Clostridium tetani} is a slender, anaerobic, gram-positive rod. It forms terminal spores that are ubiquitous in the environment. Spores often enter via traumatic wounds, including those from tail docking and castration. Sows may be infected by contamination of uterine prolapses.

**Clinical Signs and Lesions.** Tetanus is characterized by generalized skeletal muscle spasms. The incubation period ranges from several days to several weeks. In general, shorter incubation periods are associated with a more acute and fulminating course and a higher fatality rate.

The earliest sign is a stiffened gait, and disease progresses rapidly over 1–2 days. Ears become erect, the tail extends straight out, the head is slightly elevated, and the nictitating membrane may protrude. The pig becomes incapable of walking, and the skeletal muscles are very firm on palpation. Ultimately, the pig lies in lateral recumbency in opisthotonus, with front and rear legs extended posteriorly (Figure 52.7). Tetanic spasms proceed from periodic to continuous, and are noticeably heightened by sudden sensory stimuli. Tachycardia and increased respiration rate are common terminal signs, and white froth may be present around the mouth and external nares.

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In acute cases, respiratory failure resulting from severe skeletal muscle spasms is likely to be the single most important cause of death. Prolonged recumbency and nutrient deprivation may be factors in animals with a relatively long survival time. No lesions specific for tetanus are found at necropsy. Conspicuous abrasions of the skin over pressure points may be seen, and there may be pulmonary congestion and edema.

**Pathogenesis.** Development of tetanus depends upon the presence of \textit{C. tetani} in a tissue environment that will support spore germination, vegetative cell growth, and toxin production. Spores usually gain entrance via a deep penetrating wound. Spore germination is facilitated by the presence of foreign bodies, or facultative anaerobes that reduce Eh in tissue. Bacterial multiplication and tetanospasmin (TeNT) production is enhanced by tetanolysin, a cholesterol-binding toxin that inhibits neutrophil and macrophage chemotaxis and causes
local tissue necrosis. Spores may remain latent in healed wounds for 10 years or more. *Clostridium tetani* is not invasive, and remains localized at the primary site of infection. The most commonly reported locations of tetanus infection in swine are castration wounds (Kaplan 1943).

Toxin-containing vesicles pass by retrograde axonal transport along the motor nerve fibers from neuromuscular junctions at the site of the infection, acting eventually on the inhibitory neurons in the ventral horn of the spinal cord. The toxin consists of a light chain that is enzymatic (a zinc-dependent endopeptidase) and a heavy chain that binds to receptors. The L-chain cleaves synaptobrevin, a protein involved in the exocytosis of neurotransmitters by neurons, resulting in tetany.

**Diagnosis.** Diagnosis is based upon typical clinical signs. An obvious area of infection (e.g., a castration wound or umbilical abscess) may be apparent. The organism may be isolated by bacteriological culture or identified by immunofluorescence (Batty and Walker 1963), but this is usually not necessary if there is adequate antemortem clinical observation of the affected animals.

**Treatment and Prevention.** There is no practical way to eliminate spores from soil, so control is directed toward prevention of wound contamination by soil or feces. Good sanitation in the farrowing house, treatment of the umbilical cord with antiseptics soon after birth, and prompt clipping of the canine incisors are recommended preventive measures against neonatal tetanus. Sharp objects that may cause skin wounds should be removed from the environment. Most tetanus in swine follows castration, so particular emphasis should be placed on proper surgical technique, with the provision of clean quarters for the pigs after castration to prevent undue contamination of the castration wound by soil or feces.

Passive immunization with tetanus antitoxin, prophylactic use of antibiotics, and/or active immunization with tetanus toxoid may be indicated. Prophylactic use of large doses of long-acting penicillin or tetracyclines may be superior to antitoxin in preventing experimental tetanus, if treatment is instituted within a few hours after infection (Veronesi 1966). Active immunity may be obtained from a single injection of alum-precipitated tetanus toxoid, and excellent protection for a year or more can be expected if three doses are given several weeks apart.

Prognosis is poor, and there is little evidence that treatment by current practical methods is of real benefit (Kaplan 1943; Mihaljevic 1966). Various suggested treatments include reopening castration wounds and flushing them with hydrogen peroxide, administration of antitoxin in an attempt to neutralize toxin not already fixed by nervous tissue, administration of anti-biotics, and the use of tranquilizers or barbiturates as muscle relaxants.

**Clostridium botulinum (Botulism)**

*Clostridium botulinum* produces eight types of botulinum neurotoxin (BoNT) (Linial 1995; Smith 1977, 1979) that have unique geographic distribution and species susceptibility patterns (CDC 1998; Hatheway 1990, 1995; Shapiro et al. 1998; Smith 1977; Smith and Milligan 1979). Thus, botulism is a toxicosis characterized by a rapidly progressive flaccid paralysis. Swine are highly resistant to botulism.

**Etiology and Epidemiology.** *Clostridium botulinum* is strictly anaerobic and gram positive (Smith and Holdeman 1968) and forms oval, usually subterminal, spores. Growth is optimal at about 86°F (30°C). Spores are ubiquitous in soil throughout the United States (Kelch et al. 2000; Smith 1979; Whitlock and Williams 1999). Disease in other species is associated with forage (Franzen et al. 1992; Kelly et al. 1984; Kinde et al. 1991; Ricketts et al. 1984; Whitlock 1997; Wichtel and Whitlock 1991), contamination of grain by decomposing animal carcasses (Divers et al. 1986; Enfors et al. 1975; Galey et al. 2000; Whitlock and Williams 1999), or transport of BoNT by ravens or crows feeding on a decomposing carcass (Schoenbaum et al. 2000). Type D botulism has been linked to pica, in which phosphorous-deficient animals consume bones of carcasses of animals dead of botulism (Dobereiner et al. 1992). Prevalence may be associated with the quantity of organic matter in the soil, and factors such as fertilization with manure may increase bacterial numbers.

Botulism in swine is rare, so there are few recorded toxin sources, but type C disease due to consumption of dead fish (Beiers and Simmons 1967) and decomposing brewers waste (Doiurtre 1967) has been reported. Eating habits of *nonconfined* pigs should make them likely candidates for botulism, but there appears to be innate resistance to toxin administration by the oral route. The swine gastrointestinal (GI) tract may have a low permeability for botulinum toxin (Dack and Gibbard 1926b; von Scheibner 1955; Smith et al. 1971).

**Clinical Signs and Lesions.** The latent period between consumption of toxic material and onset of signs ranges from 8 hours to 3 days or more, largely determined by the amount of toxin consumed (Beiers and Simmons 1967; Smintzis and Dunn 1950). Initial signs are weakness, incoordination, and staggering, with weakness appearing first in the forelegs, followed by involvement of the hind legs and general motor paralysis and dilation of the pupils (Smith et al. 1971). The clinical effect is progressive flaccid paralysis of voluntary muscles, which manifests in the end as lateral recumbency with complete flaccidity. Other clinical signs include anorexia, reduced vision, or complete blindness,
aphonia, excessive salivation, involuntary urination, and deep labored breathing (Beiers and Simmons 1967; Smintzis and Dunn 1950).

No specific lesions are found at necropsy. Significant findings might include presence in the stomach of the material suspected as the toxin source and the occurrence of aspiration pneumonia as a result of paralysis of the muscles of deglutition (Beiers and Simmons 1967).

**Pathogenesis.** Potency of botulinum toxin varies among toxin types, and the amount of toxin produced varies with strain. Botulism occurs after ingestion of preformed BoNT or by dissemination of toxin from an infected wound or focus of clostridial multiplication in the GI tract or elsewhere (Bernard et al. 1987; Hatheway 1995; Swerczek 1980). Absorption varies among species and with different areas of the GI tract (May and Whaler 1958).

Botulinum toxin is composed of an enzymatic light chain (a zinc-dependent endopeptidase) and a heavy chain that binds to receptors and facilitates internalization. The light chain cleaves proteins involved in exocytosis of neurotransmitters by neurons. Toxin types B, D, and F cleave synaptobrevin, types A and E act on synaptosomal-associated protein 25, and type C toxin acts on syntaxin, at the myoneural junction, preventing muscular contraction. Death is generally ascribed to asphyxia resulting from paralysis of the muscles of respiration.

**Diagnosis.** A diagnosis of botulism should be considered in afebrile, alert animals with progressive weakness and recumbency. Because the pig is apparently quite highly resistant to botulism, a diagnosis should be made only after thorough investigation and exclusion of other possible diagnoses (Beiers and Simmons 1967). Toxin is detected inconsistently in serum or plasma of acutely affected animals, and this may explain the relative sensitivity of various species. Gross or histological lesions are usually absent, but inhalation pneumonia may occur due to abnormal deglutition reflex. Affected animals are dysphagic and will usually have relatively empty GI tracts.

Isolation and identification of *C. botulinum* from feed and other specimens may also be of some value in establishing the diagnosis (Muller 1967; Narayan 1967; Yamakawa et al. 1992).

**Treatment and Prevention.** If botulism is suspected, an effort should be made to find the toxin source and prevent further consumption of suspect material by the herd. Antitoxin is the only specific treatment for botulism, and it has been effective in reducing mortality in humans after consumption of toxin-containing food (Lamanna and Carr 1967). Polyvalent antitoxins incor-porating the types most commonly present in a geographic area are required for therapy in animals. Therapy aimed at reducing continued absorption of toxin from the intestine (administration of magnesium sulfate) may be helpful.

Prevention requires eliminating opportunities to consume potentially toxic material such as spoiled garbage and decomposing animal tissue. Prophylactic immunization with toxoids is not practical in swine because of the infrequent occurrence of the disease.

**REFERENCES**


Colibacillosis
John M. Fairbrother and Carlton L. Gyles

RELEVANCE

Diseases caused by Escherichia coli have been a recognized problem for as long as pigs have been raised. Early work in the 1960s and 1970s elucidated the pathogenic mechanisms of E. coli-induced neonatal diarrhea, and led to the development of maternal vaccines which effectively control this form of the disease. However, maternal vaccination does not protect piglets against the diarrhea and edema disease (ED) that occur in the postweaning period.

More recent advances in our understanding of how E. coli cause disease have led to a better classification of pathogenic strains based on the presence of virulence factors, permitting much more accurate diagnostic strategies. Antimicrobial resistance has often resulted in a crisis for pig producers because of limited treatment options and an increased public health danger due to potential transfer of drug resistance genes into the food chain. This has provided the impetus to find alternative control strategies such as novel vaccines for weaned pigs.

All over the world, E. coli is an important cause of a wide range of diseases in pigs, including neonatal diarrhea, postweaning diarrhea (PWD), ED, septicemia, polyserositis, coliform mastitis (CM), and urinary tract infection (UTI). In particular, diarrhea and ED due to E. coli may result in significant economic losses due to morbidity, mortality, decreased weight gain, and cost of treatment, vaccinations, and feed supplements. Escherichia coli PWD is also called postweaning enteric colibacillosis. ED is also known as “bowel edema” and “gut edema” because edema of the submucosa of the stomach and the mesocolon is often a prominent feature of the disease. Escherichia coli PWD and ED may occur independently, but they may also occur together in a single outbreak or in the same pig. PWD is endemic in many farms and its prevalence fluctuates over time.

In recent years, a more severe form of enteric E. coli infection has been observed in pigs. This is observed at 2–3 weeks following weaning, and is manifested as sudden death or severe diarrhea. The E. coli isolates are often resistant to a wide range of antimicrobials.

Postpartum dysgalactia syndrome (PPDS) or mastitis, metritis, agalactia (MMA) is an economically important disease complex characterized by reduced milk production postpartum, leading to pig starvation (see Chapter 18 for a more detailed discussion of this syndrome). Mastitis is the most important component, and coliforms, predominantly E. coli, are the most frequently isolated bacteria in affected sows. The term “coliform mastitis” (CM) is used to refer to puerperal mastitis in the pig.

UTI is present whenever any of the typically sterile sections of the urinary tract are colonized by microbes. UTI may or may not be accompanied by clinical or subclinical disease. In the pig, specific UTI caused by Actinobaculum suis (Chapter 48) is distinguished from nonspecific UTI caused by a variety of microbes, the subject of this chapter. According to Liebhold et al. (1995), a nonspecific UTI often predisposes for A. suis infection.

Certain E. coli, particularly those of serotype O157:H7, serogroup O26, and other non-O157 E. coli, may be present sporadically in the intestines and feces of normal pigs, and are considered as zoonotic.
ETIOLOGY

Taxonomy, Morphology, and Laboratory Cultivation

The genus *Escherichia* is named after the German pediatrician Theodor Escherich (1857–1911). It is classified with the family *Enterobacteriaceae*, which consists of gram-negative facultatively anaerobic rods. The species *E. coli* includes normal inhabitants of the gastrointestinal tract and strains causing a broad variety of intestinal and extraintestinal diseases in swine.

*Escherichia coli* are gram-negative, peritrichously flagellated rods of variable length and with a diameter of about 1 μm. Colonies on solid media reach their full size within 1 day of incubation and vary from smooth to rough or mucoid. Enterotoxigenic *Escherichia coli* (ETEC) and edema disease *Escherichia coli* (EDEC) isolates producing the F4 (K88) or F18 adhesin and certain isolates producing F6 (987P) are hemolytic on blood agar. All other ETEC from pigs are nonhemolytic. A wide range of selective media is available for growth of *E. coli*.

Species identification relies mainly on biochemical characteristics, but there is no differentiating biochemical test for which 100% of strains give a positive reaction. Commercially available identification kits therefore make use of up to 50 tests to achieve a high level of accuracy. The interpretation may be facilitated by computer-assisted processing of the data. The determination of DNA relatedness, the scientific base of discrimination between species, is restricted to research laboratories.

Classification

There are several ways to subdivide the species into types. To date, serotypes have shown the best association with certain virulence traits. Complete serotyping includes determination of O (somatic), K (capsular or microcapsular), H (flagellar), and F (fimbrial) antigens. Unlike salmonellae, only a small percentage of *E. coli* isolates are typeable with available antisera, since serotyping has been limited to isolates of proven or suspected pathogenicity. Presently, at least 175 O, 80 K, 56 H, and over 20 F antigens are officially recognized.

Bacterial traits involved in pathogenesis of disease are called virulence factors. The nomenclature for pathogenic *E. coli* has evolved over the last few years. The term “pathotype” is now used to identify types of *E. coli* on the basis of their virulence mechanism as indicated by the presence of virulence factors which characterize the way in which disease is caused. This system identifies broad classes of pathogenic *E. coli*, such as ETEC; Shiga toxin-producing *Escherichia coli* (STEC), which includes the EDEC and enterohemorrhagic *Escherichia coli* (EHEC); enteropathogenic *Escherichia coli* (EPEC); and extraintestinal pathogenic *Escherichia coli* (ExPEC) (Gyles and Fairbrother 2010). Detection of virulence factors is important for the identification of pathogenic *E. coli* and the term “virotype” is now used to refer to the combination of these factors for a particular isolate. Table 53.1 summarizes important pathotypes of *E. coli*, their common virulence factors and serogroups, as well as diseases they cause.

**Table 53.1.** Important pathotypes, adhesins, toxins, and serogroups of porcine pathogenic *E. coli*

<table>
<thead>
<tr>
<th>Pathotype</th>
<th>Adhesins</th>
<th>Toxins</th>
<th>O Serogroups</th>
<th>Diseases</th>
</tr>
</thead>
<tbody>
<tr>
<td>ETEC</td>
<td>F5 (K99), F6 (987P), F41 F4 (K88)</td>
<td>STa, STb, LT, EAST-1, α-hemolysin</td>
<td>O8, O9, O20, O64, O101 O8, O138, O141, O145, O147, O149, O157</td>
<td>Neonatal diarrhea Neonatal diarrhea Diarrhea in young pigs preweaning</td>
</tr>
<tr>
<td>ETEC</td>
<td>F4 (K88), AIDA, unknown F18, AIDA</td>
<td>STa, STb, LT, EAST-1, α-hemolysin</td>
<td>O8, O138, O139, O141, O147, O149, O157 O8, O138, O139, O141, O147, O149, O157</td>
<td>PWD</td>
</tr>
<tr>
<td>EPEC</td>
<td>Eae ( intimin) F18, AIDA</td>
<td>Stx2e (VT2e), EAST-1, α-hemolysin</td>
<td>O45, O103 O138, O139, O141, O147</td>
<td>ED</td>
</tr>
<tr>
<td>STEC (VTEC)</td>
<td>Eae ( intimin)</td>
<td>Stx1 and/or Stx2</td>
<td>O157</td>
<td>None in pigs; bloody diarrhea and hemolytic uremic syndrome in humans</td>
</tr>
<tr>
<td>ExPEC</td>
<td>P, S</td>
<td>CNF</td>
<td>O6, O8, O9, O11, O15, O17, O18, O20, O45, O60, O78, O83, O93, O101, O112, O115, O116</td>
<td>Colisepticemia/polyserositis</td>
</tr>
<tr>
<td>ExPEC</td>
<td>P, S</td>
<td>CNF</td>
<td>O1, O4, O6, O18</td>
<td>Urogenital infection</td>
</tr>
</tbody>
</table>
ETEC must attach to the apical surface of small intestinal mucosal epithelial cells or the adjacent mucous layer in order to colonize and produce enterotoxins in the microenvironment of the glycocalyx. Bacterial cells attach by fimbrial adhesins that are hairlike appendages, composed of protein subunits, extending from the bacterial cell. As viewed electron microscopically, ETEC are usually located approximately half a bacterial width away from the microvilli, and fimbriae may sometimes be visualized between the bacteria and the microvilli (Figure 53.1). Fimbriae are classified by serological reactivity.

**Neonatal Enterotoxigenic Escherichia coli.** ETEC that cause neonatal diarrhea usually produce only the heat-stable enterotoxin STa and may have one or more of the fimbriae F4 (K88), F5 (K99), F6 (987P), and F41. Of these, the F4 (K88)-positive ETEC most often belong to the serogroups O149, O8, O147, and O157 (Harel et al. 1991; Soderlind et al. 1988; Wilson and Francis 1986) and the F5 (K99)-, F6 (987P)-, and F41-positive ETEC to serogroups O8, O9, O64, and O101, respectively.

**Postweaning Enterotoxigenic Escherichia coli.** ETEC that cause diarrhea in postweaning or older suckling pigs typically produce one or more enterotoxins, including heat-stable STa and STb, heat-labile LT, and the more recently recognized enteroaggregative heat-stable enterotoxin EAST-1, originally found in enteroaggregative *E. coli* from diarrheic humans (Zhang et al. 2007). These PWD strains usually have either F4 (K88) or F18 as fimbrial adhesin (Fairbrother et al. 2005; Francis 2002; Frydendahl 2002; Mainil et al. 2002; Zhang et al. 2007). However, some F4 (K88)- and F18-negative PWD virotypes have been identified (Do et al. 2006; Frydendahl 2002). The role of these F4 (K88)- and F18-negative virotypes in the development of diarrhea has not yet been established.

Both fimbrial types in PWD have several variant subtypes, based on antigenic differences. F4 (K88) variants ab, ac, and ad have been described. However, almost all belong to F4ac (K88ac), and are often referred to simply as F4 (K88). F18 has two known variants, ab and ac. The latter is more commonly associated with PWD strains, whereas F18ab is associated with ED strains. ETEC isolates of the STb or STb:EAST-1 virotypes from weaned pigs may also produce an adhesin involved in diffuse adherence (AIDA-I) (Mainil et al. 2002; Ngeleka et al. 2003; Niewerth et al. 2001), originally detected in *E. coli* isolates from humans with diarrhea. At present in PWD, most F4 (K88)-positive isolates are O149, whereas F18 isolates are more heterogeneous and include serogroups O139, O138, O141, O147, and O157. Common serovirotypes of pathogenic ETEC from pigs with PWD are listed in Table 53.2.

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**Table 53.2.** Common serovirotypes of pathogenic *E. coli* from pigs with PWD or ED

<table>
<thead>
<tr>
<th>Fimbrial Adhesin</th>
<th>Serovirotypes</th>
</tr>
</thead>
<tbody>
<tr>
<td>F4 (K88)</td>
<td>O149: LT:STb:EAST-1</td>
</tr>
<tr>
<td></td>
<td>O149:LT:STa:STb:EAST-1</td>
</tr>
<tr>
<td></td>
<td>O149:LT:STb</td>
</tr>
<tr>
<td>F18</td>
<td>O149:LT:STb:EAST-1</td>
</tr>
<tr>
<td></td>
<td>O138:STa:STb</td>
</tr>
<tr>
<td></td>
<td>O138:LT:STb:EAST1:STx2e</td>
</tr>
<tr>
<td></td>
<td>O139: STx2e(AIDA)</td>
</tr>
<tr>
<td></td>
<td>O147:STa:STb:AIDA</td>
</tr>
<tr>
<td></td>
<td>O2:STa:STb</td>
</tr>
<tr>
<td></td>
<td>O2:STa:STb:STx2e</td>
</tr>
<tr>
<td></td>
<td>O2:STa:STb</td>
</tr>
<tr>
<td></td>
<td>O2:STb:STx2e</td>
</tr>
<tr>
<td></td>
<td>O45/O?:Eae(EAST-1)</td>
</tr>
</tbody>
</table>

Note: Virulence factors in parentheses are not always present when tested, and are not always tested for in all laboratories.

---

**53.1.** Electron micrograph of fimbrial attachment typical of enterotoxigenic *Escherichia coli* (ETEC) in the intestine. Hairlike fimbriae extend from the surface of bacterial cells that are located approximately half a bacterial width away from the microvilli of intestinal epithelial cells.
Certain strains produce both enterotoxins and Stx2e (see the section “Shiga toxin-producing Escherichia coli” below), as well as either of the fimbrial variants F18ab or F18ac. These strains are classified as ETEC rather than STEC, since they produce clinical PWD more than ED. Mixed infections of F18-positive STEC and F4 (K88)-positive ETEC may be observed. In these cases, the predominant clinical sign is often diarrhea caused by the F4 (K88)-positive ETEC, although histopathological evidence of ED may be present.

ETEC may also be associated with secondary septicaemia, particularly in older piglets. These isolates most frequently belong to the serovirotypes listed in Table 53.2.

**Enteropathogenic Escherichia coli.** Another pathotype found in pigs with PWD is known as EPEC. EPEC were initially associated with diarrhea in children, especially in developing countries. These bacteria do not attach by fimbria; rather, they have a complex secretion system which injects over 20 effector proteins into the host enterocyte, leading to intimate adherence of the bacteria to the host intestinal epithelium and to development of a characteristic “attaching and effacing” (AE) lesion (Figure 53.2). EPEC and other bacteria which induce formation of AE lesions are called attaching and effacing *Escherichia coli* (AEEC). EPEC from different animal species may have different virulence factors, but all possess a variant of the EPEC attaching and effacing factor Eae or intimin, a bacterial outer membrane protein adhesin which is responsible for intimate attachment. Hence, the presence of Eae (intimin) is indicative of an EPEC. The EPEC do not possess any virulence factors of classic ETEC PWD or ED strains (Zhu et al. 1994).

**Shiga Toxin-Producing Escherichia coli, Edema Disease Escherichia coli, and Enterohemorrhagic Escherichia coli.** STEC produce one or more of a family of cytotoxins which are known collectively as Shiga toxins (Stx) or verotoxins (VT), so-called because of their structural similarity with the Stxs of *Shigella dysenteriae*, and their lethal effect on Vero cells in culture (Mainil 1999). The two names are used interchangeably in the literature. Many STEC are probably not pathogenic, but are present in the normal intestinal microflora. However, certain STEC strains which possess additional virulence factors may be highly pathogenic. In pigs, the most important STEC are those which cause ED; these are known as EDEC. These strains produce the Stx variant Stx2e (VT2e) and may possess the fimbrial variant F18ab or F18ac (DebRoy et al. 2009). Most EDEC are serogroup 0139. Common serovirotypes of EDEC are listed in Table 53.2. Another subgroup of STEC, also known as EHEC, is highly pathogenic in humans. Most EHEC are AE, also possessing Eae and the same secretion system as EPEC.

**Escherichia coli Causing Fatal Shock.** Enteric colibacillosis complicated with shock also occurs in young pigs before and after weaning. *Escherichia coli* associated with this disease are either (1) F4 (K88)-positive ETEC that commonly belong to serogroups O149, O157, or O8, but only occasionally produce the Stx Stx2e (Faubert and Drolet 1992); or (2) Stx2e-producing *E. coli* that are associated with ED.

**Extraintestinal Pathogenic Escherichia coli.** ExPEC are a heterogeneous group of *E. coli*, so named because their normal habitat is in the intestinal tract yet they are able to invade, cause bacteremia, and induce septicaemia or localized extraintestinal infections such as meningitis or arthritis (Fairbrother and Ngeleka 1994; Morris and Sojka 1985). In contrast to ETEC, EPEC, and STEC, they are not characterized by a constant group of virulence factors. Rather, they possess a large number of virulence factors that vary greatly between strains. They often possess fimbrial antigens of the P, S, and F1C families that contribute to bacterial colonization (Dozois et al. 1997), as well as cytotoxins such as hemo-
lysins and cytotoxic necrotizing factor (CNF). They usually contain one or sometimes several iron-capturing systems, such as aerobactin, which permit them to survive in the blood and other tissues outside the intestines (Gyles and Fairbrother 2010). ExPEC possess lipopolysaccharides (LPS; O antigen) and capsules (K antigens) which protect the bacteria against killing by serum complement and phagocytes.

Only a relatively small number of E. coli serogroups have been reported in cases of septicemia. Serogroups O6, O8, O9, O11, O15, O17, O18, O20, O45, O60, O78, O83, O93, O101, O112, O115, and O116 have been most commonly identified in isolates associated with septicemia (Fairbrother et al. 1989).

Coliform Mastitis Escherichia coli. CM in sows appears to be from fecal contaminants and is noncontagious. Multiple serological types of E. coli isolates from cases of mastitis may be found within a herd, between distinct glands of one sow, or even between subcomplexes within one gland (Awad-Masalmeh et al. 1990; Morner et al. 1998). Heterogeneity of mastitis isolates has also been demonstrated by random amplified polymorphic DNA genotyping (Ramasoota et al. 2000). This great variety of coliform bacteria associated with CM indicates an abundant reservoir of potentially pathogenic bacteria. The virulence factors of E. coli from CM are not well known, although serum resistance and ability to bind to fibronectin have been associated with these isolates.

Nonspecific Urinary Tract Infection Escherichia coli. Nonspecific UTI may be caused by one or more of a number of bacterial species, including E. coli. Escherichia coli isolates from UTI in pigs have not been well characterized. Isolates from pyelonephritis in pigs differ from those of human uropathogenic isolates with respect to virulence profile, being rarely hemolytic and less frequently possessing P and F1C fimbriae (Krag et al. 2009).

Genetics of Virulence
In most E. coli diseases, virulence genes determine pathogenicity and are encoded by plasmids, bacteriophages, or pathogenicity islands (PAIs) (Gyles and Fairbrother 2010). These genes are plasmid-encoded for enterotoxins and fimbriae or pili, phage-encoded for Stx, and PAI-encoded for the AE lesion in EPEC and EHEC. In strains from most extraintestinal infections, however, the genes encoding for fimbriae, cytotoxins, and hemolysin are chromosomally located. In the laboratory, plasmids can easily be transmitted from donor to recipient strains. However, such exchanges of genetic material do not appear to play a major role in the field as the genetic makeup of pathogenic E. coli strains is remarkably stable. This may be because a whole set of virulence factors is involved in the virulence of a particular strain, and certain recipient strains may not express transmitted plasmid-determined functions. The clinically important development of antimicrobial resistance is an exception to this observation.

PUBLIC HEALTH
Certain E. coli of the STEC subgroup EHEC, particularly EHEC O157:H7, O26, and other non-O157 serogroups, may be present sporadically in the intestines and feces of normal pigs, and are zoonotic, as they may cause bloody diarrhea, hemorrhagic colitis, and/or hemolytic uremic syndrome in humans infected through food or water contaminated by animal feces (Fairbrother and Nadeau 2006). Pigs are not considered a major source of O157 EHEC, the prevalence rate being usually very low. Cattle and other ruminants are the main reservoir of zoonotic EHEC. ED bears some similarity to the human diseases caused by EHEC, which produce closely related Stx. However, the human EHEC strains colonize the intestine by a mechanism distinct from EDEC and the Stx target different organs (Gyles and Fairbrother 2010). Serotypes associated with ED are different from those of EHEC that cause disease in humans.

Escherichia coli of the intestinal ecosystem, both commensal and pathogenic, may acquire antimicrobial resistance following administration of antimicrobials either in the feed as growth promoters or as treatment for bacterial infections orally or by injection. These strains, when resistant to antimicrobials—which are preferred treatments for serious infections and for which limited alternatives are available in human medicine, such as third-generation cephalosporins (e.g., ceftiofur) and fluoroquinolones (e.g., enrofloxacin)—constitute a public health threat when they enter the food chain.

EPIDEMIOLOGY
Escherichia coli infections are widespread, occurring in both industrialized and developing countries and in temperate, subtropical, and tropical climates. Diarrhea (neonatal ETEC, postweaning ETEC, and EPEC), ED due to EDEC, systemic infections due to ExPEC, CM, and UTI occur in pigs in all countries where pigs are raised commercially.

The primary habitat of E. coli in the pig is the gastrointestinal tract. The E. coli flora of individual pigs is extremely complex, up to 25 strains being identified in the gastrointestinal tract of any one individual (Hinton et al. 1985). Dominant strains change at intervals from 1 day to several weeks, leading to successive waves of dominant strains (Katouli et al. 1995). Proliferation of E. coli takes place mainly during the passage through the small intestine, bacterial numbers remaining constant from the ileum to the rectum (McAllister et al. 1979). Numbers in the large intestine average around
10^7 colony-forming units (CFU) per gram of contents; however, *E. coli* contribute less than 1% to the total bacterial count.

Outside the intestine, *E. coli* are found in fecal-contaminated feed, water, soil, and the environment of the pig barn. Long survival times in the environment are promoted by low temperature and sufficient available water, among other factors. In slurry samples, a porcine *E. coli* O139:K82 strain remained viable for more than 11 weeks (Burrows and Rankin 1970). The spread of pathogenic *E. coli* is presumed to occur via aerosols, feed, other vehicles, pigs, and possibly other animals. Airborne transmission between pigs in wire cages 1.5 m apart was repeatedly observed in transmission experiments with an F4 (K88)-positive ETEC strain (Wathes et al. 1989).

Intestinal infections caused by ETEC, EDEC, and EPEC are considered contagious. The same strain is usually found in many sick pigs and often in consecutive batches of pigs. In contrast, infections caused by ExPEC and *E. coli*, causing CM and UTI, do not behave as communicable diseases. Mixed infections by more than one strain are frequent and are acquired by invasion of preexisting intestinal bacteria in the case of ExPEC and from fecal and/or environmental contamination of teats and the urethra in the case of CM and UTI.

Routine cleaning and disinfection are usually insufficient to break the cycle of infection by *E. coli* (Hampson et al. 1987). Under experimental conditions, however, transmission can be prevented by strict hygienic measures (Kausche et al. 1992). There are only limited data on the susceptibility of *E. coli* isolates to commonly used disinfectants. According to a Danish study, *E. coli* fecal isolates from livestock did not appear to have developed resistance to benzalkonium chloride, H2O2, chlorhexidine, formaldehyde, or zinc chloride (Aarestrup and Hasman 2004). However, Beier et al. (2005) demonstrated that reduced chlorhexidine susceptibility in virulent *E. coli* isolates from newborn pigs with diarrhea correlated with resistance to gentamicin and streptomycin. These findings underline the potential for transfer of this resistance, and the possible impact of environmental contamination.

### NEONATAL ESCHERICHIA COLI DIARRHEA

Neonatal diarrhea caused by *E. coli* is observed most commonly in pigs aged from 0 to 4 days and is caused by ETEC. The newborn pig, on leaving the uterus and before reaching the teats of the sow, encounters the heavily contaminated environment of the farrowing crate and the skin of the dam, resulting in ingestion of microbes from the intestinal flora of the sow. In conditions of poor hygiene or in a continuous-farrowing system, buildup of pathogenic strains increases risk for an outbreak.

### Pathogenesis

In the presence of the appropriate predisposing environmental conditions and host factors, ETEC proliferate in the intestine and cause disease by means of specific virulence factors (Table 53.1; see also the section “Etiology” above). Risk factors for the development of various *E. coli* diseases in pigs are summarized in Table 53.3. ETEC must be able to adhere to and colonize the intestinal mucosa to permit the release of sufficient enterotoxin to cause diarrhea. Adherence to specific receptors on mucosal epithelial cells and in the adjacent mucous layer is mediated by hair-like fimbria on the bacterial surface (Figure 53.1).

ETEC affecting neonates predominantly produce one or more of the fimbria F4 (K88), which colonizes the length of the jejunum and ileum, and F5 (K99), F6 (987P), and F41, which mostly colonize the posterior jejunum and ileum. Piglets are most susceptible to infection with F5 (K99)- and F6 (987P)-positive ETEC during the first several days of life and subsequently become more resistant. This susceptibility could be related to a reduction of the number of receptors present on intestinal epithelial cells with age or inhibition of colonization by preferential binding of bacteria to F6 (987P) receptors present in the mucus rather than to those on the intestinal epithelium.

ETEC adhering to the intestinal mucosa produce enterotoxins which change the water and electrolyte flux of the small intestine and may lead to diarrhea if the excess fluid from the small intestine is not absorbed in the large intestine. Excessive secretion leads to dehydration, metabolic acidosis, and eventually death. Most strains of neonatal ETEC produce heat-stable enterotoxin STa.

STα (ST1, ST2, and ST mouse) is a small, nonimmunogenic protein with a molecular weight (MW) of 2000 (Lallier et al. 1982). STα is active in the intestine of infant mice and young piglets of less than 2 weeks of age but is less active in older pigs. This could be due to differences in the concentration of intestinal receptors with age (Cohen et al. 1988).

STα produced by human and porcine ETEC has been designated STαh and STαp, respectively, based on differences in the genes coding for the toxin. STα binds to a guanylyl cyclase C glycoprotein receptor on the brush border of villous and crypt intestinal epithelial cells and activates guanylate cyclase, which stimulates production of cyclic guanosine monophosphate (cGMP) (Giannella and Mann 2003), leading to electrolyte and fluid secretion. Based on the concentration and affinity of the STα receptors, the posterior jejunum appears to be the major site of hypersecretion in response to STα.

In suckling pigs, the severity of ETEC-induced disease depends on antibody titers in the milk of the sow (Sarmiento et al. 1988b). Thecolostrum contains nonspecific bactericidal factors and specific antibody (immunoglobulin G [IgG] and immunoglobulin A
TABLE 53.3. Risk factors for the development of *E. coli* diseases

<table>
<thead>
<tr>
<th>Diseases</th>
<th>E. coli pathotype + others</th>
<th>Risk Factors</th>
<th>Environment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neonatal diarrhea</td>
<td>ETEC:F4, F5, F6, F41</td>
<td>• Susceptibility to F5 and F6 high, decreases with age</td>
<td>Ambient temperature less than 25°C</td>
</tr>
<tr>
<td>Septicemia</td>
<td>ExPEC</td>
<td>• Up to 50% of pigs may be resistant to F4 due to lack of receptor</td>
<td>Presence of other infections, such as rotavirus, coccidia or TGEV</td>
</tr>
<tr>
<td>Young pig diarrhea</td>
<td>ETEC:F4</td>
<td>• Some pigs are resistant to F18 due to lack of receptor</td>
<td>Rapidly growing pigs</td>
</tr>
<tr>
<td>Enteric colibacillosis</td>
<td>ETEC:F4</td>
<td>• Earlier weaning age</td>
<td></td>
</tr>
<tr>
<td>complicated by shock</td>
<td></td>
<td>• Stress</td>
<td></td>
</tr>
<tr>
<td>ED</td>
<td>STEC:F18</td>
<td>• Loss of specific antibodies from milk</td>
<td></td>
</tr>
<tr>
<td>PWD</td>
<td>ETEC:F4, F18, ETEC:AIDA, EPEC, mixed <em>E. coli</em> pathotypes</td>
<td>• Last period of pregnancy</td>
<td></td>
</tr>
<tr>
<td>Urogenital infection</td>
<td>ExPEC</td>
<td>• Parturition</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Trauma at coitus</td>
<td></td>
</tr>
</tbody>
</table>

[lgAI] that inhibit the adherence of pathogenic *E. coli* to the intestine. If the dam has not been vaccinated or exposed to the pathogenic *E. coli* present in the environment of the piglets, specific antibodies are not present in the colostrum, and the piglets are susceptible to infection. Similarly, when individual piglets do not have access to colostrum, due to injury or inability to compete or due to agalactia or insufficient teats of the sow, they are more susceptible to infection.

Low ambient temperatures in the farrowing house also impact severity of disease. In pigs kept at temperatures of less than 77°F (25°C), intestinal peristaltic activity is greatly reduced, and passage of bacteria and protective antibodies through the intestine is delayed. Increased numbers of pathogenic *E. coli* in the intestinal tract of these pigs result in a more severe diarrhea than in pigs kept at 86°F (30°C).

**Clinical Signs**

Clinical signs and age most commonly affected are summarized for the various diseases caused by *E. coli* in Table 53.4. Neonatal diarrhea may first be observed 2–3 hours after birth and may affect one or a few pigs or whole litters. Gilt litters are more often affected than sow litters. Morbidity in an affected herd is extremely variable. The average is 30–40%, but may be as high as 80% in some herds. Mortality can reach 70% in affected litters. In a low proportion of pigs the infection may be so rapid that death occurs before the development of diarrhea.

Diarrhea may be very mild with no evidence of dehydration or may be clear, watery, and profuse. The feces vary in color from clear to whitish or various shades of brown. In very severe outbreaks, a small proportion of affected animals may vomit. In severe cases, 30–40% of total body weight may be lost and result in signs of dehydration. Abdominal musculature may be flaccid, pigs may be sluggish with sunken eyes, and skin may be bluish-gray in color and parchment-like in texture. The loss of fluid and weight results in the exaggeration of bony prominences. These animals usually die. In more chronic or less severely affected cases, the anus and perineum may be inflamed from contact with the alkaline fecal material. Pigs with less severe dehydration may continue to drink and, if treated appropriately, recover with only minimal long-term effects.

**Lesions**

Few specific lesions are observed. Gross lesions may include dehydration, dilation of the stomach (which may contain undigested milk curd), venous infarcts on the greater curvature of the stomach, and dilation of the small intestine with some congestion of the small intestinal wall.
gram-negative rod-shaped bacteria colonizing the small intestinal mucosa. Colonization can be visualized in formalin-fixed, paraffin-embedded tissues by routine histopathology or \( E. coli \) organisms can be definitively identified and visualized using immunohistochemistry or in frozen sections using indirect immunofluorescence. This diagnosis is strengthened by the isolation from rectal swabs or intestines of \( E. coli \) of the appropriate serogroup or, more importantly, possessing appropriate virulence factors. Criteria used to identify causative \( E. coli \) in diarrhea in pigs are summarized in Table 53.5.

Swabs or samples of intestinal contents should be inoculated onto blood and MacConkey agar or other media which are selective for \( Enterobacteriaceae \) and allow differentiation of lactose-fermenting from lactose-nonfermenting Gram-negative enteric bacilli. Use of transport medium such as alginate swabs or Stuart’s medium should be considered if isolation cannot be done within 24 hours.

Morphology, lactose fermentation on MacConkey agar, and odor of colonies are a first indication of potential \( E. coli \). To identify the species as \( E. coli \), it is essential to determine the capacity of colonies to transform indole, since 99% of \( E. coli \) strains are indole positive. Identification can be completed by the citrate assay (\( E. coli \) are not able to use citrate as the only carbon source) and by the methyl red assay.

### Diagnosis

Neonatal diarrhea caused by ETEC must be differentiated from other common infectious causes of diarrhea, including \( Clostridium difficile \) and \( perfringens \) types A and C (Chapter 52), transmissible gastroenteritis virus (TGEV) (Chapter 35), rotavirus groups A, B, and C (Chapter 43), and porcine reproductive and respiratory syndrome virus (PRRSV; Chapter 31). In suckling pigs 5 days and older, \( Isospora suis \) (Chapter 66) must also be considered. It may be helpful to determine fecal pH as ETEC produces alkaline feces, whereas malabsorptive diarrheas produced by TGEV and rotaviruses produce acidic feces.

Diagnosis of neonatal ETEC is based on clinical signs, microscopic lesions, and the presence of small layers of \( E. coli \) are observed adhering in patches to the mucosal epithelial cells of most of the jejunum and ileum in the case of F4 (K88)-positive ETEC isolates, and of the posterior jejunum and/or the ileum in the case of other ETEC. Adhering bacteria may be found only in the crypts of Lieberkühn, or more often covering the crypts and the tips of the villi. Other lesions sometimes observed include vascular congestion in the lamina propria with hemorrhages into the intestinal lumen, increased numbers of neutrophils and macrophages in the lamina propria, and mild villous atrophy.

### Table 53.4. Age periods affected for various clinical diseases due to \( E. coli \)

<table>
<thead>
<tr>
<th>Clinical Disease</th>
<th>Age Perioda</th>
<th>Newbornb</th>
<th>Suckling</th>
<th>Postweaned</th>
<th>Grower–Finisher</th>
<th>Adult</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neonatal diarrhea</td>
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<tr>
<td>Severe watery diarrhea, dehydration, mortality up to 70% in affected litters</td>
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<td></td>
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<tr>
<td>Septicemia</td>
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<td></td>
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<tr>
<td>Shock, depression, death, polyarthritis</td>
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<tr>
<td>Young pig diarrhea</td>
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<td></td>
<td></td>
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<tr>
<td>Low mortality, diarrhea of moderate severity, decreased weight gain</td>
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<tr>
<td>Enteric colibacillosis complicated by shock</td>
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<tr>
<td>Rapid death, cyanosis of extremities, diarrhea</td>
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<tr>
<td>ED</td>
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<tr>
<td>Sudden death, possibly paralysis, and eyelid edema, sporadic mortality up to 65%</td>
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<td></td>
<td></td>
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<tr>
<td>PWD</td>
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<tr>
<td>Initially deaths, with severe to moderate diarrhea, decreased weight gain, mortality up to 25% in untreated animals</td>
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<td></td>
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<tr>
<td>Urogenital infection</td>
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<tr>
<td>Sporadic cystitis often after mating, pyelonephritis 2 weeks postpartum</td>
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<td></td>
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<tr>
<td>CM</td>
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<tr>
<td>First several days following farrowing, generally short duration. Clinically similar to lactational failure</td>
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</tbody>
</table>

a Most commonly affected age periods shaded.
b First several days.
c Most important clinical diseases.
Pathogenic *E. coli* may be identified by serotyping, since a small number of specific O groups have been associated with disease. Hence, a diagnostic laboratory can use OK typing sera against serogroups most prevalent in its geographical region to obtain a rapid presumptive diagnosis. Common O serogroups associated with disease in pigs are shown in Table 53.1. Complete O and H serotyping can only be carried out in a few reference laboratories.

Virotyping, or determination of the virulence factors, is a more definitive way of identifying pathogenic *E. coli*, as not all strains of a given serogroup are pathogenic (Table 53.1).

Until recently, detection of enterotoxins and cytotoxins was based on tests for biological activity. ST 

<table>
<thead>
<tr>
<th>F4(K88)</th>
<th>F18</th>
<th>F5(K99), F6(987P), F41</th>
<th>AIDA</th>
<th>STEC F18</th>
<th>EPEC</th>
<th>ExPEC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemolytic colonies</td>
<td>All (not discriminatory)</td>
<td>None</td>
<td>All</td>
<td>None</td>
<td>Some</td>
<td></td>
</tr>
<tr>
<td>OK serotyping</td>
<td>Most (some non-ETEC identified)</td>
<td>Not known</td>
<td>Most</td>
<td>Few</td>
<td>Some</td>
<td></td>
</tr>
<tr>
<td>F adhesin serotyping</td>
<td>All</td>
<td>Not reliable</td>
<td>Most (not reliable)</td>
<td>Not done</td>
<td>Not reliable</td>
<td>Not done</td>
</tr>
<tr>
<td>Virotyping</td>
<td>All</td>
<td>Some</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

Currently, genotypic analysis is more commonly used to define the virotypes involved in an infection. Techniques include colony or DNA hybridization and polymerase chain reaction (PCR) for the detection of genes encoding for virulence factors such as toxins and adhesins (Francis 2002; Frydendahl 2002; Wray and Woodward 1994). There is a high correlation between the results of the standard serological and biological assays and those of gene probes for the detection of fimbrial adhesins and enterotoxins of swine ETEC isolates (Harel et al. 1991). PCR may also be used to detect pathogenic *E. coli* in situ in formalin-fixed, paraffin-embedded tissues.

**Immunity**

Immunity to enteric *E. coli* infections is humoral and is initially provided through the maternal colostrum, lactogenic antibodies in the milk of the sow, and subsequently by a local intestinal immune response. Specific antibodies against fimbrial adhesins inhibit bacterial adherence to receptors on the intestinal epithelial cells and antitoxin antibodies neutralize the activity of the enterotoxins or cytotoxins produced by *E. coli*.

During the first weeks of life, the main source of immunological protection for the piglet is colostrum containing high levels of IgG, which rapidly decrease during lactation, with IgA becoming the main immunoglobulin class. The latter protects the gut against *E. coli* infection. Protective immunity is based on the presence of antibodies to surface antigens, especially the fimbrial adhesins F4 (K88), F5 (K99), F6 (987P), and F41. Antibodies to the polysaccharide capsule of ETEC may also be protective. Anti-fimbriae and anti-K antibodies function by preventing attachment of the ETEC to enterocytes. Infection with ETEC positive for one of the fimbrial antigens, for example, F5 (K99), is unlikely to result in cross-protection against infection with ETEC positive for another fimbrial antigen such as F4 (K88) or F41 unless some protection is conferred by antibodies against shared K antigens.

**Prevention and Control**

In newborn piglets, treatment with antimicrobials may be on an individual or litter basis, by mouth, or parenteral injection. It is important to confirm the diagnosis of *E. coli* infection by culture and to perform antimicrobial sensitivity tests, because antimicrobial sensitivity varies greatly among *E. coli* isolates. In vitro resistance of *E. coli* isolates to a wide range of
antimicrobial agents has dramatically increased over the last several years. Commonly used antimicrobials are ampicillin, apramycin, ceftiofur, gentamicin, neomycin, spectinomycin, furazolidone, and potentiated sulfa drugs. An alternative approach to the treatment of enteric E. coli infection is the use of bacteriophages, an approach that has been successful experimentally (Jamalludeen et al. 2009) but has not been extensively applied in the field.

Fluid therapy, consisting of electrolyte replacement solutions containing glucose given orally, is useful for the treatment of dehydration and acidosis. Drugs which inhibit the secretory effects of enterotoxin, such as chlorpromazine and berberine sulfate, may be useful for the treatment of diarrhea, although many of these drugs have undesirable side effects. The use of such antisecretory drugs as bencetamide and loperamide, alone or in combination with antibacterial agents, has also been suggested (Solis et al. 1993).

A program for the prevention of enteric E. coli infection should be aimed at the reduction of numbers of pathogenic E. coli in the environment by good hygiene, maintenance of suitable environmental conditions, and provision of a plentiful supply of colostrum at birth and a high level of immunity. Strategies commonly used for the control of enteric E. coli infections are summarized in Table 53.6.

One of the most important factors in the prevention of enteric E. coli infection and septicemia is the maintenance of piglets at an adequate environmental temperature, free of drafts, and on a low-heat-conducting floor. This is particularly true for piglets of below average weight, which lose heat more rapidly because they have a greater skin surface area per unit body weight.

Stringent biosecurity should be used to control the introduction of different E. coli virotypes or other infectious agents into the herd. Animals in the herd will have little immunity to E. coli fimbrial antigens with which they have not had contact.

Good hygiene in the farrowing area leads to a reduction in the numbers of E. coli presented to the piglet to a level that it is able to control through its own defense mechanisms. Farrowing crates should be thoroughly cleaned and disinfected between litters. An all-in/all-out farrowing system with thorough disinfection of the farrowing room between batches will reduce the E. coli population in the environment.

Farrowing-crate design is important because it affects the location at which feces are deposited by the sow. In crates that are too long, the feces are deposited over a large area of the available floor space, thereby increasing the heavily contaminated area. Ideally, the crate should be adjustable, allowing for a shorter crate for gilts than for sows. Crates on raised, perforated floors allow fecal material to drop through and away from the piglets, and litters farrowed onto such floors have a noticeably lower incidence of diarrhea than those on solid concrete floors.

A dry, warm environment reduces the moisture available for survival and growth of E. coli. This is largely affected by ventilation rates, although if room temperature is too high, sows tend to try and spread water over their lying area to cool themselves, thereby defeating other hygienic procedures. The sow should be at a temperature of approximately 72°F (22°C), necessitating a warmer creep area for the piglets. It is important to ensure that younger piglets are maintained at a constant temperature of 86–93°F (30–34°C).

Maternal vaccination has been one of the most effective ways of preventing neonatal ETEC diarrhea in piglets. One of the earliest vaccination techniques consisted of taking the small intestinal contents from a piglet with diarrhea, culturing it in milk, and feeding the culture to pregnant sows, usually about a month before parturition (Kohler 1974). This technique is effective, conferring an immunity lasting throughout the suckling period, and is still used, particularly in the United States.

Commonly used commercially available vaccines are given parenterally and may be killed whole cell bacterins or purified fimbrial vaccines. Both types of vaccines appear to work equally well. Bacterins usually contain strains representing the most important serogroups and producing the fimbrial antigens F4 (K88), F5 (K99),

Table 53.6. Strategies commonly used for the control of enteric E. coli infections

<table>
<thead>
<tr>
<th>Strategies Which Result in:</th>
<th>Reduced Number of Pathogenic E. coli</th>
<th>Increased Resistance of Animals to Infection</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Preweaning diarrhea</strong></td>
<td>- Warmth</td>
<td>- Maternal vaccination for F4(K88), F5(K99), F6(987?) F41</td>
</tr>
<tr>
<td></td>
<td>- Hygiene</td>
<td>- Pig hyperimmune γ-globulin</td>
</tr>
<tr>
<td></td>
<td>- Gate and floor design</td>
<td>- Live oral nontoxigenic F4 and F18 E. coli vaccines</td>
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<tr>
<td></td>
<td>- Quarantine</td>
<td>- Oral powdered egg yolk from F4 and F18 immunized hens</td>
</tr>
<tr>
<td></td>
<td>- All-in/all-out farrowing</td>
<td>- Stx2e toxoid vaccine (ED)</td>
</tr>
<tr>
<td><strong>PWD and ED</strong></td>
<td>- Increase weaning age</td>
<td>- Selection of F4- and F18-resistant animals</td>
</tr>
<tr>
<td></td>
<td>- Warmth</td>
<td></td>
</tr>
<tr>
<td></td>
<td>- Diet</td>
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<tr>
<td></td>
<td>- Highly digestible</td>
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<td></td>
<td>- Milk-based protein</td>
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</tr>
<tr>
<td></td>
<td>- Restricted feed intake</td>
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<td>- Hygiene</td>
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<td>- Water additive</td>
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<td>- Organic acids</td>
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<td>- Feed supplements</td>
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<td></td>
<td>- Organic acids</td>
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<tr>
<td></td>
<td>- ZnO</td>
<td></td>
</tr>
<tr>
<td></td>
<td>- Spray-dried plasma</td>
<td></td>
</tr>
<tr>
<td></td>
<td>- Probiotics</td>
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</tbody>
</table>
ED is caused by certain *E. coli*, known as EDEC, which colonizes the small intestine and elaborates an Stx, Stx2e, which enters the bloodstream and damages vessel walls resulting in edema in targeted tissues. Most notably, cerebral edema leads to the predominant nervous signs characteristic of the disease. ED strains usually have F18ab as fimbrial adhesin, or can have F18ac. Most ED strains belong to serogroups O138, O139, O141, and O147. Unlike losses due to PWD that are reported similarly worldwide, mortality due to ED does not appear to be as great in North America as is observed in Europe. ED most often manifests as sporadic cases or small outbreaks limited to a specific age group. The case mortality rate ranges from 50% to over 90% and the course of the disease in the herd varies from 4 to 14 days. The disease typically disappears as abruptly as it appears. Recurrence on premises is common.

Some F18ab- or F18ac-positive strains of *E. coli* produce both enterotoxins and Stx2e. Pigs infected by one of these strains generally exhibit clinical PWD more than ED. Outbreaks of disease may also be caused by mixed infections of both ETEC and EDEC strains. When this occurs, clinical diarrhea usually predominates, even though microscopic lesions of ED may be present.

The age group primarily affected by PWD and/or ED varies based on absolute pig age and diet. There are some differences between *E. coli* with F4 (K88) and those with F18. Receptors for F4 (K88) are fully expressed on enterocytes of pigs from birth to adult age, rendering pigs of all ages potentially susceptible to infection. F4 (K88)-positive strains most often cause outbreaks of PWD in the very first days after weaning. However, in farms where weaning rations contain higher levels of protein of animal source, plasma, acidifying agents, and zinc oxide, peaks of diarrhea may be observed at 3 weeks after weaning, or even at 6–8 weeks after weaning (J. M. Fairbrother, unpublished observations).

In contrast to F4 (K88), F18 receptors important in ED and some PWD are not yet fully expressed by piglets under about 20 days of age (Nagy et al. 1992). Hence, *E. coli* with F18 fimbriae do not cause diarrhea or ED in neonatal pigs. F18-positive strains more often cause ED or PWD between 5 and 14 days after weaning or after introduction to fattening herds. Fimbrial receptors are subject to modulation by feed lectins such as constituents of leguminous plants (Kelly et al. 1994). It is speculated that feed-induced changes of the receptor are involved in the observed reduction colonization by F18-positive *E. coli* in the first days after weaning (Bertschinger et al. 1993).

In weaned pigs, the environment of the weaner unit appears to be the most likely source of pathogenic *E. coli* strains. Older suckling pigs may acquire the infection from previously contaminated farrowing crates or their dam and develop disease and/or carry it into the

**POSTWEANING ESCHERICHIA COLI DIARRHEA AND EDEMA DISEASE**

*Escherichia coli* PWD and ED are treated in one section because they often both occur in the same age group of pigs, the causative bacteria share certain virulence factors, and some strains of *E. coli* can cause both diseases. There are also important differences in the two diseases.

Older unweaned piglets can be affected by *E. coli* diarrhea and ED as levels of protective antibody wane in maternal milk; however, loss of milk antibodies at weaning contributes significantly to susceptibility of pigs to *E. coli* enteric infections in the postweaning period (Deprez et al. 1986; Sarmiento et al. 1988). As a result, most disease is observed postweaning.

PWD and ED are caused by strains of *E. coli* that possess adhesion factors enabling the bacteria to colonize the small intestine and that elaborate one or several protein exotoxins. Nearly all of these *E. coli* are alphahemolytic. Most of them belong to a very limited number of serotypes. PWD and ED may occur independently, but they may also occur in a single outbreak or in the same pig.

PWD is most commonly caused by ETEC and mediated by enterotoxins, but can also be caused by EPEC that do not possess any virulence factors of classic PWD or ED strains. Common PWD and ED serovirotypes are summarized in Table S3.2 and virulence factors are listed in Table S3.1 and described in the etiology section above. PWD strains usually have either F4 (K88) or F18 variants F18ac or F18ab as fimbrial adhesins, although some F4 (K88)- and F18-negative virotypes exist. These strains produce one or more enterotoxins, including heat-stable STa and STb, heat-labile LT, and EAST-1.

Most PWD F4 (K88)-positive isolates are serogroup O149 and F18 isolates are O139, O138, O141, O147, or O157. PWD is observed in pigs worldwide and in a given geographical area, certain serovirotypes predominate (Do et al. 2010; Fairbrother et al. 2000; Francis 2002; Frydendahl 2002). Outbreaks of F4 (K88)-positive *E. coli* tend to involve only one strain at any one time. Occasionally, two potential pathogens are isolated, but one usually predominates in any given outbreak. Multiple outbreaks in herds involving different serogroups were detected in 47% of 84 herds (Awad-Masalmehe et al. 1988). Mortality is often 1.5–2% of pigs and can reach 25% if no treatment is given.

F6 (987P), and F41 (Nagy 1986). They are usually given parenterally at about 6 weeks and 2 weeks prior to parturition. In cases where vaccination is ineffective, it is important to identify the serotypes involved for possible inclusion in an autogenous bacterin. Further characterization of these isolates may identify new or variant fimbrial adhesins important in the pathogenesis of ETEC diarrhea.
weaner unit. Intestinal infections caused by *E. coli* are contagious and are transmitted to other pigs via contaminated feed, handlers, and drinking water and by aerosol, and infection occurs by the oral route. The same strain is usually found in many sick pigs and often in consecutive batches of pigs. For example, when ED entered Denmark in 1994, 63% of the outbreaks were traced to a single infected breeding herd (Jorsal et al. 1996). On the other hand, clinical ED occurred in no more than 5% of the herds with trading contacts. These strains are usually only shed for a few days after infection, probably due to the development of immunity. Not all infected pigs develop disease; the degree of colonization determines whether disease results from infection.

**Pathogenesis**

**Colonization.** When ingested in sufficient numbers, *E. coli* causing PWD and/or ED colonize then proliferate rapidly to attain massive numbers to the order of 10^9/g of contents. For ETEC and EDEC, colonization requires attachment of fimbrial adhesins to complementary receptors on the small intestinal epithelium or in the mucus coating in the midjejunum to ileum. Whereas, for EPEC the Eae adhesin ( intimin) binds to its complementary receptor on the apical surface of host epithelial cells in the small and large intestines, with heaviest colonization in the duodenum and cecum.

Epithelial cell receptors for pathogenic ETEC and EDEC are not present in every pig. Certain pigs do not have receptors for the F4 (K88)-adhesin on intestinal epithelial cells and are thus resistant to infection by F4 (K88)-positive ETEC. This genetic resistance to infection is inherited in a simple Mendelian way, and the allele for the receptor is dominant. Subsequent studies have demonstrated at least five pig phenotypes, based on susceptibility of brush borders of different pigs to adherence of isolates producing the variants F4ab (K88ab), F4ac (K88ac), and F4ad (K88ad) (Hu et al. 1993). The loci encoding porcine intestinal receptors for F4ab (K88ab) and F4ac (K88ac) are closely linked on chromosome 13 (Edfors-Lilja et al. 1995). A similar genetic resistance has not been observed for the other fimbriae of neonatal porcine ETEC.

The receptor for F18 fimbriae is also controlled in a single locus on chromosome 6 close to the locus for stress susceptibility, and the presence of a receptor is dominant over absence. Pigs with at least one copy of the dominant allele for the receptor are susceptible to epithelial cell adherence, and hence to intestinal colonization. There appears to be no coselection for F18 receptor and halothane sensitivity (Coddens et al. 2007).

Other factors may also affect bacterial colonization and severity of disease. Low room temperature in the weaner rooms appears to be responsible for a more severe course of PWD (Wathes et al. 1989). This is likely because chilling reduces intestinal peristaltic activity and enhances bacterial colonization. In contrast, under experimental conditions ED is not aggravated by cold stress (Kausche et al. 1992). Endogenous as well as orally administered proteases may reduce the receptor activity for F4 (K88) fimbriae (Mynott et al. 1996), thus reducing the severity of F4 (K88)-mediated diarrhea. Several predisposing factors, such as a weaner diet containing soya and field peas or (porcine reproductive and respiratory syndrome [PRRS]) virus infection, may enhance bacterial colonization and development of the AE lesions of EPEC (Neef et al. 1994).

**Mechanisms of Enterotoxigenic *Escherichia coli* Postweaning Diarrhea.** Like neonatal ETEC, postweaning ETEC adhere to the intestinal mucosa and produce enterotoxins which change the water and electrolyte flux of the small intestine and may lead to diarrhea if the excess fluid from the small intestine is not absorbed in the large intestine. Excessive secretion leads to dehydration, metabolic acidosis, and possibly death.

Postweaning ETEC strains produce one or more of the enterotoxins StA, StB, LT, and EAST-1. The properties and mechanism of action of StA are as described above for neonatal ETEC.

StB (STII, ST2, ST pig) is a small, 5000Da protein that is antigenically and genetically unrelated to StA and is poorly immunogenic (Dubreuil 1997). The intestinal epithelial cell receptor to which StB binds has been identified as sulfatide (3′-sulfogalactosyl-ceramide) (Gonçalves et al. 2008). StB does not alter cGMP or cyclic adenosine monophosphate (cAMP) levels in intestinal mucosal cells, thus differing in mechanism of action from StA and LTI. Binding of StB to its receptor leads to uptake of Ca^2+ into the cell, inducing the duodenal and jejunal secretion of water and electrolytes by unknown mechanisms (Harville and Dreyfus 1995).

LT produced by porcine PWD strains belongs to the LTI subgroup. The LT produced by human and porcine ETEC has been designated LTh and LTp, respectively, based on slight differences in the genes coding for the toxin. LTI is a high-molecular-weight toxin complex that consists of a biologically active A subunit and five B subunits that bind GM1 ganglioside receptors on the intestinal epithelial cell surface. LTI is transported across the outer membrane into the intestinal lumen and is internalized in enterocytes by receptor-mediated endocytosis (Dorsey et al. 2006). The toxin permanently activates adenyl cyclase in the basolateral border of the cell and leads to hypersecretion of electrolytes and water. Recent studies have shown that LT also promotes adherence of ETEC in vitro and in vivo (Johnson et al. 2009).

EAST-1 was first identified in enteraggregative *E. coli* isolated from humans and was subsequently reported in ETEC from pigs with diarrhea (Yamamoto...
and Nakazawa 1997). It is commonly found in F4-positive ETEC strains from pigs with diarrhea and in F18:Stx2e strains from pigs with ED (Choi et al. 2001). EAST-1 is a 38 amino acid peptide of 4100 Da that is different from STa and STb, although it shares 50% homology with the enterotoxic domain of STa (Savarino et al. 1993) and appears to interact with the STa receptor guanylate cyclase C to elicit an increase in cGMP. Hence, the mechanism of action of EAST-1 is proposed to be identical to that of STa. However, the role of EAST-1 in the development of diarrhea has not been defined.

Mechanisms of Enteropathogenic Escherichia coli Postweaning Diarrhea. Porcine EPEC attach to the intestinal mucosa and cause lesions similar to those observed for EPEC isolated from human infantile diarrhea (Hélie et al. 1991). They attach intimately to the intestinal epithelial cell membrane by means of a bacterial outer membrane protein termed “EPEC attaching and effacing factor” (Eae) or “intimin.” Eae is the product of a chromosomal PAI in EPEC composed of over 40 genes that code for proteins involved in intimate adherence to and signal transduction within the host cell (Dean and Kenny 2009; Nataro and Kaper 1998). One of these proteins is the “translocated intimin receptor” (Tir) that is injected into the host cell cytoplasm, where it reappears on the host cell surface where it acts as receptor for intimin (Gyles and Fairbrother 2010). Attached EPEC efface the microvilli and sometimes invade the epithelial cells (Figure 53.2) (Zhu et al. 1994). The mechanisms by which EPEC induce diarrhea are poorly understood. Effacement of microvilli and the consequent loss of absorptive surface area may lead to malabsorptive diarrhea (Nataro and Kaper 1998). The rapid onset of diarrhea suggests a more active secretory mechanism that may be due to EPEC signaling activity on intracellular mediators of intestinal ion transport, such as calcium, inositol phosphates, and tyrosine kinase (Gyles and Fairbrother 2010). Other possible mechanisms include an increased permeability of tight junctions between epithelial cells, a localized inflammatory response at the lesion site, or chloride secretion following polymorphonuclear (PMN) leukocyte transmigration. The clinical outcome of the infection is difficult to evaluate, as mixed infection, such as with F4 (K88)-positive ETEC, often occurs.

Mechanisms of Edema Formation by Edema Disease Escherichia coli. ED is an Stx2e toxemia that results in severe edema in specific sites in pigs that have absorbed Stx2e from the intestine following colonization by an EDEC. Highly purified Stx2e induces a dose-dependent disease indistinguishable from ED when administered intravenously to pigs (MacLeod and Gyles 1990). Colonization by EDEC develops over 3–6 days on the tips and sides of villi in the distal jejunum and ileum and is mediated by plasmid-encoded F18ab or F18ac fimbriae (Bertschinger et al. 1990b).

Stx2e produced by EDEC in the intestine is absorbed into the circulation and causes vascular damage in target organs. The toxin also binds to globotetraosyl ceramide on red blood cells. Thus, vessels are subjected to prolonged toxin exposure (Boyd et al. 1993). The toxin can be detected in endothelial cells of small blood vessels of the intestine and in microvillous membranes of enterocytes at the base of the villi by immunological methods (Waddell et al. 1996). Stx2e does not appear to be absorbed from the intestine under normal conditions, but the addition of deoxycholate to the intestine allows absorption of Stx2e to occur (Waddell and Gyles 1995), and it is possible that bile could influence absorption. Strains of EDEC may pass from the intestine to the mesenteric lymph nodes and produce Stx2e toxin there, providing another mechanism for absorption of toxin into the blood.

The most consistent injury observed in field cases, after injection of partially purified toxin (Gannon et al. 1989), and in pigs inoculated orally with live EDEC (Kausche et al. 1992), is a degenerative angiopathy of small arteries and arterioles. The edema fluid found in various tissues is low in protein and is the result of a mild increase in vascular permeability. Information on pathophysiology of ED is scarce. Clugston et al. (1974a) observed an increase in blood pressure after intravenous administration of edema disease principle (EDP), a partially purified Stx2e preparation. Hypertension developed later than clinical edema and was therefore thought to be the result of vascular injury rather than its cause. Hypertension might exacerbate the lesions in the already damaged vessels. The development of injuries in the nervous system may be due to hypoxia resulting from impaired blood flow (Clugston et al. 1974b), as well as edema.

A distinct type of ED is characterized by terminal bloody diarrhea and hemorrhagic lesions in the cardiac region of the stomach, the ileum, and the large intestine (Bertschinger and Pohlenz 1983). Gannon et al. (1989) observed acute hemorrhagic gastroenteritis in some of the pigs to which a high dose of Stx2e was administered. Epithelial necrosis secondary to necrosis of small arteries and arterioles may be responsible for luminal hemorrhage.

Clinical Signs Postweaning Diarrhea. Diarrhea in pigs from late suckling through the postweaning period is similar to that observed in neonatal piglets but tends to be less severe. Diarrhea is seen as yellowish or gray fluid, lasting up to a week and causing dehydration and emaciation. Over several days, most of the pigs in a group may be affected and mortality of up to 25% may be observed. Peaks of diarrhea may be observed at 3 weeks after...
Lesions

Postweaning Diarrhea. Pigs dead from *E. coli* PWD are generally in good condition but severely dehydrated with sunken eyes and some cyanosis. The stomach is often distended with dry feed. The gastric fundus is variably hyperemic. The small intestine is dilated, slightly edematous, and hyperemic. The contents vary from watery to mucoid, with a characteristic smell. The mesentery is heavily congested. Contents of the large intestine most often are light greenish or yellowish and are mucoid to watery. Pigs dying late in an outbreak look emaciated and exhibit a strong smell of ammonia. There are irregularly shaped superficial ulcerations in the gastric fundus and large intestine. The feces look yellow and pasty. The fluid from the anterior chamber of the eye may give a positive reaction for urea. If the causative strain of ETEC also produces Stx2e, lesions typical of ED (see below) are mild or absent.

Microscopic lesions in pigs with ETEC PWD are few. Bacterial layers are observed in patches on the apical surface of villous epithelial cells in the ileum and, less consistently, jejunum (Sarmiento et al. 1988a). The mucosa and the epithelium remain normal in appearance; however, increased numbers of neutrophils may be observed in the superficial lamina propria.

Microscopically in pigs infected with EPEC, a multifocal colonization of the brush border of mature enterocytes by *E. coli* arranged in palisades with enterocyte degeneration and light to moderate inflammation of the lamina propria is observed, mostly in the ileum (Hélie et al. 1991). Colonization is most intense in the duodenum and cecum, bacteria are sometimes observed in intracytoplasmic vacuoles in enterocytes, and colonized enterocytes swell and slough, resulting in mild to moderate villous atrophy in the small intestine. On transmission electron microscopy, bacteria are intimately attached to the cytoplasmic membrane of mature enterocytes and arranged in regular palisades, parallel to the microvilli, with effacement of adjacent microvilli. The bacterial cell wall and the apical cell membrane of the enterocyte are separated by a narrow, regular gap of 10 nm at the cupping pedestal, and apical dense regions are seen at attachment sites (Figure 53.2).

Edema Disease. Pigs dead of ED are mostly in good condition. Edema is variable in specific sites of predilection and may be absent in some animals. Subcutaneous edema may occur and is most often in the eyelids and face (Figure 53.3). Gelatinous edema that is barely detectable up to 2 cm in thickness in the submucosa of the gastric cardia and occasionally in the fundus is characteristic (Figure 53.4). The mesocolon is commonly edematous and edema of the small intestinal mesentery and gallbladder is sometimes observed. The mesenteric and colic nodes may be swollen, edematous, and congested. Pericardial, pleural, and peritoneal...

**53.3. Edematous swelling of eyelids, forehead, and lips, breathing through open mouth, and inability to rise in a weaned pig 4 days after oral inoculation with culture of Stx2e-producing edema disease Escherichia coli (EDEC) 0139:K12:H1.**

Edema Disease. ED mostly occurs in recently weaned pigs, although cases may be observed throughout to the grower barns. The disease may be sporadic or may affect an entire herd and may be first recognized as sudden death without signs of sickness. Some affected pigs become inappetent, develop swelling of the eyelids and forehead (Figure 53.3), emit a peculiar squeal, and show incoordination and respiratory distress (Sojka 1965). A portion of these will soon become recumbent and die. There is usually no diarrhea or fever. In mild cases, subcutaneous edema is accompanied by pruritus, which disappears after recovery. In some pigs with or without dyspnea, respiration is accompanied by a snoring sound. Watery diarrhea with clots of fresh blood may appear in a few pigs at the terminal stage.

Subclinical ED may occur, where pigs are clinically normal but develop vascular lesions and may have a decreased growth rate. Chronic ED occurs in a low proportion of pigs recovering from acute attacks of ED or *E. coli* PWD caused by strains that also produce Stx2e. This condition was called cerebrospinal angiopathy before its association with ED became apparent. For periods varying from days to several weeks after intestinal infection, growth stops and sick pigs often show unilateral nervous disturbances such as circling movements, twisting of the head, or atrophy of limb muscles with progressive weakness. Subcutaneous edema is rare.
cavities sometimes contain a slight increase of serous fluid that contains strands of fibrin.

The stomach is typically full of dry, fresh-looking feed, and the small intestine is relatively empty. Some think that this is a manifestation of delayed gastric emptying since some animals have a period of anorexia before death. Colonic contents may be diminished in amount and some pigs may be constipated. There may be varying degrees of pulmonary edema and a characteristic, patchy, sublobular congestion. In some cases this may be the only observable lesion. Cases with laryngeal edema have also been observed. A few epicardial and endocardial petechiae may occur. This lesion must not be confused with mulberry heart disease where hemorrhages are also throughout the myocardium.

In some pigs with ED, a form of hemorrhagic gastroenteritis occurs, which is quite different from that described with *E. coli* PWD. In addition to marked edema, the edematous submucosa of the cardiac region of the stomach and the mucosa of the lower small and upper large intestine show extensive hemorrhage. Watery diarrhea with clots of coagulated blood occurs shortly before death in some of these pigs.

Microscopically, patchy layers of bacteria are adherent to the distal jejuna and ileal mucosa early in the course of ED (Bertschinger and Pohlenz 1983). Contrasting with *E. coli* PWD, the colonization has often disappeared when pigs with ED become moribund (Smith and Halls 1968).

The hallmark microscopic lesion of ED is a degenerative angiopathy affecting small arteries and arterioles with associated edema of surrounding tissues (Clugston et al. 1974b). Sites of predilection are those mentioned above where edema is observed, as well as in the brain. The dense arterial network in the mesocolon adjacent to the colic lymph nodes is frequently affected. Vascular lesions may be subtle, affecting only some segments of arterioles, and difficult to detect in acute cases, but are more readily apparent in surviving pigs or those affected subclinically (Kausche et al. 1992).

Acute changes include necrosis of smooth muscle cells in the tunica media evidenced by pyknotic and karyorrhectic nuclear debris and hyaline change in cytoplasm. In the walls of some affected vessels, fibrinoid material is deposited (Figure 53.5). Endothelial cells may also be swollen. In acute experimental cases,
edema of the leptomeninges and perivascular spaces has been demonstrated. Affected vessels in the brain may be surrounded by eosinophilic, periodic acid–Schiff (PAS)-positive droplets (Bertschinger and Pohlenz 1974). In older lesions, there may be proliferation of adventitial and medial cells (Figure 53.6). Thrombosis is not usually a feature of uncomplicated, naturally occurring ED.

In cases of ED where hemorrhagic gastroenteritis is observed, vessels in affected areas of the gastric, lower small intestinal and upper colonic wall have changes strikingly similar to those observed in human hemorrhagic colitis caused by EHEC. These include swelling, vacuolation, and proliferation of endothelial cells, subendothelial fibrin deposition, medial necrosis, perivascular edema, and microthrombus formation.

In pigs that have recovered from natural outbreaks or survived for several days following acute signs, there may be multifocal encephalomalacia in the brain stem together with typical lesions in the small arteries and arterioles (Kausche et al. 1992). Malacia is thought to be due to ischemia secondary to vascular injury.

**Diagnosis**

In enteric *E. coli* infections, a presumptive diagnosis is made based on clinical presentation of diarrhea early after weaning (or sometimes later as previously described with certain diets), marked dehydration, and low to moderate mortality. The gross lesions, including the characteristic smell, are also helpful. Differentials should include other common causes of nonbloody diarrhea in weaned pigs, including rotaviruses (Chapter 43), TGEV (Chapter 35), salmonellosis (Chapter 60), and, in older weaned pigs, proliferative enteropathy (Chapter 59).

Likewise in ED, a presumptive diagnosis is made based on the sudden appearance of neurological disease in thriving pigs 1–2 weeks after weaning. Partial ataxia or a staggering gait is the most consistent sign and subcutaneous edema in the palpebrae and over the frontal bones is also a cardinal sign when present. Characteristic lesions of edema in gastric mucosa and mesentery are helpful when present, but may be absent in a significant number of cases, especially when severe diarrhea has preceded ED. Differentials should include other common causes of nervous signs in weaned pigs, including pseudorabies (Chapter 28), teschoviral encephalitis (Chapter 42), *Streptococcus suis* (Chapter 62), or *Haemophilus parasuis* (Chapter 55), and induced meningitis and water deprivation/salt intoxication (Chapter 70).

Definitive diagnosis of ETEC or EPEC PWD and ED is based on the presence of typical lesions and culture of the offending *E. coli* along with confirmation of appropriate serotypes and/or virulence factors (Tables 53.1 and 53.2). Criteria used to identify causative *E. coli* in diarrhea in pigs are summarized in Table 53.5. Colonization can be visualized in formalin-fixed, paraffin-embedded tissues by routine histopathology or *E. coli* organisms can be definitively identified and visualized using immunohistochemistry or in frozen sections using indirect immunofluorescence. Methods for culture and serovirotyping are discussed previously in the section on diagnosis of neonatal ETEC.

Culture of the small intestine in ETEC and small intestine and colon in EPEC and ED usually yields pure or nearly pure cultures of hemolytic (ED and ETEC) or nonhemolytic (EPEC) *E. coli*. However, in ED, bacterial numbers may have declined in more protracted cases (Bertschinger and Pohlenz 1983); therefore, a negative bacteriological result does not exclude the diagnosis of ED. Because all F4 (K88) or F18 *E. coli* causing ED and ETEC PWD are hemolytic, the presence of hemolytic colonies is often used as a rapid means for confirming a presumptive diagnosis of these conditions. However, this method would exclude EPEC that may also cause PWD, since these organisms produce nonhemolytic colonies on blood agar. This may be an important consideration when putting into place prevention strategies, such as vaccination. In addition, in cases of diarrhea with mixed infections of hemolytic and nonhemolytic ETEC, the assumption that the presence of hemolytic colonies indicated that F4 (K88) or F18 ETEC was the only causative agent may result in the nonhemolytic ETEC remaining undetected. Likewise, in cases of diarrhea mixed with subclinical ED, this assumption would result in the ED remaining undetected. These are important considerations in light of the increasing prevalence of mixed pathogenic *E. coli* infections associated with cases of diarrhea, particularly later in infection or in groups with a more endemic presentation.
In cases of subacute or chronic ED or of ED in adults, culture is of little value since offending EDEC are typically no longer a dominant strain in the intestines. Subacute or chronic ED is diagnosed by lesions, especially by the demonstration of subacute to chronic arteriopathy and possibly lesions of focal encephalomalacia. Diagnosis of ED in adult pigs may require additional effort, often requiring postmortem examination and histopathology of more than one pig. Death of adults is occasionally caused by cerebral hemorrhage (stroke) from rupture of severely damaged arterioles, with an apparent affinity for the basal ganglia, especially the corpus striatum.

**Immunity**

Protective acquired immunity is based on the presence of mucosal antibodies, predominantly secreted IgA, to fimbrial adhesins, particularly F4 (K88) and F18 in weaned pigs. There are differences in kinetics of infection and immunity in weaned pigs infected with F4 (K88)-positive and F18-positive ETEC (Verdonck et al. 2002). Infection with F4 (K88)-positive ETEC results in a more rapid intestinal colonization and induction of antifimbrial antibodies than for F18-positive ETEC, and the switch from IgM to IgA and IgG is earlier following F4 (K88)-positive ETEC infection. Pigs that have been colonized by an F18 ETEC producing StA and StB are protected against recolonization by a heterologous ETEC sharing no other antigens with the immunizing strain except F18 fimbrae. However, the cross-protection between strains with fimbrial variants F18ab and F18ac may not be very high (Bertschinger et al. 2000).

Protective acquired antibodies are produced against Stx2e in pigs that survive ED. Wieler et al. (1995) showed that pigs that had survived an outbreak of ED had antibodies reacting in an ELISA to the B subunit of Stx2e. Vaccine studies using various forms of Stx2e toxoid have demonstrated protection in ED disease models (see the section on active and passive immunization below).

**Prevention and Control**

**Treatment.** In postweaning colibacillosis, treatment with antimicrobials and electrolytes should be administered. Sick pigs must be treated parenterally since they eat and drink very little, even if they stand close to the creep and to the drinking nipple. Subsequently the antimicrobial may be given in the water or feed. Attractive rehydration fluid should be offered to counteract dehydration and acidosis or injected intraperitoneally if the pig is anorectic. Such fluids may contain glucose, glycine, citric acid, and potassium dihydrogen phosphate in an isotonic solution (Bywater and Woode 1980). Uptake should be equal to the loss (i.e., up to 25% of the body weight).

Control of bacterial proliferation therapeutically is much more effective in *E. coli* PWD than in ED, because in ED Stx2e has already been absorbed into the circulation and been bound to receptors when clinical signs become visible. In general, piglets showing neurological signs have a poor prognosis. The development of bacterial resistance against a wide range of antimicrobial drugs makes the efficacy of antimicrobial therapeutics uncertain. Antimicrobial susceptibility testing is indispensable for selection of effective drugs. Antimicrobials must be selected which reach therapeutic concentrations in the intestinal lumen, such as amoxicillin/clavulanic acid, fluoroquinolones, cephalosporins, apramycin, ceftiofur, neomycin, or trimethoprim.

For groups of pigs during outbreaks of ED, withholding the feed is thought to impair colonization and is a valuable measure still recommended to prevent new clinical cases.

**Preventive Husbandry.** Nurseries should be managed as all-in/all-out facilities and should be thoroughly cleaned of organic matter and disinfected prior to use. Water lines and water systems should likewise be disinfected using shock chlorination or a similar chemical process. Management of the weanling pig should minimize environmental and other forms of stress such as unnecessary mixing of litters, chilling, transportation, and assignment to new pens. Recently weaned pigs should be housed in a draft-free environment at a constant temperature of about 85°F (29.5°C).

**Passive and Active Immunoprophylaxis.** Various strategies involving passive immunity for the prevention of PWD and ED have been used with varying success. An improved weight gain and lower frequency of diarrhea was observed in early weaned (10 days of age) pigs fed a spray-dried porcine plasma (SDPP)-based diet, partly due to the presence of specific anti-ETEC antibodies (Owusu-Asiedu et al. 2002). Similarly, spray-dried porcine blood plasma had an inhibitory effect on *E. coli* enterotoxemia that lasted only as long as the plasma was fed (Deprez et al. 1990). Immune protection against colonization with F4 (K88)- and F18-positive *E. coli* may be attained by feeding eggs produced by vaccinated hens (Imberechts et al. 1997). Antiserum produced by injecting a horse with Stx2e toxoid was effective in protecting pigs from ED in two Danish herds with ED (Johansen et al. 2000).

Few commercial vaccines are available for the prevention of *E. coli* PWD and ED. Injectable vaccines, such as those administered to sows for the prevention of neonatal diarrhea, stimulate mostly systemic rather than mucosal immunity, giving rise to circulating antibodies which do not reach intestinal bacteria in high enough levels to be very effective (Van den Broeck et al. 1999). Such vaccines may even suppress the
mucosal immune response upon subsequent oral infection with a pathogenic *E. coli* (Bianchi et al. 1996).

Several approaches for the control of *E. coli*-associated PWD are currently being investigated. Live avirulent *E. coli* vaccine strains carrying fimbrial adhesins may be administered to weaned piglets in the drinking water or to unweaned piglets by oral dosing at least 1 week prior to the expected onset of diarrhea. Oral administration of a commercially available live nonenterotoxigenic F4 (K88) *E. coli* vaccine to pigs immediately following weaning induced a significant protection after virulent F4 (K88) ETEC challenge, resulting in normal growth rates after challenge (Nadeau et al. 2010). Current research is aimed at the oral administration of purified F4 (K88) fimbriae as a vaccine for the control of outbreaks of *E. coli*-associated diarrhea in weaned pigs (Van den Broeck et al. 1999). The use of such a subunit vaccine results in a specific intestinal mucosal immune response and a significant reduction in fecal excretion of the pathogenic F4 (K88). Hodgson and Barton (2009) have recently reviewed strategies for the prevention of ETEC diseases.

Several approaches for the control of ED have been investigated. Vaccination with various Stx2e toxoid preparations has provided the best results in preventing ED. In pigs vaccinated with detoxified purified Stx2e, mortality due to ED was significantly reduced, and daily weight gain in the nursery was significantly improved following challenge with Stx2e (MacLeod and Gyles 1991). A genetically modified Stx2e toxin was found to prevent overt and subclinical ED when vaccinated pigs were challenged with EDEC (Bosworth et al. 1996). In two Danish herds with persistent ED problems, vaccination with an Stx2e toxoid almost totally eliminated mortality due to ED (Johansen et al. 1997).

As with ETEC PWD, live avirulent F18-positive, Stx2e-negative strains carrying fimbrial adhesins have been orally administered to pigs at least 1 week prior to the expected onset of ED. Mixed results in uncontrolled trials are reported. Oral immunization with purified F18 fimbriae did not induce protective mucosal immunity against F18-positive *E. coli* infection (Verdonck et al. 2007), in contrast to observations for F4 (K88) fimbriae.

**Antimicrobial Prophylaxis.** At present, preventive feed medication with antibiotics is widely practiced in most countries despite serious drawbacks such as nonacceptance by the consumer, impaired buildup of immunity, and selection of resistant bacteria. Resistance is often induced within days or a few weeks. Isolates from *E. coli* PWD and ED show the highest rate of resistance within porcine *E. coli*. Besides the classes of antimicrobials mentioned above for parenteral therapy, the aminoglycosides and colistin are widely used for chemoprophylaxis. The latter has the advantages of high stability, low toxicity, absence of infectious resistance, and slow development of resistance. Investigators have reported that oxytetracycline reduces the adhesion of *E. coli* at concentrations below the minimum inhibitory concentration. Sarmiento and Moon (1988) reported that *E. coli* PWD induced by a tetracycline-resistant strain takes an identical course in pigs eating feed with and without tetracycline.

**Dietary Preventive Measures.** Restriction of feed intake, high-fiber diets, or ad libitum feeding of fiber have been reported as effective deterrents to the development of ED and PWD (Bertschinger et al. 1978; Smith and Halls 1968). The nutritive value of the feed may be reduced by increasing fiber content to 15–20% and reducing crude protein and digestible energy to one-half of the normal values. The addition of fiber to normal diets or feeding high-quality alfalfa coupled with restriction of daily feed intake may be beneficial. A low protein diet may decrease production of toxic protein metabolites and reduce PWD (Halas et al. 2007). Animal source proteins seem to provide protection against PWD. Addition of dairy products to feed delayed the occurrence of PWD and reduced mortality (Tzipori et al. 1980), possibly due to greater digestibility or stimulation of higher feed intake (Lalles et al. 2007).

A lower mortality due to *E. coli* enterotoxemia and improved weight gains were reported after introduction of rations with a reduced acid-binding capacity. A similar effect is ascribed to organic acids. Organic acids contribute to the maintenance of an acidic gastrointestinal tract which may control potentially pathogenic bacteria. However, mortality due to ED was not reduced by the inclusion of a mixture of organic and inorganic acids in the feed (Johansen et al. 1996). This result is not surprising in view of the highly regulated pH close to the mucosal surface (McEwan et al. 1990).

Zinc oxide offers an alternative to antimicrobials. Feeds with contents between 2400 and 3000 ppm of zinc reduce diarrhea and mortality and improve growth. The protective effects of zinc oxide may not be due to antibacterial activity but to a protection of intestinal cells from ETEC infection by inhibition of bacterial adhesion and internalization and modulation of cytokine gene expression (Roselli et al. 2003). However, environmental considerations should be included in discussions of zinc oxide at such high levels.

Several successful dietary supplements have recently been reported. Oral administration of immunostimulatory beta-glucans from the cell wall of yeasts reduces the susceptibility of weaned piglets to ETEC F4 (K88) infection (Stuyven et al. 2009). Exogenous as well as endogenous proteases lower the activity of intestinal F4 (K88) receptors. Bromelain, a protease from pineapple stems, applied orally to pigs reduced the binding of F4 (K88)-positive ETEC to brush borders in a dose-dependent manner (Mynott et al. 1996).
Addition of colicin E1, a member of a class of bacteriocins produced by and effective against *E. coli*, in the diet of young pigs decreased the incidence and severity of PWD caused by F18-positive ETEC and improved the growth performance of the piglets (Cutler et al. 2007).

Prebiotics selectively stimulate the proliferation of potentially beneficial microorganisms in the gastrointestinal tract. For example, administration of a heat-killed and dried *Enterococcus faecalis* strain significantly reduced the incidence of clinical signs due to STEC (Tsukahara et al. 2007).

Some promising results have been obtained using probiotics, potentially beneficial microorganisms. Feeding of a diet supplemented with a *Lactobacillus sobrius* strain isolated from the pig intestine resulted in a significant decrease in ETEC numbers and increased daily weight gain in weaned pigs challenged with an ETEC F4 (K88) (Konstantinov et al. 2008). On the other hand, others reported no efficacy of feeding of *Lactobacillus* spp., *Enterococcus faecium*, and *Bacillus cereus* strain “toyoi” to experimentally and/or naturally infected pigs (De Cupere et al. 1992).

Feeding of a diet supplemented with fermented soybeans (especially *Rhizopus*-fermented soybean, but also *Bacillus*-fermented soybean) reduced the excretion of ETEC and the incidence, severity, and duration of diarrhea in weaned pigs (Kiers et al. 2003).

**Breeding of Resistant Pigs.** Augmentation of the presence of both the F18 and F4 (K88) resistance loci in the pig population through breeding is an attractive approach to prevent PWD and ED. However, it will be important to avoid co-selection of unwanted traits closely linked with loci coding for the F18 and the F4 (K88) receptors. It cannot be predicted if additional types of adhesive fimbriae or new variants of known types will emerge which could bind to yet unidentified receptors. Availability of techniques for large-scale selection of resistant animals is lacking and will be the main challenge in the near future.

A polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) test detecting FUT1 M307 polymorphism, correlated with the gene controlling expression of the *E. coli* F18 receptor, could be a simple and inexpensive method for large-scale selection of resistant animals lacking and will be the main challenge in the near future. A polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) test detecting FUT1 M307 polymorphism, correlated with the gene controlling expression of the *E. coli* F18 receptor, could be a simple and inexpensive method for large-scale selection of resistant animals (Frydendahl et al. 2003). This test could be employed as a predictor of susceptibility to *E. coli* F18-associated diarrhea.

The transmembrane mucin MUC13 appears to be associated with susceptibility and resistance to ETEC F4ab/ac (K88ab/ac), as the MUC13 gene is closely linked to the genes encoding F4ab (K88ab) and F4ac (K88ac) receptors. Hence, MUC13 provides potential markers for selection of ETEC F4ab/ac (K88ab/ac)-resistant animals (Zhang et al. 2008). A mucin 4 gene polymorphism for genetic F4ab/ac (K88ab/ac) resistance that had been linked to adhesion of ETEC F4 (K88) to intestinal enterocytes has also been used in a screening assay, although it is probable that other receptors are involved (Rasschaert et al. 2007). On the other hand, the immunity aspect should be considered if breeding for resistance becomes available for F4 (K88)-positive ETEC, as resistant sows (F4 (K88) receptor-negative sows) do not develop and transfer F4 (K88)-specific antibodies in their colostrum and thus, heterozygous piglets are not passively protected from development of neonatal diarrhea due to these strains.

**Methods to Eliminate Agents from Herds.** Because most pathogenic ETEC, EPEC, and EDEC belong to a limited number of serogroups, enteric *E. coli* infection with selected pathotypes could theoretically be eliminated from herds. In Denmark, following an outbreak of ED, an eradication program involving depopulation of affected farms and disinfection of the buildings resulted in freedom from disease in most participating farms for a minimum of 4–7 months (Johansen et al. 1996). Nevertheless, the tools to prove the absence of pathogenic *E. coli* from a given herd are not yet adequate and *E. coli* are difficult to eliminate from the environment.

**ESCHERICHIA COLI CAUSING FATAL SHOCK**

Enteric colibacillosis complicated with shock occurs in young pigs before and after weaning. *Escherichia coli* associated with this disease are either (1) F4 (K88)-positive ETEC that commonly belong to serogroup O149, O157, or O8, but only occasionally produce Stx2e (Faubert and Drolet 1992); or (2) Stx2e-producing *E. coli* that are associated with ED.

In these cases of ETEC or ED, infection progresses so rapidly that death occurs due to shock before diarrhea is observed or before diarrhea proves fatal in the case of ETEC or before cerebral edema proves fatal in ED. This phenomenon is probably due to the rapid release of large amounts of LPS by the colonizing ETEC. The lipid A portion of LPS stimulates the overproduction of mediators of inflammation including tumor necrosis factor (TNF)-α, interleukin (IL)-1, and IL-6. Aggregation and subsequent degranulation of neutrophils activated by these mediators causes damage to the vascular endothelium, loss of fluid, and hypovolemic shock. Modulation of the coagulation pathway also leads to fibrin deposition and clot formation.

Clinical signs are minimal. Apparently healthy young pigs die suddenly or decline rapidly with cyanosis of the extremities. A yellowish to brownish diarrhea is sometimes observed.

Characteristic gross lesions include marked congestion of the small intestinal and stomach walls and blood-tinged intestinal contents. Microscopically, severe congestion of the gastric and small intestinal...
mucosae is commonly associated with microvascular fibrinous thrombi. Necrosis of villi with marked infiltration of neutrophils occurs in severe cases. There is only occasional hemorrhage in the lamina propria of the jejunum and the ileum (Faubert and Drolet 1992).

Immunity, diagnosis, prevention, and control are as described for EPEC and ED.

**SYSTEMIC ESCHERICHIA COLI INFECTIONS**

*Escherichia coli* may induce systemic infections, such as septicemia, or localized extraintestinal infections, such as meningitis or arthritis, resulting from bacteremia (Fairbrother et al. 1989; Fairbrother and Ngeleka 1994; Morris and Sojka 1985). Septicemia due to *E. coli* may be primary, occurring sporadically or rarely as small outbreaks, predominantly in newborn to 4-day-old pigs (Nielsen et al. 1975), or secondary, when associated with diarrhea or other compromising diseases in young pigs.

Piglets lacking immunity due to an absence of ingested colostrum or to ingestion of colostrum lacking specific antibody are most at risk for primary septicaemia. Agalactia in sows, low birth weights, an inadequate number of functional nipples, or any other factor that reduces consumption of colostrum may result in susceptible piglets. Although these piglets usually develop septicemia within the first few days of life, cases may occur throughout the suckling period, or exceptionally in pigs up to 80 days of age. Poor environmental temperature control and inadequate hygiene increase the risk of systemic infection. The intestine is considered as the major route of *E. coli* invasion. The disease can be experimentally induced by oral or intragastric inoculation (Ngeleka et al. 1993). Bacterial invasion may also be via the respiratory tract or contamination of the umbilicus. Strains involved are known as ExPEC and are a heterogeneous group that possesses a large number of varied virulence factors (discussed in the section on etiology above). Yet only a limited number of serogroups are represented (Table 53.1).

Secondary septicemia may develop after invasion by ETEC. The ETEC serovirotypes most often involved are listed in Table 53.2. Older suckling piglets suffering from secretory diarrhea caused by ETEC are most often affected. Sows may also be affected soon after farrowing. Other risk factors may include enteric viruses that damage the intestine and alter the bacterial environment or infection by PRRS virus that results in immunosuppression, permitting invasion by ETEC leading to death (Nakamine et al. 1998).

**Pathogenesis**

Bacteria pass through the mucosa of the alimentary tract, probably by endocytic uptake into intestinal epithelial cells or through the intercellular spaces formed by lateral plasma membranes of adjacent epithelial cells, to locate in the mesenteric lymph nodes before entering the bloodstream. Bacterial invasion may result in a generalized infection (septicemia, polyserositis), with bacteria disseminated in various extraintestinal organs such as lung, liver, spleen, kidney, and brain, or in a localized infection (meningitis or arthritis) (Morris and Sojka 1985).

The exact role of each virulence factor in the pathogenicity of ExPEC is not always clear, although it appears that the greater number of virulence factors present in an ExPEC isolate, the greater its pathogenic potential. LPS, K capsule and O-antigen capsule, and production of siderophores such as aerobactin are thought to allow the bacteria to invade the host and escape its defense mechanisms. These determinants increase bacterial resistance to the bactericidal effect of complement and to phagocytosis and allow bacterial growth in body fluids with low concentrations of free iron (Ngeleka et al. 1992). Fimbriae appear to be important for the survival and spread of bacteria within the host and subsequent bacterial pathogenicity, in part by promoting bacterial resistance to the bactericidal effects of phagocytosis (Ngeleka et al. 1994).

**Clinical Signs**

Clinical signs of septicemia include depression, lameness, reluctance to move, anorexia, rough hair coat, and labored respiration, due in part to the effect of bacterial endotoxin or cytotoxins or to the effects of inflammatory cytokines induced by these bacterial products (Jesmok et al. 1992; Nakajima et al. 1991). The affected piglets may show sternal recumbency and the abdomen may be somewhat distended. Sometimes piglets become unconscious, with convulsions and paddling movements; they may be in good bodily condition but cyanosis of the extremities may be observed. Some piglets are found dead, whereas others are comatose without any sign of diarrhea. These clinical signs may develop within 12 hours after birth and piglets can die within 48 hours. In older piglets, the clinical signs may include periodic scouring or other ailments which precede the onset of acute septicemia with clinical signs resembling those in the newborn pigs.

**Lesions**

In acute primary septicemia, there may be no gross lesions other than congestion of the intestine, the mesenteric lymph nodes, and the extraintestinal organs. In subacute cases, subserous or submucosal hemorrhages and fibrinous polyserositis with gross signs of pneumonia are usually observed, often accompanied by fibrinopurulent arthritis and meningitis. Histological examination of the lung reveals interalveolar interstitial pneumonia with edema and neutrophilic infiltration, but alveoli are free of exudates.
In secondary septicemia resulting from enteric colibacillosis, icterus, petechial hemorrhages in the serosal membranes, and splenomegaly accompanied by severe diarrhea and dehydration may be observed. In many cases of secondary systemic *E. coli* infection, presumably occurring in the terminal stages of the underlying disease, lesions attributable to systemic ETEC are slight or no lesions at all are recorded.

**Diagnosis**

Systemic colibacillosis should be suspected with the appearance of the clinical signs and lesions described above, especially when observed in pigs under 4 days of age. Differentials should include other causes of systemic bacterial infections. In older suckling and especially weanling pigs with polyserositis, differentials should include *H. parasuis* (Chapter 55), *Mycoplasma hyorhinis* (Chapter 57), and *S. suis* (Chapter 62).

Positive blood culture results are essential to make the diagnosis of bacteremia. However, treatment cannot be delayed until the results of blood cultures are obtained. Blood must be withdrawn from the vein aseptically and deposited into both aerobic and anaerobic blood culture bottles. Diagnosis is confirmed by the isolation in pure culture or by the predominance in extraintestinal organs such as the spleen, liver, brain, lungs, and pericardial, pleural, and peritoneal fluids of one of the typical *E. coli* serovirotypes associated with primary septicemia (Table 53.1) or, for secondary septicemia, one of the ETEC serovirotypes listed in Table 53.2. Methods used to isolate, identify, and serovirotype are as described in the diagnostic section for neonatal *E. coli* diarrhea.

**Prevention and Control**

In septicemia, treatment may be useful in subacute cases of infection but is mostly ineffective after the appearance of clinical signs. However, the remaining unaffected littermates and adjacent litters should be treated prophylactically with antibiotics. Vaccination is rarely considered for the control of septicemia due to *E. coli*. However, in the case of small outbreaks of septicemia, careful monitoring of the causative serogroup(s) and autovaccination of the pregnant sows might be beneficial. The most effective prevention is in ensuring that piglets consume adequate colostrum by focusing on management practices that enable optimal lactation and suckling. Additionally, facility design and husbandry practices to minimize environmental contamination by feces will reduce exposure and transmission of offending strains of *E. coli* to suckling pigs.

**COLIFORM MASTITIS**

The term “coliform mastitis” (CM) is used to refer to mastitis in swine, underlining the parallel of this disease with CM in the cow. Up to 80% of dysgalactic sows may have gross lesions of mastitis (Ross et al. 1981). Wegmann et al. (1986) reported that *E. coli* or *Klebsiella pneumoniae* were isolated from 79% of mammary complexes in sows with mastitis. Mortality among affected sows is low, but in piglets nursing multiparous sows with PPDS it has been reported to be as high as 55.8%, compared to 17.2% in piglets nursing healthy sows (Bäckström et al. 1984).

The fecal microflora is a reservoir for extraintestinal coliform infections such as mastitis and UTI. Various coliform strains are found in the intestine of each individual pig, and mixed infections of more than one strain are often found in any one pig. In about one-third of the sows with mastitis, identical isolates were found in mastitic glands, the uterine contents, and the urinary bladder (Bertschinger et al. 1977a). The intestinal flora of the sow, the oral flora of the neonatal piglet, and environmental bacteria may significantly contribute to contamination of the nipples. Awad-Masalmeh et al. (1990) found identical O serogroups of *E. coli* in mammary secretion and in feces of about one-fourth of sows with CM. The bedding of the sow is very important, permitting dung and urine to contaminate the udder (Muirhead 1976). *Klebsiella* spp. may also originate from wood shavings used for bedding. Counts of coliform bacteria on teat ends and incidence of intramammary *E. coli* infections were considerably greater in sows farrowing in conventional farrowing crates as compared to those observed in sows farrowing in pens where they could lie down in a clean resting area (Bertschinger et al. 1990a).

A great heterogeneity of isolates, evidenced by multiple serological types and by random amplified polymorphic DNA genotyping, may be cultured in cases of mastitis within a herd, between distinct glands of one sow or even between subcomplexes within one gland (Awad-Masalmeh et al. 1990; Morner et al. 1998; Ramasoota et al. 2000).

**Pathogenesis**

Mastitis has been reproduced in the sow by intramammary instillation of as few as 120 organisms of a strain of *K. pneumoniae*. External contamination of the nipples was as successful on gestation day 111 as at 2 hours after completion of farrowing (Bertschinger et al. 1977b). However, it is not clear when spontaneous invasion of the cistern takes place. Significant numbers of coliform bacteria have been observed in about one-fourth of mammary glands cultured immediately prior to parturition (McDonald and McDonald 1975). New infections seem to most commonly occur from before the birth of the piglets to 2 days postpartum (Bertschinger et al. 1990b).

The bacteria are located in the ductular and alveolar lumina, either free or within phagocytic cells. Little adhesion to surfaces is observed. At postmortem examination the causative bacteria are frequently isolated...
from regional lymph nodes, whereas isolations from the liver, spleen, or kidney are rare (Ross et al. 1981).

Multiplication of bacteria in the mammary secretion is controlled by antimicrobial mechanisms, including a variety of inhibitors acting in concert and conferring on the dry udder a nearly total resistance to coliform proliferation. A lower opsonic activity observed in mammary secretions of sows at parturition (Osterlundh et al. 1998), as well as a lower phagocytic capacity of PMN cells in colostrum as compared to milk (Osterlundh et al. 2001), may explain the increased susceptibility to the development of CM at parturition. CM is a self-limiting disease. The bacteria generally disappear between 1 and 6 days after parturition (Bertschinger et al. 1990b), but in severe cases they may persist in necrotic foci throughout lactation (Löpfe 1993).

CM in the sow is associated with massive accumulation of neutrophils in the lumina of affected glands and may be accompanied by a severe leukopenia (Bertschinger et al. 1977b). Severe reaction is the consequence of massive and persistent multiplication of inoculated bacteria, with susceptibility to infection being associated with impaired function of circulating neutrophils (Löfstedt et al. 1983). The cause of the impaired neutrophil function is not well understood. Magnusson et al. (2001) showed that sows were more susceptible to experimental infection immediately prior to parturition than at 4 days before parturition, the number of neutrophils in the blood being greater in the former. These findings suggest a role for the number of circulating neutrophils at the time of infection in the development of clinical CM in the sow. Nevertheless, Osterlundh et al. (2002) have shown in experimental inoculation studies that impaired chemotactic or phagocytic capacity of blood granulocytes does not appear to be involved in susceptibility of sows to develop clinical CM at parturition.

The systemic signs of CM are induced by bacterial endotoxin (Bertschinger et al. 1990b). The development of clinical signs of CM, like high fever, is associated with the degree of local expression of regulatory cytokines such as IL-1 and IL-6 (Zhu et al. 2008).

**Clinical Signs**

In CM, the initial signs are most often detected on the first or second day and more rarely on the third day after farrowing, although they may be observed as early as during parturition. The first clinical signs are pyrexia, listlessness, weakness, and loss of interest in the piglets. Affected sows prefer sternal recumbency. In severe cases they become stiff, do not stand, and may even become comatose. Consumption of feed and water is either reduced or absent. Body temperature is moderately elevated and only rarely exceeds 107°F (42°C). Afebrile cases have been reported. On the other hand, many normal sows will have rectal temperatures that exceed the 103°F (39.7°C) limit on the day of parturition and for 2 days thereafter. In affected sows, respiratory and heart rates are increased. In general, clinical signs do not last for more than 2–3 days.

The clinical findings in sows with proven CM may be quite similar to those in sows with lactational failure. Interpretation of clinical parameters is also rendered difficult by the presence of subclinical CM in apparently healthy sows. The behavior of the piglets is very helpful in the early detection of lactational failure. Undernourished piglets look gaunt. They frequently try to suck, move from nipple to nipple, nibble at litter, and lick urine from the floor. If access to the nipples is given by the sow, the periods of suckling are shortened. After suckling, the piglets wander instead of resting in close contact with their littermates.

Precise localization of mammary lesions is often not possible because reddening and heat of the skin extend over several subcomplexes. The reliable clinical assessment of the state of the mammary tissue is difficult because of subcutaneous fat and considerable subcutaneous edema. If palpable, the mastitic tissue is firmer and palpation may cause pain. The red color of the skin is blanched by finger pressure, which causes a depression of the tissue lasting for some time. Mere clinical examination will at best detect some of the affected subcomplexes (Persson et al. 1996). The inguinal lymph nodes may be swollen.

**Lesions**

Lesions of CM are confined to the mammary glands and regional lymph nodes. Grossly, the exudate from inflamed subcomplexes looks serous to creamy pus. It may contain clots of fibrin or blood. The subcutaneous tissue may be edematous over affected parts of the udder. Irregularly scattered foci of mastitis may be detected in different subcomplexes. The appearance of affected mammary tissue varies from slightly increased firmness and grayish discoloration to sharply demarcated, red-mottled, hard, and dry areas (Figure 53.7).

Microscopically, an acute purulent exudative mastitis with congestion is observed. The severity varies greatly, both between and within subcomplexes, ranging from a small number of neutrophils in the alveolar lumina to severe purulent infiltration with necrosis. Acute purulent lymphadenitis may be present in the inguinal and iliac lymph nodes. Large necrotic foci surrounded by fibrous tissue may persist through the lactation in severe cases (Löpfe 1993).

**Diagnosis**

Any hypogalactia at the beginning of lactation arouses suspicion of CM. The diagnosis may be supported by fever, anorexia, reluctance to stand, lying on the gland, and disinterest in the piglets. In severe cases some affected glands may be reddened, swollen, and firm, and the secretion may look abnormal.
A reliable rapid test for use on the farm is not available. Due to the higher cell content of sow milk, tests developed for use with the cow cannot be recommended. Bacteriological and cytological examinations of the secretion are only valuable if all glands are sampled or if affected glands are known. The pH is of limited diagnostic value (Persson et al. 1996), but cytological examination allows differentiation between healthy and mastitic complexes, at least during the first 48 hours after parturition (Wegmann and Bertschinger 1984). Because mastitis is a local process, samples must be taken from individual complexes and not pooled. A suggested threshold value for the total cell count is $5 \times 10^6$ cells per milliliter of milk and fewer than 70% PMN. Culture of the secretion may be necessary in unclear cases. Methods for culture are as described for neonatal *E. coli* diarrhea.

**Immunity**

CM apparently does not result in protection against homologous reinfection (Bertschinger and Bühlmann 1990). Ringarp (1960) reported a higher incidence in sows than in gilts, as well as repeated occurrence, up to 10 times, in individual sows.

**Prevention and Control**

In CM mastitis, therapeutic measures are usually not undertaken before the sow shows signs of dysgalactia. Thus, treatment may at best shorten the period of underfeeding of the piglets. Antimicrobial therapy is complicated by the heterogeneous pattern of antimicrobial susceptibility of individual isolates not only within a herd but also within a sow. Therefore; sensitivity testing is of little value in individual cases.

Much attention should be given to the piglets. They may either be fostered by other sows or remain with their mother and receive a milk substitute. A sterile 5% glucose solution can be repeatedly injected intraperitoneally every several hours, or a more concentrated solution may be administered intragastrically. When the piglet obtains insufficient amounts of milk, protection against chilling is particularly important.

Strategies for prevention of CM include protection of the teats from bacterial contamination (Muirhead 1976). Optimal prophylaxis is achieved by designing farrowing accommodation in which the sow is prevented from lying down in her own excreta. On the other hand, washing and disinfection of the pen and of the newly housed sow contribute much less to efficient prophylaxis. If cases of CM accumulate, the bedding materials should be checked. Drastic reduction of the sow’s ration shortly before parturition is a widespread practice. Reduction of the daily feed allowance may substantially lower the incidence of agalactia (Persson et al. 1989). Feed reduction might act through reduced exposure of the teats to microbial infection due to the much smaller amounts of feces and urine contaminating the lying area.

Chemoprophylaxis appears to be the most promising method of control for CM mastitis where accommodation cannot be improved. The prevalence of drug resistance and the wide variety of bacteria associated with the disease in a herd must be considered when the drug is selected. Feed medication should be replaced by individual application of the drug in a small amount of feed because the feed consumption of the sow in the periparturient period is quite variable. Minimizing the period of treatment helps to postpone the emergence of drug resistance. Antimicrobials demonstrating some effectiveness include a combination of trimethoprim, sulfadimidine, and sulfathiazole; apramycin; and enrofloxacin.

**NONSPECIFIC URINARY TRACT INFECTION**

Like CM, nonspecific UTI behaves like a noncontagious infectious disease of endogenous origin. The fecal microflora is a reservoir for extraintestinal infections such as mastitis and UTI. Contamination and ascent of the urethra by fecal flora is more likely in females than in males. Under intensive confinement conditions, sows’ vulvas are often placed in direct contact with feces (Smith 1983). The dog-sitting position helps to force fecal material into the vagina. Sows resting for long periods void urine at longer intervals, and urinary stasis favors bacterial ascent in the urethra and proliferation in the bladder. However, housing conditions have not been studied with respect to UTI. The age distribution of UTI favors the concept of continuous exposure to fecal contamination, since the prevalence of UTI increases with sow parity and increasing parity.
is associated with greater vulvar and urethral trauma and laxity (Becker et al. 1985). Low water consumption may also have a role in predisposing to UTI.

Pathogenesis
In humans and in dogs, colonization of the lower genital tract and of the urinary tract by uropathogenic E. coli is greatly facilitated by fimbrial adhesins, particularly type 1 and P fimbriae (Gyles and Fairbrother 2010). Similarly, P fimbriae and mannose-sensitive hemagglutination indicative of type 1 pili were found in E. coli isolates from the urine of pigs with bacteriuria (de Brito et al. 1999). Serovirotypes of E. coli commonly isolated from UTI are listed in Table 53.1. Bacteria most likely ascend through the urethra. Invasion is favored by the short, wide urethra of the female pig, the relaxation of the sphincter muscle in late pregnancy and puerperium, trauma to the urethra and bladder at coitus and parturition, abnormal bacterial colonization of the sinuses urogenitalis and the genital organs, incomplete closure of the vulva, and catheterization of the bladder. Asymptomatic bacteriuria may result in cystitis with spontaneous remission. Nonspecific infection may promote colonization of the bladder by A. suis (Liebhold et al. 1995). Bacterial colonization may lead to shortening and deformation of the ureteric valve, promoting vesicoureteric reflux (Carr et al. 1990).

UTI may predispose to MMA due to ascending invasion of the uterus at parturition and of the mammary glands from contamination of the lying area. Identical OK serotypes of E. coli have been found in the urinary bladder and uterus, or in the bladder and mammary gland of sows with MMA (Bertschinger et al. 1977a).

Clinical Signs
In the vast majority of nonspecific UTI cases there are no clinical signs. Sows with a significant bacteriuria tend to wean small litters, have increased intervals between litters, show a lower fertility rate, and exhibit an inferior body condition (Akkermans and Pomper 1980). Sows with cystitis may void urine in small quantities with straining, or be observed in a dog-sitting position (Becker et al. 1988).

Vulvar discharge may appear as dried deposits around the vulva, on the underside of the tail, or more often as a pool on the floor underneath the sow (Dial and MacLachlan 1988a). The discharge may be mucoid, mucohemorrhagic, or purulent and is observed most often during the final phase of urination. However, discharge may result from inflammation of any part of the urogenital tract. Significant discharge is more often the consequence of endometritis than of UTI.

Severe pyelonephritis becomes clinically manifest during the first 2 weeks postpartum in 40% of the cases (Stirnimann 1984). Typical cases exhibit a rectal temperature below 100°F (38°C), a heart rate over 120, polypnea, cyanosis, ataxia, and, more rarely, general-ized tremor. The blood concentrations of urea and creatinine are higher than normal.

Lesions
The gross lesions of cystitis begin as focal or diffuse mucosal hyperemia (Dial and MacLachlan 1988b). Subsequently, there may be mucosal ulceration with fibrinopurulent exudate over affected areas. The bladder wall becomes thickened. Similar lesions occur in the ureters and the renal pelvis if infection ascends the urinary tract. In pyelonephritis the inflammatory process extends into the renal parenchyma. Multiple unevenly distributed foci of inflammation, mostly affecting the renal poles, are observed (Isling et al. 2010). Wedge-shaped foci extend from the distorted pelvis to the cortex. Fibrosis of the kidneys may occur with time.

Microscopic bladder lesions can be found even in sows with nonspecific UTI and no proteinuria. They consist of a prominent goblet cell proliferation and of intraepithelial cysts containing a few granulocytes. The epithelial layer is infiltrated with neutrophils, whereas mononuclear cells dominate in the lamina propria (Liebhold et al. 1995). In kidneys, tubulointerstitial infiltration with neutrophils and mononuclear cells and tubular destruction are the main findings (Isling et al. 2010).

Diagnosis
Clinical examination of the animal is of little value in the diagnosis of UTI. Bacterial culture of the urinary tract is complicated by the presence of the normal flora colonizing the vagina and the distal part of the urethra. Therefore, distinction between contamination and infection is based on the number of bacteria in the urine. A viable count of 10^5 CFU/mL is interpreted as indicative of infection and 10^4 CFU/mL as suspicious. Catheterization of the sow is possible but does not circumvent contamination and involves the risk of setting up a new UTI.

Immunity
Serum antibody against the infecting E. coli strain can regularly be detected in sows with pyelonephritis, less often in sows with cystitis, and rarely in sows with asymptomatic bacteriuria (Wagner 1990). Escherichia coli strains may persist in the urinary tract despite high antibody concentrations in the urine.

Prevention and Control
Treatment of urogenital infections of swine is frustrating (Dial and MacLachlan 1988b). For UTI, therapy with broad-spectrum or combined antimicrobials, such as trimethoprim–sulfonamides, is recommended, due to the variable susceptibilities of the diverse bacteria involved and the frequent acquisition of resistance (Berner 1990). Prolonged parenteral treatment may be
recommended, although subclinical UTI often persists after treatment (Becker et al. 1988). Treatment of affected sows with specific antimicrobial drugs before parturition may be helpful.

Strategies for control of UTI include decreasing environmental exposure by improving fecal drainage and housing conditions. Frequency of urination may be increased by giving access to an exercise yard and by increasing water intake, being achieved by a salt content of 1% in the diet (Smith 1983). Water accessibility and flow rates in water lines and nipples should also be evaluated. Decreased water consumption can also be caused by poor palatability.

REFERENCES

RELEVANCE
The causative organism of swine erysipelas, *Erysipelothrix rhusiopathiae*, was first isolated from a pig in 1882 by Louis Pasteur. In 1885, *E. rhusiopathiae* was isolated from pigs in the United States (Smith 1885). The first complete description of the disease in experimentally infected pigs was published in 1886 by Friedrich Löffler. For the first 40 years after its initial recognition, swine erysipelas was reported to occur sporadically in the swine population. In the early 1930s, an epidemic was observed in North America, leading to the first organized efforts and successes in the development of treatment, prevention, and control programs. Since the first reported epidemic, there is some evidence that more severe and prevalent swine erysipelas outbreaks tend to occur in recurring intervals of approximately 10 years.

Swine erysipelas, when uncontrolled, is an economically significant disease capable of affecting all stages of pork production. The greatest losses usually manifest as cases of sudden death or acute septicemia in grow–finish pigs. The sequel of surviving an acute infection is often chronic lameness and arthritis, resulting in poor growth. Both erysipelas-associated septicemia and arthritis are responsible for significant production losses and decreased carcass value.

ETIOLOGY
In 1876 *Erysipelothrix muriseptica* was isolated from the blood of a mouse with septicemia by Koch. The name was changed to *E. rhusiopathiae* in 1966. Until recently, the genus was thought to contain only one species, *E. rhusiopathiae*. The genus *Erysipelothrix* is now subdivided into two major species: *E. rhusiopathiae* (Migula 1900; Skerman et al. 1980) and *Erysipelothrix tonsillarum* (Takahashi et al. 1987). In addition, there are other strains that constitute one or more additional species currently known as *Erysipelothrix* sp.-1 (Takahashi et al. 1992, 2008), *Erysipelothrix* sp.-2 (Takahashi et al. 1992, 2008), *Erysipelothrix inopinata* (Verbarg et al. 2004), and *Erysipelothrix* sp.-3 (Takahashi et al. 2008). Based on heat-stable cell wall antigens, *Erysipelothrix* spp. strains can be differentiated by precipitation reactions using hyperimmune rabbit antiserum into at least 28 serotypes (Kucsera 1973; Wood and Harrington 1978). Strains that do not possess heat-stable cell wall antigen are referred to as serovar N. *Erysipelothrix rhusiopathiae* includes serotypes 1a, 1b, 2, 4, 5, 6, 8, 9 (a majority of strains), 11, 12, 15, 16, 17, 19, 21, and N; *E. tonsillarum* contains serotypes 3, 7 (a majority of strains), 10 (a majority of strains), 14, 20, 22, 23, 24, 25, and 26; *Erysipelothrix* sp.-1 contains serotype 13; *Erysipelothrix* sp.-2 contains serotype 18 and few strains of serotypes 9 and 10; *Erysipelothrix* sp.-3 contains some strains of serotype 7; and *E. inopinata* has not been characterized serologically (Takahashi et al. 1987, 1992, 2008; Verbarg et al. 2004).

Field cases of swine erysipelas throughout the world are predominantly caused by *E. rhusiopathiae* serotypes 1a, 1b, or 2, while less common serotypes of *E. rhusiopathiae* typically have lower virulence for swine. *Erysipelothrix tonsillarum* is not generally considered to be pathogenic in pigs as are also the few strains comprising potential new species. Uncommonly, strains of *E. tonsillarum* have been isolated from cases of chronic arthritis and vegetative valvular endocarditis (Bender et al. 2011; Takahashi et al. 1984, 1996), suggesting potential for pathogenicity. However, inoculation studies in pigs have so far not demonstrated *E. tonsillarum* to be a significant pathogen (Harada et al. 2011; Takahashi et al. 1987, 1992, 2008).
Members of the genus *Erysipelothrix* are nonmotile, nonsporulating, non-acid-fast, slender gram-positive rods (Brooke and Riley 1999). They are facultative anaerobes and grow between 41°F (5°C) and 111°F (44°C), with optimal growth occurring between 86°F (30°C) and 98.6°F (37°C) (Brooke and Riley 1999; Carter 1990; Sneath et al. 1951). Colonies of *Erysipelothrix* spp. are either smooth or rough with rough colonies being slightly larger with an irregular edge (Grieco and Sheldon 1970). On agar media, colonies are clear, circular, and very small (0.1–0.5 mm in diameter) after 24 hours of incubation at 95°F (35°C) or 81°F (27°C), with increased size (0.5–1.5 mm in diameter) after 48 hours (Carter 1990). Most strains induce a narrow zone of hemolysis on blood agar media, usually with a greenish color. Rough colonies are not typically associated with hemolysis (Carter 1990). The organisms favor an alkaline pH ranging from 7.2 to 7.6 (Sneath et al. 1951). The genus *Erysipelothrix* is generally inactive and does not react with catalase, oxidase, methyl red, or indole (Cottral 1978), but does produce acid and hydrogen sulfide in triple-sugar iron agar (Vickers and Bierer 1951; White and Shuman 1961).

Other gram-positive, nonsporulating rod-shaped bacteria that can be confused with *Erysipelothrix* spp. include members of the genera *Bronchothrix*, *Corynebacterium*, *Lactobacillus*, *Listeria*, *Kurthia*, and *Vagococcus* (Bender et al. 2009; Brooke and Riley 1999; Dunbar and Claridge 2000).

**PUBLIC HEALTH**

*Erysipelothrix rhusiopathiae* is zoonotic. Most cases occur via scratches or puncture wounds of the skin (Wood 1975) as an occupational disease in those whose jobs are closely related to infected animals or their products. At highest risk are butchers, abattoir workers, veterinarians, farmers, fisherman, fish handlers, and housewives (Reboli and Farrar 1989). The most common form of the disease manifests in humans as an acute localized painful cellulitis with reddening of the skin known as “erysipeloid” (Rosenbach 1909). Systemic clinical signs such as fever, joint pain, and lymphadenopathy occur in some cases. Most cases are self-limiting in 1–2 weeks. Given its occupational associations, erysipeloid has historically been known by such names as whale finger, seal finger, speck finger, blubber finger, fish poisoning, fish handler’s disease, and pork finger (Reboli and Farrar 1989; Wood 1975). Occasionally, erysipeloid develops into a generalized cutaneous form with simultaneous eruptions at multiple sites and consistent systemic clinical signs (Klauder 1938). In such cases, the clinical course is longer than for localized erysipeloid and recurrence is frequent. Rarely, *E. rhusiopathiae* causes septicemia, often resulting in endocarditis that is frequently fatal (Gorby and Peacock 1988). These conditions should not be confused with a different disease in humans that is sometimes called “erysipelas” and is caused by group A beta-hemolytic streptococci.

**EPIDEMIOLOGY**

*Erysipelothrix rhusiopathiae* is worldwide in distribution and is ubiquitous. The domestic pig is considered the most important reservoir. Besides pigs, at least 30 species of wild birds and 50 species of mammals are known to harbor the organism, providing a broad reservoir (Shuman 1970). Notably potential reservoirs include: sheep, cattle, horses, dogs, mice, rats, fresh and saltwater fish, marine mammals, turkeys, chickens, ducks, geese, sparrows, starlings, and blackbirds (Bricker and Saif 1997; Grieco and Sheldon 1970; Reboli and Farrar 1989; Wood 1975). Studies have demonstrated that *E. rhusiopathiae* can easily be recovered from tonsils of clinically normal cattle (Hassanein et al. 2001, 2003).

It is estimated that approximately 30–50% of apparently healthy pigs harbor *E. rhusiopathiae* in their tonsils and other lymphoid tissues (Stephenson and Berman 1978). These carriers can shed the organism in their feces or oronasal secretions, creating an important source of infection. *Erysipelothrix rhusiopathiae* is believed to be transmitted directly via oronasal secretions and feces and indirectly via environmental contamination. Pigs can be infected by ingestion of contaminated feed or water or contamination of skin wounds. In indoor production systems, contamination of floors with feces and urine from infected animals is the likely source of infection.

Swine affected with acute erysipelas shed *E. rhusiopathiae* profusely in feces, urine, saliva, and nasal secretions for extended periods of time. *Erysipelothrix rhusiopathiae* can be easily isolated from oral fluids of an acutely infected population for several weeks (unpublished observation). Survival of *Erysipelothrix* spp. in soil is less than 35 days (Wood 1973). When growth and survival of *E. rhusiopathiae* were investigated under various conditions of temperature, pH, moisture, and organic matter, no evidence of establishment of a stable viable population in the soil was found (Wood 1973). *Erysipelothrix rhusiopathiae* is killed by moist heat at 131°F (55°C); however, it is resistant to salting and many other food preservation methods (Conklin and Steele 1979).

*Erysipelothrix* spp. can be inactivated by commonly available disinfectants (Conklin and Steele 1979). Several commercially available home disinfectants have been found to be highly effective; however, structurally complex equipment which contained organic matter was more difficult to disinfect especially without cleaning (Fidalgo et al. 2002). Due to the inability of disinfectants to fully remove the organism from the environment, a multifaceted approach comprised of sound husbandry, herd management, sanitation, and immunization is recommended.
PATHOGENESIS

There are marked differences in virulence between strains of *E. rhusiopathiae*, modulated by virulence factors that are partially characterized and have been recently reviewed (Wang et al. 2010). Most important are neuraminidase, capsular polysaccharides, and surface proteins. The amount of secreted neuraminidase is proportional to the degree of virulence in strains of *E. rhusiopathiae* (Krasemann and Muller 1975; Nikolov et al. 1978); none is secreted by nonpathogenic *E. tonsillarum* (Wang et al. 2005). Neuraminidase is an enzyme that cleaves sialic acids from glycoproteins, glycolipids, and polysaccharides on host cell walls, providing bacterial nutrients and aiding in bacterial adhesion and tissue invasion (Nakato et al. 1986, 1987; Schauer 1985). The polysaccharide capsule of *E. rhusiopathiae* is important in resistance to phagocytosis by host cells (Shimoji et al. 1994). Surface proteins on the bacterial cell wall that may contribute to virulence include novel adhesins RspA and RspB (Shimoji et al. 2003) that are important in early biofilm formation. Other important surface proteins include the 64–66kDa proteins that are expressed in higher amounts in highly virulent strains (Galan and Timoney 1990), later named surface protective antigens (Spa) due to their role as the major immunizing antigen of *E. rhusiopathiae* (Imada et al. 2003; Makino et al. 1998). Spa proteins resemble choline-binding proteins of *Streptococcus pneumonia*, suggesting a potential role in virulence (Jedrzejas 2001).

Route of exposure to *E. rhusiopathiae* in pigs is primarily oral with initial infection of the tonsils or gastrointestinal mucosa. Bacteria may also enter through skin abrasions by direct contact or by bites of arthropods that can serve as mechanical vectors (Chirico et al. 2003). Usually, bacteremia develops within 24 hours in the absence of an effective immune response. Subsequent septicemia results in distribution of the organism throughout the body. In the early septicemic stage, damage occurs to capillaries and venules of most body organs and synovial tissue (Schulz et al. 1975a, 1977). At 36 hours after subcutaneous inoculation, endothelial swelling, monocyte adherence to vascular walls, and hyaline thrombosis occur (Schulz et al. 1975b). This process is referred to as a shock-like generalized coagulopathy that leads to fibrinous thrombosis, diapedesis, and invasion of vascular endothelium by bacteria and deposition of fibrin in perivascular tissues (Schulz et al. 1975a, 1976a). Eventually, there is connective tissue activation in predisposed sites of infection, including joints, heart valves, and skin (Schulz et al. 1976b). Hemolysis and ischemic necrosis can occur in severe cases. Sequestration of *E. rhusiopathiae* in the cytoplasm of chondrocytes of articular cartilage is reported (Franz et al. 1995), and likely provides protection from host immunity, contributing to chronic arthritis.

It was found that pigs less than 3 months of age (due to a protective effect of passively acquired immunity) or pigs older than 3 years of age (due to repeated subclinical disease) are generally least predisposed to erysipelas. The degree and duration of passively acquired immunity are thought to be related to the immune status of the dam and colostrum uptake of the offspring.

There is no experimental evidence that susceptibility to swine erysipelas is related to the genetics of the host. Sudden changes in weather, especially hot summer weather, or other stressors have been implicated in increased incidence of disease.

CLINICAL SIGNS

Three clinical forms of swine erysipelas are described: acute, subacute, and chronic (Conklin and Steele 1979; Grieco and Sheldon 1970). The acute form is septicemic disease that manifests as a sudden onset of any combination of the following: acute death; abortions; depression; lethargy; pyrexia (104–108°F [40–42°C] or greater); withdrawal; lying down; painful joints evidenced by stiff, stilted gait; reluctance to move and/or vocalization during movement; partial or complete inappetence; and characteristic pink, red, or purple raised firm rhomboid or squared “diamond skin” lesions. In dark-skinned animals, the skin lesions are best appreciated by palpation or by observing areas with raised hairs. In nonfatal cases, the skin lesions will gradually disappear within 4–7 days.

The subacute form is also septicemic but is clinically less severe than the acute form. Animals do not appear as sick, temperatures are not as high or persistent, appetite may remain unaffected, skin lesions may be few in number or absent, mortality will be lower, and animals will recover more rapidly. There may be infertility, litters with increased numbers of mummies or small litters, and pre- or postparturient vulvar discharges. Some cases may be so mild as to remain unnoticed (subclinical).

Chronic erysipelas follows acute, subacute, or sometimes subclinical septicemic erysipelas in a proportion of surviving animals. The most economically significant form is chronic arthritis that may appear as soon as 3 weeks after the initial outbreak. Affected animals are mildly to markedly lame with associated reduction in feed intake. Firm enlargement of hock, stifle, or carpal joints may be observed. A second manifestation of chronic erysipelas is vegetative valvar endocarditis, which may lead to cardiac insufficiency and consequent pulmonary edema and respiratory signs, lethargy, cyanosis, or sudden death.

Morbidity and mortality varies depending on the immune status of a herd. In outbreaks of acute swine erysipelas in naive herds, mortality can quickly rise to 20–40%. In subclinically or chronically affected herds
the morbidity and mortality associated with *Erysipelothrix* spp. varies and is dependent on herdsmanship, environment, and other concurrent infections.

**LESIONS**

The nearly pathognomonic gross lesions of acute swine erysipelas consist of multifocal pink to purple rhomboid (diamond-shaped) slightly raised skin lesions (Figure 54.1) predominately around the snout, ears, jowls, throat, abdomen, and thighs. Skin of extremities can also be purple. In addition to skin lesions, other lesions typical of septicemia are observed, including enlarged and congested lymph nodes, enlarged spleen, and edematous and congested lungs. Petechiae and ecchymoses may be found in the renal cortex (Figure 54.2), heart (epicardium and atrial myocardium), and occasionally elsewhere. Joints may be slightly enlarged and the synovium and periarticular tissues are typically distended by serofibrinous exudates that may also fill the joint cavity.

In pigs that survive acute clinical or subclinical disease, chronic gross lesions may develop. Chronic arthritis may involve joints of one or more legs or the intervertebral articulations. Proliferative synovial membranes (Figure 54.3) and serosanguinous effusion in the joint cavity are observed. The joint capsule is often hyperemic. There may be proliferation and erosion of the articular cartilage that lead to fibrosis, ankylosis, and spondylitis. Valvular endocarditis (Figure 54.4) can be seen as proliferative, granular growth on the heart valves (mitral valve most common). Ischemic necrosis
often dilated and congested. Microthrombi and bacterial emboli may occlude vessels, leading to circulatory stasis and focal necrosis (Figure 54.6). Neutrophils infiltrate the affected dermis. Similar hyperemia, vasculitis, neutrophilic infiltrates, and focal necrosis can also be observed in brain, heart, kidneys, lungs, liver, spleen,
and synovial membranes. Acute interstitial and exudative pneumonia is observed as alveolar septal vessels are affected and serous exudates expand the alveolar septa and flood the alveoli that also often contain aggregates of macrophages. Damage to glomerular vessels may result in hemorrhages that are grossly visible on the renal cortex. Affected lymph nodes are hyperemic, hemorrhagic, and infiltrated with neutrophils. Segmental hyaline and granular necrosis of muscle fibers, which is followed by fibrosis, calcification, and regeneration, have also been observed. As lesions become subacute, infiltrates of monocytes, lymphocytes, and macrophages are also observed in sites of inflammation.

Chronic arthritis is characterized by marked hyperplasia of synoviocytes, resulting in thickened villus proliferations on synovial membranes that also have stromal thickening due to infiltrates of lymphocytes, plasma cells, and macrophages as well as neovascularization. In later stages, marked fibrosis may be observed in synovial membranes and periarticular tissues. Articular cartilage may be focally to extensively necrotic with associated fibrinous to fibrinopurulent exudates.

Chronic vegetative valvular endocardial lesions are composed of irregular lamina composed of fibrin, necrotic cellular debris, mixed inflammatory cells, bacterial colonies, and granulation tissue.

DIAGNOSIS

Differentials for acute swine erysipelas include septicaemia and sudden death in grow-finish pigs due to Salmonella choleraesuis (Chapter 60), Actinobacillus suis (Chapter 48), Actinobacillus pleuropneumoniae (Chapter 48), Haemophilus parasuis (Chapter 55), Streptococcus suis (Chapter 62), and other bacteria. Skin lesions resembling swine erysipelas can also be observed with classical swine fever virus (Chapter 38), porcine dermatitis and nephropathy syndrome (PDNS) (Chapter 17), or A. suis septicemia.

Timely and accurate diagnosis of erysipelas is important as effective treatments are available. For diagnosis of Erysipelothrix spp. a variety of tests are available (Table 54.1). Selection of diagnostic assays should be based on cost, required turnaround time, and availability in different regions.

Isolation of Erysipelothrix spp. from tissues (heart, lungs, liver, spleen, kidneys, joints, skin) with morphological lesions provides a definitive laboratory diagnosis of erysipelas infection. Direct culture from noncontaminated specimens is usually quick and easy and can be conducted using basic laboratory equipment. Chronic cases and contaminated specimens often require prior enrichment with selective culture broth. Several enrichment methods have been described in the literature (Cross and Eamens 1987; Harrington and Hulse 1971; Wood 1965) and are very effective.

### Table 54.1. Application of different diagnostic assays for identification of Erysipelothrix species

<table>
<thead>
<tr>
<th>Assay</th>
<th>Preferred Sample</th>
<th>Estimated Turnaround Time (hours)</th>
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<tr>
<td></td>
<td>Fresh Tissues&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Fluids or Blood</td>
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<tr>
<td>Direct isolation</td>
<td>X&lt;sup&gt;b&lt;/sup&gt;</td>
<td>X&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td>Enrichment</td>
<td>X</td>
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<tr>
<td>Fluorescent antibody assay</td>
<td>X&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>Immunohistochemistry</td>
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<tr>
<td>Conventional PCR</td>
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<tr>
<td>Real-time PCR</td>
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</table>

<sup>a</sup>Tissues include spleen, lungs, liver, affected sections of skin, kidney, and lymph nodes.<br>
<sup>b</sup>Not treated with antibiotics.
techniques recently adapted for use in veterinary diagnostic laboratories (Bender et al. 2009). *Erysipelothrix rhusiopathiae* is relatively inactive in commonly used biochemical tests (Cottral 1978). The organism produces acid but no gas from certain fermentable carbon compounds and produces hydrogen sulfide in triple-sugar iron agar (Vickers and Bierer 1958; White and Shuman 1961).

The usage of a fluorescent antibody assay in frozen tissue sections for rapid identification of *E. rhusiopathiae* has been reported; however, it was found that the assay was not as sensitive as culture methods (Harrington et al. 1974). An immunohistochemical assay was also developed utilizing hyperimmune serum against serotypes 1a, 1b, and 2 produced in rabbits and was found to be highly sensitive and specific when compared to direct culture techniques, especially in treated animals (Opriessnig et al. 2010).

Several polymerase chain reaction (PCR) methods have been developed for the rapid detection of *Erysipelothrix* spp. Among conventional PCR assays, genus-specific methods (Makino et al. 1994; Shimoji et al. 1998a), a conventional differential PCR assay capable of distinguishing among all four species of *Erysipelothrix* (Takeshi et al. 1999), and a conventional multiplex PCR assay capable of differentiating between *E. rhusiopathiae* and *E. tonsillarum* (Yamazaki 2006) have been described. Most recently a real-time multiplex PCR assay was described, which detects and differentiates *E. rhusiopathiae*, *E. tonsillarum*, and *Erysipelothrix* spp. strain 2 (Pal et al. 2009).

Differences in genetic or antigenic properties of *Erysipelothrix* spp. isolates can be utilized to gain insight into the origin and relatedness of individual isolates on a farm or across farms within a production system or geographic region. Methods include serotyping, genomic fingerprinting, and pulsed-field gel electrophoresis (PFGE).

The standard method for serotyping used today utilizes a double agar-gel precipitation test with type-specific rabbit antisera and antigen recovered through the use of hot aqueous extraction (Kucsera 1973; Wang et al. 2010). Two surface proteins, a heat-labile protein which is species specific and an acid-stable polysaccharide antigen, form the basis for differentiation of isolates by serotyping. Serotyping depends on the availability of antiserum and the test requires about 3 days for completion.

Genomic fingerprints can be created for species for which minimal information is available by random amplified polymorphic DNA (RAPD) analysis. In 2000, this method was applied to differentiate 81 *Erysipelothrix* spp. isolates and a total of 14 patterns were identified (Okatani et al. 2000). Genetic variation among strains of *Erysipelothrix* spp. was identified and different strains within the same serotype could be differentiated (Okatani et al. 2000, 2004).

Among the current DNA-based typing methods, PFGE has been considered the gold standard by some (Olive and Bean 1999); however, a disadvantage of this assay is the long turnaround time as the test requires approximately 3–4 working days to complete. Strains of *Erysipelothrix* spp. were analyzed in different studies using restriction enzyme *SmaI* and the majority of the isolates had a distinct PFGE pattern allowing differentiation among isolates (Okatani et al. 2001; Opriessnig et al. 2004).

A variety of serological tests have been used to detect antibodies in attempts to diagnose swine erysipelas. These include plate, tube, and microtitration agglutination; passive hemagglutination; hemagglutination inhibition; complement fixation; enzyme-linked immunosorbent assay (ELISA); and indirect immunofluorescence. Serological diagnosis may be useful in evaluation of vaccination in breeding herds, but has limited practical application in the diagnosis of acute swine erysipelas.

**IMMUNITY**

Both humoral and cell-mediated immunity play a role in host defense against *E. rhusiopathiae* infection. A significant role of humoral immunity is implied since therapy with antiserum has been widely used as an effective treatment for acute septicemia. Shimoji et al. (1994, 1996) demonstrated that *E. rhusiopathiae* bacteria opsonized with immune serum are readily eliminated by neutrophils, peripheral mononuclear cells, or macrophages in contrast to nonopsonized bacteria. This suggests that the protective activity of antiserum is mediated by the opsonic activity of immunoglobulin G (IgG) antibodies in type I phagocytosis (Shimoji 2000), and that participating antigens are on the bacterial cell surface. Mice immunized with *E. rhusiopathiae* capsular antigen were not protected from subsequent challenge (Shimoji 2000). In contrast, antiserum raised against the surface protein SpaA induced protection in mice (Makino et al. 1998) and pigs (Shimoji et al. 1999). The Spa proteins are now recognized as the major immunizing antigen of *E. rhusiopathiae* (Imada et al. 2003).

The role of cellular immunity in protection is less clear. Studies in which mice were immunized with acapsular *E. rhusiopathiae* YS-1 strain demonstrated protective antibodies as well as a cell-mediated response evidenced by significant proliferation in spleen cells harvested on 7, 15, and 21 days postimmunization in response to *E. rhusiopathiae* antigen (Shimoji et al. 1998b). The relative contribution of cell-mediated immunity to protection and the bacterial antigens involved in inducing cell-mediated immunity is unknown.

A significant level of cross protection is observed between strains in different serotypes of *E. rhusiopathiae*. 
Pigs vaccinated with a live serotype 2 vaccine were protected against clinical swine erysipelas when challenged with serotypes 1a, 1b, 2, 4, 5, 6, 8, 11, 12, 15, 16, 17, and 21 but developed local dermal lesions with serotype 9 and 10 challenge (Takahashi et al. 1984). In an earlier study (Wood 1979), similar conclusions were reached; again, swine remained susceptible to serotypes 9 and 10 after vaccination with a serotype 2 isolate, whereas they were protected when challenged with serotypes 1, 2, 4, and 11.

Cross-protection is best understood in light of later studies elucidating the role of Spa proteins. These 64–66kDa cell surface proteins are known to elicit highly protective antibodies (Galán and Timoney 1990; Groschup et al. 1991; Lachmann and Deicher 1986). So named as Spa by Makino et al. (1998), when the gene was sequenced and the first Spa characterized as SpaA. Later, an additional two Spa-related genes were described (SpaB and SpaC), and Spa genes were identified in serotype reference strains as follows: SpaA in serotypes 1a, 1b, 2, 4, 5, 6, 8, 9, 12, 15, 16, 17, and 21; SpaB in serotypes 4, 6, 11, 19, and 21, and SpaC in the unclassified serotype 18 strain (To and Nagai 2007). Later work by Ingebritson et al. (2010), which evaluated several strains of *E. rhusiopathiae* per serotype from swine, fish, or cetacean origin, revealed a more complex picture. The type of Spa (A, B or C) is not confined exclusively to specific serotypes; some strains of aquatic origin contained more than one Spa type and in some strains no Spa could be detected, likely indicating that additional types or subtypes of Spa exist.

In a mouse cross-protection model it was demonstrated that the recombinant SpaA, SpaB, or SpaC proteins elicited complete protection against challenge with strains containing homologous Spa proteins but heterologous protection varied (To and Nagai 2007). Similarly, mice immunized with an *E. rhusiopathiae* serotype 2 SpaA strain and challenged with various *E. rhusiopathiae* isolates were completely protected against strains exhibiting the homologous Spa type; however, protection varied against strains possessing a heterologous Spa or more than one Spa type (Ingebritson et al. 2010). Because Spa classifications based on genetic relatedness are predictive of cross-protection and because reagents for serotyping are not readily available and results are not easily reproducible between laboratories, a new classification scheme for *E. rhusiopathiae* isolates-based Spa genes has been suggested (Ingebritson et al. 2010).

**PREVENTION AND CONTROL**

*Erysipelothrix rhusiopathiae* is highly susceptible to penicillin, which is the treatment of choice. However, most strains are also susceptible to ampicillin, cloxacillin, benzylpenicillin, ceftriaxone, tylosin, enrofloxacin, and danofloxacin (Yamamoto et al. 2001). Treatment early in the course of infection usually results in a good response within 24–36 hours. The use of antiserum for suckling pigs or on a whole herd basis during an acute outbreak is a fairly common and effective practice in some parts of the world. Pigs given antiserum subcutaneously are immediately protected and this passively acquired protection lasts up to 2 weeks.

Prevention of swine erysipelas is best accomplished by immunization programs. Current vaccines are based on *E. rhusiopathiae* serotypes 1 or 2 and are either inactivated bacterins for intramuscular injection or attenuated (avirulent live) vaccines designed for whole herd mass treatment via drinking water. Most bacterins are serotype 2 (Eamens et al. 2006; Wood 1979) and most attenuated live vaccines contain serotype 1a isolates (Opriessnig et al. 2004). Vaccination is generally effective in preventing swine erysipelas and the duration of immunity varies between 6 and 12 months for both correctly administered bacterins and avirulent vaccines (Swan and Lindsey 1998). Vaccination may not be as effective in preventing chronic arthritis since sequestration of *E. rhusiopathiae* in the cytoplasm of chondrocytes of articular cartilage may provide protection from host immunity. Vaccination of breeding animals reportedly reduces the incidence of periparturient vulval discharge, decreases farrowing intervals, and increases the numbers of live-born pigs in clinically affected herds (Gertenbach and Bilkei 2002).

There are risks involved with the use of any live vaccine and this has been documented in Japan for *E. rhusiopathiae*. In 1932, an acriflavine-resistant attenuated live vaccine was developed (Kondô et al. 1932) and used intensively during and after swine erysipelas outbreaks in 1966 and 1967. In a recent study involving 800 strains of *Erysipelothrix* spp. isolated from pigs in Japan with erysipelas, it was found that, in the 381 serotype 1a isolates, the incidence of acriflavine resistance was 97.7%. It was concluded that the intensive use of the live vaccine led to approximately 2000 cases of vaccine-induced swine erysipelas annually since the 1990s (Imada et al. 2004).

Negative herds can be established by cesarean derivation or medicated early weaning. However, given the ubiquity of *E. rhusiopathiae*, it is unlikely that negative herds can be maintained for extended periods of time.

**REFERENCES**


Glässer’s Disease
Virginia Aragon, Joaquim Segalés, and Simone Oliveira

RELEVANCE
In 1910, K. Glässer discovered a bacillus in exudates from pigs with fibrinous polyserositis. The isolation and characterization of this fastidious bacillus required several years and, after some changes, it is now named *Haemophilus parasuis* (Hps). The disease of fibrinous polyserositis and arthritis caused by Hps is known as Glässer’s disease.

Glässer’s disease is present in all major swine-raising countries and remains a significant disease in modern age-segregated production systems. The prevalence of Glässer’s disease is nowadays rising and severely affects high health status systems. In the United States, for example, it is considered one of the main infectious problems in the nursery, also affecting growing pigs and sows (Holtkamp et al. 2007).

ETIOLOGY
The etiological agent of Glässer’s disease was formally identified in 1931 by Lewis and Shope as *Haemophilus influenzae* (variety *suis*), since it was similar to the human pathogen *H. influenzae*, but of porcine origin. Between 1942 and 1952, several authors reproduced Glässer’s disease with pure cultures of this bacillus (Bakos et al. 1952; Hjärre 1958; Hjärre and Wramby 1943). Later, in 1969, Biberstein and White determined that, contrary to *H. influenzae*, the swine bacterial species did not require the X factor (hemin) to grow. Following the standard nomenclature in this group of microorganisms, *H. influenzae* (variety *suis*) was renamed Hps (Biberstein and White 1969).

Hps is a gram-negative bacterium and member of the family *Pasteurellaceae*, but its location within this family is uncertain. The classification of some members of this family by 16S rRNA gene sequencing shows that *Haemophilus*, as well as *Actinobacillus* and *Pasteurella*, do not form monophyletic groups. In fact, high diversity in 16S rRNA gene sequence within the Hps species and segregation into two distinct clusters has been reported (Angen et al. 2007; Olvera et al. 2006a, 2007a).

Classification of Hps Strains
Hps strains are heterogeneous in phenotypic and genotypic traits, including virulence. Strain classification is of special interest in Hps diagnosis and control, since it is essential to differentiate between colonizing nonvirulent strains and pathogenic virulent strains.

The first efforts to classify Hps strains focused on antigenic proprieties as defined by agar gel precipitation test (AGPT) using heat-stable antigens and rabbit polyclonal antisera (Bakos et al. 1952; Kielstein et al. 1991; Morozumi and Nicolet 1986; Rapp-Gabrielson and Gabrielson 1992; Schimmel et al. 1985). Isolates sharing similar antigens were grouped into 15 serovars (Kielstein and Rapp-Gabrielson 1992). The high percentage of nontypeable (NT) isolates using AGPT led to the development of an indirect hemagglutination serotyping method, which was more efficient in assigning Hps isolates into the 15 recognized serovar groups (del Río et al. 2003b; Tadjine et al. 2004b). However, reduction in the number of NT isolates was not always achieved and discrepancy in results has been reported (Turri and Blackall 2005). Inconsistent serotyping results are likely due to differences in methods, antisera, and reacting antigens.
Haemophilus parasuis experimental inoculation with strains from different serovars of which generates genomic fingerprints based on random DNA with restriction endonucleases and the analysis of the resulting fragments. This method allowed the identification of several Hps strains in a single animal or farm; also, this study suggested that strains isolated from systemic and respiratory sites represented two distinct groups. These observations were further confirmed using enterobacterial repetitive intergenic consensus-polymerase chain reaction (ERIC-PCR), which generates genomic fingerprints based on random amplification by PCR (Oliveira et al. 2003a; Rafiee et al. 2000; Ruiz et al. 2001).

There is no direct association between genotype and serovar. Isolates from the same serovar may include different strains, whereas strains with identical genotypes may differ regarding their serovars (Neil et al. 1969; Oliveira et al. 2003a; Turni and Blackall 2010). These differences may be associated with different expression of heat-stable antigens in vitro or, most likely, lack of reproducibility of the serotyping techniques. Interlaboratory reproducibility is also an issue for ERIC-PCR, which restricts data sharing among laboratories (Olvera et al. 2007c). Lack of association between genotype and serovar has also been confirmed by three different restriction fragment length polymorphism-PCR (RFLP-PCR) protocols using tbpA (de la Puente Redondo et al. 2003), 16s rRNA (Lin 2003) and aroA genes (del Rio et al. 2006a).

To exchange genotyping information among laboratories, sequencing methods were developed (Olvera et al. 2006a,b). Multilocus sequence typing (MLST) detected one cluster of strains associated with systemic lesions and a cluster of nasal strains from healthy animals (Aragon et al. 2010b; Olvera et al. 2006b, 2007b).

### Genomic Organization and Gene Expression

Recently, Yue et al. (2008) reported the complete genome sequence of Hps strain SH0165 (GenBank accession number CP001321), a virulent serovar 5 Chinese isolate. The Hps genome size is about 2.3 Mb and it is composed of more than 2000 predicted genes. Several putative virulence-associated genes were detected in the SH0165 strain; however, little is known about the function of these genes in Hps pathogenesis.

The identification of virulence genes in Hps is of practical interest for the differentiation of strains with high pathogenic potential and the improvement of vaccine development. Two approaches have been used: analysis of genetic differences between virulent and nonvirulent strains and analysis of the gene expression of virulent strains under in vivo conditions.

The gene dhps, which confers sulfonamide resistance, has been found in serovar 2, while it was absent in serovars 1, 3, and 5 (del Rio et al. 2005b). However, the relationship of this gene with virulence is not clear and it may be strain specific, since dhps can be carried by plasmids. Comparison of the virulent Nagasaki strain from serovar 5 and a nonvirulent strain from serovar 11 detected several serovar 5-specific genes: the hemolysin hhdAB, the iron acquisition gene cirA, and phage genes (Sack and Baltes 2009). Although these genes were not present in nonvirulent strains, they were not always found in virulent strains, indicating that they are not strictly necessary for virulence. A similar approach identified 15 genes that were present in the Nagasaki strain and absent in the nonvirulent strain SW114 from serovar 3 (Zhou et al. 2010a). Apart from a fimbria-related gene (fimB), which could play a
role in colonization, the majority of genes belonged to metabolism and hypothetical or unknown categories, lacking a clear relationship with virulence.

Comparative analysis of strains from different clinical backgrounds detected a group of 13 genes coding for \textit{vtaA} or virulence-associated trimeric autotransporter (Pina et al. 2009). These proteins have been shown to be immunogenic and expressed in vivo (Olvera et al. 2010). Finally, differences in outer membrane proteins P2 and P5 have been reported, but no clear relationship with virulence was observed (Mullins et al. 2009).

In order to identify the virulence factors of Hps, several authors have studied the gene expression of virulent strains under conditions mimicking the in vivo environment; that is, high temperature, acidic conditions, iron restriction, and/or incubation with cerebrospinal fluid (Hill et al. 2003; Melnikow et al. 2005; Metcalf and MacInnes 2007; Xie et al. 2009). However, the majority of the genes identified in these studies were involved in metabolic adaptation to the stress conditions or coded for hypothetical proteins. The role of these genes in virulence is difficult to predict since they were present in strains from all serovars and may be involved in adaptation and survival in the host, which is a requirement for both nonvirulent and virulent strains of Hps. The genes more frequently detected were iron acquisition genes \textit{hxuCBA}, \textit{tonB-exbBD-thpAB}, and \textit{yfeACD}, and protease genes (Hill et al. 2003; Melnikow et al. 2005; Xie et al. 2009). Interestingly, a hydrolase was associated to strains with high virulence, and \textit{cpdB}, involved in nucleotide use, was detected in two different variants, but a clear relationship with virulence was not found (Metcalf and MacInnes 2007). Xie et al. (2009) detected the upregulation of \textit{pilA} under iron restriction, indicating that iron restriction can induce pilus biogenesis and could be a signal for colonization.

Finally, several genes with homology to putative virulence factors, such as a putative large adhesin (or \textit{vtaA}), \textit{sidB} (involved in sialic acid utilization), or a protease, were expressed in vivo in the infected lung (Jin et al. 2008). In addition, genes with putative function in biofilm formation were detected, supporting a role of biofilm in Hps infection and colonization (Jin et al. 2006).

**Laboratory Cultivation**

Hps is a small, nonmotile, pleomorphic (from single coccobacilli to filamentous chains) gram-negative bacterium in the family \textit{Pasteurellaceae}, which requires V factor (nicotinamide adenine dinucleotide, NAD) but not X factor (hemin) for growth. In the laboratory, Hps grows on enriched chocolate agar but not on blood agar. However, it can also be cultured on blood agar with a \textit{Staphylococcus} nurse streak as a source of V factor, showing the characteristic satellitic growth. Hps requires 1–3 days to produce small brown to gray colonies on chocolate agar plates or small, translucent non-hemolytic colonies on blood agar.

**EPIDEMIOLOGY**

Hps is a member of the normal respiratory microbiota and, as such, is ubiquitous in swine herds. It is an early colonizer of the upper respiratory tract of piglets and the initial acquisition of this bacterium takes place through contact with the sow following birth. Attempts to isolate Hps from the vagina of the sows have failed and this is in agreement with the production of Hps-free piglets by snatch-farrowing and subsequent artificial feeding. Snatch-farrowed colostrum-deprived piglets have been useful to experimentally reproduce disease by Hps (Blanco et al. 2004; Oliveira et al. 2003b).

Hps has been detected in nasal swabs of piglets up to 6 months after birth, with a maximum prevalence of colonization occurring at 60 days of age (Angen et al. 2007; Cerdà-Cuéllar et al. 2010). Different strains of Hps can be isolated from the nasal cavities of healthy piglets and a single animal can carry more than one strain. Recently, it was demonstrated that there is a high diversity and turnover of strains within one herd (Cerdà-Cuéllar et al. 2010). Four to five strains can be isolated from a herd at a given time (Oliveira et al. 2003a; Olvera et al. 2006b, 2007b; Smart et al. 1993), with a total of 16 different strains being identified in a herd over a period of 5–6 months (Cerdà-Cuéllar et al. 2010). In spite of this high variety of strains within a herd, usually one single prevalent strain is associated with an outbreak (Rafiee et al. 2000).

In a healthy scenario, colonization by Hps develops when piglets are still protected by the maternal immunity and a balance between colonization and immunity is achieved. Disease can emerge when this balance is disturbed. Different factors can trigger the onset of disease, including management practices such as unstable room temperature, poor ventilation or early weaning, immune status of the piglets, presence of other pathogens, and virulence of Hps strains present in the herd or introduction of a new virulent strain.

Domestic pig and wild boar are the only known hosts for this bacterium. Hps has been isolated (Olvera et al. 2007a) and specific antibodies have been detected (Vengust et al. 2006) in wild boar, but Glässer’s disease has not been reported in this species. The role of wild boar as a reservoir for Hps infection needs to be further evaluated.

Transmission of Glässer’s disease occurs through contact of carrier or diseased pigs with susceptible animals. Thus, mixing pigs from different origins is a risk factor that has to be considered when controlling disease. Hps is very labile in the environment. Although there are not many studies on its resistance to disinfectants, efficacy of several formulations, including
chloramine-T and quaternary ammonium compounds, has been reported (Rodríguez-Ferri et al. 2010).

**PATHOGENESIS**

Disease caused by Hps has been reproduced by different inoculation routes (Amano et al. 1994; Blanco et al. 2004; Hjärre 1958; Neil et al. 1969; Rosendal et al. 1985). After inoculation using the natural route of infection (i.e., intranasal inoculation), Hps was detected in the nasal mucosa where supplicative rhinitis and epithelial cell degeneration were observed (Vahle et al. 1997). This may represent the first step in the pathogenesis, and biofilm formation may play an important role at this stage, especially in the normal colonization by nonvirulent strains (Jin et al. 2006). Bacterial adhesion to epithelial cells, induction of apoptosis, and cytokine release can also be important events for Hps colonization (Bouchet et al. 2009). After nasal colonization, the bacterium was isolated from lung and later from internal organs after a short passage through blood (Vahle et al. 1995). Support for the lung as the primary site of entry for systemic infections is lent by findings in a study by Rosendal et al. (1985), where systemic infection was produced following deposition of bacterial inoculum in the lower airways. In the lung virulent strains of Hps survive the phagocytic activity of porcine alveolar macrophages (PAMs), while nonvirulent strains are efficiently engulfed and killed by PAMs (Olvera et al. 2009). Phagocytosis resistance is likely associated with the expression of capsule (Olvera et al. 2009), in agreement with the detection of capsule after in vivo passage (Rapp-Gabrielson et al. 1992).

Hps invades endothelial cells and induces apoptosis and production of proinflammatory interleukin (IL)-6 and IL-8 (Aragon et al. 2010a; Bouchet et al. 2008; Vanier et al. 2006). These phenomena may play a role in the passage to the blood and across the blood–brain barrier. Lipooligosaccharide (LOS) has a partial role in endothelial adhesion and induction of inflammation (Bouchet et al. 2008). Virulent strains are able to survive the bactericidal effect of the blood complement (Cerdà-Cuèllar and Aragon 2008) and can reach systemic sites, including the brain. Finally, Hps reaches internal organs where it replicates in serosal surfaces, producing deposits of fibrin and accumulation of fluids characteristic of Glässer’s disease.

Bacterial survival during infection requires the effective acquisition of nutrients. Inside the host, microorganisms face a limited concentration of free iron. Thus, Hps can acquire iron through surface receptors, such as TbpA (Charland et al. 1995; del Río et al. 2005a; Morton and Williams 1989) or FhuA (del Río et al. 2006b). Besides, Hps possess a neuraminidase (Lichtensteiger and Vimr 1997, 2003), which operates as scavenger of sialic acid for nourishment or modification of the bacterial surface to evade the immune system.

Hps infection induces a reaction in the pig that was studied in the spleen of infected animals (Chen et al. 2009). Genes involved in immune response were differentially expressed during infection and included inflammatory molecules, acute-phase proteins, adhesion molecules, complement, and genes with functions in antigen processing and presentation.

Even though domestic pig and wild boar are the only natural hosts for Hps, the guinea pig model is suitable to reproduce lesions (Morozumi et al. 1982; Rapp-Gabrielson et al. 1992), and the mouse model has been proven useful to study the protective capacity of experimental vaccine candidates (Tadjine et al. 2004a; Zhou et al. 2009a).

Severity of disease depends on the virulence of the Hps strain, the immunity of the piglets, the concomitant presence of other pathogens in the herd, and the genetic resistance of the host. Hps can act as primary or secondary pathogen. Immunosuppressive events allow strains of Hps that are usually restricted to the respiratory tract to invade and be isolated from systemic sites (Olvera et al. 2009). An epidemiological association between Hps infection and porcine reproductive and respiratory syndrome virus (PRRSV), porcine circovirus type 2 (PCV2), and influenza virus type A has been reported (Li et al. 2009; Palzer et al. 2008). Earlier work by Brockmeier (2004) linked higher nasal colonization by Hps to prior colonization by Bordetella bronchiseptica. The influence of genetic background on resistance to Hps systemic infection has been evaluated. Blanco et al. (2008) reported that piglets obtained from six different sire boars significantly varied regarding their resistance, but the exact genetic traits associated with resistance remain to be defined.

**CLINICAL SIGNS**

Clinical signs are mainly observed in 4- to 8-week-old pigs, although the age of affected animals may vary, depending on the level of acquired maternal immunity and colonization. Although rare, Glässer’s disease outbreaks have been described in adult pigs coming from naïve herds, usually after commingling with healthy Hps-colonized animals (Wiseman et al. 1989). Based on experimental infections using Hps-free piglets (Aragon et al. 2010b), the incubation period varies depending on the infecting strain and ranges from less than 24 hours to 4–5 days postinoculation.

Peracute disease has a short course (<48 hours) and may result in sudden death without characteristic gross lesions (Peet et al. 1983). The typical clinical signs of acute Glässer’s disease include high fever (41.5°C), coughing, abdominal breathing, swollen joints with lameness, and central nervous signs such as lateral decubitus, paddling, and trembling (Neil et al. 1969; Nielsen and Danielsen 1975; Riley et al. 1977; Vahle et
SECTION IV  BACTERIAL DISEASES

SEROSITIS AND USUALLY DOES NOT CONTRIBUTE ADDITIONAL USEFUL INFORMATION EXCEPT THE DETECTION OF POTENTIAL FIBRINOPURULENT MENINGITIS (FIGURE 55.1C), WHICH CAN BE FOUND IN 80% OF PIGS CLINICALLY AFFECTED BY HPS (THOMSON 2007).

CHRONICALLY AFFECTED ANIMALS USUALLY SHOW SEVERE FIBROSIS OF THE PERICARDIUM, PLEURA, AND/OR PERITONEUM, AS WELL AS CHRONIC ARTHRITIS.

DIAGNOSIS

THE CLINICAL SIGNS AND LESIONS DESCRIBED FOR HPS SYSTEMIC INFECTION ARE NOT PATHOGENOMONIC OF THIS AGENT

LESIONS

PERACUTELY DISEASED PIGS USUALLY DIE WITHOUT CHARACTERISTIC gross lesions, but may show petechial hemorrhages in some tissues (Peet et al. 1983; Riley et al. 1977). Histologically, those pigs show septicemia-like microscopic lesions such as disseminated intravascular coagulation (fibrinous thrombi in different tissues such as renal glomeruli, liver sinusoids, and pulmonary capillaries) and microhemorrhages (Amano et al. 1994). Increased serosanguinous fluid in the thoracic and abdominal cavities, without fibrin, can also be seen in peracute cases of Hps infection.

Acute systemic infection is characterized by the development of fibrinous or fibrinopurulent polyserositis, polyarthritis, and meningitis (Figure 55.1). The fibrinous exudate can be observed on the pleura, pericardium, peritoneum, synovia, and meninges, and is usually accompanied by an increased amount of fluid (Oliveira et al. 2003b; Riley et al. 1977; Vahle et al. 1995). Fibrinous pleuritis may be found with or without cranioventral consolidation due to catarrhal-purulent bronchopneumonia or, rarely, fibrinohemorrhagic pneumonia (Little 1970; Narita et al. 1994). Lack of characteristic gross lesions is also common in a number of pigs showing neurological clinical signs. Histopathological examination of typical Glässer’s disease reveals fibrinous-to-fibrinopurulent


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55.1. Gross and microscopic lesions found in Haemophilus parasuis systemic infection. (A) Polyserositis characterized by fibrinopurulent exudate on serosal membranes in peritoneal and thoracic cavities. Note the significant amount of fluid in the thorax. (B) Fibrinopurulent exudate on pericardial surface. (C) Brain, fibrinopurulent meningitis.
and other agents need to be included in a differential diagnosis. Fibrinous polyserositis may be caused by other gram-negative bacteria such as nonhemolytic Escherichia coli (Nielsen et al. 1975; Wilkie 1981). Polyserositis caused by nonhemolytic E. coli is sporadic and usually affects suckling piglets (Nielsen et al. 1975). Beta-hemolytic Shiga toxin 2e (Stx2e)-positive and usually affects suckling piglets (Nielsen et al. 1975). beta-hemolytic Shiga toxin 2e (Stx2e)-positive E. coli can cause central nervous system signs in recently weaned pigs similar to those observed with Hps systemic infection. However, microscopically, Stx2e does not cause fibrinopurulent meningitis typical of Hps, but does cause necrosis of endothelial and smooth muscle cells in small arteries and arterioles which are not observed in Hps-infected pigs (Moxley 2000). Mycoplasma hyorhinis is another important cause of fibrinous polyserositis in nursery pigs (Friis and Feenstra 1994), and it is frequently found with Hps, coinfected pigs (Rovira 2009). Streptococcus suis usually affects pigs at the same age as Hps and may cause similar lesions (Reams et al. 1994; Vecht et al. 1989). Other agents involved in development of lameness and arthritis include Erysipelothrix rhusiopathiae and Mycoplasma hyosynoviae. However, both agents are more likely to cause chronic nonpurulent arthritis in finishing pigs (Hagedorn-Olsen et al. 1999; Hariharan et al. 1992).

Considering that Hps is a colonizer of the upper respiratory tract of healthy pigs, detection of this microorganism in the nasal cavity and trachea does not imply disease development. Systemic isolates are more likely to carry important virulence factors and, therefore, those isolates should be pursued for laboratory confirmation of morbidity and mortality caused by this agent.

Hps can be isolated from the fibrinous exudate and parenchyma of affected internal organs and from lung lesions in pneumonia cases (Pijoan et al. 1983; Turni and Blackall 2007). It is a fastidious microorganism with limited survival at room temperature and special requirements for in vitro growth (Biberstein et al. 1977; Morozumi and Hiramune 1982). The chances of isolating Hps can be considerably improved by utilizing swabs with Amies transport media and submitting samples under refrigeration to the laboratory (del Río et al. 2003a; Oliveira 2007). Although Hps isolation can be a difficult task, it is important to obtain an isolate for antibiotic sensitivity assessment and typing.

Hps can also be detected directly in the lesions of affected tissues by immunohistochemistry (Amano et al. 1994; Segales et al. 1997; Vahle et al. 1995) or in situ hybridization (Jung et al. 2004). However the specificity of the in situ hybridization has not been fully clarified and cross-reaction with Actinobacillus pleuropneumoniae in immunohistochemistry has been reported (Segales et al. 1997).

PCR is a sensitive and specific method to detect Hps. Several methods are described in the literature, varying from conventional gel-based tests to real-time detection (Angen et al. 2007; Jung et al. 2004; Oliveira et al. 2001; Turni et al. 2010). PCR detects Hps even when the organism is no longer viable and it represents a great advantage in sensitivity compared to bacterial isolation. A recently described real-time PCR reported a detection limit of 0.83–9.5 colony-forming units (CFU) per reaction and was more sensitive and specific than previously reported gel-based methods (Turni et al. 2010). PCR can be used also for differentiation between virulent and nonvirulent isolates. A recently described multiplex test based on the detection of vtaAs can be used to further characterize isolates regarding invasiveness potential (Pina et al. 2009). This information is relevant, especially when selecting isolates for antibiotic sensitivity or autogenous vaccine production.

Antibodies against Hps can be detected utilizing complement fixation and enzyme-linked immunosorbent assay (ELISA). Complement fixation has been mostly used on a research basis to characterize the antibody responses in experimental infections (Nielsen 1993). ELISAs have also mostly been used for research purposes. Whole-cell indirect ELISAs have been used to characterize the transfer of maternal immunity from sows to piglets and to demonstrate seroconversion postvaccination (Baumann and Bilkei 2002; Cerdà-Cuéllar et al. 2010; Solano-Aguilar et al. 1999). Blanco et al. (2004) utilized a commercial ELISA to characterize the decay of maternal antibodies and identify a window of susceptibility in conventional pigs for experimental infection. The specificity of experimental and commercially available Hps ELISAs has not been extensively evaluated, and their ability to detect antibodies against different Hps serovars and strains has been poorly characterized.

**IMMUNITY**

Hps, as any gram-negative bacteria, interacts with the immune system at varied levels, including the innate and the specific immune system. The literature offers a vast characterization of antibody-mediated immune response, whereas little information is currently available for cell-mediated responses.

**Immune Responses**

Immunohistochemistry and in situ hybridization methods have demonstrated that following infection, Hps is phagocytosed by neutrophils and macrophages and can be found as degenerated bacteria in dilated phagosomes (Amano et al. 1994; Segales et al. 1999). Nonvirulent strains are promptly phagocytosed by PAMs, whereas virulent strains require prior opsonization with specific antibodies. If successfully internalized Hps strains will be killed by PAMs regardless of their virulence potential (Olvera et al. 2009).
Hps stimulates the production of proinflammatory cytokines IL-8 and IL-6 by newborn pig tracheal cells and porcine brain microvascular endothelial cells upon adhesion (Bouchet et al. 2009; Bouchet et al. 2008). Interleukin-8 is a potent chemoattractant for leukocytes, whereas IL-6 is an important mediator in the development of acute phase response. Increased levels of IL-1alpha have been reported in pigs undergoing severe disease following experimental infection, whereas IL-4, IL-10, tumor necrosis factor-α (TNF-α), and interferon-γ (IFN-γ) were expressed in significantly higher levels by survivors (Martín de la Fuente et al. 2009a).

Development of antibodies against Hps following exposure to this agent has been demonstrated in colonized, convalescent, and vaccinated pigs by complement fixation, Western blot analysis, and ELISA testing (Miniats et al. 1991b; Nielsen 1993; Solano-Aguilar et al. 1999). Pigs exposed to Hps live cultures or vaccinated with killed bacterins generate a transient immunoglobulin M (IgM) response followed by a solid and progressively increasing immunoglobulin G (IgG) antibody response. Pigs with high IgG titers are protected against challenge (Martín de la Fuente et al. 2009b). Pigs exposed to live Hps cells develop antibodies against VtaAs, whereas pigs vaccinated with killed vaccines lack antibodies against these antigens. The role of anti-VtaA antibodies in protective immunity remains to be defined (Olvera et al. 2010).

Protective Immunity and Cross Protection
Protective immunity against Hps infection has been linked mainly to the development of specific antibodies following exposure to live cells or vaccination. Specific antibodies are known to successfully opsonize Hps and facilitate phagocytosis by PAMs (Olvera et al. 2009). Complete protection against challenge is usually obtained with homologous vaccination, whereas partial protection is generally observed with heterologous challenges (Martín de la Fuente et al. 2009b; Miniats et al. 1991a,b; Nielsen 1993; Smart and Miniats 1989; Takahashi et al. 2001). Autogenous vaccines are highly effective in protecting susceptible pigs, especially during introduction into populations harboring virulent strains (Smart et al. 1993).

Exposure and colonization with live Hps cells are also known to generate protective immunity. Pigs given an aerosol with serovars 2, 3, 4, or 7 developed circulating antibodies and resisted challenge with a virulent serovar 5 strain (Nielsen 1993). Controlled exposure of 5-day-old pigs with a low dose of a herd-specific live Hps culture significantly reduced mortality compared to vaccination using inactivated commercial and autogenous vaccines (Oliveira et al. 2004). Inactivated Hps vaccines represent incomplete antigens and do not generate antibodies against important virulence-associated factors that are only expressed in vivo by Hps.

Cross-protection between different serovars and even within the same serovar is variable and difficult to predict (Rapp-Gabrielson et al. 1997). Therefore, different approaches are now used for the design of new universal vaccines. Proteomic and immunoproteomic approaches are currently being used for the identification of surface proteins with protective capacity (Martínez et al. 2010; Zhou et al. 2009a,b).

Maternal Immunity
Maternal antibodies are an important source of protective immunity for susceptible piglets. Pigs lacking maternal immunity are susceptible to systemic infection using low doses of Hps, whereas pigs with maternal antibodies are protected against challenge (Blanco et al. 2004; Oliveira et al. 2003b).

Levels of maternal antibodies directly influence the susceptibility of the offspring to Hps systemic infection. Solano-Aguilar et al. (1999) demonstrated that pigs from vaccinated gilts had significantly higher maternal antibody titers compared to those from nonvaccinated gilts. Pigs from vaccinated gilts were protected against challenge at 28 days of age, whereas pigs from nonvaccinated gilts were fully susceptible to systemic infection at 21 days of age. Maternal immunity decay was evident in pigs from nonvaccinated gilts at 16 days of age. A slight decay was observed at 38 days of age in pigs from vaccinated gilts. A recent study by Cerdà-Cuéllar et al. (2010) demonstrates the dynamics of maternal immunity versus levels of colonization using pigs obtained from vaccinated and nonvaccinated sows. According to this study, maternal immunity decay was observed at 20 days in pigs from nonvaccinated sows and at 60 days in those from vaccinated sows. These timings coincide with high levels of isolation and detection of Hps in the nasal cavities of these pigs. Pigs from vaccinated sows tended to have a lower amount and reduced variety of Hps strains compared to pigs from nonvaccinated sows.

PREVENTION AND CONTROL
Vaccination and antibiotics can be used to prevent and control Hps infections. In some countries, regulations restrict the use of antibiotics as a preventative measure, only allowing these drugs for treatment purposes. In light of these regulations, vaccination becomes a valuable option to prevent systemic infection and mortality.

The use of antibiotics is recommended to treat individual pigs and control severe outbreaks due to Hps systemic infection. Individual treatments tend to be more effective than water or feed medication, since pigs clinically affected by Hps systemic infection are prostrated and less likely to ingest the required antibiotic dose via food and water intake.
Antibiotic susceptibility profiles are variable in different countries and reflect the selection of drugs used in each region. Danish and British isolates, for example, are reportedly susceptible to most antibiotics, whereas high rates of resistance to commonly used antibiotics are reported for Chinese and Spanish isolates (Aarestrup et al. 2004; Martín de la Fuente et al. 2007; Zhou et al. 2010b). Resistance to tetracycline and beta-lactam antibiotics has been linked to the presence of resistance genes in plasmids carried by Hps (Lancashire et al. 2005; San Millan et al. 2007).

Hps is a commensal of the upper respiratory tract and colonizes virtually every pig. The early colonization of pigs with virulent strains in the presence of maternal immunity actually prevents disease and mortality postweaning (Oliveira et al. 2004). Herds that have chosen to eliminate Hps are highly susceptible to severe outbreaks with up to 60% mortality if exposed to virulent Hps owing to a lack of immunity (Torrison and Rossow 2004). Strict biosecurity that forbids introduction of Hps-positive swine is essential to prevent high mortality in such cases. Natural colonization during the suckling period usually is not protective against the wide variety of strains circulating in different swine populations owing to poor heterologous protection (Oliveira et al. 2004). Therefore, commingling of pigs from different sources is an important cause of increased mortality due to Glässer’s disease and should be avoided when possible.

Vaccination is an effective measure to prevent mortality (Miniats et al. 1991a; Smart and Miniats 1989; Smart et al. 1988). Vaccines should contain the virulent strain(s) endemic to the farm to be most effective. Strains to be included in farm-specific vaccines should be isolated from systemic sites in diseased pigs such as from serosal fibrin, exudates from joints, or meninges, and not from nasal swabs, tonsils, or lung parenchyma.

REFERENCES
Leptospirosis
William A. Ellis

RELEVANCE
Leptospirosis is a cause of reproductive loss in breeding herds and has been reported in swine from all parts of the world; however, knowledge of the incidence and economic impact of the disease is largely confined to the intensive pig industries of the northern hemisphere, Australia, New Zealand, where its importance has apparently declined, and Argentina and Brazil.

Endemic infection in a herd of swine may produce little evidence of clinical disease, but when it is first introduced into a susceptible breeding herd, or during periods of waning herd immunity, it can cause very appreciable losses through abortion, or the full-term birth of dead pigs, or weak pigs of reduced viability or infertility.

Leptospires persist in the kidneys and genital tracts of carrier swine and are excreted in urine and genital fluids. Survival outside the host is favored by warm moist conditions. Transmission is by direct or indirect contact with a carrier animal. Interruption of transmission from infected pig or other host to the pig is the critical factor in control.

ETIOLOGY
Leptospirosis of swine is a disease caused by a variety of morphologically similar, but antigenically and genetically distinct, small, motile, aerobic spirochetes belonging to the genus *Leptospira*. They are thin, helical, motile, gram-negative organisms, which are often hooked at one or both ends. They range in length from about 6 to 20µm, with amplitude of approximately 0.1–0.15µm and a wavelength of about 0.5µm. Under adverse nutritional conditions, leptospires may be greatly elongated, while under conditions such as high salt concentrations, aging culture, or in tissues, leptospires may form coccoid forms of about 1.5–2µm diameter. They divide by binary fission. They stain poorly with aniline dyes. Unstained cells are visible only by dark field microscopy. In a suitable liquid environment, motility is accomplished by rotating along the long axis, but an undulating action is observed in semisolid media. They require special media containing mammalian serum or albumin for cultivation (Faine et al. 1999).

The major structural components are an outer membrane, which surrounds a double membrane structure in which the cytoplasmic membrane and peptidoglycan cell wall are closely associated. Two flagella with polar insertions are located in the periplasmic space and are responsible for motility (Adler and de la Peña Mocetzuma 2010). Within the outer membrane, the lipopolysaccharide (LPS) constitutes the main antigen for *Leptospira*. In addition to LPS, structural and functional proteins form part of the leptospiral outer membrane. A large proportion of such proteins are lipoproteins with LipL32, LipL21, and LipL41 being the most abundant on the cell surface (Cullen et al. 2005). Integral membrane proteins such as the porin OmpL1 are also located in the outer membrane (Shang et al. 1995).

The genus *Leptospira* includes both saprophytic and pathogenic species. The pathogenic family consists of 13 pathogenic species: *L. alexanderi*, *L. alstonii*, *L. borgpetersenii*, *L. inadai*, *L. interrogans*, *L. fainei*, *L. kirschneri*, *L. licerasiae*, *L. noguchi*, *L. santarosai*, *L. terpstrae*, *L. weilii*, and *L. wolffii*, with more than 260 serovars. There...
are six saprophytic species (Adler and de la Peña Moctezuma 2010). There are differences in the global distribution of some of the Leptospira species: L. interrogans, L. borgpetersenii, and L. kirschneri have a worldwide distribution, whereas L. noguchii and L. santarosai are found mainly in North and South America while L. weilli is found mainly in China and eastern Asia. Strains that cause disease in pigs are found mainly in the L. interrogans and L. borgpetersenii species.

At the subspecies level, serovar classification of Leptospira is still widely used as it facilitates serodiagnostic, epidemiology and prevalence studies. It is based on the expression of the surface-exposed epitopes in a mosaic of the LPS antigens, while the specificity of these epitopes depends on their sugar composition and orientation (Adler and de la Peña Moctezuma 2010). In addition, the concept of serogroups, which cluster antigenically related strains, is still used as the basis of selecting cross-reactive strains for serological tests.

**PUBLIC HEALTH**

Leptospirosis is an important occupational zoonosis of those who work with pigs, especially farmers, veterinarians, and abattoir workers (Faine et al. 1999). Infection is by direct or indirect contact of mucous membranes or skin wounds with the urine of an infected pig. A majority of cases are asymptomatic or present as sudden onset of an approximately weeklong febrile sickness with any combination of chills, conjunctival suffusion, severe headache, myalgia of lower back and legs, abdominal pain, vomiting, and diarrhea. In approximately 5–10% of cases, the clinical course progresses rapidly to include icterus, renal failure, cough, dyspnea, and hemoptysis that may be fatal.

**EPIDEMIOLOGY**

The epidemiology of swine leptospirosis is potentially very complicated, since swine can be infected by any of the pathogenic serovars. Fortunately, only a small number of serovars will be endemic in any particular region or country. Furthermore, leptospirosis is a disease that shows a natural nidality, and each serovar tends to be maintained in specific maintenance hosts. Therefore, in any region, pigs will be infected by serovars maintained by pigs or by serovars maintained by other animal species present in the area. The relative importance of these incidental infections is determined by the opportunity that prevailing social, management, and environmental factors provide for contact and transmission of leptospires from other species to pigs.

Pigs act as maintenance hosts for serovars belonging to the pomona and australis serogroups while icterohaemorrhagiae, grippotyphosa, and tarassovi serogroups are among the more commonly identified incidental infections in swine.

**Pomona Infection**

Serovar Pomona and the closely related serovar—Kennewicki—have been the most common serovars isolated from pigs worldwide. Infections with these serovars have been extensively studied and provide a suitable model with which to illustrate general concepts of swine leptospirosis. Many strains of serovars Pomona and Kennewicki, especially those found in the United States and Canada, are adapted to swine. They have been the cause of widespread clinical disease in swine in North and South America, Australia, New Zealand, parts of Asia, and eastern and central Europe, and are endemic in many of these regions. There is now evidence of high levels of infection in parts of Africa (Agunloye 2001) and Southeast Asia (Al-Khleif et al. 2009). Such strains are apparently absent from the more westerly parts of Europe where a rodent maintained serovar Mozdok may cause occasional outbreaks of clinical disease (Barlow 2004; Rocha 1990; Zieris 1991).

In parts of North America, the prevalence of Pomona infection in pigs has fallen from the high levels observed in the 1950s and early 1960s. No carriers were detected in a 1989 meat-plant survey carried out in Iowa (Bolin and Cassells 1992). In contrast, Baker et al. (1989) recovered serovar Kennewicki from almost 10% of pigs in a small survey in Canada.

Leptospires have a particular affinity for the kidneys of infected pigs, where they persist, multiply, and are voided in the urine. This characteristic is very important in the transmission of infection. Infection is introduced into a susceptible herd by three possible routes: the introduction of infected stock, exposure to a contaminated environment, or contact with an alternative infected animal vector. Carrier pigs are probably the most common route of introduction. Replacement gilts or infected boars have been identified as important means of introducing infection (Ellis 2006).

The importance of free-living species as possible sources of Pomona infection of pigs depends on geographical location. In North America, the skunk has been incriminated as a source of Pomona outbreaks in pigs (Mitchell et al. 1966), and the move to indoor housing has reduced the opportunity for skunk-to-pig transmission.

Once Pomona has been introduced into a pig population, a high prevalence of infection is established. Only low infective doses are required to transmit infection (Chaudhary et al. 1966a,b). If direct contact is prevented, indirect contact through contaminated effluent, water, or soil ensures transmission. The presence of moisture is critical for indirect transmission; the organisms cannot withstand desiccation, but when infected urine is deposited in damp soil or water with a neutral or slightly alkaline pH, the organisms may survive for extended periods (Ellis 2006).
During the initial herd infection, clinical disease may occur in all ages of sows. Following the initial establishment of infection, an endemic cycle typical of that in a maintenance host population is set up (Hathaway 1981). Piglets are passively protected in the first weeks of life by colostrum-derived immunoglobulins from infected dams (Bolt and Marshall 1995a; Fish et al. 1963). The duration of this passive protection depends primarily on the quantity of immunoglobulins received in colostrum (Chaudhary et al. 1966b). A study of grower pigs in New Zealand has shown that *Leptospira* infection becomes apparent in piglets from 12 weeks of age and by slaughter up to 90% may be infected. The intensity of shedding in urine is greatest in the first 3–4 weeks of infection after which it declines and becomes intermittent (Bolt and Marshall 1995a,b). Infection between groups of fattening pigs is often by urine-contaminated effluent from a common drainage system (Buddle and Hodges 1977). In herds with endemic infection, clinical disease is usually restricted to gilts that have either been reared in isolation since weaning and reintroduced into the herd, or more commonly brought in from an uninfected herd.

**Australis Infection**

Serovar Bratislava and, to a lesser extent, the closely related serovar Muenchen have emerged as major swine-maintained leptospiral infections but remain poorly understood due to difficulties in culturing these strains. Serological data have indicated widespread infection in Europe, the United States, Canada, Australia, Brazil, South Africa (Ellis 2006), Nigeria (Agunloye 2001), Korea (Choi et al. 2001), and Japan (Kikuchi et al. 2009) among others.

In contrast to the high seroprevalences reported worldwide, serovar Bratislava has only been recovered from pigs in a few countries, namely The Netherlands (Hartman et al. 1975), United Kingdom (Ellis et al. 1991), the United States (Bolin and Cassells 1990, 1992; Ellis and Thiermann 1986), Germany (Schönberg et al. 1991), the United States (Bolin and Cassells 1990, 1992; Ellis et al. 1986b,c; Power 1991). Venereal transmission is thought to play an important role in the spread of Bratislava infection.

**Tarassovi Infection**

There is much less information available on the epidemiology of Tarassovi infection in pigs. The pig was previously thought to act as a maintenance host for some strains of Tarassovi found in eastern Europe and Australia, but declining seroprevalences would suggest that this is not so (Wasinski 2005). High seroprevalences associated with reproductive failure have recently been reported from Vietnam (Boqvist et al. 2007).

Many strains of Tarassovi have been recovered from free-living animals further supporting the view that Tarassovi infections are incidental infections of pigs resulting from wildlife contact. Work in the United States supports this view: Tarassovi has not been recovered from swine, but there is serological evidence of infection in pigs in the southeastern states (Cole et al. 1983) where it has been isolated from raccoons, skunks, and opossums (McKeever et al. 1958; Roth 1964).

**Canicola Infection**

Although organisms belonging to this serogroup have been recovered from swine in a number of countries, little is known of the epidemiology of serovar Canicola infection in pigs. Conventional wisdom has been that infection is acquired from dogs—the recognized maintenance host for this serovar—although wildlife may also be a source (Paz-Soldan et al. 1991). The long period of urine shedding observed in infected pigs (at least 90 days) and the ability of Canicola to survive for up to 6 days in undiluted pig urine (Michna 1962) suggest that intraspecies transmission could occur.

**Icterohaemorrhagiae Infection**

Serological evidence of icterohaemorrhagiae serogroup infection has been reported in many countries, but few isolations have been made from pigs. Both serovars Copenhageni and Icterohaemorrhagiae may be involved and are probably introduced to susceptible stock via an environment contaminated with infected urine from the brown rat (*Rattus norvegicus*)—the maintenance host for these serovars. Urinary excretion is less than 35 days (Schnurrenberger et al. 1970), and intraswine transmission is inefficient (Hathaway...
1985). It is believed, in the absence of supporting isolation data, that vaccine induced antibodies are responsible for the seroprevalences to Icterohaemorrhagiae observed in the United States. High seroprevalences to Icterohaemorrhagiae associated with clinical disease have been detected in some pig populations in Brazil (Osava et al. 2010).

**Grippotyphosa Infection**

Serovar Grippotyphosa infection is maintained by wildlife hosts, and incidental infection of pigs gives rise to low prevalences of antibodies in swine in various regions, particularly eastern and central Europe and the United States, and it has been recovered from pigs in Russia and the United States in the past (Ellis 2006). A high seroprevalence has been reported in Thailand (Puchadapirom et al. 2006).

**Hardjo Infection**

Serovar Hardjo infection is maintained by cattle worldwide, and where cattle and pigs come in close contact, the opportunity arises for infection in pigs to occur. There have been reports of the isolation of Hardjo from pigs in the United Kingdom (Ellis et al. 1986a; Hathaway et al. 1983) and the United States (Bolin and Cassells 1992), but persistence in renal tissue was not a feature of experimental infection (Hathaway et al. 1983) and therefore, intraspecies transmission is unlikely.

**PATHOGENESIS**

The most important route of natural infection has not been determined. It is thought to be via the mucous membranes of the eye, mouth, or nose. Infection via the vaginal route is also possible, and transmission through milk from an infected dam has been demonstrated experimentally. A period of bacteremia, which may last for a week, begins 1 or 2 days after infection. During this period, leptospires can be isolated from most organs of the body and also from the cerebrospinal fluid (Ellis 2006). This primary bacteremic phase ends with the appearance of circulating antibodies, which are detectable usually after 5–10 days (Hanson and Tripathy 1986). A secondary bacteremic period (after 15–26 days) has been reported in experimental Hardjo infection (Hathaway et al. 1983).

Antileptospiral agglutinins appear at detectable levels in the blood at approximately 5–10 days after infection and reach maximum levels at around 3 weeks. Peak titers vary considerably (1:1000 to 1:100,000 in the MAT), and these may be maintained for up to 3 weeks, after which a subsequent gradual decline occurs. Low titers may be detectable for several years in many animals.

Following the period of leptospiremia, leptospires localize in the proximal renal tubules where they multiply and are voided in the urine. The duration and intensity of urinary shedding varies from pig to pig and with the infecting serovar. In the case of Pomona infection, the intensity of excretion is highest during the first month of shedding, when more than a million leptospires may be present in each milliliter of urine; urine shedding is very constant during this period. A variable period of intermittent, low-intensity leptospiruria then ensues, and this may last for up to 2 years in some cases.

Leptospires also localize in the uterus of pregnant sows and abortion, production of stillborn pigs, and neonatal disease frequently result from intrauterine infections occurring in the last half of the gestation period. Abortions and stillbirths usually occur 1–4 weeks following infection of the gilt or sow (Hanson and Tripathy 1986), by which time most sows have developed detectable antibody titers. Since pig fetuses are capable of producing antibodies during the latter stages of gestation, some stillborn piglets will have detectable titers.

The pathogenesis of reproductive disease is poorly understood, but some authors believe that transplacental infection, occurring during the very limited period of maternal leptospiremia, is the sole cause. While this may be true for systemic infections such as Pomona, the low antibody titers detected in sows aborting Bratislava-infected fetuses has led to the hypothesis that infection occurs as a result of waning uterine immunity being unable to prevent transplacental infection by leptospires present in the genital tract. Horizontal transmission to littermates not infected during the period of maternal leptospiremia may also occur. Once the placental barrier is breached, septicemia results in large numbers of leptospires in all fetal tissues (Ellis 2006).

An additional feature seen in Bratislava infection but not reported for the other swine leptosporal infections is the persistence of leptospires in the oviduct and uterus of nonpregnant sows and in the genital tracts of boars (Ellis 2006; Oliveira et al. 2007).

**CLINICAL SIGNS**

The vast majority of swine leptospiral infections are subclinical. Two groups of pigs are most likely to experience clinical infections: the young piglet and the pregnant sow.

**Acute Leptospirosis**

This phase usually coincides with the period of bacteremia. In experimental infections, many pigs exhibit transient anorexia, pyrexia, and listlessness at this time.
(Hanson and Tripathy 1986). However, the mild nature of these signs means that in natural infections, especially in endemically infected herds where perhaps only one or two animals may be affected, this phase of infection usually goes unrecognized.

There have been a few reports of jaundice and hemoglobinuria in naturally occurring outbreaks (Ferguson et al. 1956), particularly in cases of infection in piglets under 3 months of age by Icterohaemorrhagiae (Field and Sellers 1951; Modric et al. 2006). A high proportion of these undergo spontaneous recovery within a week of when clinical signs develop. The small number of such reports suggests that this more severe form of disease is rare.

**Chronic Leptospirosis**

Abortions, stillbirths, the birth of weak piglets of reduced viability, and reduced litter size are primary signs of chronic leptospirosis, particularly Pomona infection, and it is this aspect of leptospirosis that can cause considerable economic loss (Azevedo et al. 2008; Ellis 2006).

Information as to the importance of leptospirosis as a cause of abortion in national swine herds is not available, and if it were, it must vary from country to country depending on prevalence, and epidemiological and management factors, including the implementation of control measures. From the limited and dated information available, it would appear that even in countries where vaccination has been widely practiced, leptospirosis is a common cause of swine abortion. In Ontario for example, 6% of swine abortions were attributed to Pomona infection (Anonymous 1986). Endemic Tarassovi infection was considered to be the cause of a 3% abortion rate in herds in Poland investigated by Wandurski (1982). Fearnley et al. (2008) found 4 of 24 fetal diagnostic submissions to be polymerase chain reaction (PCR) positive. Acute outbreaks can still give rise to severe losses; Saravi et al. (1989) described an outbreak in a herd in which 19% of pregnant sows aborted, while the number of dead piglets/sow rose from 8% prior to the outbreak to 28% during the outbreak. Differences in strain pathogenicity also contribute to different prevalences of clinical abortion in infected herds (Nagy 1993).

A very high prevalence of serovars belonging to the australis serogroup in aborted pig litters has been observed in parts of the United Kingdom (Ellis et al. 1986a). Similar strains have also been recovered from aborted piglets in the United States (Bolin and Cassells 1990; Bolin et al. 1991). Rehmtulla et al. (1992) reported fetal Bratislava infection following abortions in 16% of sows in a herd in Ontario. Egan (1995) reported fluorescent antibody test (FAT)-positive prevalences ranging from 5% to 23% in diagnostic submissions in Ireland. Published experimental evaluations of the significance of such microbiological findings are not available. There has, however, been an absence of significant isolations of other abortifacient agents from these cases, and the farrowing rate and the number of live piglets born/sow improve significantly following either Bratislava vaccination (Frantz et al. 1989) or the use of an antibiotic medication program (Ellis 1989).

Following abortions due to Pomona, there does not appear to be any subsequent limitation on reproductive performance, even in pigs that remain infected for long periods (Ferguson and Powers 1956; Kemenes and Suveges 1976; Mitchell et al. 1966).

Infertility is a feature of Bratislava infection (Hathaway and Little 1981; Jensen and Binder 1989; Van Til and Dohoo 1991). Split-herd trials, carried out using a Bratislava bacterin, have demonstrated significant improvements in sow fertility (Frantz et al. 1989).

**LESIONS**

The main pathological changes are essentially the same for all infections, with the primary lesion being damage to the membranes of the endothelial cells of small blood vessels.

In acute leptospirosis, there are no pathognomonic gross changes. Pathological changes in acute Pomona infection are very limited, reflecting the mild nature of acute clinical disease. Hanson and Tripathy (1986) reported little gross or histopathological change in swine killed during the acute phase of leptospirosis. Burnstein and Baker (1954) reported that petechial and ecchymotic hemorrhages could be seen in the lungs of some pigs and histological examinations have revealed minor renal tubular damage, focal liver necrosis, lymphocytic infiltration of the adrenal glands, and meningoencephalitis with perivascular lymphocytic infiltration (Burnstein and Baker 1954; Chaudhary et al. 1966a; Sleight et al. 1960).

In chronic leptospirosis, lesions are confined to the kidneys and consist of scattered small gray foci, often surrounded by a ring of hyperemia. Microscopic examination shows these lesions to be a progressive focal interstitial nephritis (Burnstein and Baker 1954; Cheville et al. 1980; Langham et al. 1958). The interstitial leukocytic infiltrations, which consist mainly of lymphocytes, macrophages, and plasma cells, may be extensive in some areas. Focal damage may also involve glomeruli and renal tubules. Some affected glomeruli are swollen, some atrophic, and others are replaced by fibrosis. The Bowman’s capsule may be thickened, containing eosinophilic granular material (Langham et al. 1958). Tubular changes involve atrophy, hyperplasia, and the presence of necrotic debris in the lumen in some areas. Occasionally, petechial hemorrhages may be present in interstitial spaces. Older lesions mainly
consist of fibrosis and interstitial infiltration. Chronic lesions with accompanying acute inflammatory changes are still noticeable as long as 14 months postinfection (Morter et al. 1960).

Experimental studies indicate that leptospires can invade the mammary gland of pigs and produce mild, focal nonsuppurative mastitis (Tripathy et al. 1981).

The gross pathology of fetuses aborted as a sequela of Pomona infection is nonspecific and includes edema of various tissues, serous or bloodstained fluid in body cavities, and sometimes petechial hemorrhages in the renal cortex (Fennestad and Borg-Petersen 1966; Ryley and Simmons 1954). These changes are probably the result of intrauterine autolysis. Jaundice may be seen in some aborted piglets (Hathaway et al. 1983). Focal necrosis, presenting as small grayish-white spots, is a frequent finding in the liver (Fennestad and Borg-Petersen 1966; Fish et al. 1963; Ryley and Simmons 1954). Histological examination may reveal small foci of interstitial nephritis. Placentas from aborted fetuses aregrossly normal (Fennestad and Borg-Petersen 1966; Fish et al. 1963).

**DIAGNOSIS**

A diagnosis of leptospirosis in swine may be required not only for the clinician to confirm leptospirosis as a cause of clinical disease but also for other reasons, such as (1) the assessment of the infection and/or the immune status of a herd for the purposes of a control or eradication program on either a herd or national basis; (2) epidemiological studies; and (3) an assessment of the infectivity status of an individual animal to assess its suitability for international trade or for introduction into an uninfected herd.

The mild, often inapparent, clinical signs of acute leptospirosis make clinical diagnosis difficult; therefore, diagnosis is usually based on the results of laboratory procedures. Laboratory diagnostic procedures for leptospirosis fall into two groups. The first group consists of tests for antibody detection; the second contains the tests for the demonstration of leptospires in pig tissues. The selection of tests to be carried out depends on the purpose for which a diagnosis is to be made and the resources available.

**Serological Tests**

Serological testing is the most widely used method for diagnosing leptospirosis, and the MAT (OIE 2008) is the standard serological test. The minimum antigen requirements are that the test should employ representative strains of all the serogroups known to exist in the particular country, plus those known to be maintained by pigs elsewhere.

The MAT is used primarily as a herd test. To obtain useful information, at least 10 animals or 10% of the herd, whichever is greater, should be tested. A retrospective diagnosis of both acute leptospirosis and Pomona abortion may be made when the majority of affected animals have titers of 1:1000 or greater. Increasing the sample size and sampling a number of different cohorts markedly improves epidemiological information, investigations of clinical disease, assessments of vaccination needs, and public health tracebacks.

As an individual animal test, the MAT is very useful in diagnosing acute infection; rising antibody titers in paired acute and convalescent serum samples are diagnostic. The presence of antibody in fetal serum is diagnostic of leptospiral abortion.

The MAT has severe limitations in the diagnosis of chronic infection in individual pigs, both in the diagnosis of abortion and in the identification of renal or genital carriers. Infected animals may have MAT titers below the widely accepted minimum significant titer of 1:100 (Ellis et al. 1986b,c). In these cases, a competitive ELISA has proved useful (Frizzell et al. 2004).

**Demonstration of Leptospires in Pig Tissues**

The isolation of leptospires from, or their demonstration in, the internal organs (such as liver, lungs, brain) and body fluids (blood, cerebrospinal, thoracic, peritoneal) of clinically affected animals gives a definitive diagnosis of acute clinical disease, or in the case of a fetus, a diagnosis of leptospiral abortion and probable chronic infection of its mother.

Their presence in the male or female genital tract, the kidney, or urine, in the absence of evidence of generalized infection, is diagnostic of chronic infection. Failure to demonstrate leptospires in the urine of a pig does not rule out the possibility of the animal being a chronic renal carrier; it merely indicates that the pig was not excreting detectable numbers of leptospires at the time of testing.

**Isolation**

Isolation, especially from clinical material, is difficult and time-consuming, and is a job for laboratories specializing in the identification of isolates. Isolation from renal carriers is very useful in epidemiological studies to determine which serovars are present in an animal species, or in a particular group of animals or geographical location.

Isolation is the most sensitive method provided that antibiotic residues are absent, that tissue autolysis is not advanced, and that tissues for culture have been stored at a suitable temperature (39°F [4°C]) and, in the case of urine, at a suitable pH since collection.

Culture should be carried out in a semisolid (0.1–0.2% agar) bovine serum-albumin medium containing either Tween 80 (Johnson and Harris 1967) or a
combination of Tween 80 and Tween 40 (Ellis 1986), and preferably with a small amount of fresh rabbit serum (0.4–2%). A dilution culture method should be used (Ellis 1986). Contamination may be controlled by a variety of selective agents, for example, 5-fluorouracil, nalidixic acid, fosfomycin, and a cocktail of rifampicin, polymyxin, neomycin, 5-fluorouracil, bacitracin, and actidione. Culture media containing 5-fluorouracil at levels between 200 and 500µg/mL should be used as transport media for the submission of samples (Ellis 1990). Cultures should be incubated at 84–86°F (29–30°C) for at least 12 weeks, preferably for 26 weeks (Ellis 1986). They should be examined by dark-ground microscopy every 1–2 weeks.

Other Methods of Demonstrating Leptospires
Leptospires do not stain satisfactorily with the aniline dyes, and silver-staining techniques lack sensitivity and specificity (Baskerville 1986). Dark-ground microscopy of fetal fluids or urine leads to false-positive diagnoses and should be avoided.

The demonstration of leptospires by immunochi-

cal methods (immunofluorescence, immunoperoxidase, immunogold) is more suited to most laboratory situations; however, these tests are “number-of-organisms” dependent and lack the sensitivity of culture. They provide no information as to the infecting serovar (Ellis 1990) and require high immunoglobulin G (IgG) titer antileptospire sera. Immunofluorescence is the method of choice for the diagnosis of fetal leptospirosis.

Identification of leptospiral DNA by PCR should be the most sensitive method of detecting leptospires in tissues and fluids since PCR is not dependent on viable organisms. There have been few reported uses of PCR as a diagnostic tool in the diagnosis of swine leptospiro-

risis and poor correlations with culture have been reported (Miraglia et al. 2008), but some studies have shown promise (Oliveira et al. 2007).

PREVENTION AND CONTROL
Interruption of transmission from infected pig or other host to pig is the critical factor in control. Control of leptospirosis is dependent on the combined use of three strategies: antibiotic therapy, vaccination, and management. Unfortunately, not all these options are available in every country; for example, vaccines are not available in many western European countries, while problems of antibiotic residues may make the use of antibiotic therapy difficult in other situations. In the United States, the most useful antibiotic for leptospiral control/treatment programs, streptomycin, is no longer available for veterinary use. Control programs must therefore be modified to meet local conditions.

Vaccination induces immunity of relatively short duration. Immunity to infection is probably never 100% and, at best, lasts little more than 3 months (Ellis et al. 1989; Kemenes and Suveges 1976); immunity to clinical disease is believed to last somewhat longer, although exact duration is not known. Vaccination will markedly reduce the prevalence of infection in a herd (Kemenes and Suveges 1976; Wrathall 1975) but will not eliminate infection (Cargill and Davos 1981; Edwards and Daines 1979; Hodges et al. 1976). Given the developments that have occurred with bovine leptospirosis vaccines where microbiological immunity of around a year has been achieved, there is a need for the development of comparable swine vaccines containing only appropriate serovars.

Antibiotics alone will not eliminate pig-maintained leptospiral infections from the individual carrier animal or control infection in herds. Despite claims by some authors that either systemic streptomycin at 25mg/kg body weight (Alt and Bolin 1996; Dobson 1974) or oral tetracyclines at levels of 800g/t of feed (Stalheim 1967) will eliminate carriers, others have reported that these regimes do not work (Doherty and Baynes 1967; Hodges et al. 1979). Work on the use of alternative antibiotic therapy regimes indicates that oxytetracycline (40mg/kg for 3 or 5 days), tylosin (44mg/kg for 5 days), or erythromycin (25mg/kg for 5 days) may be effective in eliminating Pomona from the kidneys of experimentally infected pigs (Alt and Bolin 1996).

The main management factor in the control of leptospirosis is the prevention of direct or indirect contact with free-living vectors or other domestic stock. Strict biosecurity should be implemented, and rodent control programs should be instigated in and around the production complex. When faced with an outbreak of clinical disease, the best option is to treat both affected and at-risk stock with streptomycin at 25mg/kg body weight, to immediately vaccinate the at-risk stock, and then to introduce a regular vaccination program. If vaccination is not an available option, then a feed medication program, using either chlor- or oxytetracycline at 600–800g/t of feed, should be introduced. This ratio is fed either continuously or on a 1 month on/1 month off basis. Alternatively, it may be fed for two periods of 4 weeks in a year, preferably, one in the spring and the other in the autumn.

The use of artificial insemination is an important tool in the control of Bratislava infection.

REFERENCES
OVERVIEW OF MYCOPLASMAL DISEASES

Mycoplasmas are members of the class Mollicutes, a group of bacteria that lack cell walls and infect a wide variety of plants and animals (including humans). Mollicutes consist of low G + C-content eubacteria that are phylogenetically related to the gram-positive eubacteria bacilli, clostridia, enterococci, lactobacilli, staphylococci, and streptococci. The first mycoplasma, Mycoplasma mycoides, was cultured in 1898 by Edmund Nocard and Emile Roux and described as the microbe of pleuropneumonia (Nocard and Roux 1990). Since then, 119 species in the Mycoplasma genus have been identified, and an additional 109 species in 14 other genera compose the class Mollicutes. Though related taxonomically, members of the Mollicutes differ significantly in their habitats, their growth requirements, and their overall structure (Pollack et al. 1997; Razin et al. 1998).

Mycoplasmas are the smallest known cells that are able to propagate in a cell-free medium, and all mycoplasmas have small genomes with a limited number of genes resulting in a lack of biosynthetic pathways (Pollack et al. 1997). The lack of biosynthetic pathways requires that they obtain amino acids, purines, pyrimidines, and membrane components from their growth environment. For the members of the family Mycoplasmataceae, the animal pathogens, this occurs primarily at the host mucosal surface where damage to host cells occurs through a variety of mechanisms.

The mycoplasma genome is not only small in size, but it also has unusual features. Mycoplasmas contain a relatively low G + C content of 27–32 mol%. The low G + C content of the genome is thought to result from a strong A + T-biased mutation pressure that has operated during the evolution of mycoplasmas (Muto and Ushida 2002). The genetic systems of mycoplasmas have unusual features as well. For example, they utilize UGA as a tryptophan codon rather than as a stop codon (Muto and Ushida 2002). They also contain a minimal set of tRNAs (approximately 20 of the 62 potential), but yet can translate all available codons (Muto and Ushida 2002). How gene expression is controlled has yet to be defined in mycoplasmas. A comparison of their genome sequences indicates independent evolution for each species. With few exceptions, mycoplasma genomes are extensively different in gene content and organization.

Mycoplasma hyopneumoniae (Mhyo) is a concern to the swine industry throughout the world. Mhyo-initiated pneumonia, also known as enzootic pneumonia, plays a primary role in the porcine respiratory disease complex (PRDC), a leading cause of economic loss to swine producers. Other important pathogenic mycoplasmas found in swine include Mycoplasma hyorhinis (Mhr) that induces polyserositis and arthritis; Mycoplasma hyosynoviae (Mhs), also a cause of arthritis in grow–finish pigs; and Mycoplasma suis (Ms), formally known as Eperythrozoon suis, the cause of infectious anemia in pigs. Other swine mycoplasmas, including Mycoplasma flocculare, Mycoplasma sualvi, Mycoplasma hyopharyngis, and several species of Acholeplasma can be isolated from swine, but appear to be nonpathogenic.

Mycoplasma hyopneumoniae

Mare and Switzer (1965) in the United States and Goodwin (1985) in the United Kingdom isolated Mhyo. Since that time, the role of Mhyo in respiratory disease
and consequent reduced productivity in pigs has been increasingly recognized. Mhyo initiates a chronic insidious bronchopneumonia known as “enzootic pneumonia” by suppression of innate and acquired pulmonary immunity allowing upper respiratory commensal bacteria such as Pasteurella multocida, Streptococcus suis, Haemophilus parasuis, and/or Actinobacillus pleuropneumoniae to proliferate in the lungs and contribute to disease. In addition, when combined with viral pathogens as part of PRDC, Mhyo can also potentiate disease caused by certain viral pathogens including porcine respiratory and reproductive syndrome virus (PRRSV) and porcine circovirus type 2 (PCV2). Likewise, some viral infections potentiate disease caused by Mhyo. Although not significant as a sole cause of pulmonary disease, Mhyo is notorious as an augmenter of other pulmonary pathogens and, as such, a major cause of economic loss.

**Etiology**

Culture and isolation of Mhyo are slow and complex. It can be grown in a specialized medium; however, culture and identification are tedious, time-consuming, and often unsuccessful (Fris 1975). Contamination by other bacteria or other mycoplasmas, especially Mhr, will often preclude successful culture and isolation of the organism. Media and methods for the isolation of Mhyo were reviewed by Ross and Whittlestone (1983).

In culture, Mhyo grows slowly compared with other porcine mycoplasmas, producing turbidity and an acid color shift to the media 3–30 days after inoculation of the media. Inoculation of solid agar medium and incubation in a 5–10% carbon dioxide atmosphere results in barely visible colonies after 2–3 days of incubation. The organism must be differentiated from other swine mycoplasmas and the nonpathogenic M. flocculare has many morphological, growth, and antigenic similarities to Mhyo. More recently, polymerase chain reaction (PCR) assays are used for detecting and confirming the presence of Mhyo and will be discussed later under diagnostics.

Strains of Mhyo are antigenically and genetically diverse. Antigenic diversity was first identified by Frey et al. (1992) and further supported by Artiushin and Minion (1996) and Kokotovic et al. (1999). The genome of Mhyo was first sequenced by Minion et al. (2004) for strain 232 and then by Vasconcelos et al. (2005) for strains J and 7448, and genetic variability was confirmed (Djordjevic et al. 2004). Multilocus sequence typing was used by Mayor et al. (2007, 2008) to estimate genetic diversity, and Madsen et al. (2007) showed genetic diversity in a series of U.S. midwest field isolates through comparative genomic hybridization. Their studies revealed a single genomic region with a high degree of variability. Finally, a series of studies using pulsed-field gel electrophoresis followed by amplified fragment length polymorphism (AFLP), random amplified polymorphic DNA (RAPD) analysis, restriction fragment length polymorphism (RFLP) of the gene-encoding lipoprotein P146, and the variable number of tandem repeats (VNTRs) of the P97 encoding gene demonstrated significant variation at the genetic level in Mhyo (Stakenborg et al. 2005b, 2006). The importance of these antigenic and genetic differences between isolates is unknown in relation to their virulence or potential cross-protection between isolates. A study demonstrated that infection with a low-virulence Mhyo isolate did not prevent infection and disease from a high-virulence isolate (Villarreal et al. 2009).

Studies over the years have shown variation in surface proteins between field strains, but the molecular basis for this has only recently been revealed (Calus et al. 2007; Ro and Ross 1983). A series of studies revealed that surface antigens of Mhyo are proteolytically processed upon translocation across the membrane (Burnett et al. 2006; Djordjevic et al. 2004; Wilton et al. 2009). Unlike many other mycoplasma pathogens, Mhyo does not have variable surface proteins that undergo size and phase switching through random genetic changes. Instead, Mhyo varies its surface proteins through varied proteolytic events resulting in altered immunoblot protein patterns, confounding evaluation of strain relatedness by immunoblot analysis.

**Epidemiology**

Transmission of Mhyo through nose-to-nose contact with carrier swine is the most common source of infection in field conditions. Isolation of the organism in nasal samples from infected pigs was first demonstrated by Goodwin (1972). More recently, PCR assays confirmed the presence of Mhyo in nasal secretions of infected pigs (Calsamiglia et al. 1999, 2000; Kurth et al. 2002; Mattsson et al. 1995). Transmission of the organism can occur between penmates, independent of age (Etheridge et al. 1979; Piffer and Ross 1984). The slow growth characteristics and fastidious nature of Mhyo suggest that transmission between herds would be difficult. However, numerous researchers have documented the infection or reinfection of herds. As early as 1985, Goodwin found that herds that were less than 3.2 km apart could infect each other. A number of studies of the Danish specific pathogen-free (SPF) systems found that herd reinfections often occurred in the autumn and winter or when SPF herds were close to non-SPF herds (Jorsal and Thomsen 1988). More recently, risk factors identified with reinfection of Mhyo-free herds in Switzerland include the close proximity of finishing farms, large mixed breeding–finishing farms, infected neighbors, and the close proximity of pig transport parking sites to the farm (Hege et al. 2002). Airborne transmission of Mhyo has been documented (Fano et al. 2005a). Recent work demonstrated successful aerosol transmission of Mhyo in an experi-
mental system at 1, 75, and 150 m (Cardona et al. 2005) and aerosol transport of Mhyo for up to 5.7 mi (9.2 km) (Otake et al. 2010).

Infection with Mhyo is maintained in most herds by transmission via nose-to-nose contact from sow to pig (Cal samiglia and Pijoan 2000; Rautiainen and Wallgren 2001). The proportion of sows that shed Mhyo in nasal secretions decreases by parity. In one study, 73% of parity 1, 42% of parities 2-4, 50% of parities 6-7, and 6% of parities 8-11 were shedding the organism (Cal samiglia and Pijoan 2000). Use of early weaning strategies, where pigs are weaned at 7-10 days of age and removed to an isolated site, significantly reduces but does not always completely eliminate vertical transmission from sows (Dritz et al. 1996).

Once infection is established in some suckling piglets, transmission to littermates and later to pen mates occurs. Mhyo infects individuals for long periods and has been isolated from the respiratory tract of individual pigs for 119 (Fano et al. 2005b) and 214 (Pieters et al. 2009) days. Generally, nose-to-nose transmission is inefficient and spread is slow. In one study, it was estimated that each infected nursery pig transmitted Mhyo to one other pig during a 6-week period (Meyns et al. 2004). Investigation of differences between farms and production systems have identified numerous factors important in the dynamics and severity of Mhyo disease on a herd level, including housing styles, ventilation systems, management practices including stocking density, climatic conditions, and type of system, that is, one-, two-, or three-site production systems (Sibila et al. 2004; Vicca et al. 2002). In a majority of herds, significant transmission among penmates begins at weaning, although Mhyo can be isolated as early as 1 week of age (Sibila et al. 2007). In continuous-flow systems, Mhyo may be transmitted in large numbers in the nursery not only from the few infected pigs in the weaned cohort, but especially from older infected pigs already in the nursery. However, overt signs of mycoplasmal pneumonia are typically not observed in pigs under 6 weeks of age, although all ages of pigs are susceptible (Pieters and Ross 1984). Concurrent infection with PRRSV may shorten the incubation (Thacker et al. 1999) and may in some cases result in outbreaks of clinical Mhyo at younger ages.

The incidence of mycoplasmal pneumonia varies between countries. A recent survey of the U.S. swine populations in the National Animal Health Monitoring Survey (NAHMS 2000) found that swine producers in the United States felt Mhyo was a concern in 19.6% of nursery pigs on 29% of the farms surveyed. In large swine operations with more than 10,000 pigs, mycoplasma-associated disease was important in 52.7% of the sites with nursery pigs and 68% sites with finisher pigs. A diagnosis of Mhyo was made in over 50% of the sites. Other countries have indicated the prevalence of herds with pneumonia consistent with mycoplasma ranging between 38% and 100% (Guerrero 1990). The exact prevalence of pneumonia due to Mhyo is often difficult to ascertain due to the complication of inaccurate diagnostics and the presence of coinfections with other respiratory pathogens including P. multocida, PRRSV, swine influenza virus (SIV), and PCV2.

Eradication strategies for Mhyo have been implemented in various countries with differing success. A strategy used by the Swiss involved emptying the facility completely of all animals. One partial depopulation strategy involves a piglet and gilt free period for approximately 10 months and providing medicated feed to the remaining animals for 10-14 days. Antibiotics used include tiamulin or a combination of chlortetracycline, tylosin, and sulfa drugs. An eradication program using partial depopulation consisting of a 2-week period during which no animals younger than 10 months were allowed on the farm, has been used successfully in a number of European countries including Switzerland, Denmark, Sweden, and Finland (Baekbo et al. 1996; Heinenon et al. 1999, 2011; Rautiainen et al. 2001; Zimmerman et al. 1989). In a follow-up to the Swiss eradication program, it was determined that 2.6% of the farms became reinfected, which is a considerable reduction over the number of infected farms prior to the implementation of the eradication program (Hege et al. 2002). Continued monitoring of the country found that in 2005, the incidence of clinical cases was less than 1% (Stark et al. 2007). Several of the difficulties observed in the eradication of Mhyo from herds are the diagnostic challenges, which will be discussed later and the long-term persistence of the organism in the pig.

Economic losses due to mycoplasmal pneumonia have been associated with reduced daily weight gain (DWG), increased mortality, reduced feed efficiency, and increased costs due to medication. DWG was reduced from 2.8% to 44.1% in 24 different studies (Straw et al. 1989). Pointon et al. (1985) found that the growth rate of pigs in contact with Mhyo-infected pigs was reduced by 12.7%. Information related to the exact amount of economic loss and percentage of pneumonia can often be difficult to interpret. Scheidt et al. (1990) found no correlation between average daily gain and severity of pneumonia at slaughter. Paisley et al. (1993) reported that mycoplasma-like pneumonia, as well as other respiratory lesions, was related to reduced mean DWG. However, they concluded that lesions present at slaughter were only responsible for 9-27% of the variation and suggested that the remaining variation was due to factors such as environment, feed, genetics, and management systems.

**Pathogenesis**

The pathogenesis of Mhyo is complex and involves long-term colonization of airway epithelium, stimulation of a prolonged inflammatory reaction, suppression
and modulation of the innate and adaptive immune responses, and interaction with other infectious agents. Colonization of the airways by Mhyo begins with the binding of the organism to the cilia of epithelial cells in the airways of the pig (Zielinski and Ross 1993). While the exact method of adherence of the organism to the cilia has not been fully elucidated, a number of proteins involved with adhesion have been identified. The P97 protein is involved in adherence of Mhyo to cilia, as monoclonal antibodies to this protein block adherence of the organism in vitro (Zhang et al. 1994). The P97 gene has been cloned and the binding region has been identified (Hsu et al. 1997; Hsu and Minion 1998). However, it has been determined that variation in this gene, due to the addition or subtraction of repeated amino acids, may result in alteration of the protein that interferes with recognition by the immune system, thus making effective immunization against P97 difficult (Wilton et al. 1998). While P97 has been recognized as important in adherence to the cilia, vaccination against this protein alone was not protective against clinical disease or colonization in vivo (King et al. 1997).

Other glycoproteins and cell surface features are probably also involved in the binding of the organism to the cilia (Chen et al. 1998; Zielinski and Ross 1992). The P97 protein is a member of a family of proteins as is also the protein coded by the adjacent gene, P102 (Minion et al. 2004). The combined actions of products of these two gene families have been shown to contribute to cellular adherence (Burnett et al. 2006; Wilton et al. 2009).

The colonization of respiratory cilia by Mhyo results in ciliostasis, clumping and loss of cilia (DeBey and Ross 1994), and loss of bronchial epithelial and goblet cells (DeBey et al. 1992). This results in a significant reduction in the efficiency of clearance of debris and invading pathogens, especially bacteria, by the mucociliary apparatus. As a consequence of this and other immunosuppressive effects of Mhyo (see below), upper respiratory commensal bacteria such as P. multocida, S. suis, H. parasuis, A. pleuropneumoniae, and others are able to establish and proliferate in the alveoli as secondary pathogens. This bronchopneumonia caused by coinfection of Mhyo as primary pathogen and other bacteria as secondary pathogens is known as enzootic pneumonia.

The virulence factors associated with Mhyo are largely unknown and appear to be extremely complex. The genome of virulent strain 232 has recently been sequenced along with strains J and 7448, which should facilitate the identification of the genes and proteins that are important in the induction of disease and immunity (Minion et al. 2004; Vasconcelos et al. 2005). Virulence factors that contribute to attachment, colonization, cytotoxicity, competition for substrates, and evasion and modulation of the respiratory immune system remain unknown. The various mechanisms of pathogenesis involved with Mhyo infection are probably not due to a single gene, but a multitude of genes that will need to be identified.

Colonizing Mhyo also modulate the innate and adaptive respiratory immune response (Thacker 2001). Although this altered immune response prevents systemic spread of Mhyo, it is unable to rapidly clear infection resulting in chronic colonization of airways and a prolonged pulmonary inflammatory response. Immunopathological events play a significant role in the development of lesions; however, the exact mechanisms of immune modulation/alteration and immunopathology are incompletely understood. As cilia are colonized, peribronchial and adjacent perivascular connective tissues are infiltrated by macrophages and both B and T lymphocytes. Over time, lymphoid nodules with germinal-like centers form. Within this lymphoid response, CD4+ T cells are more prevalent than CD8+ cells (Sarradell et al. 2003).

Macrophages play a prominent role. Even though macrophages are a first line of defense in the innate immune system via their phagocytic and killing capacity, they are rendered less effective by Mhyo. In a study by Caruso and Ross (1990), macrophages harvested from Mhyo-infected pigs were less able to phagocytose A. pleuropneumoniae bacterial cells than were macrophages from control animals. This impaired phagocytic capacity is likely important in the reduced clearance of Mhyo as well as other secondary bacterial pathogens. Mhyo infection also induces macrophages to produce proinflammatory cytokines, including interleukin (IL)-1, IL-6, IL-8, and tumor necrosis factor-α both in vivo (Ahn et al. 2009; Asai et al. 1993, 1994; Choi et al. 2006; Lorenzo et al. 2006; Thacker et al. 2000) and in vitro (Thanawongnuwech et al. 2001). Additionally, IL-10 and IL-12 levels are increased in the bronchoalveolar lavage fluid 28 days after inoculation with Mhyo (Thanawongnuwech and Thacker 2003). IL-18, another proinflammatory cytokine, is also increased in Mhyo infection; however, the expected IL-18-induced secretion of interferon-γ, a potent inducer of the cell-mediated immune response, is inhibited (Muneta et al. 2006). This suggests that Mhyo downregulates cell-mediated immunity. Production of these proinflammatory cytokines stimulates inflammation, which in turn, causes tissue injury in the lungs. In fact, tissue injury in Mhyo infections seems to be caused more by the host inflammatory response than by direct action of mycoplasmal cells.

In addition to macrophages, Mhyo alters the function of both B and T lymphocytes. In a study by Kishima and Ross (1985), cell membranes of Mhyo reduced lymphocyte transformation in response to nonspecific T-cell mitogen phytohemagglutinin (Kishima and Ross 1985), suggesting a general immunosuppressive effect on the cell-mediated immune response by Mhyo.
A subsequent study found nonspecific mitogenic stimulation of lymphocytes by Mhyo (Messier and Ross 1991), suggesting that the accumulated lymphocytes in pulmonary lesions of mycoplasmosis are, at least in part, not sensitized to mycoplasmal antigens and thus are not directed against Mhyo. Tajima et al. (1984) further confirmed the role of the immune system in the pathogenesis of mycoplasmal pneumonia by demonstrating that the pneumonia was less severe in thymectomized pigs treated with antithymocyte serum and inoculated with Mhyo. These results suggest that a T-cell-dependent mechanism may be important in the development of pneumonia. However, in the same study, Mhyo was isolated from the spleen of one of the thymectomized pigs, suggesting that T lymphocytes are crucial to prevent the systemic spread of the organism.

Not all respiratory infections with Mhyo result in clinical pneumonia. Development of clinical pneumonia is dependent on the number of organisms that colonize the respiratory tract, the virulence of the infecting strain(s) of Mhyo, and on the subsequent involvement of secondary bacterial pathogens in the case of enzootic pneumonia and also of viral pathogens in the case of PRDC. The number of organisms colonizing a pig is likely dependent on cumulated infectious doses, capacity of the Mhyo strain(s) to multiply in the lungs, and time. Strains of Mhyo also differ in virulence with high-virulence strains inducing more severe pneumonia in a larger proportion of pigs (Meyns et al. 2007; Vicca et al. 2003). Higher pathogenicity in high-virulence strains is attributed to a higher capacity to multiply in the lungs and induction of a more severe inflammatory process (Meyns et al. 2007). Experimentally, onset of coughing occurs within 7–14 days postinoculation; however, in natural infection, the incubation period is less predictable and often longer (Roberts 1974; Sorensen et al. 1997; Vicca et al. 2002). Disease has been reported to occur as early as 2 weeks of age (Holmgren 1974), but more commonly, it spreads slowly within the herd and clinical disease begins at 2–6 months of age.

The interaction of Mhyo with other pathogens is important in the pathogenesis of enzootic pneumonia and PRDC. An infection with Mhyo alone typically causes a mild chronic pneumonia; however, in conjunction with other pathogens, respiratory disease often becomes severe. In numerous experimental coinfections, Mhyo has caused other respiratory pathogens to elicit more severe disease often of longer duration. Interaction of Mhyo with various secondary bacterial respiratory pathogens has been reviewed (Ciprian et al. 1994). In enzootic pneumonia, the combination of primary infection by Mhyo and secondary infection by any number of upper respiratory bacterial commensal organisms, pneumonia is more severe than with Mhyo alone. For example, studies demonstrated that Mhyo increases the severity of clinical disease with A. pleuropneumoniae serotype 9 (Marois et al. 2009).

Mhyo also interacts with viral respiratory pathogens, contributing to the development of PRDC. In a first study investigating the interaction of Mhyo and PRRSV, Van Alstine et al. (1996) did not see a strong interaction between the two. However, in later studies, Thacker et al. (1999) demonstrated that the viral pneumonia induced by PRRSV was significantly increased in both severity and duration when both pathogens were present. It was also found that the presence of PRRSV resulted in increased acute mycoplasmal pneumonia. In another study, pseudorabies virus (PRV) also increased mycoplasmal pneumonia (Shibata et al. 1998). Mhyo was also shown to potentiate the severity of PCV2-associated lung and lymphoid lesions as well as increase the amount and prolong the presence of PCV2 antigens (Opreiessnig et al. 2004). PCV2 may also increase the severity of respiratory disease in pigs infected with Mhyo (Dorr et al. 2007; Wellenberg et al. 2010). In contrast, the co-infection of Mhyo with SIV lacks the interaction observed with the other viruses, and while the severity of pneumonia at the peak of coinfection is increased, the potentiation observed with PRRSV and PCV2 did not occur (Thacker et al. 2001a; Yazawa et al. 2004).

**Clinical Signs**

Two forms of the disease occur: epidemic and endemic. Epizootic Mhyo disease is uncommon and occurs when the organism is introduced into a negative immunologically naïve herd. Animals of all ages are susceptible and disease spreads relatively rapidly, likely owing to rapid colonization in nonimmune animals and heavy nasal shedding. Morbidity may reach 100%, and coughing, acute respiratory distress, pyrexia, and death may be observed. Typically, infection transitions to an endemic pattern within 2–5 months.

Endemic mycoplasmosis is the form most commonly observed. Depending on the aforementioned management factors, clinical signs may be first observed in nursery- or finishing-age animals. Onset is insidious, affecting first a few animals and slowly spreading to involve most. Affected pigs exhibit a dry, nonproductive cough that is most evident when animals are roused. Affected individuals may cough only 2–3 weeks or coughing may persist throughout the growing period. More severe clinical signs including fever, decreased appetite, labored breathing or prostration are due to secondary pathogens. Generally, in a population of pigs with respiratory mycoplasmosis, pigs will appear fairly healthy but variably reduced feed intake will result in greater dispersion in size.

**Lesions**

Gross lesions in acute cases of epidemic respiratory mycoplasmosis include cranial ventral or diffuse
increased firmness, failure to collapse, and marked edema of the lungs. The more common gross lesions seen in chronically affected enzootic pneumonia consist of purple to gray rubbery consolidation of the cranial ventral portions of the lungs in a lobular pattern. In uncomplicated infections, the lesions affect a smaller portion of the lungs and on cut surface, the parenchyma is relatively uniform in color, and the catarrhal exudate is expressed from the airways. In contrast, lungs affected by enzootic pneumonia complicated by secondary pyogenic bacterial pathogens have a larger portion affected, are more firm and heavy, and on cut surface are mottled by arborized clusters of gray-to-white exudate-distended alveoli, and mucopurulent exudate can be expressed from the airways. In chronic recovered lesions, interlobular connective tissue in the cranial ventral lungs is thickened by firm white connective tissue. Tracheobronchial lymph nodes are usually firm, moist, and enlarged.

Microscopically, lesions in clinically affected lungs are subacute to chronic. Lymphocytes and fewer macrophages form cuffs around the airways, and adjacent blood vessels and lymphocytes expand the propria-submucosa of the airways. The epithelium of the airways and in scattered alveoli may be hyperplastic. Alveoli and airway lumens contain serous fluid and fluid-distended macrophages as well as fewer neutrophils, lymphocytes, and plasma cells. In more chronic lesions, lymphocytic cuffs are more prominent and contain lymphoid nodules. Increased numbers of goblet cells and hyperplasia of submucosal glands may be observed in bronchi. In enzootic pneumonia, exudate in alveoli and airway lumens is more extensive, predominantly neutrophilic, and may contain dense aggregates of secondary bacteria. In recovering lesions, alveoli are collapsed and/or emphysematous, and lymphoid nodules as well as fibrosis are common in peribronchial regions.

Assessment of the lungs is commonly performed at slaughter to evaluate the incidence of pneumonia within a herd. There are a number of different methods for scoring lungs. These can be based on points or percentage of affected lungs (Christensen et al. 1999; Hannan et al. 1982; Morrison et al. 1985; Straw et al. 1986). To determine the incidence of pneumonia in a system, it is important that an adequate number of lungs are assessed for an accurate picture of pneumonia on the farm. It has been recommended that the lungs of approximately 30 animals be evaluated and scored; however, this may vary with the size of the production and number of animals at slaughter (Davies et al. 1995). A disadvantage of lung assessment at slaughter is that pneumonia from Mhyo can be missed if the pigs have recovered by that time. If it appears that enzootic pneumonia is a problem in the herd, periodic necropsy of pigs with respiratory disease clinical signs should be performed and the cause of pneumonia should be determined.

**Diagnosis**

Typical herd epidemiology and dry hacking cough with highest prevalence at the onset of exercise are the basis for a presumptive diagnosis of respiratory mycoplasmosis. Other causes of coughing should be included in a differential diagnosis, especially influenza virus (Chapter 40). Gross and microscopic lesions can lend support to a diagnosis, but are also nonspecific and can be similar to subacute viral pneumonia especially when secondary bacterial pathogens complicate either primary disease, that is, Mhyo, or respiratory viral pathogen. Definitive diagnosis requires the demonstration of Mhyo in the lungs with typical lesions. Because the colonization of the airways by Mhyo can be patchy and segmental, it is important to collect from areas with typical gross lesions several samples of lungs that contain grossly visible airways for testing.

The traditional reference standard for the demonstration of Mhyo in the lung tissue is culture. However, culture of Mhyo is difficult and impractical due to its requirement of 4–8 weeks to grow to measurable levels (Friis 1975). In addition, it requires specialized media that includes swine serum negative for Mhyo antibodies for optimal growth and is prone to overgrowth by more rapidly growing mycoplasma, especially Mhr. Culture is not typically used in routine diagnostics, and failure to isolate Mhyo should not be the basis for concluding absence of Mhyo.

Detection of Mhyo in lung tissue by either fluorescent antibody (FA) or immunohistochemistry (IHC) assays is rapid, cheap, and often used in veterinary diagnostic laboratories (Amanfu et al. 1984; Opriessnig et al. 2004). Mhyo is also less commonly detected in lung tissue by in situ hybridization (ISH) (Kwon and Chae 1999). IHC and FA utilize the specificity of anti-Mhyo antibodies, and ISH the specificity of nucleic acid probes, respectively, to tag and visualize Mhyo on respiratory epithelium lining airways. As a result, tissues without postmortem degeneration must be used, since the epithelium of airways sloughs as tissues degenerate. Therefore, tissues must be collected soon after death and either fixed in 10% neutral buffered formalin (NBF) for IHC or ISH testing or chilled and shipped on ice for arrival in a laboratory within 24 hours for FA testing. Both tests suffer from relatively low sensitivity, so a negative test should not be construed as eliminating Mhyo infection.

The development of various PCR assays has provided a sensitive and specific method for confirmation of Mhyo in a variety of samples (Artiushin et al. 1993; Calsamiglia et al. 1999; Harasawa et al. 1991; Mattsson et al. 1995; Stark et al. 1998; Stemke 1997; Stemke et al. 1994; Verdin et al. 2000) and is increasingly used routinely by veterinary diagnostic laboratories. Various
samples and potential uses of PCR to accurately detect Mhyo have been investigated (Cai et al. 2007; Calsamiglia and Pijoan 2000; Calsamiglia et al. 1999; Fablet et al. 2010; Kurth et al. 2002; Sorensen et al. 1997). Lung tissue, bronchial swabs, or bronchial washings are the most useful samples, while detection of Mhyo from nasal swabs is more variable. To increase the sensitivity of the assay, nested PCR assays using two sets of primers are typically used. This results in an assay capable of detecting as few as four to five organisms. In addition, a multiplex PCR assay has been developed to identify multiple relevant porcine mycoplasmas in culture broth (Stakenborg et al. 2006). More recently, a number of real-time PCR assays have been developed for the detection of Mhyo (Dubosson et al. 2004; Marois et al. 2010; Strait et al. 2008a). Real-time PCR assays allow for detection as well as quantification of Mhyo in samples. One potential problem with PCR assays is the genetic diversity of Mhyo that may result in false-negative tests. As a result, assays that simultaneously target several genes have been used (Marois et al. 2010; Strait et al. 2008b). The use of these sensitive and specific assays improves the accuracy of the detection of Mhyo. Additionally, the use of PCR enables detection of Mhyo earlier and often more accurately than assessing seroconversion.

While the elegant sensitivity of PCR assays allows detection of low numbers of Mhyo organisms, the potential for contamination during collection or at the laboratory during testing may be problematic. This is particularly true for the more sensitive assays such as nested PCR. In fact, it has been documented that PCR assays are capable of detecting the organism in the air of production units housing pigs infected with Mhyo (Stark et al. 1998), and it is likely that contamination arising from the testing laboratory surfaces or air supply can also occur (Kurth et al. 2002). As a result, positives should be confirmed in several samples or by different test technologies before interpreting as a true positive.

Differences in the genome exist between Mhyo strains (see the section “Etiology” under “Overview of Mycoplasmal Diseases”). As the genomes of more isolates have been published, use of molecular typing to assess the number and relatedness of Mhyo strains within and between herds has increased in order to investigate clinical disease and vaccine efficacy. Reports have described farms infected by a single or multiple Mhyo strains (Mayor et al. 2007; Stakenborg et al. 2005b). As more molecular studies are performed, greater understanding of the impact of genetic and antigenic variation on diagnostic assays and vaccine efficacy should aid in improvements in the detection and prevention of Mhyo.

Serology is the most common tool used to determine the positive or negative Mhyo status of a population. However, the interpretation of serological results can be challenging. Serology is best suited for determining herd status, and care must be used in drawing conclusions regarding the Mhyo status of an individual animal or for vaccine compliance determination. Likewise, serology is not suitable for detecting recent infections in herds (see below). Numerous studies have compared the various assays as well as their association with lung lesions and protection against disease. Originally, complement fixation assays were used to detect antibodies to Mhyo. However in several comparison studies, it was determined that an indirect enzyme-linked immunosorbent assay (ELISA) was more accurate in detecting Mhyo antibody than the complement fixation assay (Bereiter et al. 1990; Okada et al. 2005; Piffer et al. 1984). Currently, ELISAs are most commonly used.

Three ELISAs are currently used in the United States to detect mycoplasmal serum antibodies, including the Tween 20 assay (Bereiter et al. 1990; Nicolet et al. 1980), the HerdCheck Mycoplasma hyopneumoniae ELISA assay (Idexx Laboratories, Westbrook, ME), and the Oxoid Mycoplasma hyopneumoniae ELISA (Oxoid Limited, Basingstoke, UK), a blocking ELISA based on an antigenic internal protein. A study using serum from experimentally infected pigs found that all three assays had excellent specificity in recognizing antibody-negative samples and thus few false-positive results are observed (Erlandson et al. 2005). In contrast, the sensitivity of the assays was low and ranged from 37% to 49%. Antibodies are first detected in serum of pigs by ELISA at 3–6 weeks postexposure and may still be detected in some animals for at least a year (Bereiter et al. 1990; Okada et al. 2005; Sorensen et al. 1993). This low sensitivity results in a low negative predictive value and a high percentage of false-negative results. Of the assays assessed, the DAKO ELISA was the most consistent in identifying infected pigs; however, a combination of assays appeared to improve the predictive ability of the tests. Sorensen et al. (1994) found similar results of high specificity and low sensitivity in Mhyo ELISAs. In addition, research has found that the assays varied in their ability to detect antibodies induced in pigs experimentally infected with different field isolates of the organism (Strait et al. 2008b; Vicca et al. 2002). To further complicate the serological diagnosis of Mhyo, M. flocculare antibodies have been reported to cross-react with a number of the serological assays and must be considered in the diagnostic workup within a farm (Bereiter et al. 1990).

A study of nine naturally infected Danish swine herds found that most pigs seroconverted in the grower or finishing units, and the association between lung lesions at slaughter and seroconversion was complex (Andreasen et al. 2000, 2001). It was demonstrated that pigs seroconverting to Mhyo close to slaughter had the highest percentage of pneumonia at slaughter and early seroconversions appeared to be related to pleuritic lesions in the cranial ventral regions of the lungs.
In addition, concurrent infection with PRRSV (Thacker et al. 1999), SIV (Thacker et al. 2001b), or PCV2 (Opiensnig et al. 2004) appears to increase Mhyo antibody levels. Antibody levels following vaccination with Mhyo bacterins may vary depending on the vaccine, the infection status of the pig, and the serological assay used (Erlandson et al. 2005; Thacker et al. 1998b, 2000c). No correlation between vaccine-induced antibody levels and protection from colonization and disease has been observed (Djordjevic et al. 1997; Thacker et al. 1998a).

In addition to assessing serum samples for the presence of antibodies to Mhyo, colostrum has been used to document freedom of a herd from infection (Rautiainen et al. 2000). Detection of antibodies in colostrum occurred weeks prior to clinical outbreak of mycoplasmal pneumonia in one case (Sorensen et al. 1993). However, sampling colostrum within the first 2 hours after farrowing is required for the most accurate detection of antibodies, which decreases the practicality under typical field situations. In addition, it has been demonstrated that parity is important in the accurate detection of antibodies in colostrums, with high-parity sows being a better source for the detection of antibodies to assess the herd status (Rautiainen et al. 2000).

Ultimately, the assay used to detect the presence of Mhyo is dependent on whether it is to determine infection status as an aid in determining intervention strategy timing or to assess whether a herd is free of the organism in eradication protocols. Most diagnostics are best used on a herd basis, not an individual pig basis. Serology alone would be a poor choice to confirm that a herd is negative for Mhyo, while PCR assays are not usually required to determine timing of vaccination or therapy, which can be based primarily on the occurrence of clinical disease. Sorensen et al. (1997) compared the duration of disease and evaluation of four diagnostic assays including serology and PCR following experimental challenge. He reported similar predictive values between all of the assays. Thus, for the greatest degree of accuracy on detecting the organism, multiple diagnostic procedures are probably required. Thus, interpretation of tests should consider their sensitivity and specificity, and overall interpretation on a herd basis should include results of all assays as well as clinical signs and lesions.

**Treatment**

Antibiotics against Mhyo can help control the disease but may not eliminate the organism from the respiratory tract nor heal existing lesions. A number of studies have assessed the efficacy of a number of antibiotics in vitro including various quinolones, tylosin, oxytetracycline, tilmicosin, and tulathromycin using a number of different testing systems (Cooper et al. 1993; Godinho 2008; Hannan et al. 1989; Tanner et al. 1993; Ter Laak et al. 1991; Thacker et al. 2001b; Williams 1978; Wu et al. 1997). In early studies, the quinolones tended to be highly effective, while tiamulin, danofloxacin, chlorotetracycline, lincomycin, tilmicosin, and other antibiotics were active against the organism. A study of 21 field isolates found resistance by one isolate to lincomycin, tilmicosin, and tylosin, while 5 isolates demonstrated resistance to fluoroquinolone (Vicca et al. 2004). However, care must be taken when comparing in vitro antibiotic studies on Mhyo to their performance in the pig due to the location of the organism on the cilia within the airways. In order for an antibiotic to be effective against the organism, it must be able to achieve significant levels within the mucus and fluids of the respiratory tract.

Studies assessing antibiotic efficacy in vivo have also been performed, occasionally with conflicting results. Mhyo lacks a cell wall, which precludes the effectiveness of antibiotics that would interfere with cell wall synthesis such as penicillin, ampicillin, amoxicillin, and cephalosporin. Other antibiotics that have little efficacy against Mhyo include polymyxin, erythromycin, streptomycin, trimethoprim, and sulfonamides. The frequency of the development of antibiotic resistance by Mhyo is unknown; however, it has been reported in the field that resistance to the tetracyclines, macrolides, lincosamides, and fluoroquinolones occurs (Le Carrou et al. 2006; Maes et al. 1996; Stakenborg et al. 2005a; Vicca et al. 2007).

Often results of antibiotic therapy in vivo are variable. Tiamulin has been reported to reduce the severity of experimentally induced and naturally acquired mycoplasmal pneumonia (Hannan et al. 1982). In a separate study, Ross and Cox (1988) failed to observe beneficial effects of tiamulin on macroscopic or microscopic lesions or Mhyo antigens detected by FA. These differences may be due to differences in susceptibility of the Mhyo isolates, experimental design, parameters measured, and the presence or absence of other secondary pathogens.

Research has demonstrated that use of chlorotetracycline in the feed administered prior to challenge reduces both the severity of pneumonia as well as the number of organisms. In contrast, administration of the drug after the onset of clinical signs of coughing was significantly less effective (Thacker et al. 2006). Other studies have demonstrated the beneficial effects of tilmulin, tilmicosin and tylosin, tulathromycin, and doxycycline on weight gain and clinical disease (Bousquet et al. 1998; Hsu et al. 1983; Mateusen et al. 2001; Nanjiani et al. 2005; Nutsch et al. 2005). However, these were field trials and pigs were infected with multiple pathogens making the assessment of antibiotic impact on Mhyo more difficult to interpret. In addition, infection with secondary pathogens makes therapy more challenging and often results in the need to use multiple antibiotics to control all of the various pathogens associated with respiratory disease. The successful use of
Combination therapies with antibiotics has also been reported (Burch et al. 1986; Stipkovits et al. 2001).

Antibiotics as a therapy for mycoplasmal pneumonia are best used during periods of stress in the life of the pig, including weaning or mixing. Knowing the other pathogens present in the respiratory tract is critical for the success of therapy as well as determining the optimal timing for the best results. Strategic administration of medication prior to or early following exposure to the organism is also important for success in using medication as an aid in controlling mycoplasmal pneumonia (Thacker et al. 2006). Pulse medication during critical times in a pig's life can also be used successfully (Le Grand and Kobisch 1996). However, extended pulse medication should be minimized to reduce the risk of increasing antibiotic-resistant isolates. Overall, prevention of the development of mycoplasmal pneumonia is the most effective method for decreasing the economic impact of Mhyo within a swine herd.

**Prevention**

Effective prevention and control of mycoplasmal pneumonia, enzootic pneumonia, or PRDC are based on providing an optimal environment for the pigs that includes adequate quality of air, ventilation, ambient temperature, and the appropriate number of animals housed in the available space. An excellent review of strategies to control Mhyo infection was provided by Maes et al. (2008). In this review, recommended management practices such as all-in/all-out pig flow, medicated and segregated early weaning, and multisite operations further facilitate control of respiratory disease associated with Mhyo infection (Maes et al. 2008). Other management strategies that help to limit the impact of Mhyo on pig production include a balanced and stable sow herd with fewer than 30% replacement gilts, closing the herd or minimizing the number of sources used to procure pigs, multisite production, biosecurity to prevent the spread and introduction of disease, reduction of stress on pigs, optimal stocking density and ventilation, and optimal room temperature with minimal fluctuation.

Eradication has become a goal for many production systems and was discussed earlier. The Swiss have used a program referred to earlier to eradicate the organism from the country (Zimmerman et al. 1989). Other protocols for eradication of the organism within a herd include medicated early weaning protocols where the sow is treated with antibiotics and the pigs weaned at 6 days of age (Alexander et al. 1980), and segregated early weaning with the use of multisite operations to significantly decrease the number of organisms transmitted from the sow to the pigs (Harris 1990). Using pigs that have been produced by cesarean-derived, colostrum-deprived pigs to repopulate a herd is often the only method guaranteed to produce Mhyo-free pigs consistently. However, a number of genetic systems have successfully eradicated Mhyo from their herds, making them a potential source of negative pigs. Careful assessment of the status of herds used for replacement animals is critical in maintaining Mhyo-free status. In all cases, reexposure and reinfection have been problems in maintaining Mhyo-free pigs.

Vaccines against Mhyo produced from adjuvanted whole-cell or membrane preparations are commonly used to control the clinical disease associated with mycoplasmal pneumonia. Numerous commercial vaccines are now used throughout the United States and the world. More than 85% of the herds in the United States vaccinate with a mycoplasma vaccine (NAHMS 2000). Molecular technologies including subunit vaccines and oral vaccine strategies are being investigated, although no vaccines utilizing these technologies are available commercially at this time.

Numerous studies have been performed, demonstrating the efficacy of existing vaccines under both field and experimental settings. Currently, in the United States, both single and dual dose vaccination protocols are used successfully to control disease. The appropriate use of either of these strategies is based on a number of factors including overall health of the farm, time of clinical disease associated with Mhyo, level of maternal antibodies, and the circulation of PRRSV in the herd.

The economic benefit of mycoplasma vaccination has been demonstrated in a number of studies (Dohoo and Montgomery 1996; Jensen et al. 2002; Maes et al. 1999). Analysis of the immune response induced by Mhyo bacterins has demonstrated reduction in percentage of lung lesions, production of serum antibodies, production of local immunoglobulin G (IgG) and immunoglobulin A (IgA) in the respiratory tract, and reduction of proinflammatory cytokines (Djordjevic et al. 1997; Kobisch et al. 1987; Kristensen et al. 1981; Messier et al. 1990; Ross et al. 1984; Sheldrake et al. 1993; Thacker et al. 1998b, 2000c). In addition, the effectiveness of antibiotic use in conjunction with vaccination has been demonstrated as an efficacious method to reduce the clinical disease associated with Mhyo infection (Mateusen et al. 2001, 2002).

Sow vaccination strategies remain controversial, and a study demonstrated no impact of sow vaccination on Mhyo colonization in the piglets, but the severity of pneumonia in pigs from vaccinated sows was reduced (Sibila et al. 2008). It appears that maternal antibody levels against Mhyo inhibit vaccine efficacy only if levels of antibodies are very high (Jayappa et al. 2001; Thacker and Thacker 2001; Thacker et al. 1998b, 2000c). In another study, piglets from vaccinated sows demonstrated no immune responsiveness until after natural infection with a field isolate, and while vaccination primed the immune system, the passively acquired antibodies demonstrated no anamnestic response.
Etiology

If present, Mhr is typically the first mycoplasma that grows in culture when investigating mycoplasmal diseases in pigs. The protocol and media for isolation and growth of Mhr is well summarized by Ross and Whittlestone (1983). The presence of the organism in pigs frequently prevents the isolation of other mycoplasmas.

Epidemiology

Mhr is a common pathogen in swine production units with pigs becoming infected from either sows or older pigs in the system. Ross and Spear (1973) demonstrated that the organism could be isolated from the nasal secretions of 10% of sows and 30–40% of weanling pigs. It is considered a normal member of the upper airways of young pigs (Ross and Young 1993). Following exposure, the organism spreads quickly through the upper respiratory tract and can frequently be isolated from the lungs and eustachian tube. The majority of infected pigs have no apparent clinical disease, although many clinical diseases including pneumonia, arthritis, polyserositis, conjunctivitis, eustachitis, and otitis media have been described in conjunction with Mhr infection.

Pathogenesis

Little is known about the virulence factors of Mhr or about the pathogenesis of Mhr-induced diseases. Like Myho, Mhr adheres to ciliated epithelial cells within the upper and lower respiratory tract of pigs. Within the respiratory tract, pneumonia has been reported to be associated with some strains of Mhr (Lin et al. 2006). In addition, infection with Mhr can result in arthritis (Morita et al. 1999). The presence of the organism in the eustachian tube may impair the mucociliary apparatus as the organism adheres to the cilia of the epithelial cells, enabling ascending infections by other bacteria such as P. multocida and Arcanobacterium pyogenes. Coinfection of the respiratory tract with other pathogens, including PRRSV or Bordetella bronchiseptica (Gois et al. 1977; Kawashima et al. 1996), has been suggested as being important for the increased respiratory disease that occasionally occurs with Mhr infection.

While Mhr is a common inhabitant of the respiratory tract of pigs, most disease is associated with invasion of the organism systemically resulting in polyserositis and arthritis. The mechanism that enables Mhr to leave the respiratory tract and induce systemic disease is currently unknown, although the presence of other pathogens or stress may facilitate the spread of the organism systemically. Once systemic, the organism produces polyserositis and polyarthritis in pigs less than 8 weeks of age, while infection in 3- to 6-month-old pigs typically results in only arthritis (Potgieter and Ross 1972; Potgieter et al. 1972).

Isolation of the organism from areas of polyserositis or arthritis is most successful during the acute stages of disease. It may be possible to isolate the organism later in infection, and the organism has been shown to persist as long as 6 months in some joints. In a study of arthritis in Canada, 56 of 153 joints with arthritis were positive for bacteria and five of those were a Mycoplasma sp., with three confirmed as Mhr (Harirharan et al. 1992). This suggests that, while infection with the
organism is potentially capable of inducing arthritis in pigs, it is not a frequent cause. Genetic differences in the susceptibility of pigs to experimental inoculation with Mhr have been suggested and appear to be related to the production of proinflammatory cytokines (Magnusson et al. 1998; Reddy et al. 2000).

**Clinical Signs**
The polyserositis associated with Mhr infection generally occurs in 3- to 10-week-old pigs, although occasionally, it can occur in older animals. Typically, evidence of disease occurs 3–10 days after exposure. Pigs become unthrifty in appearance with roughened hair coat, slight fever, depression, reduced appetite, reluctance to move, difficulty breathing, abdominal tenderness, lameness, and swollen joints. The acute clinical signs begin to resolve after 10–14 days depending on the severity of the clinical disease. Some pigs continue to do poorly or die acutely. If arthritis is involved, lameness and swollen joints will persist for 2–3 months, although many pigs will remain lame for up to 6 months.

In cases of Mhr pneumonia, clinical disease is uncommon, but when present is characterized by a dry nonproductive cough indistinguishable from Myxo. Clinical signs are usually absent or head tilt is seen in pigs with otitis media from which Mhr as well as other bacteria may be isolated. There are no clinical signs typically observed due to the Mhr colonization of respiratory epithelium in the upper and lower respiratory tracts. Conjunctivitis caused by Mhr can be observed as reddening of the conjunctiva, crusting of the margins of lids by exudate, and tearing (Friis 1976).

**Lesions**
Acute gross lesions observed in septicemic Mhr consist of fibrinopurulent pericarditis, pleuritis, and occasionally peritonitis. Over time, affected serosal membranes are thickened, cloudy, and rough, often with fibrous adhesions. Joints affected by acute Mhr arthritis are often swollen with increased amounts of serosanguinous and sometimes fibrinous synovial fluid. The synovial membranes are swollen and hyperemic. Over time, the synovial fluid increases in amount, and pannus, erosion of articular cartilage, and fibrous adhesions may occur. Lesions in the lungs caused by Mhr are as described for Myho (see above) but are typically milder. Otitis due to Mhr is characterized by the appearance of mycoplasmas among the cilia in the auditory canal and middle ear, and purulent exudate may fill the middle ear when secondary bacterial pathogens, such as *A. pyogenes* or *P. multocida*, are also involved.

**Diagnosis**
A presumptive diagnosis of Mhr can be made based on typical clinical signs and gross lesions. However, common other causes of fibrinous polyserositis and arthritis should be included in a differential diagnosis including *H. parasuis* and *S. suis*. Diagnosis of Mhr is confirmed by demonstration of the organism from a site with typical lesions. Samples should be collected from untreated animals with acute or, less preferably, subacute clinical signs. Swabs of serosal surfaces or joints with typical exudate, or fibrin from these locations are ideal samples. One should not sample lung parenchyma, airways, or the upper respiratory tract in cases of septicemic Mhr, as demonstration of Mhr in these locations merely confirms commensal infection.

Mhr can be confirmed in samples using culture methods described by Ross and Whittlestone (1983) or by molecular methods (Taylor et al. 1984, 1985). PCR assays capable of detecting Mhr have been used to assist in differentiating the various mycoplasmal species isolated from field cases (Stakenborg et al. 2006; Strait et al. 2008a) and are in use as routine diagnostic tests in some veterinary diagnostic laboratories.

**Treatment**
In vitro antibiotic susceptibility for Mhr has been shown for many antibiotics. However, treatment of clinically affected animals is usually unsuccessful as the majority of the lesions are chronic in nature and elimination of the organism does little to reduce the adhesions and inflammation. Treatment with tylosin or lincomycin may be beneficial (Ross 1992).

**Prevention**
Control programs should stress preventing the other medical conditions that may predispose the animals to the systemic spread of Mhr. No published information is available on the ability of antibiotics to reduce clinical disease, and no vaccine is currently available on a commercial basis.

**MYCOPLASMA HYOSYNOVIAE**
Arthritis caused by Mhs has been recognized throughout the world with reports from the United States, England, Germany, and Denmark (Blowey 1993; Nielsen et al. 2001; Roberts et al. 1972; Ross and Duncan 1970; Ross et al. 1977). It was demonstrated in 1995 that 8–9% of synovial fluid samples from Danish slaughter pigs with nonpurulent arthritis were positive for the organism, and Friis et al. (1992) isolated the organism from 20% of arthritic lesions of pigs in a Danish abattoir (Buttenshon et al. 1995).

**Etiology**
A review of isolation techniques and the media required for isolating Mhs are reported in Ross and Karmon (1970). Isolation of the organism is often complicated because of overgrowth by Mhr and bacteria. A selective
medium has been described that facilitates the ability to isolate Mhs in the presence of Mhr (Friis 1979). Research identified genetic variation between isolates of the organism with several genotypically distinct variants occurring in a single herd, but no further research on the diversity has been reported since those studies (Kokotovic et al. 1999, 2002).

**Epidemiology**
Mhs colonizes the respiratory tract of pigs and is primarily located in the upper portions (Friis et al. 1991; Ross and Spear 1973). The organism can persist in carrier swine indefinitely in the tonsils (Friis et al. 1991; Ross and Spear 1973). Although present in infected sows, the organism is not transmitted to the pigs until 4–8 weeks of age (Ross and Spear 1973). Large numbers of the organism are shed only during the acute phase of infection and only intermittently from persistently infected sows (Ross and Spear 1973). It is unknown why the organism cannot be isolated from pigs younger than 4 weeks of age; however, it does allow pigs to be obtained that are free of the organism.

Following infection of a few pigs at 4–8 weeks of age, it is thought the organism is spread throughout the pens from either acute or chronically infected animals (Hagedorn-Olsen et al. 1999a). The rate of spread may be related to environmental factors as well as stocking density. Pigs of all ages were found to be susceptible to infection and clinical disease from Mhs (Lauritsen et al. 2008).

**Pathogenesis**
The acute phase of infection with Mhs lasts 1–2 weeks, during which time the organism spreads systemically to the joints and various tissues throughout the body. Arthritis can occur after an incubation period of 4–9 days and Mhs can be isolated from the joints during the acute phase, which is 1–2 weeks after the occurrence of lameness and typically 2–3 weeks postexposure. The subacute and chronic phases occur 3–16 weeks after clinical arthritis, during which time the tonsils remain infected and viable organisms can persist in joints and lymph nodes. Infection of pigs from chronically infected animals through pen contact may not result in the systemic spread of the organism but infect the tonsils only. This suggests that animals are initially infected through the tonsils, which may then result in systemic spread (Hagedorn-Olsen et al. 1999b). In a study by Hagedorn-Olsen et al. (1999c), septicemia was found in 90% of experimentally infected pigs; 12 of 23 pigs developed clinical arthritis and 20% of the joints from which the organism was isolated appeared normal demonstrating that infection does not always result in clinical disease. Differences in genetics, body structure, management practices, and environment appear to play a role in determining whether clinical arthritis will result following infection with Mhs (Ross 1973). Osteochondrosis or trauma-induced bursal lesions may predispose pigs to arthritis induced by Mhs (Nielsen et al. 2001).

**Clinical Signs**
Clinical lameness associated with Mhs typically occurs in 3- to 5-month-old pigs. The lameness appears acutely and may occur in more than one leg. One study found that only hind legs were involved in clinical lameness associated with the organism (Nielsen et al. 2001). Rectal temperatures remain normal, and pigs may exhibit a slight reduction in appetite with an accompanying weight loss. Joints may remain normal in size or can be swollen, soft, and fluctuating.

Acute signs persist for 3–10 days, after which the lameness gradually decreases in severity. Many animals recover with no further lameness or may exhibit stiffness of motion. Continued clinical signs are often due to osteochondrosis as well as Mhs-induced arthritis. Mortality is low, and morbidity ranges from 1% to 50% in affected herds (Ross 1992).

**Lesions**
Proliferation, swelling, edema, and hyperemia of synovial membranes are common in Mhs-infected joints. Small amounts of fibrinous or fibrinopurulent exudate may coat synovial membranes. Increased volumes of synovial fluid that is serofibrinous, serosanguinous or cloudy, and brownish are frequently observed. The periarticular tissues surrounding the affected joint are often edematous. In chronic phases, the joint membranes may be thickened by fibrosis. Cartilaginous changes may be associated with either Mhs infection or due to osteochondrosis. Pseudocysts or calluses on the cranial surface of the carpal joint or the plantar and lateral surface of the tarsal joint may occur (Nielsen et al. 2001). Microscopically, acute lesions in synovial membranes are characterized by edema, hyperemia, hyperplasia of synovial cells, and perivascular infiltration with lymphocytes, plasma cells, and macrophages. As the infection progresses, increased numbers of plasma cells and lymphocytes are present, occasional lymphoid follicles form and fibrosis may be observed (Hagedorn-Olsen et al. 1999c).

**Diagnosis**
Acute lameness in 10- to 20-week-old pigs that is not responsive to penicillin is suggestive of Mhs (Ross 1992). Demonstration of Mhs from the joint that exhibits lesions consistent with Mhs infection is required for a definitive diagnosis. Animals with a disease profile consistent with Mhs infection and in the acute phase of the disease should be selected for diagnostic procedures. Joint fluid or synovial membranes aseptically collected from affected joints in acutely
have found that PCR assays for Mhyo do not cross-react demonstrated in samples by culture or PCR. Studies lame untreated animals should be tested. Mhs can be represented in the absence of disease, so the use of paired serum samples collected during the acute and subacute or chronic phase of disease should be used to ascertain that the antibody levels are increasing with the clinical disease (Nielsen

Serology can be used to detect antibodies to the organism. Both complement fixation assay and ELISA have been described, although these assays are not generally available in the United States (Hagedorn-Olsen et al. 1999a; Zimmermann and Ross 1982). An indirect ELISA developed in Denmark appears to have improved sensitivity and specificity compared with the earlier serological assays; however, this assay is not available in the United States. Pigs with subclinical infections may develop antibodies in the absence of disease, so the use of paired serum samples collected during the acute and subacute or chronic phase of disease should be used to ascertain that the antibody levels are increasing with the clinical disease (Nielsen et al. 2005).

**Treatment**

An in vitro study demonstrated that enrofloxacin, lincomycin, tetracycline, and tiamulin are all active against Mhs (Aarestrup and Friis 1998). In the same study, isolates collected between 1968 and 1971 appeared to be highly susceptible to tylosin activity, while isolates collected in 1995–1996 were divided between highly susceptible and relatively resistant, suggesting the development of resistance to tylosin by some isolates (Aarestrup and Friis 1998). Earlier studies had documented that Mhs was sensitive to tylosin, lincomycin, and valnemulin (Hannan et al. 1997; Zimmermann and Ross 1975). In addition, Burch and Goodwin (1984) demonstrated that the use of tiamulin and lincomycin was effective in improving production parameters and reducing lameness in a herd that was experiencing clinical disease associated with Mhs. However, in a study of nine Danish herds, treatment had no apparent impact on the outcome of clinical disease, with the majority of lameness resolving independent of the antibiotic therapy used (Nielsen et al. 2001).

No commercial vaccine for prevention of disease caused by Mhs has been developed. It is unknown whether the use of autogenous vaccines would aid in preventing disease.

**MYCOPLASMA (EPERYTHROZOON) SUIS**

With the advent of molecular biology, *Eperythrozoon suis* has been renamed *Mycoplasma suis* (Ms) and reclassified as a member of the *Mollicutes* family based on the physical characteristic of the organism and the 16S ribosomal RNA gene sequences (Neimark et al. 2002). Independent of name, the organism remains the cause of anemia in pigs.

**Etiology**

Ms was originally observed as “a rickettsia-like or anaplasmosis-like disease in swine” characterized by icterohaemoglobinuria, respiratory distress, weakness, and fever occurring in 2- to 8-month-old pigs (Doyle 1932). In 1950, Splitter and Williamson described the organism responsible for the clinical disease observed earlier by Doyle and named the organism *E. suis* because of its similarity to similar organisms in cattle and sheep (Splitter 1950a). Originally described as two species, *E. suis* and *Eperythrozoon parvum* due to differences in appearance, it was later determined that they are the same organism at different stages of maturity (Liebich and Heinritzi 1992; Zachary and Bagshall 1985).

Ms was originally classified in the family *Anaplasmataceae* due to biological and phenotypical characteristics that were not consistent with regular bacteria (Moulder 1974). However, it was suspected of being a member of the class Mollicutes based on lack of intracellular parasitism, small size, lack of cell wall, resistance, and susceptibility to tetracyclines (Tanaka et al. 1965). This was confirmed in 1997 when Rikihisa et al. (1997) sequenced the 16S rRNA gene sequences of the organisms. The gene sequences were found to have little in common with other rickettsial organisms, instead being closer to other mycoplasmal species (Johansson et al. 1999). As a result, it was proposed that *E. suis* was named *M. suis* (Neimark et al. 2002).

Ms is round to oval with an average diameter of 0.2–2.0 µm that adheres to the surface of erythrocyte membranes (Liebich and Heinritzi 1992; Zachary and Bagshall 1985) and may invade erythrocytes, residing in membrane-bound vacuoles or free in the cytoplasm (Groebel et al. 2009). It has not been cultured in media in the absence of cells to date.

**Epidemiology**

The disease has been reported to be widespread in the midwestern United States (Splitter 1950a,b). Smith and Rahn (1975) tested 10,000 swine sera and found approximately 20% of the animals were seropositive at a titer of 40 or more using an indirect hemagglutination assay (IHA). Morbidity ranges from 10% to 60%, and mortality may reach up to 90% in association with acute disease (Anthony et al. 1962). Morbidity due to subclinical infection is much lower as is mortality and difficult to measure. Recently, a PCR assay was developed and a small study of 60 pigs found that 29% of the serum tested positive for the organism (Messick et al. 1999). Clinical disease is often closely associated with outbreaks of other infectious diseases present in the herd.

Transmission of the organism can be through direct exposure by oral uptake of blood and blood components from such practices such as licking wounds, cannibalism, or uptake of blood-contaminated urine. Indirect transmission also occurs by means of
vectors including ectoparasites and bloodsucking insects, and by nonliving vectors such as contaminated needles, surgical instruments, or snares. Transmission by semen occurs only in the case of blood contamination and thus is rare (Heinritzi 1999). Ms can be transmitted from dam to piglets in utero (Henderson et al. 1997).

The incubation period in experimentally infected splenectomized pigs is between 3 and 10 days. This results in an acute phase of the disease. However, a carrier state that can recrudesce is also possible (Splitter 1950b). The incubation in naturally infected animals is highly variable with some infected pigs never exhibiting clinical disease. Infected animals can remain normal for months prior to exhibiting clinical disease often related to stress or individual susceptibility.

**Pathogenesis**

The acute phase of infection is characterized by heavy bacteremia that may result in severe anemia that can be fatal. The initial decrease in packed cell volume, total red blood cell (RBC) count, and hemoglobin concentration observed with Ms infection is due to massive parasitism of RBCs. It has been demonstrated that the parasites bind by fibrillary structures to the membrane of the RBCs (Zachary and Baggall 1985). This results in damage to the RBCs. Recently, it was found that a certain isolate of Ms can invade cells, resulting in life-threatening disease (Groebel et al. 2009). It is unknown how commonly Ms invades RBCs. Intracellular habitat for Ms may protect the organism from the host’s immune system and increase the likelihood of a persistent infection with Ms. Reduction in RBC numbers may result in anemia and bilirubinemia. Infected RBCs are more fragile, have altered membranes and are recognized as abnormal, and removed from the circulation by the spleen. In addition to the direct damage to RBC membranes, the host immune response appears to play a role in both acute and chronic forms of the anemia associated with Ms. Autoantibodies may be produced against host RBCs (Smith 1992), resulting in cold-reacting agglutinins directed at sialoglycoconjugates on the RBC membranes (Feizi and Loveless 1996). It has been suggested that the host’s immune response may exacerbate the hemolytic episodes. A prerequisite for RBC agglutination is some type of membrane injury and blood from pigs with both spontaneous and experimentally induced Ms infection agglutinated in the cold (Hoffman et al. 1981). The mechanism used by Ms to adhere to RBCs is unknown.

In the acute phase, an increased bleeding potential is observed occasionally resulting in a consumptive coagulopathy. The greater the number of RBCs affected by the organism, the more striking the changes. Similar effects on blood coagulation are not observed with latent infection with Ms (Plank and Heinritzi 1990). During acute infection, hypoglycemia and blood aci-

**Clinical Signs**

Ms infection can cause acute hemolytic disease and death in young pigs, prepartum sows, and stressed weaned and feeder pigs (Henry 1979; Smith 1992). Pigs of any age can exhibit disease in association with Ms. Pallor, fever, occasional icterus, and cyanosis of the extremities, especially the ears, are observed clinically during the acute phases of the disease. More commonly, mild anemia and poor growth rates are observed in weaned and feeder pigs. Infection of sows may result in fever, anorexia, lethargy, decreased milk production, and poor maternal behavior. Clinical disease in sows typically occurs within 3–4 days of introduction to the farrowing room or immediately after farrowing.

Chronic infections in animals with low or undetectable numbers of parasites results in unthriftness, pallor, and occasionally skin hypersensitivity characterized by urticaria. Chronic Ms infection has been associated with decreased reproductive efficiency evidenced by sows with anestrus, delayed estrus, early embryonic death, and abortions. However, Zinn et al. (1983) found no appreciable impact on sow reproductive performance but did see a reduced rate of gain in pigs farrowed from sows with high IHA titers.

In all cases, secondary bacterial or viral infections, poor management strategies including overcrowding, poor environmental conditions, and the presence of parasites contribute to the severity of disease associated with Ms infection. Frequent injections and vaccina-
tions can also be an important factor in spread of the organisms as well as subsequent reinfection. The use of oral treatment with tetracycline in treatment of other diseases can mask the clinical signs.

**Diagnosis**

The inability to culture Ms makes the development of diagnostic tests difficult. Diagnosis is based on clinical signs, hematology results, and the demonstration of the organism. Initially, the best diagnostic test to detect or confirm latent infection in carrier animals was by splenectomizing a potentially infected pig or by inoculating a splenectomized pig with blood from suspected pigs. The development of improved assays, especially PCR, has made this practice obsolete for the most part.

Detection of serum antibodies has been done using complement fixation, indirect hemaggulination and more recently ELISAs. The use of whole cell ELISAs has proven unreliable in accurately detecting infection with Ms and due to the difficulty in obtaining consistent antigen has rarely been used. Recently, a new ELISA has been developed using two recombinant antigens, rMSG1 and rHspA1 (Hoelzle et al. 2007a). This assay has a sensitivity level of 84.8%, 83.8%, and 90.6%. However, the specificity is lower ranging from 74.0% to 58.1%. The production of antibodies occurs in waves, with each reinfection or recrudescence episode resulting in the production of new antibodies. However, the antibody titers may persist only 2–3 months resulting in frequent false-negative results (Heinritzi 1999).

More recently, PCR assays have been developed, which are more sensitive and allow increased detection of pigs that are either carriers or subclinically infected (Hoelzle et al. 2003; Messick et al. 1999). A sensitive and specific real-time PCR assay has been developed and is currently the best assay for detecting the organism (Hoelzle et al. 2007b). One study found that the real-time PCR assay was much more sensitive than blood smears in detecting infected pigs (Ritzmann et al. 2009). Based on the results of PCR assays, infection of pigs with Ms may be more common than previously thought (Ritzmann et al. 2009).

**Treatment**

The treatment of choice for infection is oxytetracycline at a dose of 20–30mg/kg administered parenterally (Heinritzi 1999). Acutely ill pigs require parenteral treatment due to lack of adequate feed consumption. Administration of oxytetracycline at times of stress or treatment in infected herds may help prevent acute disease. However, treatment does not necessarily eliminate the organism from the pig. Oral chlortetracycline therapy can reduce the incidence of anemia, although will not prevent outbreaks. Supportive therapy and iron injections (200mg iron dextran/pig) will help recovery and minimize mortality.

**Prevention**

Supportive and prophylactic measures for treating Ms-infected pigs with clinical disease should be included with therapy (Claxton and Kunish 1975). Stopping the spread of the organism and preventing reinfection are critical to controlling the herd infection status. Parasitic control and hygiene are critical for disease control. Transmission by needles and surgical instruments must be minimized by changing needles between sows and litters.

No vaccine is currently available, and vaccine development is complicated by the lack of ability to culture Ms and knowledge of the virulence factors. An attempt to produce a vaccine from a recombinant protein produced in E. coli, while inducing a humoral and cellular immune response, failed to protect against challenge (Hoelzle et al. 2009). As a result, if a herd is Ms-free, new additions should also be from herds negative for the organism. Negative status can be assumed if serological or PCR tests from serum of pigs in the farrowing unit are negative or if transfusion from at least 10 blood samples into splenectomized pigs has no effect.

**OTHER MYCOPLASMA SPP. FROM SWINE**

A number of other mycoplasmas are present in swine that are of less importance to the industry than Mhyo, Mhr, Mhs, and Ms. These include mycoplasmas that are typically in other species, strains that are not normally associated with disease in swine, and acholeplasmas that are common in a wide variety of animals and plants.

A species of mycoplasma that is frequently isolated from swine, but considered nonpathogenic is M. flocculare. It was first isolated from the respiratory tract of pigs in Denmark (Friis 1972). Since that time, the organism has been isolated by researchers in the United Kingdom, Sweden, and the United States (Armstrong and Friis 1981). It was demonstrated that M. flocculare is capable of inducing lymphocytic infiltrations in the nasal tissues and peribronchial areas (Friis 1973). These findings were later confirmed by Armstrong et al. (1987). However, the role of M. flocculare in respiratory disease in the field remains poorly defined. Mycoplasma flocculare is of primary importance to the swine industry due to its antigenic similarities to Mhyo, which can complicate their differentiation both antigenically following culture and isolation and more importantly, serologically (Bereiter et al. 1990). However, it has been confirmed using molecular techniques that the two organisms differ genetically, and PCR developed for detecting the pathogenic mycoplasmas of swine can differentiate between species (Blank and Stemke 2001; Stakenborg et al. 2006; Stemke et al. 1994; Strait et al. 2008a).

Infection of the genitourinary tract with mycoplasmas in many animal species is common; however, little
REFERENCES


Pasteurellosis
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RELEVANCE
Bacteria of the genus Pasteurella were recognized as disease-causing organisms in 1880 when Louis Pasteur established Pasteurella multocida (Pm) as the etiological agent of fowl cholera (Pasteur 1880); the genus was subsequently named in his honor. The importance of Pm as a respiratory disease agent in pigs has been recognized for more than 125 years, yet this bacterium continues to significantly impact swine health worldwide as a cause of progressive atrophic rhinitis (PAR) and pneumonia.

Atrophic rhinitis in pigs characterized by stunted development or total disappearance of the nasal turbinates was first reported in Germany (Franque 1830) where it became prevalent in several areas. Following considerable debate for more than a century over the precise etiology of the disease, it is now recognized that a severe and irreversible form, referred to as PAR, is caused by toxigenic strains of Pm either alone or in combination with Bordetella bronchiseptica (de Jong and Nielsen 1990). PAR may negatively affect growth rate and the efficiency of feed conversion (Pedersen and Barfod 1981; Riising et al. 2002) although variable observations have been made in herds infected naturally (Coward et al. 1990; Donkó et al. 2005). PAR is of global economic significance to swine production and moderate to severe outbreaks can be of considerable economic importance (Muirhead 1979; Pedersen and Nielsen 1983). Bordeletella bronchiseptica alone may also cause rhinitis and turbinate atrophy in young pigs accompanied by minor effects on growth but this reversible condition, known as nonprogressive atrophic rhinitis (NPAR) is distinct from PAR (see Chapter 49).

Pneumonic pasteurellosis, the result of Pm infection of the lungs, is the common final stage of enzootic pneumonia or porcine respiratory disease complex (PRDC). This syndrome is one of the most widespread and costly diseases of pigs, especially those raised under confinement. In the United States, pneumonia is the leading cause of death in nursery and grower–finisher pigs (USDA 2007) and one of the most frequent reasons for live market pig condemnations (USDA 2008). Reduced weight gain and the cost of treating sick animals add further to the economic impact. Pm is one of the bacterial agents most frequently isolated from pneumonic lungs with a particularly high prevalence in finishing pigs (Choi et al. 2003; Hansen et al. 2010). Pm is rarely a primary agent of pneumonia in pigs but rather is an opportunist that follows infections with other primary predisposing bacterial and viral agents.

Sporadic outbreaks of fatal, acute septicemia in young and adult pigs in the absence of respiratory disease have also been attributed to Pm in several countries of the eastern hemisphere (Kalorey et al. 2008; Mackie et al. 1992; Townsend et al. 1998b).

ETIOLOGY
Pm includes four subspecies: multocida, septica, gallicida, and tigris. The vast majority of swine isolates appear to be Pm subsp. multocida, but subspecies gallicida and septica are also occasionally reported (Blackall et al. 2000; Bowles et al. 2000; Cameron et al. 1996; Davies et al. 2003; Varga et al. 2007).

Pm is a nonmotile gram-negative rod or coccobacillus approximately 1.0–2.0µm in length. Initial or low-passage isolates may exhibit a distinct bipolar staining not usually observed following serial subculture. The bacterium is a facultative anaerobe and grows well at 98.6°F (37°C) in most enriched media. On blood agar plates, it forms grayish, nonhemolytic colonies, often mucoid, with a characteristic “sweetish” odor. It does

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not grow on MacConkey agar, is oxidase and catalase positive, and produces indole.

Five capsular serotypes, A, B, D, E, and F, are recognized (Carter 1955; Rimler and Rhoades 1987) with A and D comprising most swine isolates. A small proportion of isolates from the respiratory tract are noncap-sulated and, therefore, untypeable. Serotype A is most commonly cultured from pneumonic lungs while most PAR isolates are serotype D, but either serotype may be isolated from either condition (Bethe et al. 2009; Choi et al. 2001; Davies et al. 2003; Ewers et al. 2006; Høie et al. 1991; Pijoan et al. 1983, 1984; Varga et al. 2007). Serotype F has also rarely been recovered from pigs. The most prevalent capsular serotype associated with acute septicemic pasteurellosis in swine is B, but types D and A have also been reported (Kalorey et al. 2008; Mackie et al. 1992; Townsend et al. 1999b).

An additional “somatic” serotyping method was later developed to further distinguish isolates on the basis of lipopolysaccharide (LPS) antigens (Heddleston et al. 1972). There are 16 known somatic serotypes, designated 1–16, and strains expressing multiple types are frequently encountered. Types 3, 5, and 12 are most commonly detected in the swine respiratory tract (Pijoan et al. 1983; Rimler and Brogden 1986). The combined results of capsular and somatic serotyping have traditionally been the basis for characterization of isolates, but alternative genetic typing techniques are increasingly employed, partly due to the difficulty of generating consistent, high quality, and readily available reagents for serotyping. DNA-based methods offer superior discriminatory power and are more highly informative in the investigation of outbreaks. Various techniques based on pattern analysis of genomic DNA fragments, including restriction endonuclease analysis (REA), pulsed-field gel electrophoresis, and ribotyping, have been employed for typing swine isolates of Pm (Dziva et al. 2008). A polymerase chain reaction (PCR) approach, random amplification of polymorphic DNA, has also shown promise and requires minimal equipment and reagents. A significant limitation of all these methods is the lack of standardization and the associated difficulty in comparing data across laboratories.

Multilocus sequence typing (MLST), based on DNA sequences obtained from short internal fragments of a defined set of housekeeping genes, has replaced other typing schemes for many pathogenic bacteria. MLST provides definitive and objective results that are easily evaluated, compared, and archived. Subaaharan et al. (2010) have recently described an MLST scheme for avian isolates of Pm that is highly discriminatory and predicts a population structure generally concordant with alternative typing methods. Although not yet evaluated using swine isolates, the authors have proposed the method as the new “gold standard” for typing Pm.

Only a single annotated genome sequence is currently available for Pm, that of fowl cholera strain Pm70 (May et al. 2001). The Pm70 genome is ~2.25 Mb with a G + C content of 41%. It is predicted to encode ~2000 gene products of which 104 potentially contribute to virulence. No function could be assigned for 546 coding regions suggesting considerable gaps in our understanding of the biology of this agent. Whole-genome studies monitoring changes in gene expression under various conditions designed to mimic the environment in vivo have been carried out with Pm70 and a few other strains not of swine origin. The relevance of those data to disease in pigs caused by Pm is unclear.

PUBLIC HEALTH

Pm is an important zoonotic agent and is responsible for most human infections related to animal bites or scratches. Dogs and cats are the predominant source, but infection following bites from pigs, rabbits, rats, and various wild animals has been reported (Holst et al. 1992; Hubbert and Rosen 1970; Migliore et al. 2009). Human pasteurellosis most often presents as skin or soft tissue infection, typically with rapid onset, characterized by inflammation, swelling, and purulent exudate. More serious manifestations generally limited to immunocompromised patients include septicemia, osteomyelitis, endocarditis, pneumonia, meningitis, and peritonitis.

Pm is not a usual constituent of the human upper respiratory tract, but strains genetically identical to those found in the swine reservoir are frequently isolated from pig farmers and from inhabitants of regions with intensive pig breeding (Donnio et al. 1999; Marois et al. 2009). Most human carriers remain healthy, but Pm may also be associated with acute or chronic respiratory disease. It has been proposed that pneumonic pasteurellosis be considered an occupational disease. Appropriate precautions should be observed by persons who have contact with swine infected with Pm, particularly those who may be immunocompromised.

EPIDEMIOLOGY

Pm has been isolated throughout the world from a wide array of wild and domesticated mammals and birds, including aquatic mammals (Smith et al. 1978). It causes acute or chronic disease of importance in poultry, cattle, water buffalo, swine, sheep, and rabbits. Pm often occurs as part of the normal flora and clinically inapparent infections that spark disease outbreaks in immunologically naïve animals are common in many hosts. It has no known environmental reservoir.

The epidemiology of Pm in swine is not well understood. The organism is present in practically all herds and can be detected in the nose and tonsils of healthy animals. Aerial transmission has been postulated but
only low numbers of airborne Pm (144 CFU/mL) could be recovered in fattening herds suffering from PAR (Bækbo and Nielsen 1988). Although the bacterium may occasionally be spread via aerosols, nose-to-nose contact is probably the common route of infection. Introduction of Pm into a herd may occur by vertical transmission, with breeding stock serving as a reservoir from which disease spreads rapidly among seronegative animals, but within farms, most transmission appears to be horizontal (Dritz et al. 1996; Fablet et al. 2011; Zhao et al. 1993). Pm can persist in a herd for months or even years, sometimes with little evidence of disease. Spread related to contaminated fomites or intermediate hosts has been suggested (Goodwin et al. 1990). Some evidence suggests occasional interspecies spread of avian, bovine, ovine, and porcine strains (Davies et al. 2004). Rodents, cats, dogs, and other hosts that commonly carry Pm should be considered possible sources of exposure to pigs. Whether healthy human carriers can transmit Pm to swine is unknown.

Molecular typing techniques have been used to better understand the epidemiology of Pm in pigs but comparisons among studies are problematic because there is no widely adopted and standardized method, and many studies fail to provide a quantitative measure of diversity. Nonetheless, it appears that there is limited genetic diversity among swine pneumonic isolates. One or a few strains often predominate in closed herds or in those with minimal introductions, although strains differ between operations (Blackall et al. 2000; Marois et al. 2009; Rúbies et al. 2002; Zhao et al. 1993). Multiple strains are more likely to be involved in herds where pig movement occurs (Bowles et al. 2000). It has been hypothesized that highly pathogenic clones preferentially cause pneumonia, but this seems doubtful since no traits associated exclusively with virulence have been identified, and commensal isolates from healthy and diseased pigs possess a similarly limited degree of genetic heterogeneity (Bethe et al. 2009; Ewers et al. 2006).

Only a few investigations using DNA-based typing methods have evaluated strains associated with PAR, and these similarly document relatively limited genetic diversity. Results of REA and ribotyping demonstrated that multiple PAR strains may be found within a herd and that single strains may be found in more than one herd (Bethe et al. 2009; Gardner et al. 1994; Harel et al. 1990).

Pm survives for ∼1 week at 39°F (4°C) following growth in liquid or on solid medium but may remain viable for up to several months when stored at 59°F (15°C) or 98.6°F (37°C). It is culturable for up to 6 days in pig slurry and more than 49 days in nasal washings obtained from piglets, with maximal survival at 59°F (15°C) or higher (Thomson et al. 1992). Aerosolized organisms suspended in nasal washings remain viable for at least 45 minutes. The half-life of Pm in a rotating aerosol chamber at 73°F (23°C) and 75% relative humidity is 21 minutes (Müller et al. 1992).

Several disinfectants suitable for farm use are bactericidal for Pm, some under high organic matter conditions (Thomson et al. 2007). The bacterium is inactivated by heating to 140°F (60°C) for 10 minutes or by overnight incubation at 39°F (4°C) in 0.5% phenol or 0.2% formalin.

### PATHOGENESIS

#### Colonization

Pm poorly colonizes the swine respiratory tract in the absence of preexisting damage to the mucosa (Pedersen and Elling 1984). In vitro studies using swine turbinate explants or epithelial cells from the nasal cavity or trachea consistently demonstrate little or no attachment (Chung et al. 1990; Frymus et al. 1986; Jacques et al. 1988; Nakai et al. 1988).

Pm can bind to components of porcine respiratory tract mucus (Jacques et al. 1993; Letellier et al. 1991. When mucociliary clearance is compromised, interaction of bacteria with components of the extracellular matrix may facilitate colonization of the mucosal epithelium. Attachment of Pm to porcine tracheal rings previously infected with \textit{B. bronchiseptica} increased by several orders of magnitude as compared with tracheal rings infected with Pm alone (Dugal et al. 1992). Enhanced binding was credited to \textit{B. bronchiseptica}-induced mucus accumulation and ciliostasis. This phenomenon was observed with numerous Pm isolates from healthy and diseased pigs representing capsular types A and D with or without toxin production.

Homologs of the outer membrane proteins OmpA and OmpH, known or putative adhesins in other pathogens, have been identified in swine isolates of Pm (Davies et al. 2003; Lugtenberg et al. 1986; Marandi and Mittal 1996). OmpA from a bovine isolate has been shown to mediate in vitro attachment to tissue culture cells and to bind to components of the eukaryotic extracellular matrix (Dabo et al. 2003), but no studies have yet addressed whether OmpA or OmpH function as adhesins in swine.

LPS, a major component of the outer membrane, has been implicated in attachment of Pm. LPS purified from a toxigenic type D swine isolate binds to porcine respiratory tract mucus and blocks adherence of the bacterium to porcine tracheal rings (Jacques et al. 1993). Elimination of the capsule by serial passage in vitro significantly increased the number of adherent organisms, suggesting that type D capsule may interfere with binding to the trachea. The authors hypothesized that during the early stages of infection, organisms express a minimal amount of capsular material, thereby exposing otherwise masked outer membrane components (LPS and perhaps others) involved in attachment. A later study showed that cells grown...
under iron-restricted conditions, such as those encountered in vivo, are covered by a thinner layer of capsular material and display increased adherence to porcine respiratory tract mucus and to frozen sections of porcine lung and trachea as compared with cells cultivated under iron-replete conditions (Jacques et al. 1994).

Many isolates of Pm have hemagglutinating activity but there is no correlation with in vitro binding or in vivo colonization (Fortin and Jacques 1987; Pijoan and Trigo 1990; Vena et al. 1991). Likewise, fimbriae are produced by many toxigenic and nontoxigenic capsular types A and D strains, but appear to play no role in adherence to cells or tissues of swine origin (Isaacson and Trigo 1995; Pijoan and Trigo 1990).

The mucociliary apparatus operative in the upper respiratory tract is absent at the alveolar level, where phagocytes, antimicrobial peptides, complement, and other innate immune defenses are important. This difference, as well as the general association of PAR with toxigenic type D strains in the upper respiratory tract and pneumonia with nontoxigenic type A strains in alveoli, suggests that the mechanism(s) of colonization may differ between the two capsular types. Vena et al. (1991) reported preferential binding of type A strains to swine primary lung cell cultures as compared with type D strains. But, other studies provide little evidence that strains of different capsular types employ distinct adherence strategies (Dugal et al. 1992; Frymus et al. 1986; Letellier et al. 1991; Pijoan and Trigo 1990). Neither does there appear to be a relationship between toxigenicity and differential attachment. Interestingly, in a study of 158 swine isolates, Davies et al. (2003) found that most pneumonic isolates shared outer membrane profiles that were distinct from those shared by PAR isolates, irrespective of capsular type. However, it is unknown whether any of the discriminatory outer membrane proteins has a role in attachment or colonization.

The tonsil, particularly the tonsillar crypt, appears to be the preferred habitat of Pm in swine and may protect bacteria from inflammatory cells or act as a physical barrier to removal by swallowing (Ackermann et al. 1994). Colonization of the tonsil by type A and type D strains in the absence of prior mucosal damage and persistence for up to 60 days postinfection has been reported in experimentally infected piglets (Ono et al. 2003; Pijoan and Trigo 1990). The bacterial products essential for the colonization of this site are unknown, but the tonsil may serve as a reservoir from which strains capable of causing PAR or pneumonia subsequently spread when innate defenses of the upper or lower respiratory tract are weakened or incapacitated.

**Progressive Atrophic Rhinitis**

The common predisposing factor in pigs naturally affected by PAR is prior infection with *B. bronchiseptica* (Pedersen and Barfod 1981). The mechanism facilitating colonization by Pm is unproven, but a tracheal cytotoxin released by *B. bronchiseptica* that causes ciliostasis and destruction of the mucosal epithelium is likely of primary importance (Dugal et al. 1992; Flak et al. 2000). Other toxins and effectors produced by *B. bronchiseptica* also probably play a role (see Chapter 49).

Elaboration by Pm of a 146-kDa protein toxin, referred to as Pm toxin (PMT), is the essential virulence factor for the pathogenesis of PAR. PMT produces progressive snout shortening and turbinate atrophy when given to pigs intranasally (Il’ina and Zasukhin 1975) and by a variety of parenteral routes (Rutter and Mackenzie 1984). It produces lesions in the turbinates, liver, and urinary tract characterized by degenerative and hyperplastic changes. The toxin interferes with normal remodeling and formation of bone in the turbinates (Dominick and Rimler 1988; Fogel et al. 1987; Martin-Doizé et al. 1990) and can decrease physeal area in the long bones of pigs (Ackermann et al. 1996), which perhaps contributes to slow growth associated with PAR. PMT may have direct access to the turbinates when toxigenic strains are growing in the nasal cavity but the capacity to act systemically suggests that its effects may also be exerted by bacteria colonizing the tonsil or other anatomical sites.

PMT interferes with G-protein and Rho-dependent signaling pathways and stimulates mitogenesis (Lax et al. 2004). The biologically active region has been localized to the C terminus, while a cell-binding and/or internalization domain appears to reside in the N terminus (Busch et al. 2001; Pullinger et al. 2001). PMT is encoded by the *toxA* gene (Petersen and Foged 1989), which has a G + C content significantly different than that of the Pm genome (May et al. 2001), indicating that it may have been horizontally acquired. Further study by Pullinger et al. (2004) revealed the gene is located within an inducible prophage. PMT lacks a typical signal sequence and is not secreted during growth in vitro leading to the suggestion that, like some other bacterial toxins, phage-induced cell lysis triggered by contact with host cells or by some environmental factor encountered in vivo is the mechanism of export. Phage-mediated transduction could potentially lead to acquisition and expression of *toxA* by nontoxigenic strains of Pm, or even other bacteria, but whether this occurs is not known.

Several environmental, management, and husbandry factors can influence the occurrence and presentation of PAR (Penny 1977). More severe disease is generally associated with intensive indoor production systems that have high stocking density, poor hygiene, and poor ventilation. Exposure to high levels of dust and ammonia may facilitate colonization of the upper respiratory tract by Pm and/or exacerbate disease (Andreasen et al. 2000; Hamilton et al. 1999).
Continual pig throughput and frequent moving and mixing of pigs are also predisposing factors.

Pigs infected within the first few weeks of life with toxigenic Pm are the most severely affected with PAR but mild to moderate turbinate lesions occur in those infected as late as 16 weeks of age (Rutter et al. 1984). Apparently healthy 3-month-old pigs can develop PAR when introduced into a production unit where severe disease is occurring (Nielsen et al. 1976). Age-dependent disease severity may be partly related to changes in the amount and type of mucins present in the nasal cavity and altered cellular distribution within the epithelial lining that occur as pigs mature (Larochelle and Martineau-Doizé 1990, 1991). The impact of aerial pollutants may also be greatest in young pigs (Robertson et al. 1990).

Feed consumption may be influenced by PAR, since piglets with an acute rhinitis may accept feed less readily and become stunted and weak. Growing pigs with conchal damage may also have reduced feed intake.

**Pneumonia**

The virulence factors of Pm that contribute to the development of pneumonia are unknown and may differ depending on the number and identity of other agents present in the lungs. There is no convincing evidence in support of a role for PMT, but its importance remains unresolved. Pijoan et al. (1984) were the first to isolate toxigenic strains from lungs and some investigators have since reported an unusually high percentage of toxigenic strains from this site (Høie et al. 1991; Iwamatsu and Sawada 1988), sometimes preferentially associated with acute cases (Kielstein 1986). Nonetheless, nontoxigenic strains continue to comprise the majority of lung isolates, and there is no consistent evidence for a change in their overall prevalence.

Coinfection with other respiratory disease agents is the most significant factor contributing to swine pneumatic pasteurellosis. Implicated agents include *Mycoplasma hyopneumoniae* (Amass et al. 1994; Ciprián et al. 1988) and pseudorabies virus (Carvalho et al. 1997; Fuentes and Pijoan 1986), sometimes preferentially associated with acute cases (Kielstein 1986). Nonetheless, nontoxigenic strains continue to comprise the majority of lung isolates, and there is no consistent evidence for a change in their overall prevalence.

Some strains of Pm produce pleuritis and abscessation (Pijoan and Fuentes 1987). The factors that distinguish these strains from less virulent pneumonic strains are not defined. However, there is some suggestion that PMT may be involved in the formation of pulmonary abscesses (Ahn et al. 2008; Iwamatsu and Sawada 1988; Kielstein 1986).

Large swine herds with high stocking density and poor air quality are associated with a higher prevalence of pneumonia (Done 1991). Excessive aerial ammonia was reported to facilitate pulmonary infection of unweaned piglets with Pm (Neumann et al. 1987). Other investigators have reported little effect of ammonia on the development of lung lesions in pigs coinfected with *M. hyopneumoniae* and Pm but noted lower slaughter weights in those exposed to higher concentrations (Andreasen et al. 2000; Diekman et al. 1993). Oral exposure to endotoxin or fumonisins, possibly via contaminated feed, has also been shown to potentiate pneumonia caused by Pm (Halloy et al. 2005a,b).

**CLINICAL SIGNS**

**Progressive Atrophic Rhinitis**

Sneezing in baby pigs is often the first clinical sign of PAR but also results from infection with other pathogens that produce acute rhinitis. Pigs may continue to sneeze, snuffle, and snort throughout the growing period and a variable amount of serous to mucopurulent nasal and ocular discharge may be observed. Tear staining that radiates from the medial canthus of the
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feature is that pleuritic pasteurellosis rarely results in sudden death. Rather, pigs become extremely emaciated but may survive for a long time.

Septicemic Pasteurellosis
Septicemic pasteurellosis has a sudden onset with mortality ranging from 5% to 40% and has been observed in all ages of pigs. Clinical signs include high fever, dyspnea, labored breathing, prostration, edema of the throat and lower jaw, and purplish discoloration of the abdomen, suggesting endotoxic shock.

Pneumonia
Pneumonic pasteurellosis most commonly occurs in grower and finisher age swine where it exacerbates primary mycoplasmosis or contributes to PRDC. These polymicrobial disease processes typically cause high morbidity and low mortality but can greatly lengthen the time to market and increase the number of culls. Clinical signs can vary depending on the pathogens involved and stage or severity of the disease but often include coughing, intermittent fever, depression, anorexia, labored breathing or thumping, and in severe cases, cyanosis or blue discoloration especially in the tips of the ears. More severe disease and clinical signs may be associated with certain strains of Pm that produce abscesses and pleuritis. Clinically, this form is very similar to pleuropneumonia due to A. pleuropneumoniae (see Chapter 48). The main distinguishing feature is that pleuritic pasteurellosis rarely results in sudden death. Rather, pigs become extremely emaciated but may survive for a long time.

LESIONS

Progressive Atrophic Rhinitis
The gross lesions of PAR are restricted to the nasal cavity and adjacent structures of the skull, although in advanced cases, pigs may also be stunted. Atrophy of the ventral and dorsal turbinates, present in variable degrees, is the hallmark lesion. In mild to moderate cases, the ventral scrolls are by far the most consistently and extensively affected area; severe cases may progress to total loss of the turbinate and deviation of the nasal septum (Figures 58.3–58.7). Mucopurulent exudate may be found in the nasal cavity, occasionally with associated hemorrhage.

Depending on the stage of disease at the time of necropsy, acute, subacute, or chronic histological
58.3. Cross section of the snout of an 18-week-old pig showing normal anatomy of the turbinates.

58.4. Cross section of the snout of an 18-week-old pig. Slight distortion of the ventral scrolls of the turbinates is present, a common finding.

58.5. Cross section of the snout of an 18-week-old pig showing modest but definite turbinate atrophy.

58.6. Cross section of the snout of an 18-week-old pig showing severe bilateral turbinate atrophy.

58.7. Cross section of the snout of a 22-week-old pig showing total atrophy of all turbinate structures with severe bending of the nasal septum.

changes may be observed. The principal microscopic lesion of PAR is the replacement of the boney trabeculae of the nasal turbinates with fibrous connective tissue (Pedersen and Elling 1984). Increased numbers of osteoclasts are observed contributing to this process. The degree of epithelial damage or inflammation depends on the stage of disease and whether coinfection with *B. bronchiseptica* is present (Duncan et al. 1966; Elling and Pedersen 1985). Subacute cases in conventional pigs will show various mixtures of degenerative, inflammatory, dystrophic, and reparative processes.

**Pneumonia**
Pneumonic lesions vary since Pm is often only one component of a complex infection. Macroscopic lesions include typical red-to-gray firm consolidation with a
cranial–ventral lobular distribution characteristic of bacterial pneumonia (Figure 58.8). Abscesses, pleuritis with visceral and parietal pleural adhesions, and pericarditis also occasionally occur (Figures 58.9 and 58.10).

Microscopic lesions consist of suppurative bronchopneumonia characterized by neutrophil infiltration of bronchial and alveolar spaces and interstitial thickening. Fibrinosuppurative pleuritis and fibrous encapsulation of necrosuppurative areas will be seen in cases where pleuritis and abscesses are present. Additional lesions may be apparent depending on the other agents involved. For example, peribronchiorial lymphocyte infiltration may be seen when *Mycoplasma* is present, while interstitial pneumonia may be a feature when PRRSV is a component.

**Septicemic Pasteurellosis**

Macroscopic lesions may include subcutaneous hemorrhagic edema, hemorrhage, and congestion on serosal surfaces, patchy hemorrhagic consolidation of the lungs, and generalized congestion of the abdominal viscera (Mackie et al. 1992). Fibrin may be present in the peritoneal and pleural cavities. Extensive epicardial hemorrhage has also been observed in neonatal pigs. Microscopically, intravascular thrombosis and changes consistent with widespread vascular damage are apparent, and bacteria can be observed in the vessels and throughout the affected tissues.
**DIAGNOSIS**

**Progressive Atrophic Rhinitis**
A definitive diagnosis of PAR depends on clinical and pathological observations and demonstration of toxigenic Pm. A preliminary diagnosis of PAR can be made based on a pattern of typical clinical signs. Animals showing lateral deviation of the snout and/or marked brachygnathia superior almost always have pronounced turbinate atrophy, but snout deformation alone is not pathognomonic. When these signs are not apparent or are of decreasing prevalence, for example, following treatment, it is not possible for even experienced observers to assess the extent of turbinate atrophy in the live animal. Radiography (Done 1976) and computed tomography (Magyar et al. 2003) can provide objective observations from live animals and are non-invasive but require that pigs be sedated or physically immobilized. Tomography reveals more subtle changes that may not be apparent by radiography.

The prevalence and severity of turbinate atrophy are best estimated by examination of nasal turbinates during regular slaughter checks. Snouts should be transversely sectioned at the level of the first/second upper premolar; sectioning cranial to this may give a false-positive result. Atrophy may be scored subjectively by various systems (Cowart et al. 1990). Although there may be considerable interobserver variation within a system, subjective scoring is highly useful for monitoring herd status and evaluating treatment. Objective methods better suited for data analysis are also available (Gatlin et al. 1996). Samples from the tonsils and nasal cavity should be evaluated for the presence of toxigenic Pm to ensure a proper diagnosis. When severe PAR is first detected, infection may have actually occurred weeks or months earlier and evidence of toxigenic Pm may be difficult to obtain. In such cases, it is recommended to also examine and culture less severely affected (usually younger) pigs.

Tonsil swabs or biopsies provide the highest isolation rates for Pm (Ackermann et al. 1994), but nasal swabs suffice. Live pigs should be adequately restrained, and the external nares should be cleaned. Swabs with flexible shafts are preferred to avoid breakage in the event of sudden movements of the animal. Mini-tipped swabs are available that facilitate sampling in young pigs. The surface of the tonsil should be swabbed or, when sampling the nasal cavity, a single swab should be inserted with slight rotation deep into both sides of the nasal cavity. Swabs should be transported to the laboratory within 24 hours, preferably in a transport medium under cooled conditions (39–46°F [4–8°C]). Nutrient transport media that support the growth of fast-growing contaminants are best avoided, but sterile phosphate buffered saline is suitable.

Toxigenic and nontoxigenic strains of Pm share many cross-reacting antigens, and at present, there are no satisfactory serological tests for identification of animals infected with only toxigenic strains. PMT-specific enzyme-linked immunosorbent assays (ELISAs) designed for use with the bacterium (Bowersock et al. 1992; Foged et al. 1988) can be adapted for use with serum, but PMT is a weak immunogen in naturally infected pigs and PMT-specific antibodies are often undetectable. Use of toxoid-containing vaccines limits the diagnostic value of this approach to herds with no history of vaccination or to detection of vaccine response in immunized herds.

Sneezing in young pigs occurs in active PAR but also during infection with *B. bronchiseptica* or porcine cytomegalovirus, both widely prevalent. Brachygnathia superior develops naturally in certain lines of the Large White/Yorkshire breed but can generally be distinguished from PAR by the absence of turbinate atrophy. Sows and gilts kept in stalls often bite, chew, or play with bars or drinkers, and this can give rise to asymmetric bone development causing protrusion of the lower jaw or mandibular misalignment. These conditions can be confused with the facial deformity of PAR, especially in the older pig, but careful inspection should reveal that the lower jaw is abnormally placed rather than that the snout is shortened or laterally deviated. The presence of mild turbinate atrophy in a herd may represent either NPAR or developing PAR. Definitive diagnosis requires culture.

**Pneumonia**
Lung lesions caused by Pm are not pathognomonic and cannot be used as the only criteria to establish a definite diagnosis. The history of the outbreak, histopathology, and isolation of the organism should be used to confirm the original presumptive diagnosis.

Optimal specimens include swabs of tracheobronchial exudate and affected lung tissue obtained from the border area between affected and normal tissue. Nasal swabs may also be good samples for the isolation of Pm (Schöss and Alt 1995). Swabs should be immersed in an appropriate transport medium, such as Stuart’s. Lung samples should be obtained as aseptically as possible. All samples should be refrigerated (but not frozen) until cultured. There is no serological test routinely available to assist in the diagnosis of Pm lung infection.

Differential diagnosis should include other causes of purulent bronchopneumonia or pleuropneumonia such as *M. hyopneumoniae*, *Streptococcus suis*, *Haemophilus parasuis*, *Arcanobacterium pyogenes*, *B. bronchiseptica*, *Salmonella choleraesuis*, *Actinobacillus suis*, and *A. pleuropneumoniae*. Accurate clinical differentiation is often difficult, requiring bacterial culture, histopathology, and other testing as needed for a definitive diagnosis.

**Septicemic Pasteurellosis**
Diagnosis depends on the detection of Pm in blood and affected tissues, the presence of multifocal thrombosis
and necrosis, and the absence of other agents causing sudden death.

Identification and Characterization of *Pasteurella multocida*

**Culture and Phenotypic Methods.** Pm grows readily on blood agar, but nonselective media are appropriate only when specimens are obtained aseptically from a normally sterile site, for example, the lungs. For most specimens, a selective medium is preferred to prevent masking of colonies by overgrowth of other bacteria often present in higher numbers. Various formulations have been used, but comparison of studies in the literature indicates that the highest isolation rates are obtained with modified Knight medium (Lariviere et al. 1993) or KPMD (Ackermann et al. 1994). A selective medium for simultaneous isolation of both Pm and *B. bronchiseptica* from swine has also been described (de Jong and Borst 1985). Once isolated, conventional biochemical tests can be used to identify suspect colonies. Vera Lizarazo et al. (2008) recently evaluated the performance of some commonly used commercial systems for the identification of porcine Pm isolates.

Capsular typing of Pm is useful for epidemiological purposes. Serotyping by indirect hemagglutination has traditionally been used (Carter 1955), but the hyaluronidase test (Carter and Rundell 1975) and acriflavine test (Carter and Subronto 1973) are simpler methods for the detection of types A and D strains, respectively.

Classification of Pm as PMT positive or PMT negative is critical for a diagnosis of PAR and may also be informative in other disease manifestations. Tests based on dermonecrotic or lethal effects of the toxin in rodents were initially used but toxigenicity can be more easily and humanely demonstrated in vitro by assessing cytopathic effects in embryonic bovine lung cells or vero cells (Chanter et al. 1986; Pennings and Storm 1984). ELISAs based on the use of PMT-specific monoclonal antibodies have now generally replaced biological assays and are more rapid, sensitive, and specific (Bow- ersock et al. 1992; Foged et al. 1988).

**DNA-based Methods.** Several species-specific PCR assays for detection of Pm have been developed, but relatively few have been evaluated for use with swine isolates (Dziva et al. 2008). On the basis of published reports, the PCR of Townsend et al. (1998a), which targets the predicted esterase/lipase gene *kmt1*, appears to be most widely used and offers both high specificity and sensitivity. The *kmt1* gene has also been successfully used as a target for loop-mediated isothermal amplification (Sun et al. 2010). This easy-to-perform method has the potential to be used as a screening assay in the field since a thermal cycler is not required and the amplification products can be visualized directly without the need for electrophoresis.

A multiplex capsular PCR (Townsend et al. 2001) has largely replaced serological typing methods. It has a high correlation with serological results, with the exception of antigenically related types A and F for which PCR typing was shown to be more accurate. Because there is often a reduction or complete loss of capsular material when Pm is passaged on media fewer isolates may be untypeable by PCR than by serology. However, false-positive PCR results may arise from strains that fail to synthesize a capsule due to point mutations or other minor changes in the DNA sequences targeted by the primers.

A number of assays based on the use of DNA probes (Kamps et al. 1990; Register et al. 1998) or PCR (Kamp et al. 1996; Lichtensteiger et al. 1996; Nagai et al. 1994) have performed well for detection of toxigenic swine isolates. A multiplex PCR for simultaneous identification of *B. bronchiseptica* and both toxigenic and non-toxigenic Pm also appears promising for use in diagnostic laboratories (Register and DeJong 2006).

**IMMUNITY**

**Progressive Atrophic Rhinitis**

PMT is the major protective antigen in PAR, and administration of PMT toxoid alone alleviates the associated lesions (Foged et al. 1989). Antibody appears to be important in immune defense as evidenced by passive transfer experiments (Chanter and Rutter 1990) and protection of piglets via antibody passively acquired from vaccinated sows (Foged et al. 1989). Bacterins made from toxigenic strains of Pm elicit antibacterial antibodies but vary in efficacy since they often fail to induce adequate toxin-specific antibodies (Chanter and Rutter 1990). Therefore, vaccines with added toxoid are superior to bacterins alone. Because PMT is difficult to purify and attenuate in large quantities, methods of producing recombinant detoxified PMT have been developed that rely on either truncation (Nielsen et al. 1991; Petersen et al. 1991) or genetic modification via amino acid substitution at two key sites (To et al. 2005). It is important to provide maternal colostral protection of piglets at an early age since exposure most often occurs during the first few weeks of life. Vaccination of sows between 4 and 8 weeks and again between 2 and 4 weeks before farrowing is an effective means for controlling disease. Vaccines for PAR often contain both *B. bronchiseptica* and Pm to protect against the combined effects of these pathogens. Vaccination does not provide sterilizing immunity but will reduce the pathogen load and significantly diminish or abolish clinical disease and lesions associated with PAR (Riising et al. 2002).

**Pneumonia**

Identification of immunogens or immune responses important for protection against pneumonic pasteurellosis in swine has been elusive. Studies comparing
parenteral to aerosol delivery of vaccines indicate that a respiratory mucosal immune response is important for clearance of the organism and protection from pulmonary lesions (Müller et al. 2000). Much of the research devoted to vaccines for pneumonic pasteurellosis has been performed in hosts other than swine. The absence of a reliable pulmonary disease model in pigs makes vaccine efficacy difficult to assess. Maternally derived immunity is probably not as important as an adaptive immune response for protection against pneumonia caused by Pm since this is typically a disease of older swine. Overall, the effectiveness of vaccines in controlling pneumonic pasteurellosis in swine is questionable.

PREVENTION AND CONTROL

Progressive Atrophic Rhinitis
Effective treatment of PAR requires a selected combination of management, environmental, chemotherapeutic, and vaccination procedures. No single approach is equally applicable to all affected herds. The overall aims of treatment are threefold. The first aim is to reduce the prevalence and load of Pm in young pigs, with or without *B. bronchiseptica*, by sow vaccination, medication of feed, and antibiotic treatment of piglets. The second aim is to treat growing pigs suffering from acute rhinitis in order to reduce the burden of infection and severity of the hypoplastic changes as well as to maintain efficient growth and feed utilization. The final aim is to manipulate housing, ventilation, and management to improve the overall environment.

To reduce the prevalence and severity of nasal infection acquired from dams, the sow’s feed can be medicated during the final month of gestation. Sulfonamides and tetracyclines are most widely used. Increasing resistance to some sulfonamides has been reported for both *B. bronchiseptica* and Pm, as has an increase in resistance of Pm to oxytetracycline and some other potentially therapeutic antibiotics (Lizarazo et al. 2006; Tang et al. 2009). Thus, determining the antibiotic susceptibility profile for isolates from a particular herd is prudent. Suckling piglets are best medicated by strategic injections of antibacterial agents in therapeutic dosages through the first 3–4 weeks of life. Drugs used commonly for treatment of Pm are ceftiofur, enrofloxacin, and tulathromycin. However, because of resistance, ceftiofur should not be the first choice if *B. bronchiseptica* is a factor in PAR. PAR in weaned pigs that leads to marked turbinate atrophy at slaughter can be controlled to some extent by medication of weaner and/or grower rations or by the addition of antibiotics to drinking water. Such medication also assists in the maintenance of growth and feed efficiency in the face of active PAR. Medication is most effective when environment and management are improved and vaccination is employed.

Sow vaccination induces a significant degree of passive colostral protection against PAR (Riising et al. 2002). Vaccines often contain a combination of *B. bronchiseptica* and Pm bacterins. PMT is an important component of vaccines for PAR, and those with added PMT toxoid offer superior protection (Foged et al. 1989; Hsuan et al. 2009; Nielsen et al. 1991; To et al. 2005). Two doses should be given 6 and 2 weeks prior to initial farrowing followed by revaccination 2 weeks before each subsequent farrowing. Vaccination of nonimmune piglets from unvaccinated dams at 1 and 4 weeks of age can be of value; however, passive antibody from the sow can interfere with parenteral vaccination of the piglet. Vaccination of older pigs undoubtedly produces an active humoral response but its value is debatable since the main effects of the infection occur in younger animals.

Medication and vaccination should never be introduced without concurrent attempts to improve management and husbandry. All-in/all-out systems are favored for farrowing, weaner, and preferably fattener units. Stocking density should be reduced, strict hygiene measures should be implemented, and ventilation rates should be maintained to reduce airborne bacteria, noxious gases, and dust. The age of the sow herd can be allowed to rise to avoid the introduction of large numbers of infected new gilts. Steps should also be taken to reduce factors that stress young pigs, including temperature variations, chilling, and drafts. Infection can be eradicated by depopulation and restocking, and clean herds may be maintained free from PAR by isolation, herd monitoring, and the use of clean breeding stock.

Pneumonia
Treatment of pulmonary Pm infections with antibiotics is challenging because of the difficulty of achieving therapeutic concentrations in consolidated, pneumonic lungs. Parenteral antibiotics are preferable including long-acting oxytetracycline, ampicillin, ceftiofur, enrofloxacin, and tulathromycin. Since strains vary in susceptibility and an increase in resistance to some antibiotics has recently been reported (Lizarazo et al. 2006; Tang et al. 2009), antibiograms should be performed before instituting treatment. In-feed antibiotics, such as chlortetracycline, are best used on a preventative basis.

Under field conditions, the effectiveness of vaccination against pneumonia with Pm is dubious. Since pneumatic pasteurellosis is often the final stage of enzootic pneumonia or PRDC, which are polymicrobial infections, control of the primary pathogens such as *M. hypopneumoniae*, *B. bronchiseptica*, or PRRSV through vaccination, medication, or management practices may be the most efficient method of controlling the disease. Management changes that reduce the spread of the pathogens involved may have value in decreasing the
incidence of pneumonia. These include segregated early weaning, all-in/all-out production, limiting the introduction of outside pigs and determining the health status of the farm from which they are purchased, minimizing mixing and sorting, reducing the building and pen size, and reducing animal density.

REFERENCES


CHAPTER 58 PASTEURELLOSIS 809
Proliferative Enteropathy

Steven McOrist and Connie J. Gebhart

RELEVANCE
Proliferative enteropathy ([PE] also known as ileitis) forms a commonly occurring group of acute and chronic conditions of widely differing clinical signs but with a single underlying pathological change visible at necropsy: a thickening of the mucosa of the small intestine and colon. Histologically, the affected tissues show marked proliferation of immature epithelial cells of the intestinal crypts, forming a hyperplastic to adenoma-like mucosa. These proliferating cells always contain numerous intracytoplasmic *Lawsonia intracellularis*, an obligate intracellular bacterium.

In growing pigs with uncomplicated proliferation of the mucosa, the condition is a chronic PE, also known as porcine intestinal adenomatosis (PIA). Case lesions and clinical signs can vary from mild and subclinical, through to clinical diarrhea and weight loss. In more severe cases, additional changes can be superimposed on this basic lesion, including a more necrotic enteritis, or an acute proliferative hemorrhagic enteropathy (PHE) (Rowland and Lawson 1975). All these forms of PE remain common and important enteric diseases. Estimates across the global swine industry show that around 96% of farm sites are infected, wherein around 30% of weaner-to-finisher pigs have detectable lesions at some point, causing clear economic losses (McOrist et al. 2003; Stege et al. 2000).

The lesions of PE were first described in pigs in Iowa in the 1930s (Biester and Schwarte 1931) and were subsequently found to occur in all other major pig-raising areas throughout the world. In 1973, Alan Rowland and Gordon Lawson investigating major U.K. outbreaks developed a productive research program. They found that whenever these proliferative changes in pigs were studied, either ultrastructurally or using silver stains, intracellular bacteria were consistently present within the abnormal proliferating cells (Rowland and Lawson 1974). The identity of these bacteria and their etiological role in PE in pigs were later resolved in 1993 with successful co-culture of the intracellular organism and the reproduction of the disease in pigs using a pure culture of this agent, fulfilling Koch’s postulates (Lawson et al. 1993; McOrist et al. 1993). Also in 1993, its taxonomic position was clarified (Gebhart et al. 1993); its definitive name is *L. intracellularis* (McOrist et al. 1995a). The disease has also been detected in wild Eurasian pigs, *Sus scrofa* (Tomanova et al. 2002).

The economic losses due to PE have been estimated from its negative impacts on slaughter weight, feed conversion efficiency, space utilization, breeding problems, and morbidity–mortality effects, totalling at U.S. $1 to U.S. $5 per affected growing pig, depending on the variable prices for pigs, building spaces, and feed (McOrist 2005; McOrist et al. 1997b; Veenhuizen et al. 2002).

ETIOLOGY
The cause of PE is the obligately intracellular bacterium *L. intracellularis*, which preferentially grows within the cytoplasm of intestinal epithelial cells. This bacterial growth is invariably accompanied by localized proliferation of infected immature crypt epithelial cells. It has not as yet been cultivated in cell-free media, due to unique metabolic requirements, including a need for preformed mitochondrial triphosphates (Schmitz-Esser et al. 2008). Some earlier literature refers to the intracellular bacterium as a *Campylobacter*-like organism; however, that designation was only based on its morphological similarity to that genus.
Lawsonia intracellularis forms vibrioid-shaped (curved to straight) rods with either tapered or rounded ends and measure 1.25–1.75 \( \mu \)m in length by 0.25–0.43 \( \mu \)m in width. It has a typical gram-negative trilaminar outer envelope. No fimbriae or spores have been detected. It has a small, single genome and three plasmids, totaling 1.72 million bp and 1324 protein encoding regions. A monopolar flagellum and darting motility has been observed in some isolates co-cultured in vitro, but only when the bacteria are located extracellularly. It possesses the small genome, low G + C%, and significant expression of heat shock proteins commonly seen in other symbiont intracellular bacteria (Dale et al. 1998). Lawsonia forms a distinct and single branch in the Desulfovibrionaceae family of anaerobic vibrioids, but it has gained cell-dependent respiration and lost its sulfate reduction capacity (Schmitz-Esser et al. 2008).

Initial in vitro co-cultures of L. intracellularis used monolayers of intestinal epithelial cells, in a microaerobic atmosphere of 82.2% nitrogen, 8.8% carbon dioxide, and 8% oxygen at 98.6°F (37°C), which attempted to mimic the conditions of in vivo growth (Lawson et al. 1993). However, it was subsequently found that L. intracellularis could be somewhat more easily grown using co-cultures of McCoy mouse fibroblast cells at 98.6°F (37°C) in low oxygen, hydrogenated atmospheres (Guedes and Gebhart 2003a).

Pathological changes closely resembling porcine PE and caused by L. intracellularis are now commonly recognized in young horses and certain laboratory-housed animals, such as rabbits and hamsters (Cooper and Gebhart 1998; Pusterla et al. 2009). Case reports have also described the organism and related pathology in certain other host species, such as dogs, deer, foxes, monkeys, and ratite birds (Cooper and Gebhart 1998; Klein et al. 1999). The common infection among laboratory rabbits complicates simple production of specific polyclonal antibody (Duhamel et al. 1998). All isolates of L. intracellularis from pigs and other host species show a very high degree (>99%) of similarity among their 16S rDNA sequences and outer membrane proteins. However, the clear variations in infectivity when cross-species transmission is attempted (Jasni et al. 1994; Murakata et al. 2008), has suggested that there may be at least two biovars, one strain for pigs and one other for the other host species. The existence of only a single strain of L. intracellularis affecting pigs globally is consistent with it being both a recently evolved bacterial species (Schmitz-Esser et al. 2008) and spread by global movement of breeding pigs, such as commonly occurred in both the 1880s and 1970s. The differing clinical expression of PE cases in pigs are therefore due to dosage and host response differences, not to separate bacterial strains, as also confirmed by experimental challenge results using isolates from various clinical forms (Mapother et al. 1987).

Experimental transmission studies using pure cultures of porcine-origin L. intracellularis as oral-challenge inocula for conventional pigs and using gnotobiotic pigs predosed with a minimal bacterial flora of non-pathogenic enteric species have resulted in reproduction of the specific and characteristic lesions of PE (McOrist et al. 1993, 1994). Exposure of pigs to crude or partially filtered, homogenized diseased mucosa resulted in reproduction of specific intestinal lesions and clinical disease in some early challenge trials (Mapother et al. 1987; McOrist and Lawson 1989; Roberts et al. 1977). The difficulties in routine culture of L. intracellularis meant that this strategy was later revived into the mucosal homogenate challenge exposure model for reproduction of PE in conventional pigs (Boutrop et al. 2010; Guedes and Gebhart 2003b; Winkelman et al. 2002). Intestinal lesions that develop as a result of artificial exposure to various isolates have had all the characteristics of the field disease, including the presence of mucosal proliferation and intracellular bacteria (Guedes and Gebhart 2003b; McOrist et al. 1993).

PUBLIC HEALTH

Despite obvious infection opportunities, no case of PE or Lawsonia infection has been detected in humans. Investigations of Crohn’s disease and other mucosal inflammatory conditions of humans have consistently failed to find typical PE lesions or L. intracellularis (Crohn and Turner 1952; Michalski et al. 2006).

EPIDEMIOLOGY

The disease is worldwide in distribution and occurs commonly in all pig-raising regions and in all pig farm management systems, including outdoor ones (McOrist et al. 2003). The incidence of gross lesions in pigs at normal slaughter age is generally low at 0.25–2.0% and therefore unreliable for farm monitoring (Christensen and Cullinane 1990; Suto et al. 2004). The wide use of serology and fecal polymerase chain reaction (PCR) diagnostics has led to a fuller understanding of epidemiology patterns of PE. As stated above, surveys indicate that around 4% of farm sites show no detectable infection; usually, these are isolated breeding farms with closed herds.

There are three basic patterns of farm infection, which occur in relation to the management system and antibiotic usage (Figure 59.1). On single-site farms with a continuous pig flow between different farm areas (farrow-to-finish systems), infection usually occurs a few weeks after weaning, presumably when maternal antibodies fade (pattern 1). The infection can then amplify via oral–fecal infections over the next few weeks in groups of weaner–nursery and early grower pigs (Stege et al. 2004). Where group housing and straw
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ably less common following the wide usage of live attenuated *Lawsonia* vaccine for breeding pigs.

*Lawsonia intracellularis* can remain viable in feces at 5–59°F (15°C) for 2 weeks (Collins et al. 2000). The infectious dose is relatively low (Guedes et al. 2003; McOrist et al. 1993), and fecal excretion may be high in some infected “spreader” pigs (Guedes et al. 2002a; Smith and McOrist 1997). Studies tracking PE infection on breeding farms have indicated that infected gilts or sows do not readily transmit the infection to their progeny in the farrowing area (Guedes et al. 2002a; Jacobson et al. 2010).

Improved farm hygiene measures will reliably reduce levels of PE. Quaternary ammonium-based compounds have effective anti-*Lawsonia* disinfectant activity (Collins et al. 2000; Wattanaphasak et al. 2010), but isolates appeared somewhat resistant to phenolic- or iodine-based mixtures. Rigorous washing and cleaning of feces from all parts of pig pens, facilities, boots, and equipment on both single-site and multisite farms, as well as insect and mouse control, is likely to be more effective at reduction of PE than reliance on slatted floors and sunken pits for feces removal (Bronsvoort et al. 2001; Smith et al. 1998).

**PATHOGENESIS**

PE can be reproduced by exposing susceptible pigs to *L. intracellularis* or to diseased mucosa containing these intracellular bacteria (Guedes and Gebhart 2003a,b; McOrist and Lawson 1989; McOrist et al. 1993, 1994; Roberts et al. 1977). In typical oral challenge exposure studies of naïve postweaned pigs with a standard inoculum of 10⁸ *L. intracellularis* bacteria, numerous intracellular bacteria can be visualized in the developing proliferative intestines and feces 1–3 weeks following inoculation with a peak of infection and lesions 3 weeks after challenge. In most of these pigs, intestinal infection, proliferative lesions, and excretion persists
for approximately 4 weeks, but in some exposed pigs, excretion may persist for at least 10 weeks (Guedes et al. 2002a; Smith and McOrist 1997). At the peak of infection, 3 weeks after challenge, moderate diarrhea and histological lesions of PE are usually observed in about 50% and 100%, respectively, of animals challenged with this inoculum. Infection and lesions in the large intestine generally occur a week or two after small intestinal infection following oral challenge (Guedes and Gebhart 2003b). Naïve pigs of a wide age range (neonates to grower–finishers) are susceptible to oral challenge.

Proliferative enteritis develops initially as a progressive proliferation of immature epithelial cells populated by numerous intracellular bacteria. In most cases, no significant inflammatory reaction occurs and the organisms remain in the epithelium at this stage. In severe cases of PE, *L. intracellularis* can also be observed in the mesenteric lymph node and tonsils (Jensen et al. 2000; Roberts et al. 1980), but these appear to be secondary sites. In vivo and in vitro studies have elucidated some of the early events in bacteria cell interaction (Boutrop et al. 2010; Lawson et al. 1995; McOrist et al. 1989, 1995b). Bacteria associate with the cell membrane and then quickly enter the enterocyte via an entry vacuole. Specific adhesins or receptors have not been identified, but *L. intracellularis* possesses a type III secretion system, which facilitates cell entry (Alberdi et al. 2009). The entry vacuole rapidly breaks down (within 3 hours) and the bacteria flourish and multiply free (not membrane bound) within the cytoplasm. The intriguing mechanism whereby the bacteria cause infected cells to fail to mature but continue to undergo mitosis and form the hyperplastic-to-adenomatous crypts is not fully understood, despite the full * Lawsonia* genome being available for study (GenBank AM180252, AM180253, AM180254, and AM180255). This adenomatous effect probably reflects a *Lawsonia*-specific inhibition of the normal crypt cell differentiation process, as regulated locally at the neck of the crypts (McOrist et al. 2006; Oh et al. 2009). *Lawsonia intracellularis*-infected intestinal crypts can become enormously elongated and often branched. Loss of body protein and amino acids into the intestinal lumen and the reduced nutrient absorption by the intestinal mucosa lacking mature enterocytes are the likely causes of the reduction in weight gain and feed conversion efficiency seen in pigs and hamsters affected with chronic uncomplicated PE lesions (Gogolewski et al. 1991; Rowan and Lawrence 1982; Vanucci et al. 2010).

Degenerative, necrotic, and reparative changes may be superimposed on the basic enterocyte proliferation. Acute hemorrhagic PE, also known as PHE, is marked by severe bleeding into the lumen of the intestine, but with typical underlying lesions of PE. The hemorrhage occurs concurrently with the widespread degeneration and desquamation of many epithelial cells and leakage from the capillary bed. The pathogenesis of this acute lesion has not yet been determined fully.

**CLINICAL SIGNS**

Clinical cases of chronic PE are observed most commonly in the postweaned pig between 6 and 20 weeks of age. In many cases of chronic PE in growing pigs, the clinical signs are slight to subclinical, and little more is seen than variation in pig performance with a failure to sustain growth despite normal feed intake. Ileal lesions are a consistent feature of these pigs. In some pigs, there may be a degree of anorexia, characterized by curiosity about food but refusal to eat. Thus, affected animals vary from the clinically unremarkable to those showing marked dullness and apathy. Diarrhea, when present, is generally moderate, with loose, sloppy to watery stools of normal gray-green color. This is probably a feature of only a proportion of pigs affected with chronic PE (Moller et al. 1998). Blood or mucus is not a feature of chronic PE diarrhea. When chronic or subclinical PE is suspected in a herd, milder cases can be relatively common but difficult to detect (Jacobson et al. 2003). Therefore, such farms should be inspected for apparent wasting of growing animals with anorexia and irregular diarrhea and variable sizes of pigs in a group. Records should be carefully examined to detect changes in average weight gain and feed conversion efficiency in the postweaned group (Gogolewski et al. 1991; Roberts et al. 1979). Those pigs that develop necrotic enteritis show severe loss of condition and often scour persistently. These severe cases may occur more often in pigs on straw bedding, which facilitates oral–fecal intakes and secondary bacteria, such as salmonellosis.

Unlike chronic PE, cases of acute hemorrhagic PE occur more commonly in young adults 4–12 months old, such as breeding gilts, and present a clinical picture of acute hemorrhagic anemia. Black tarry feces are often the first visible clinical sign, and these may become loose. However, some animals die without fecal abnormality and show only marked pallor. Probably around half of the animals clinically affected will die, the remainder recovering over some weeks. Pregnant animals that are clinically affected may abort, the majority within 6 days of the onset of clinical signs, with some residual reproductive losses possible (McOrist et al. 1999). Progenies from acutely affected breeding females are not fully protected from acquiring PE (Guedes et al. 2002a; Jacobson et al. 2010).

In most cases of uncomplicated PE, recovery occurs 4–10 weeks after the onset of clinical signs with a return of appetite and growth rate to normal levels. Even though pigs usually progress to slaughter weight despite extensive lesions (Suto et al. 2004), average daily weight gain and feed efficiency will be reduced and more days to reach market weight will be required, with a conse-
quent cost burden. Average weight gains are reduced 6–20% in affected pigs, and the increase in feed required per unit gain is 6–25%, compared with normal pigs (Gogolewski et al. 1991; McOrist et al. 1996b, 1997a). The costs in increased “variation” in a group of pigs destined for breeding programs or a specific market target can also be significant.

**LESIONS**

Chronic PE in growing pigs occurs most commonly in the terminal 60 cm of the small intestine and the upper third of the proximal colon. In severe cases, the lesions will extend to the jejunum, cecum, and lower large intestine. The magnitude of the proliferation varies widely, but in the developed lesions, the wall is visibly thickened and the overall diameter is increased. In minor lesions, the area of the terminal ileum that is 10 cm proximal from the ileocecal valve should be carefully examined as the most likely site of infection. Care is needed to distinguish minor lesions from contracted mucosa over Peyer’s patches. Some subserosal and mesenteric edema is common, and the normal corrugated pattern of the serosal surface is emphasized. The mucosal surface is moist but not mucoid, sometimes with flecks of inflammatory exudate loosely adherent. The affected mucosa itself is thrown into deep folds, longitudinal or transverse (Figure 59.2). Similar changes in the large intestine occur and may result in thick plaques.

Histologically, the mucosa is composed of enlarged, branching crypts lined with immature epithelial cells. Compared with normal crypts, which are 1 cell layer thick, affected crypts are often 5, 10, or more cells thick (Figure 59.3). Numerous mitotic figures occurring throughout the crypt are evident (Lomax and Glock 1982; Rowland and Lawson 1974). Other nuclei of affected cells may appear as enlarged vesicular structures or densely staining elongated spindles. Goblet cells are absent, and their reappearance in deep glands is an indication of impending resolution. In uncomplicated disease, the lamina propria is normal.

Silver staining, specific immunostaining, or electron microscopy of affected intestinal sections reveals intracellular *L. intracellularis*, often in considerable numbers, lying in the apical cytoplasm of the affected epithelial cells (Figure 59.4). In recovering lesions, the organisms become aggregated and may be extruded in degenerate cells into the lumen or be consumed by activated macrophages in the lamina propria. Many cases show little evidence of inflammatory reaction. The recovering

![Image 59.2](image1.png)  **59.2.** Chronic proliferative enteropathy. Ileum with thickened, ridged mucosa.

![Image 59.3](image2.png)  **59.3.** Chronic proliferative enteropathy. High-magnification micrograph showing marked enlargement of affected intestinal crypts, 5–10 epithelial cells thick, compared with adjacent normal crypt (hematoxylin and eosin [H&E]; original magnification ×400).
lesions are notable for the resumption of development of a population of mature epithelium, with goblet cells in the deep crypts and a rapid disappearance of the adenomatous cells from the surface (McOrist et al. 1996a).

In more severe cases, a coagulative necrotic enteritis may occur, with marked inflammatory exudation superimposed on an established lesion of PE. Yellow-gray cheesy masses that adhere tightly to the jejunal–ileal mucosa are present. Histologically, the coagulative necrosis is clearly defined, with fibrin deposits and degenerative inflammatory cells. Diagnosis is confirmed by the presence of remnants of the *Lawsonia*-infected proliferative epithelium in the deep layers.

In acute hemorrhagic PE or PHE, the affected intestine is thickened, dilated, and somewhat turgid with serosal edema. The lumen of the ileum and colon usually contains one or more formed blood clots (Figure 59.5), often with no other bloody fluids or feed contents evident (Lawson et al. 1979; Rowland and Lawson 1975). The rectum may contain black, tarry feces of mixed blood and digesta. The mucosal surface of the affected portion of intestine shows little gross damage except for the marked hyperplastic thickening. No bleeding points, ulcers, or erosions are observed. Histological examination demonstrates extensive degeneration, congestion, and hemorrhage within the proliferative epithelium. There is marked accumulation of bloody cellular debris containing numerous *L. intracellularis* organisms above the affected mucosa and in the lumina of affected intestinal crypts.

### DIAGNOSIS

The differential diagnosis for clinical cases of PE varies with the particular form of the disease. Chronic PE or PIA is most likely to be confused with endemic forms of coronavirus (Chapter 35) or rotavirus (Chapter 43) infections, milder forms of brachyspiral colitis (Chapter 50), salmonellosis due to *Salmonella typhimurium* (Chapter 60), porcine circovirus-associated disease (Chapter 26), and nutritional diarrheas (Chapter 68). Mixed infections of these endemic agents can often occur. Acute hemorrhagic PE or PHE is most likely to be confused with esophagogastric ulceration (Chapter 15), or acute swine dysentery (Chapter 50). Acute hemorrhagic PE is also considered separate from the “hemorrhagic bowel syndrome,” which are blood-filled intestinal torsion cases (Straw et al. 2002).

The difficulty in routinely culturing *L. intracellularis* has led to several alternative methods for PE diagnosis. Confirmation of a clinical diagnosis of PE may be
obtained by demonstration of *L. intracellularis* in feces, usually by a PCR assay using *L. intracellularis*-specific primers (Jacobson et al. 2004; Jones et al. 1993), or by using a specific antibody incorporated into fecal immunoassay techniques. Pigs with active lesions are usually found to be excreting the agent over several weeks (Guedes et al. 2002b; Jacobson et al. 2010; Knittel et al. 1998). However, fecal analysis is not sufficiently sensitive for the diagnosis of all infections (Jacobson et al. 2004, 2010). The PCR assay can detect 10^5–10^6 organisms per gram of feces, depending on the DNA extraction method and type of assay used. Animals 6–10 weeks old usually have the highest prevalence rates for screening of single-site farms. Older animals are usually only sampled during outbreaks of acute PE. Feces should be stored at 39°F (4°C) or below for either test.

Methods described for the serological diagnosis of PE have employed whole bacterial antigen incorporated into an indirect immunofluorescence assay (Knittel et al. 1998) or an immunoperoxidase monolayer assay (Guedes et al. 2002b). Several enzyme-linked immunosorbent assays (ELISAs) have been described incorporating differing antigen extracts (Boesen et al. 2005; Kroll et al. 2005; Watarai et al. 2004). Results from serological assays suggest that the serum antibody response in pigs to *L. intracellularis* is specific and involves immunoglobulin M (IgM) and immunoglobulin G (IgG). While detectable antibody responses relate well to the presence of lesions, exposure may not induce significant seroconversion in all cases. Although blood collection can be more time-consuming than feces collection, the serotests are cheaper to perform and more suitable for initial group testing.

At necropsy, the use of modified Ziehl–Neelsen stain or the Giminez stain on mucosal smears to demonstrate the intracellular organisms is a simple presumptive technique (Love et al. 1977). Histopathological examination of affected tissues will reveal the distinctive morphology of the proliferative lesions. Porcine circovirus infections may produce grossly evident mucosal thickening of the intestines, but distinctive systemic lymph node lesions and granulomatous (non-adenomatous) enteritis differentiate it from PE (Jensen et al. 2006). Specific identification of *L. intracellularis* in PE lesions is best achieved by immunohistochemical staining of fixed embedded tissues (Guedes and Gebhart 2003a; Ladinig et al. 2009; McOrist et al. 1987). In the absence of specific immunological reagents, silver-staining techniques will clearly show the presence of intracellular bacteria (Figure 59.6). The affected crypts need to be examined carefully at high magnifications due to the small size of *L. intracellularis*. Where electron microscopic facilities are available, the presence of the intracellular organism can be confirmed.

Cultivation of the obligately intracellular *L. intracellularis* requires the establishment of a suitable cell line, such as IEC-18 rat enterocytes and the addition of purified *L. intracellularis* from pig intestines in the presence of antibiotics to retard the growth of other bacteria (Lawson et al. 1993; McOrist et al. 1995b). Maintenance and passage of the organism in intestinal cell co-culture require suitable microaerobic atmospheres and cell lysis conditions, respectively (Lawson et al. 1993). Most cells in a monolayer are each typically infected with over 30 cytoplasmic bacteria (Figure 59.7), causing no apparent cytopathic effect, in particular, no abnormal cell proliferation. Culture attempts have regularly been more successful from PHE cases than from chronic PE cases.

**59.6. Chronic proliferative enteropathy. Micrograph of enlarged mucosal crypt showing numerous intracellular bacteria (arrows) in the apical cytoplasm of epithelial cells (Warthin–Starry silver stain; original magnification ×2000).**

**IMMUNITY**

Early lesions contain few infiltrating inflammatory cells, not above the normal for pig intestines (McOrist et al. 1992), indicating the initial epithelial cell nature of the infection. Affected epithelial cells contain a large accumulation of intracellular IgA (Lawson et al. 1979; McOrist et al. 1992), and intestinal lavages contain a detectable level of *Lawsonia*-specific IgA (Guedes et al. 2002a). Macrophage ingestion of *L. intracellularis* in developing lesions probably leads to a typical Th-1-type immune cell response in the lamina propria (MacIntyre et al. 1998).
per kilogram of body weight (McOrist et al. 1996b, 1997a; Schwartz et al. 1999; Walter et al. 2001). In the United States, some quinoxalines (such as carbadox) are also available and effective. Acquired resistance to these active drug groups has not been demonstrated in L. intracellularis (McOrist 2000). Apparent medication failures with these drugs are most likely to occur in pigs with ileitis that are underdosed on a body weight basis, such as breeding pigs with a low feed intake, or when pigs are medicated before or too long after actual peaks of exposure. Antimicrobial drugs now known to be inherently ineffective against L. intracellularis in clinical cases of PE include the penicillins, bacitracin, aminoglycosides such as neomycin, virginiamycin, and the ionophores. Nonantibiotic therapies such as copper or zinc compounds or feed acidifiers have also shown no evidence of efficacy.

Various approaches to medication are possible, depending on the age of pigs involved. Treatment of acute PE in breeding herds requires a vigorous approach, including both the clinically affected and the in-contact animals (which may be the whole herd). A preferred treatment would be tiamulin (120 ppm) or tylosin (100 ppm) for 14 days, delivered orally via a water-soluble formulation or an in-feed premix or by intramuscular injection of an equivalent dose to affected and in-contact pigs (McOrist et al. 1999).

Severe chronic clinical disease manifests as wasting pigs will often appear to be moderated by the use of tylosin or tiamulin (or carbadox where available). If sufficient numbers of clinical cases are occurring in growing pigs, then the removal of affected animals to separate accommodation, with supportive therapy, may limit losses. Controlled field trials now suggest that incorporation of in-feed or water-soluble antibiotics for control achieves best results if given early in the course of infections. On many single-site farms, this is around 8–11 weeks of age. Medication of older pigs, such as breeding stock, is not likely to eliminate the infection. Therefore, partial depopulation and medication-based eradication attempts have been largely unsuccessful. Because PE disease can vary in the time of onset on different farms and between batches on the same farm, in-feed antibiotics for treatment might be added too late to stop damaging clinical signs and poor performance (Hammer 2004). Alternatively, if they are added too early, groups of pigs may not get the chance to develop active immunity and may remain naïve and susceptible to later severe acute PHE cases.

Vaccination is a widely used and effective control method given the endemic nature, major economic impact, and variable time of onset of PE. Oral administration of a single dose of an attenuated live vaccine (Enterisol® Ileitis, Boehringer Ingelheim, Ingelheim, Germany) to young pigs provided significant levels of protective immunity against subsequent challenge with virulent heterologous L. intracellularis (Kroll et al. 2003; McOrist et al. 1992). The entry of Lawsonia into host cells induces a nonspecific host cell “alarm response” of interferon and related immunity and apoptosis genes (Oh et al. 2009). Both cell-mediated and humoral responses occur in the blood of affected pigs (Guedes and Gebhart 2003a; Knittel et al. 1998; McOrist and Lawson 1993). These are first detectable 2 weeks after exposure and can persist for some 3 months in acutely infected pigs (Guedes et al. 2002a). It is therefore likely that most animals exposed to L. intracellularis show a specific immune response. This response appears to correlate with long-lasting protective immunity to reinfection (Kroll et al. 2004).

**PREVENTION AND CONTROL**

In vitro evaluations via a co-culture approach have compared the minimum inhibitory concentrations for antibiotic groups with potential activity against L. intracellularis (McOrist et al. 1995c; Wattanaphasak et al. 2009). Challenge exposure and controlled field evaluations of treatment and prevention measures in commercial pigs over numerous years and sites indicate that macrolides and pleuromutilins are the most effective antibiotics, when given at an adequate dosage rate

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**59.7. Immunohistochemistry micrograph of Lawsonia intracellularis in co-culture within intestinal epithelial cells in monolayer.**
This protection was independent of the route of oral administration chosen (individual oral drench or drinking water delivery to a group). Significantly improved weight gains and reduced fecal shedding of *L. intracellularis* were also noted after vaccine administration, particularly if given via drinking water. This vaccine has proven useful and effective in repeated global usage (Hardge et al. 2004; McOrist and Smits 2007) and has assisted reduction of antibiotic usage on farms for PE (Bak and Rathkjen 2009). Killed or subunit vaccine types are not available.

This attenuated vaccine is particularly important for the introduction of replacement breeding stock into new premises. Previous use of acclimation and medication programs in this situation led to many failures resulting in PE outbreaks. It remains a matter of concern that acute and chronic PE continue to be serious problems in high-health-status, minimal-disease herds, often with early weaning and high-quality commercial breeding lines in place. It cannot be overemphasized that in most conventional herds, the absence of clinical PE, even over a period of years, is no guarantee of freedom from the infection and disease. Apparently, clean animals from such herds may be responsible for the introduction of PE into a hitherto uncontaminated environment, often followed by an explosive outbreak of acute hemorrhagic PE and later by a low level of endemic chronic PE.

REFERENCES


Salmonellosis
Steven A. Carlson, Alison E. Barnhill, and Ronald W. Griffith

RELEVANCE
Members of the genus *Salmonella* are notorious for their ability to infect a broad range of hosts. Taylor and McCoy (1969) observed that salmonellae have been isolated from virtually all vertebrate hosts from which they have been sought. Many of the more than 2400 *Salmonella* serotypes have a broad host range, but several serotypes are quite adapted to a single host species including *Salmonella typhi* (humans), *Salmonella dublin* (bovine), *Salmonella pullorum/gallinarum* (poultry), and *Salmonella choleraesuis* (swine).

*Salmonella* infections of swine are of concern for two major reasons. The first is clinical disease in pigs (salmonellosis) and the second is that swine can be infected with a broad range of *Salmonella* serotypes that can potentially contaminate pork products and pose a threat to human health.

ETIOLOGY
*Salmonella choleraesuis* is a type of species for the genus *Salmonella*, and the hydrogen-sulfide-producing variant *kunzendorf* appears to be restricted to swine. There is considerable disagreement over the nomenclature of the genus *Salmonella*, and several proposals for altering it have been made. Convention is to refer to each of the over 2400 distinct serotypes as though it is a species. The new proposal would designate *S. choleraesuis* as *Salmonella enterica* serotype *choleraesuis*.

Disease in swine is almost always caused by either *S. choleraesuis* variety *kunzendorf* or *S. typhimurium*. The former produces septicemia with lesions in a variety of organs and has historically been and continues in some parts of the world to be the most frequent serotype causing disease in swine (Lawson and Dow 1966; Levine et al. 1945; Mills and Kelly 1986; Morehouse 1972; Schwartz and Daniels 1987; Wilcock et al. 1976). An emerging concern is illustrated by a recent study that identified multiple antibiotic-resistant isolates of *S. choleraesuis* exhibiting hypervirulence after the bacterium has escaped from waterborne protozoa (Xiong et al. 2010).

*Salmonella typhimurium* has emerged as the most frequently isolated serotype from diseased swine in North America (Foley et al. 2008), causing diarrheal disease as a consequence of enterocolitis. Disease caused by *S. typhimurium* occurs with greater than expected frequency in what could be considered unusually clean herds such as purebred breeding herds (Gooch and Haddock 1969; Heard et al. 1968; Lynn et al. 1972) because of introduction into a previously immunologically naïve population. This organism is also frequently isolated as a sequel to other enteric or debilitating diseases. As with *S. choleraesuis*, some multiresistant *S. typhimurium* can be hypervirulent following exposure to common waterborne protozoa (Xiong et al. 2010).

Localized epidemics of disease caused by the biochemically atypical *S. typhisuis* have been historically reported in the American Midwest (Andrews 1976; Barnes and Bergeland 1968) and Europe (Barnes and Sorensen 1975). This organism grows poorly in standard selective media for *Salmonella* isolation, but the disease produced by *S. typhisuis* is so characteristic that outbreaks are not likely to remain unnoticed (Barnes and Bergeland 1968). This serotype was recently demonstrated to be able to subsist on antibiotics as a sole carbon source (Barnhill et al. 2010), a concern given the potential for antibiotics present in manure lagoons.
Other serotypes are occasional causes of disease in swine but are usually transient and associated with predisposing factors including debilitation, immunocompromise, other intestinal disturbances, or circumstances, which allow immunologically naïve pigs to be exposed to very large doses. *Salmonella heidelberg* has been associated with postweaning diarrhea with mild lesions more typical of enterotoxigenic diarrheal disease than of the typical fibrinonecrotic enterocolitis observed with *S. choleraesuis* or *typhimurium* (Reed et al. 1985). *Salmonella dublin* (Lawson and Dow 1966; McErlean 1968) and *enteritidis* (Reynolds et al. 1968) have been rarely reported as causes of meningitis in suckling pigs.

**PUBLIC HEALTH**

Nontyphoid salmonellosis is a worldwide health problem and is the leading cause of food-borne illnesses in the United States and many parts of the world. The topic has been recently reviewed (Doyle et al. 2009; Foley and Lynne 2008; Foley et al. 2008). Although less common than contaminated food, direct contact with clinically or subclinically infected animals can also be a source of human infection (Hoelzer et al. 2011). Among the top 20 *Salmonella* serotypes isolated from diseased humans, four are commonly isolated from swine, that is, *S. typhimurium*, *heidelberg*, *agona*, and *infantis*. Even so, human salmonellosis is more commonly acquired in the United States through consumption of contaminated poorly cooked poultry, eggs, or beef than by consumption of pork or direct contact with pigs. *Salmonella choleraesuis* is an uncommon human pathogen in the United States but remains a predominant human disease in many Asian countries.

Nontyphoid human salmonellosis usually manifests as self-limiting diarrhea, abdominal cramps, vomiting, and fever that begins 6–12 hours after oral exposure and resolves after 2–7 days. In a low percentage of affected individuals, septicemic disease develops leading to osteomyelitis, pneumonia, or meningitis that requires antibiotic therapy. An exception is *S. choleraesuis* that consistently produces severe septicemic disease. Mortality may result from dehydration or effects of septicemic disease. Severe disease and mortality are most common in infants, the elderly, or immunocompromised individuals.

**EPIDEMIOLOGY**

The reservoir for salmonellae is the intestinal tract of warm- and cold-blooded animals. Salmonellae have virtually all of the attributes necessary to ensure wide distribution, including abundant reservoir hosts, efficient fecal shedding from carrier animals, persistence within the environment, and the effective use of transmission vectors (feed, fomites, vehicles, etc.). Inapparent, long-term carriers that can shed salmonellae in feces continuously or intermittently, often in high numbers, are common in most host species. Shedding of the organism can be exacerbated by a long list of stressors, including commingling of pigs, transportation, concurrent diseases, antibiotic therapy, and food deprivation.

Infection of swine with persistent shedding by one or more serotypes is common, but primary clinical disease caused by serotypes other than *S. choleraesuis* or *typhimurium* is uncommon. Put differently, clinically normal swine may be infected and shed a variety of serotypes that pose little threat to pigs, but may pose risk for humans through direct exposure or contamination of pork products. Thus, shedding of salmonellae by clinically and subclinically infected pigs has implications both for contamination of pork carcasses and food-borne human illness as well as for the development of clinical salmonellosis in swine populations.

**Salmonellae in Pork**

Although much of the *Salmonella* contamination of pork products occurs by fecal contamination during slaughter within abattoirs, infected pigs leaving the farm are considered the original source of abattoir contamination. The stress of transport and feed deprivation increases shedding from inapparent carriers, which then contaminate the environment of the truck and abattoir (Isaacson et al. 1999; Williams and Newell 1970). The prevalence of infection within the group continues to increase with increasing length of stay in the pens prior to slaughter, rising by about 50% for each 24-hour period (Craven and Hurst 1982; Morgan et al. 1987).

Although *Salmonella* contamination of poultry and beef products exceeds that of pork, *Salmonella* control programs in swine will continue to be a primary focus of food safety initiatives. *Salmonella* reduction programs are becoming commonplace, with long-range goals to include the production and marketing of salmonellae-free pork products. Numerous, dynamic programs are in place utilizing hazard analysis and critical control point principles. Those programs that have been in place for a sufficient period of time, such as the Danish program, have significantly reduced the rate of *Salmonella* infection in pork products (Nielsen et al. 1995). Fortunately, most of the methods useful for preharvest *Salmonella* reduction in swine populations are related to sound management practices that also improve the overall health of a swine operation. The Danish program, which uses serology to identify *Salmonella*-infected herds and thereby control the introduction of shedding swine into the abattoir, has not been as successful as originally hoped.
Salmonellosis in Swine

Most salmonellosis outbreaks occur in intensively reared weaned pigs, and although disease in adults and suckling pigs is infrequent, infection is not (Gooch and Haddock 1969; Wilcock et al. 1976). The low frequency of salmonellosis in suckling pigs presumably results from lactogenic immunity, since naïve neonatal swine are susceptible to oral challenge with salmonellae and develop a disease comparable with that in weaned pigs (Wilcock 1978). Disease occurs worldwide but varies markedly in estimated prevalence, morbidity, and mortality.

Host-adapted *S. choleraesuis* is isolated almost exclusively from diseased swine and is usually manifested as septicemia. Midwestern U.S. veterinary diagnostic laboratories and veterinarians reported an increasing frequency of salmonellosis due to *S. choleraesuis* from 1981 to 1990 and a decreasing frequency from 1991 to 1997 (Schwartz 1997). The decrease in the Midwest in the mid-1990s is likely due to improvements in swine management and husbandry and the advent of efficacious attenuated live vaccines.

*Salmonella typhimurium* has worldwide distribution, is not host specific, and is now the most frequently isolated serotype from clinically ill pigs in the United States (Foley and Lynne 2008). Clinically ill pigs develop enterocolitis and exhibit diarrhea and dehydration. Disease most commonly develops in pigs with concurrent debilitating diseases, in conditions of poor hygiene that allow exposure to high doses of the organism, or in immunologically naïve pigs. The latter are frequently encountered in modern production systems utilizing age-segregated production.

Caution should be exercised before incriminating other serotypes as primary pathogens. Most other serotypes are transient, sporadic causes of disease, and often disease cannot be reproduced experimentally without unique qualifying criteria. *Salmonella heidelberg* has been implicated in watery diarrhea in recently weaned pigs thought to be caused by an enterotoxin-mediated secretory mechanism and associated with only mild catarrhal enterocolitis (Reed et al. 1985). This is in sharp contrast to the severe lesions of fibrino necrotic enterocolitis observed in *S. typhimurium*-associated disease.

Sources of Infection

The number of potential sources of *Salmonella* infection for a population of swine is seemingly endless. A task force study in the United States did not reach a consensus as to the most important source of salmonellae for pigs (Bixler 1978), in large part due to the diversity and the biology of the genus *Salmonella*. *Salmonella choleraesuis* is a frequent isolate from clinically ill pigs, but it is a very infrequent isolate from pig feeds or nonporcine *Salmonella* reservoirs. Therefore, infected shedding pigs and contaminated environments are the major sources of new infections of *S. choleraesuis*. Vertical (dam to offspring) and horizontal transmissions both occur.

The source of infection for other serotypes is less clear since the host and vector range for salmonellae is broad, and they have amazing capability to persist in environments outside the host. For serotypes other than *S. choleraesuis*, pigs should be thought of as biological filters for the low numbers of various *Salmonella* serotypes present in feed, water, or litter contaminated by birds, rodents, or other animals. There may be exceptions to this. For example, *Salmonella derby* seems to be very common in some abattoir surveys of swine. Evidence linking sources of contamination to primary clinical outbreaks, without other concurrent diseases or predisposing conditions, is generally lacking. Feed containing ingredients of animal origin is widely accepted as a source of *Salmonella* infection to herds, but it should be emphasized that ingredients of vegetable origin can also be a source of salmonellae-contaminated feed. Water is not as likely a source of infection unless surface water is used for consumption or pigs have access to recycled lagoon water. Birds, insects, rodents, and pets can all act as carriers, as can bedding and litter (Allred et al. 1967; Nape and Murphy 1971; Williams et al. 1969). The isolation of salmonellae from feed was significantly associated with the lack of bird proofing, with on-farm feed preparation, and with the housing of pigs in facilities other than total confinement, for all stages of production.

Transmission, Shedding, and Carrier State

Definitive statements regarding the transmission, shedding and carrier states of Salmonellae are difficult due to the dynamic and complex relationships among salmonellae, hosts, and environment. *Salmonella* transmission and shedding within differing populations of animals in an endless variety of environmental, feeding, and management situations result in countless unique situations that cannot be experimentally reproduced.

In general, fecal–oral transmission is the most likely mode of transmission of virulent salmonellae. *Salmonella* can be recovered from the intestinal tract of pigs within several minutes of oral exposure. Transmission can occur from pig to pig, contaminated environment to pig, or dam to offspring. Oral–pharyngeal secretions may contain salmonellae, largely due to the fact that tonsils become rapidly contaminated with salmonellae following oral transmission. This may allow nose-to-nose transmission. Aerolized secretions, feces, or contaminated dust particles make the potential for aerosol transmission for short distances quite real. In fact, experimentally esophagostomized pigs can acquire systemic *Salmonella* following aerosol introduction of the pathogen (Fedorka-Cray et al. 1995).

*Salmonella* infection of swine herds is much more common than disease. Longitudinal Dutch studies
suggest that about 25% of herds are never infected, 24% are constantly infected, and 50% are infected most of the time. There appear to be infection cycles, with the endemic salmonellae having an ecological advantage over newly introduced Salmonella serotypes. Infection occurs in the first weeks after arrival or commingling and reaches a maximum of 80–100% prevalence within another 2–3 weeks. About 5–30% of the pigs are still excreting salmonellae at the end of the finishing period. In 2006, the National Animal Health Monitoring Service (NAHMS) reported 52.6% of herds sampled across the United States were Salmonella positive, up from data obtained in 1995 (38.2%) and 2000 (32.8%).

During acute disease, pigs will shed up to \(10^6\) S. choleraesuis per gram of feces (Smith and Jones 1967) or \(10^7\) S. typhimurium per gram of feces (Gutzmann et al. 1976). The minimum disease-producing dose of either serotype has not been established in field situations, but disease is difficult to reproduce experimentally at low doses. There is one report of moderate disease following oral inoculation of \(10^6\) microbes (Dawe and Troutt 1976), but most authors report successful experimental disease production with doses of \(10^6–10^{11}\) organisms unless pigs are artificially stressed by injection of dexamethasone or in some other manner. Pigs infected with \(10^5\) organisms remained clinically normal but uninoculated pigs in the same pen did become clinically ill (Gray et al. 1996). It is likely that dose (and perhaps virulence) is magnified when pigs are infected and sequential (pig to pig) transmission occurs in field situations, so that the initial infective dose in the field is considerably less than that required in experimental situations. High animal density, stress of transport, and intercurrent nutritional or infectious disease are assumed to increase the shedding by carriers as well as the susceptibility of exposed pigs. Pigs with nondetectable shedding of salmonellae can begin shedding within hours of an applied stress. The transmission demonstrated between feeder pigs also occurs between pigs during market transport and lairage at abattoirs, with infection rates proportional to time spent in transport and lairage (Hurd et al. 2001a,b). It is likely that catecholamines are released in association with stress, resulting in decreased gastric acid production and increased intestinal motility. Increases in stomach pH increase the likelihood that salmonellae will survive passage through the stomach and will access and replicate in the intestine and colon.

Outbreaks of salmonellosis are usually characterized by spread from pen to pen. Situations of spread from pen to distant pen are likely because of vectors or caretaker transmission. When all animals sicken simultaneously, a common source such as feed, bedding, water, or a contaminated environment should be suspected. Salmonella infections tend to be more prevalent in continuous-flow systems than in barns managed by principles of all-in/all-out. Prevalence is also higher in barns with open flush gutters than in those with slatted floors, with the highest rates of infection observed in outdoor finishing systems (Davies et al. 1997).

Numerous studies in a variety of host species with a variety of serotypes have demonstrated prolonged carrier states following infection. The pattern of shedding and the duration of the carrier state after clinically apparent disease have been studied only in group-housed pigs with no barrier to repeated reinfection (Wilcock and Olander 1978; Wood et al. 1989). After experimental infection, S. typhimurium was isolated from feces daily during the first 10 days postinfection and frequently during the next 4–5 months. When slaughtered 4–7 months after initial infection, over 90% of pigs were positive for S. typhimurium in the mesenteric lymph node, tonsil, cecum, or feces (Fedorka-Cray et al. 1994; Wilcock and Olander 1978; Wood and Rose 1992; Wood et al. 1989). Salmonella newport has been shown to persist in mesenteric lymph nodes for 28 weeks. Infection of individual animals may be relatively short-lived (less than 8 weeks), but organisms may circulate within a population and between pigs and the environment for extended periods of time. Salmonella choleraesuis has been shown to persist for at least 3 months in wet feces and 6 months in desiccated feces.

The influence of antibiotics on the frequency and duration of shedding of salmonellae in pigs has received little attention. In human enteric salmonellosis, the use of antibiotics has long been recognized to prolong the carrier state (Aserkoff and Bennett 1969; Dixon 1965). In pigs with enterocolitis, antibiotics do not reduce the duration or the magnitude of fecal shedding, but neither are they reported to prolong or intensify shedding (Deggeet et al. 1976; Finlayson and Barnum 1973; Gutzmann et al. 1976; Jacks et al. 1988; Jones et al. 1983; Wilcock and Olander 1978). In contrast, vigorous antibacterial therapy early in the course of septicaemia caused by S. choleraesuis may significantly reduce the magnitude and duration of fecal shedding (Jacks et al. 1981).

**PATHOGENESIS**

The clinical and pathological features of Salmonella infections are extremely variable. Severity is influenced by serotype, virulence, natural and acquired host resistance, and route and quantity of the infective dose. Over 200 virulence factors have been associated with salmonellae but few have been completely characterized. Generally, those that promote virulence in pathogenic salmonellae are involved in adhesion, invasion, cytotoxicity, and resistance to intracellular killing, often working in combination to promote disease. Despite distinct differences in clinical signs, many parallels can be drawn between S. choleraesuis and S. typhimurium when discussing pathogenesis.
Although large doses (greater than $10^7$ microbes) are required to induce disease experimentally, intraluminal replication may be important with small inocula. Disease is facilitated by factors such as peristaltic impairment, interference with intestinal flora, and elevation of gastric pH (Clarke and Gyles 1993). Replication to about $10^7$ organisms per gram of intestinal content is required for lesion production in pigs infected with *S. typhimurium*, a finding that probably also applies to other serotypes causing enterocolitis. Alterations in normal intestinal defenses by antibiotic-induced changes in normal flora or cold-induced alteration in intestinal motility may reduce the amount of replication required for disease or increase the ease of *Salmonella* replication (Bohnhoff et al. 1954).

The ability to invade is a requirement for pathogenesis and is encoded by a serotype-specific plasmid (Helmuth et al. 1985). Removal of this plasmid results in a lack of ability to invade but has no effect on ingestion or killing by murine macrophages, lipopolysaccharide (LPS) production, or serum resistance (Gulig and Curtiss 1987). During the invasion process, there is induction of synthesis of new proteins that enhance intracellular survival (Finlay et al. 1989). While many epithelial cell types (enterocytes, M cells, goblet cells) in the jejunum and ileum may be invaded, the predominant portal of entry into the submucosa may occur at Peyer's patches (Meyerholz and Stabel 2003; Meyerholz et al. 2002; Schausser et al. 2004). *Salmonella choleraesuis* locates preferentially in the colon on the luminal surface of ileal M cells of Peyer's patches (Pospischil et al. 1990). Invasion into M cells appears to be the preferred route given the shorter glycosylation that coats these cells. Attachment of the bacteria to epithelial receptors triggers microfilament-controlled uptake, vacuole formation, vacuole transport through the cell cytoplasm, and entry into the lamina propria via exocytosis through the basement membrane (Takeuchi 1967; Takeuchi and Sprinz 1967). Passage through the epithelium causes mild and transient enterocyte damage. Salmonellae can synthesize over 30 proteins that are selectively induced during infection of macrophages, making them facultative intracellular bacteria (similar to *Brucella*, *Mycobacteria*, and *Listeria* organisms) that can survive within macrophages and neutrophils in the lamina propria (Roof et al. 1992a,b). Spread to mesenteric lymph nodes is rapid, occurring within 2 hours of inoculation of ligated intestinal loops or 24 hours after oral challenge (Reed et al. 1985, 1986). Knockout mice (CD18-/-) have been used to show that CD18+ phagocytes are important in the dissemination of the organism to the spleen and liver (Vazquez-Torres et al. 1999). Two leading cell candidates for this systemic transport are the macrophages and dendritic cells (Vazquez-Torres et al. 2000). Recent in vitro work has suggested that dendritic cells are capable of producing tight junction proteins to penetrate the epithelium and sample luminal bacteria including *Salmonella* (Rescigno et al. 2001). Concurrent with bacillary spread is the appearance of an acute, predominantly macrophagic, inflammatory reaction and prominent microvascular damage with thrombosis within the lamina propria and submucosa. Nonenteric routes of systemic invasion may be important since *S. choleraesuis* demonstrated primary colonization of the lungs within 4 hours after intranasal administration to esophagectomized pigs (Fedorka-Cray et al. 1995; Gray et al. 1995).

Early intestinal inflammation is considered a key feature of pathogenesis for enteric forms of salmonellosis. Neutrophil recruitment and transmigration across the epithelium is considered the most significant component (McCormick et al. 1995). Host-derived caspase-1 can act as a proinflammatory agent by cleaving interleukin-1 beta and interleukin-18 into active molecules (Fantuzzi and Dinarello 1999). SipA, a protein that *Salmonella* injects into host cells, has also been shown to contribute to the inflammatory response by activation of phosphokinase C (Lee et al. 2000). *Salmonella*-induced activation of inflammatory mediators such as nuclear factor-kappaB and phosphokinase C results in basolateral secretion of interleukin-8 and apical secretion of pathogen-elicted epithelial chemoattractant (Eaves-Pyles et al. 1999; Lee et al. 2000). These molecules act as chemokines promoting the transepithelial migration of neutrophils into the intestinal lumen (Gewirtz et al. 1999). In contrast, several studies using rabbits, monkeys, calves, or pigs have demonstrated fluid secretion independent of mucosal necrosis or inflammation (Clarke and Gyles 1987; Giannella et al. 1973; Kinsey et al. 1976; Rout et al. 1974). These studies present evidence that, at least early in the disease, diarrhea is the result of decreased sodium resorption and increased chloride secretion due to cholera-like and Shiga-like enterotoxins. Secretion stimulated by prostaglandins elaborated by endotoxin-stimulated neutrophils may also be important (Stephen et al. 1985). Toxic effects of certain *Salmonella* outer membrane proteins, as well as lipid A associated with the LPS, are also important mediators of cell damage. Survival within phagocytes is an important attribute of virulent salmonellae, the mechanism of which is not clear. Salmonellae that possess smooth LPS, O side chains, and a complete LPS core are more resistant to phagocyte killing.

Mucosal inflammation and necrosis, as well as septicemia, occur in concert with the diarrhea but perhaps independently of this response. Microvascular thrombosis and endothelial necrosis in the submucosa and lamina propria are consistent early lesions in porcine salmonellosis (Brown et al. 2007; Lawson and Dow 1966; Reed et al. 1986; Wilcock et al. 1976), probably in response to locally produced endotoxin. Salmonellae are not directly associated with the damaged vessels but direct the events from the protected intracellular niche.
of macrophages in the surrounding submucosa or lamina propria (Takeuchi and Sprinz 1967). Mucosal ischemia as a result of the microvascular thrombosis is probably a major contributor to the mucosal necrosis so typical of salmonellosis in all species. The second major contribution to mucosal necrosis is probably from the chemical products of mucosal inflammation. The systemic signs and lesions of septicemic salmonellosis, in swine almost exclusively S. choleraesuis infection, are most commonly attributed to endotoxemia from bacterial dissemination. The complex biology of endotoxin is beyond the scope of this chapter, and readers should consult Cybulsky et al. (1988), Elin and Wolff (1976), or Wolff (1973). Briefly, endotoxin interacts with plasma and with leukocytes to initiate inflammation and fever. Most of the effects are mediated by interleukin-1, a lymphokine produced by macrophages stimulated by the endotoxin (Rubin and Weinstein 1977). Endotoxins have either direct effects on tissue or effects via an array of cytokine mediators that are likely a result of interactions with toll-like receptor 4 (McGettrick and O’Neill 2010).

**CLINICAL SIGNS**

**Salmonella choleraesuis**

Septicemic salmonellosis caused by S. choleraesuis is most often observed in weaned pigs less than 5 months of age but may be seen occasionally in market swine, suckling piglets, or adult breeding stock. Clinical signs observed are initially from generalized sepsis and later may be from localization in one or more organs/systems. Initially affected animals are inappetent, lethargic, and febrile with temperatures of 105–107°F (40.5–41.6°C), and may have a shallow, moist cough with slight expiratory dyspnea. Icterus may be apparent. The first evidence of disease may be finding pigs reluctant to move, huddled in the corner of a pen, or even dead, with cyanosis of extremities and abdomens. Diarrhea is not usually a feature of septicemic salmonellosis until the third or fourth day of disease, when watery yellow feces may be seen. Infrequently, nervous signs may be observed that resemble classical swine fever or pseudorabies (Reynolds et al. 1968; van der Wolf et al. 2001; Wilcock and Olander 1978) as a result of necrotizing and histiocytic vasculitis leading to encephalitis and/or meningitis. In pregnant sows, abortions may be observed. In most outbreaks, the case fatality rate is high while morbidity is variable but is usually less than 10%. The duration of the disease in individual pigs, as well as the duration and severity of each epidemic, is unpredictable but will be prolonged without successful intervention.

**Salmonella typhimurium**

The initial clinical sign in pigs infected with S. typhimurium is watery yellow diarrhea initially without blood or mucus. The disease may spread rapidly to involve most pigs in a pen within a few days. The initial diarrhea in an individual pig usually lasts 3–7 days, but it typically may recur for second and third bouts, giving the impression of a waxing and waning diarrheal disease of several weeks’ duration. Blood may appear sporadically in the feces but rarely with the profuseness typical of swine dysentery or hemorrhagic porcine proliferative enteropathy. Affected pigs are febrile, have decreased feed intake, and are dehydrated, paralleling the severity and duration of the diarrhea. Mortality is usually low and occurs only after several days of diarrhea, presumably as the result of hypokalemia and dehydration. Most pigs make complete clinical recovery but a portion may remain as carriers and intermittent shedders for at least 5 months. A few pigs may remain unthrifty and chronically waste. Occasional pigs may develop rectal strictures, resulting in obstipation and marked distention of the abdomen. Rectal strictures have been ascribed to defective healing of ulcerative proctitis caused by S. typhimurium (Wilcock and Olander 1977a,b). The stricture reportedly represents fibrosis in an area of persistent ischemia, with the rectum predisposed because of a normally precarious blood supply.

**Other Serotypes**

*Salmonella typhitis* is an infrequent cause of chronic diarrhea and wasting with characteristic caseating lesions in affected pigs. *Salmonella heidelberg* has been infrequently associated with outbreaks of acute watery diarrhea in weaned pigs. *Salmonella dublin* and *enteritidis* rarely cause nervous signs in suckling pigs as a consequence of supplicative meningitis.

**LESIONS**

**Salmonella choleraesuis**

Gross lesions in pigs dying in the acute phase of septicemia include cyanosis of the ears, feet, tail, and ventral abdominal skin. Lymph nodes, especially the gastrohepatic and mesenteric, are typically enlarged, moist, and congested, and the spleen is enlarged, dark purple, and pulpy (Figure 60.1). The liver may be slightly enlarged with scattered small 1- to 2-mm foci of parenchymal necrosis, and the wall of the gall bladder may be thickened and edematous. There are often renal cortical petechiae and ecchymoses. Acute interstitial pneumonia evidenced by moist, slightly firm, resilient, noncollapsing lungs that often have red (hemorrhagic) fluid separating lobules is usually observed. The gastric mucosa is often markedly congested. Additionally, in pigs that survive a few days, there may be infarction of the skin on the tips of ears that appears dry and dark purple sometimes with portions that slough (Figure 60.2). Icterus is inconsistently severe. Bronchopneumonia may be observed as consolidation of cranial ventral
ized swelling of the endothelial cells and histiocytosis typical of gram-negative sepsis. Diffuse histiocytic interstitial pneumonia or suppurative bronchopneumonia is often observed. Segmental necrotizing vasculitis with perivascular histiocytic infiltrates, sometimes with localized necrotizing encephalitis is uncommonly observed. Lesions in small and large intestines are the same as for *S. typhimurium* and are described below.

More complete discussions of the pathology of septicemic salmonellosis are available (Brown et al. 2007; Lawson and Dow 1966).

**Salmonella typhimurium**

The most consistent gross lesion in pigs suffering from *S. typhimurium* is enterocolitis most often involving the ileum, cecum, and spiral colon, and occasionally extending to involve the descending colon and rectum. Affected segments typically have thickened edematous walls and the mucosa is red and roughened with a granular appearance and may have multifocal or coalescing erosions and ulcers that are covered with adherent gray-yellow fibrinonecrotic debris (Figure 60.4). Sharply delineated deep button ulcers may be observed in more chronic lesions. Mesenteric lymph nodes, especially ileocecal nodes, are consistently markedly enlarged and moist. Stomach and intestinal contents are usually scant and bile stained. Often cecal and colonic contents contain black or sand-like gritty material. Of note is that the ileal mucosa in uncomplicated cases of *S. typhimurium* is usually reddened and slightly roughened with occasional adherent fibrin. It should not be confused with the markedly thickened and necrotic ileal mucosa that was observed in the

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**60.1.** Splenomegaly, hepatomegaly, and swollen mesenteric lymph nodes in a pig infected with *S. choleraesuis*.

**60.2.** Ischemic necrosis of the skin on the distal portions of the ears in a finishing-age pig infected with *S. choleraesuis*. Courtesy of Dr. Greg Stevenson, Iowa State University.

**60.3.** Focal coagulative necrosis infiltrated with neutrophils and macrophages, a “paratyphoid nodule,” in the liver of a finishing-age pig infected with *S. choleraesuis*. Courtesy of Dr. Darin Madson, Iowa State University.
the lamina propria and are less frequent in larger vessels in the submucosa. Necrosis, likely a sequel to infarction, sometimes extends through the mucosa into the submucosa and lymphoid patches creating grossly visible ulcers (Figure 60.6). Fibrinonecrotic debris that may contain myriad opportunistic bacteria and numerous Balantidium coli organisms is often adherent to the luminal surface of damaged epithelium. Submucosal lymphoid patches may be necrotic in acute disease, but later in pigs dying of the naturally occurring disease, lymphoid hypertrophy or even regenerative hyperplasia is more common. Regional lymph nodes are typically edematous and contain neutrophils acutely and predominantly macrophages by days 2–3 in sinuses. In some nodes, there may be focal necrosis. More complete discussions of lesions are available (Brown et al. 2007; Wilcock et al. 1976).

Other Serotypes
Lesions observed in wasting pigs infected with S. typhimurium are characteristic (Andrews 1976; Barnes and Bergeland 1968; Fenwick and Olander 1987). Chronic ulcerative colitis is observed with deep multifocal-to-coalescing mucosal ulcers that have caseous necrotic material in their centers. Likewise, regional lymph nodes are enlarged and contain caseous necrotic debris, forming the base. Bronchopneumonia with caseous abscesses, resembling tuberculosis, may also be observed.

Lesions in weaned pigs with diarrhea associated with S. heidelberg are mild or absent. Small and large intestines are typically fluid filled, and the mucosa may have sparse-to-moderate amounts of adherent mucous. In the few reports of nervous disease in suckling pigs infected with S. dublin or enteritidis, the leptomeninges
are distended by fibrin and aggregates of neutrophils and fewer macrophages.

**DIAGNOSIS**

Clinical signs and lesions may lead to a presumptive diagnosis of salmonellosis, but are insufficient to confirm a diagnosis given the number of other diseases that resemble the various forms of salmonellosis. The clinical signs observed with *S. choleraesuis* are similar to other septicemias such as caused by *Erysipelothrix rhusiopathiae* (Chapter 54), *Streptococcus suis* (Chapter 62), *Actinobacillus suis* (Chapter 48), or to those observed with classical swine fever (Chapter 38) or *Actinobacillus pleuropneumoniae* (Chapter 48). Gross lesions of splenomegaly, hepatomegaly, lymphomegaly, interstitial pneumonia, and focal hepatic necrosis are very suggestive of septicemia by *S. choleraesuis*, but are not seen in every case and closely resemble lesions of classical swine fever.

Likewise the differential diagnosis of diarrhea in weaned pigs, in addition to *S. typhimurium* and *choleraesuis*, should include swine dysentery (Chapter 50), proliferative enteropathy (Chapter 59), rotaviruses (Chapter 43), coronaviruses (Chapter 35), circovirus type 2 (Chapter 26), colibacillosis (Chapter 53), coccidiosis (Chapter 66), and trichuriasis (Chapter 67). Combined infections in diarrheal disease are commonplace. Gross lesions in enteric salmonellosis, swine dysentery, and proliferative enteropathy are similar in that all three may present as fibrinonecrotic typhlocolitis. However, presumptive differentiation at necropsy is possible in many cases by recognition of differences in lesion distribution. Salmonellosis is usually in the spiral colon and occasionally small intestine, may be focal or diffuse, may include mucosal ulcers, and always involves marked mesenteric lymphadenopathy. The lesion of swine dysentery is diffuse, shallow (no deep ulceration), and restricted to cecum and spiral colon. In addition, lymph node enlargement is absent or mild. In proliferative enteropathy, ileal involvement usually overshadows the milder colonic lesions, and the mucosa underlying any fibrinonecrotic exudate is markedly hyperplastic.

The lesions of caseating button ulcers in the spiral colon, caseous lymphadenitis, and bronchopneumonia with caseating abscesses in wasting pigs with chronic diarrhea are strongly presumptive of infection with *S. typhimurii*. However the intestinal lesions may heal in these pigs, leaving the lymphoid and pulmonary lesions to be distinguished from tuberculosis and infection with *Arcanobacterium pyogenes* (Barnes and Sorensen 1975).

A definitive diagnosis of salmonellosis is confirmed by bacterial isolation and identification in conjunction with demonstration of compatible lesions. The wide distribution of environmental salmonellae and the incidence of subclinical infection and shedding of various *Salmonella* serotypes make isolation alone unreliable for disease diagnosis. A positive isolation should always be supported by appropriate lesions before a diagnosis of salmonellosis is made.

To isolate *S. cholerae suis* from suspected cases, samples of the lung, liver, or spleen often yield pure cultures of the organism on brilliant green, bismuth sulfite, blood agar, or MacConkey agar. Enrichment techniques are seldom required unless the organs have been contaminated by feces or careless handling or have autolysis, in which case tetraphionate broth at 107.6–109.4°F (42–43°C) is the enrichment medium of choice. Selenite broth is inhibitory for *S. cholerae suis* and should be avoided (Edwards and Ewing 1972). Attempts to isolate salmonellae from animals that have received antimicrobial therapy are often unrewarding. Intestine or feces are not reliable specimens for isolation of the organism in pigs with acute septicaemia (Schwartz 1991).

In cases of diarrhea in which salmonellosis is suspected, a pool of ileum, colon, and ileocecal lymph node should enable detection of virtually all active or recently recovered cases, although tissues such as tonsil or cecal wall will usually yield positive cultures as well (Wilcock et al. 1976; Wood et al. 1989). From live animals, large (10 g) aliquots of feces or pharyngeal tonsil scrapings are preferable to rectal swabs for isolation, with tetraphionate enrichment the method of choice.

Other tests using more sophisticated technology, including polymerase chain reaction (PCR), are not required for routine diagnosis. PCR currently has value as a screening tool but has a relatively high cost and currently lacks sensitivity without pre-enrichment. PCR-based detection of salmonellae does not constitute diagnosis of salmonellosis since this assay can detect DNA from dead *Salmonella* and the organism may be present without causing clinical disease.

Serology is becoming increasingly available, usually in the form of an enzyme-linked immunosorbent assay (ELISA) test. Most tests use surface antigens such as LPS. These tests, some of which use mixed antigens containing both LPS or antigens from several serotypes, thus far appear to lack specificity and sensitivity for individual-animal diagnosis but are useful for herd screening (Baum et al. 1996). A mixed-ELISA test using meat juice at slaughter to detect antibody to a broad range of serotypes has been useful in categorizing the level of *Salmonella* infection in herds in Denmark (Mousing et al. 1997; Nielsen et al. 1995).

**PREVENTION AND CONTROL**

**Prevention**

Prevention of infection of swine with salmonellae is not currently possible. Infection does not necessarily
result in disease, and pigs may not sicken with disease until severely stressed long after initial exposure. The control of disease expression rests on efforts to minimize the exposure dose and to maximize pig resistance. The carrier pig and contaminated feed or environment are the most significant sources of infection to pigs, and pigs are most likely to develop disease during periods of stress or when exposed to massive numbers of salmonellae. The commingling and transport of weanling pigs from different sources to finishing farms enhances activation of latent carriers and ensures exposure of stressed pigs to salmonellae (Allred 1972). The source of host-adapted S. choleraesuis, which is rarely isolated from feed or feed ingredients, would seem to be limited to carrier pigs and facilities previously contaminated with this serotype. The fact that many outbreaks occur in facilities with good sanitation suggests that other stresses probably contribute to occurrence of the disease. Management practices that allow filling of grower and finishing rooms with single-source and single-age pigs are beneficial. Minimizing the variety of stresses often involved in acute outbreaks requires constant attention to details of management and husbandry, including proper animal density; dry, comfortable pens and temperatures; and adequate ventilation. On farms with endemic disease, modifications to the facility and environment as well as implementation of management practices that emphasize all-in/all-out production should precede a prophylactic drug program. Antibiotics are probably useful as aids in preventing occurrence of disease when used prophylactically, but their use will not prevent infection and when relied upon for prevention of disease will eventually fail.

As with other facultative intracellular bacteria, live vaccines that stimulate cell-mediated immunity are the most likely to be protective for salmonellae in swine. Historically, an attenuated live S. choleraesuis vaccine was used widely in the United Kingdom for many years but was withdrawn when S. choleraesuis infection decreased in that country to negligible proportions. Recently, the introduction of effective and safe modified live attenuated vaccines for S. choleraesuis has been credited with a major reduction in the occurrence of systemic salmonellosis in North America. The isolates used in these vaccines are either naturally occurring avirulent S. choleraesuis or are derived from repeated passage through porcine neutrophils, the product of which was demonstrated to have been cured of a 50-kb virulence plasmid necessary for intracellular survival (Kramer et al. 1987, 1992; Roof et al. 1992b). When given at weaning, vaccine protected pigs for at least 20 weeks (Roof and Doitchinoff 1995) against homologous serotypes, with some cross-protection suggested with heterologous serotypes.

Partial protection can be obtained with bacterins, primarily because of the nonspecific mitogenic and immunostimulant effect of LPS (Fenwick et al. 1986) and the immunodominance of Salmonella O antigens. Killed vaccines for S. typhimurium are safe, but the bulk of the evidence suggests that they have little efficacy in preventing disease following strong challenge because resistance to disease rests primarily on cell-mediated immunity (Collins 1974; Davies and Kotlarski 1976). Extrapolation of information from experience in humans (Hornick et al. 1970; Welliver and Ogra 1977) and calves (Bairey 1978) suggests, however, that use of a potent killed vaccine may increase the dose necessary to cause disease and may offer some protection from septicemic salmonellosis, in which humoral immunity may play a role.

Monitoring herds for salmonellae has not been commonly practiced. The detection of carrier animals is difficult because of the unpredictability of fecal shedding. The detection of salmonellae by bacterial culture of feces and tonsils of diarrheic pigs in the nursery would likely be the most rewarding for identification of infected herds. However, even repeated negative fecal or tonsillar cultures do not guarantee that a herd or individual is not a Salmonella carrier and thus a potential shedder. The use of Salmonella serology can determine if an animal has had previous exposure to salmonellae, but this has little relevance to the carrier status or to the probability of shedding. Food safety concerns have stimulated renewed interest in serology as a method to determine the Salmonella infection status of groups of market swine. This technology offers the possibility of sensitive and specific methods to identify infected herds, but it is not yet useful for determining the infection status of individual pigs.

**Control**

With either septicemic or enteric salmonellosis, the goals of treatment in an outbreak of salmonellosis are to minimize the severity of clinical disease, to prevent the spread of infection and disease, and to prevent the recurrence of the disease in the herd. With salmonellosis, the attainment of these goals is particularly difficult. Both S. typhimurium and S. choleraesuis are often resistant in vitro to many antibacterial agents used in swine (Barnes and Sorensen 1975; Blackburn et al. 1984; Schultz 1989; Schwartz 1997; Wilcock et al. 1976). During clinical disease, the organism inhabits a protected intracellular niche inaccessible to many common antibacterials. The use of various antibiotics to treat enteric salmonellosis is widely advocated (Barnes and Sorensen 1975; Morehouse 1972; Radostits et al. 2007), but much of the information to support this recommendation has been taken from trials designed to test the prophylactic efficacy of drugs, not their therapeutic efficacy. Thus, pigs on medicated feed, when inoculated orally with salmonellae, have the antibiotic already present in the gastrointestinal tract.
to interact with the salmonellae, resulting in milder disease because of what amounts to a decreased inoculum. In the few trials designed specifically to test antibacterial drug efficacy against clinical enteric salmonellosis, such therapy was considered to have little merit (Gutzmann et al. 1976; Heard et al. 1968; Olson et al. 1977; Wilcock and Olander 1978). Although not therapeutic, oral medications may decrease the efficiency of transmission and have a prophylactic effect on pigs not yet affected. Antimicrobials are ordinarily administered at maximum permissible levels in feed or, preferably, water. Ideally, the choice of antibacterial agent should be based on in vitro susceptibility testing of isolates from each outbreak. Since medication must often be initiated before such results are available, choices must be based on previous experience and results of controlled trials.

In contrast, vigorous therapy early in the course of septicemia caused by *S. choleraesuis* has been reported to significantly reduce the duration and severity of disease (Jacks et al. 1981). In that report, therapy was initiated after inoculation but prior to the onset of clinical signs. Evaluation of efficacy under field conditions is difficult because of the unpredictability of the disease and husbandry changes often accompany the use of antibacterials in an outbreak. Reports and practitioner communications from the American Midwest, however, suggest that visibly affected animals respond to aggressive therapy with parenteral antimicrobials (Schwartz 1991). Mass medication of the population at risk to decrease severity of disease and transmission of salmonellae is also widely practiced. The choice of an appropriate antimicrobial is aided by antibiograms and previous herd experience. In the absence of either, amikacin, gentamicin, apramycin, ceftiofur, and trimethoprim–sulfonamide are effective in vitro against most isolates (Barnes and Sorensen 1975; Mills and Kelly 1986; Schultz 1989; Schwartz 1997; Wilcock et al. 1976). Anti-inflammatory agents are sometimes administered to critically ill animals to combat the effects of endotoxin (Schultz 1989; Schwartz and Daniels 1987). Dipyrdone was the anti-inflammatory of choice in swine, but this drug is now banned in food-producing animals. Flunixin was recently approved for use in swine, and this drug has potent antiendotoxin effects.

Most *Salmonella* antimicrobial resistance is plasmid mediated. Of concern is the recent emergence of an *S. typhimurium* definitive type 104 (DT 104), isolated primarily from bovine and human populations, that has chromosomally integrated multiple antimicrobial resistance (Low et al. 1997). This isolate has a higher morbidity and mortality in humans than other *S. typhimurium* organisms and is increasing in prevalence in human and bovine populations. A recent study (Xiong et al. 2010) identified a DT-104-like resistance gene cassette in *S. choleraesuis* exhibiting hypervirulence in vivo. However, this isolate has yet to be implicated in field outbreaks of swine salmonellosis.

In addition to antimicrobial therapy, the successful treatment of salmonellosis relies heavily on routine husbandry procedures recommended for control of infectious diseases. The diarrheic pig contaminates its environment and is the single most important source of infection for other pigs. Removal and isolation of sick animals, minimizing exposure to infective material by scrupulous pen sanitation, frequent cleaning of water bowls, and restriction of animal or staff movement from potentially contaminated to clean areas are necessary. Efforts to modify management and environment to decrease crowding and stress and increase pig comfort are essential adjuncts to specific therapy.

REFERENCES


Staphylococci are prevalent in pigs and in swine facilities. They are essentially ubiquitous. The most common disease-causing organisms are *Staphylococcus hyicus*, the cause of exudative epidermidis, and *Staphylococcus aureus* that causes abscesses and other conditions. On occasion other staphylococcal species such as *Staphylococcus chromogenes*, *Staphylococcus epidermidis*, *Staphylococcus sciuri*, *Staphylococcus warneri*, and *Staphylococcus xylosus* may be isolated from lesions, but their role as primary pathogens should be viewed with caution. However, careful investigation of such cases may be warranted since reports of potential pathogenic strains of some of these species have been published and are discussed in this chapter.

**STAPHYLOCOCCUS HYICUS: EXUDATIVE EPIDERMIS**

Exudative epidermitis (EE) is the most common staphylococcal skin disease in swine. Also known as greasy pig disease and Marmite disease, EE is seen most frequently in newborn piglets up to about 8 weeks of age. In the classic form, affected animals develop a generalized nonpruritic dermatitis/epidermitis with loss of fluid that may lead to dehydration and death. EE is seen worldwide and has been reported in all major swine-producing countries. Although described clinically nearly 170 years ago by Spinola (1842), it was not until 1953 that the cause of EE was attributed to *Micrococcus hyicus*, now known as *S. hyicus*. Exfoliative toxins appear to play a major role in causing disease, but other factors that diminish host defenses are contributory. Treatment primarily consists of fluid replacement, topical disinfectants, and antimicrobial therapy. Isolation of strains with increased drug resistance is becoming more frequent. Localized forms of EE affecting ear tips, head, flanks, and extremities are seen primarily in older animals. *Staphylococcus hyicus* has also been associated with polyarthritis and reproductive failure (Duncan and Smith 1992; Hill et al. 1996).

**Etiology**

*Staphylococcus hyicus* is a gram-positive coccoid that is considered normal bacterial flora on the skin of adult swine. The organism can also be recovered from the environment in swine facilities. Virulent and avirulent strains of *S. hyicus* can be found on the skin of EE-affected animals as well as healthy pigs (Park 1986; Wegener et al. 1993). Virulence is closely associated with the production of exfoliative toxins (Andresen et al. 1997; Futagawa-Saito et al. 2007; Sato et al. 1991b; Wegener et al. 1993). At least six exfoliative toxins, ExhA, ExhB, ExhC, ExhD, ShetA, and ShetB, have been found in *S. hyicus* strains from diseased pigs (Ahrens and Andresen 2004; Andresen 1998; Andresen et al. 1997; Sato et al. 2000; Watanabe et al. 2000). These toxins target the cells of the stratum granulosum in the epidermis and are similar to toxins produced by strains of *S. aureus* that are isolated from staphylococcal scalded skin syndrome in humans. In addition to the presence of toxigenic *S. hyicus*, other factors predispose animals to EE. These include viral diseases, nutritional deficiencies, ringworm infection, pityriasis rosea, parasitism, poor hygiene, poor ventilation, high humidity, trauma, and genetic susceptibility.

Initially described as *M. hyicus* in 1953 (Sompolinsky 1953), *S. hyicus* was placed in the *Staphylococcus* genus in 1965 (Baird-Parker 1965). Later *S. hyicus* was divided into subspecies *hyicus* and *chromogenes* (Devriese et al. 1978). Elevation in 1986 of *S. hyicus* subsp. *chromogenes*...
to *S. chromogenes* resulted in *S. hyicus* as a singular species (Hajek et al. 1986). Colonies of *S. hyicus* on blood agar appear nonhemolytic, creamy white, convex, and circular. Biochemically, *S. hyicus* is catalase positive, oxidase negative, and Voges–Proskauer negative. *Staphylococcus hyicus* produces DNase, phosphatase, hyaluronidase, gelatinase, and lecithinase (Gillespie and Timoney 1981). Some strains produce coagulase, which is enhanced by porcine plasma (Lammler 1991). Aerobic fermentation of fructose, glucose, lactose, mannose, and trehalose is seen with most strains (Lammler 1991). A small zone of hemolysis can be seen on chocolate agar, and a CAMP-like zone of complete lysis in the zone of incomplete lysis of the staphylococcal beta-lactamase can be seen on sheep blood agar (Lammler 1991). Expression of protein A-like receptors for immunoglobulin G (IgG) is common in porcine strains (Lammler 1991). Although *S. hyicus* does not form spores, it is resistant to drying and can remain viable in the environment for long periods.

**Public Health**

*Staphylococcus hyicus* causes no disease in humans.

**Epidemiology**

*Staphylococcus hyicus* is distributed globally and is present in many herds without causing disease. In the United States, it was reported to cause sickness or mortality in 27.5% of sites with nursery-age pigs (USDA 2007) and 16.5% of pig herds in the United Kingdom (Taylor 2004). Although EE occurs sporadically, it tends to occur more frequently in piglets from start-up herds or from gilts when they are introduced into an infected herd or environment. Entire litters may be affected with high mortality. Offspring from infected carriers or animals where *S. hyicus* is endemic is generally unaffected. This scenario suggests that immunity plays an important role in preventing disease. Outbreaks are usually self-limiting and generally last 2–3 months but may persist for 12–18 months. *Staphylococcus hyicus* can be recovered from multiple skin sites on healthy pigs (Hajsig et al. 1985). Inoculation of piglets as they pass through the vagina during farrowing is suspected as a means of vertical transmission. *Staphylococcus hyicus* strains isolated from the skin of piglets within 24 hours after birth were the same type as their dams and identical to strains isolated from the same litter 3 weeks later (Wegener and Skov-Jensen 1992). Many predisposing factors for the development of EE have been suggested. These include trauma induced by fighting or biting behaviors as well as environmental hazards, mineral and vitamin deficiencies, high humidity and poor ventilation, skin parasites, ringworm, and viral infections such as poxvirus. EE has been associated with porcine circovirus type 2 (PCV2) and porcine parvovirus (PPV) (Kim and Chae 2004; Wattrang et al. 2002). In summary, the development of EE likely involves interplay of immune status, prior exposure, nutrition, housing conditions, and commitment disease, combined with strain virulence and skin entry site.

*Staphylococcus hyicus* has been found in other animal species such as cattle, goats, horses, and chickens where it may have a role in diseases such as mastitis, dermatitis, and tenosynovitis (Birgersson et al. 1992; Devriese et al. 1983; Jarp 1991; Kibenge et al. 1983; Myllys 1995; Takeuchi et al. 1985). However, it appears that species-specific differences exist, which preclude other animal sources of *S. hyicus* from causing EE in pigs (Devriese et al. 1978; Shimizu et al. 1987, 1997; Takeuchi et al. 1987).

**Pathogenesis**

Although *S. hyicus* may directly penetrate the skin, a traumatic breach of the epidermis is the most common inciting factor for EE. Initial lesions are reddening with focal erosions in the stratum granulosum. As the bacteria multiply, the lesions extend causing multifocal epidermal ulcerations, which may extend to the stratum germinativum with an accompanying supplicative folliculitis. The lesions enlarge and coalesce. Excessive sebaceous secretions and serum exudate accumulates over lesions and surrounding skin, giving rise to a greasy, moist appearance. Crusts with cracks and fissures form as the exudates dry. High numbers of bacteria are present in the adherent material. Neutrophils invade and degrade, releasing proinflammatory enzymes. The loss of fluids and electrolytes lead to dehydration and death in severely affected piglets. Older animals may develop subcutaneous abscesses, polyarthritis, and necrosis of the ears and tail.

Several virulence factors are described for *S. hyicus*. A homolog of *S. aureus* protein A with multiple binding sites for IgG is expressed that helps the bacterium resist phagocytosis (Rosander et al. 2011). Coagulase is produced which aids in the formation of a fibrin clot that can protect the *S. hyicus* from host defenses. Surface fibronecint-binding proteins are likely important in bacterial adhesion to fibronecin (Lammler et al. 1985). Additionally, *S. hyicus* produces staphylokinase and excretes lipase; these cleave proteins and phospholipids, respectively. The exact role of these factors in enhancing virulence is unclear.

Exfoliative toxins are considered the most important virulence factor in the development of EE in pigs. In 1979, Amtsberg (1979) demonstrated that culture filtrate of *S. hyicus* could cause exfoliation in the skin of piglets and suggested this was due to exotoxin production. So far, six exfoliative toxins have been identified in *S. hyicus*. Four of these were indentified in Denmark, EhA-D (Ahrens and Andresen 2004; Andresen 1998; Andresen et al. 1997), and two in Japan, SHETA and SHETB (Sato et al. 2000; Watanabe et al. 2000). Toxins EhA, EhB, EhC, EhD, and SHETB are very similar to serine proteases ETA, ETB, and ETD that are produced.
by strains of \textit{S. aureus} and cause scaled skin syndrome in humans (Ahrens and Andresen 2004). The target for this group of toxins is desmoglein (Dsg1), a cell–cell adhesion molecule found in desmosomes (Fudaba et al. 2005). Cleavage of extracellular domains of Dsg1 leads to cell separation in the stratum spinosum, exfoliation of the skin, and serous exudation. Subcutaneous injection of purified exfoliative toxins from \textit{S. hyicus} caused exfoliation in piglets and chickens (Sato et al. 1991a; Tanabe et al. 1996). Additionally, piglets experimentally inoculated with toxin-producing strains of \textit{S. hyicus} developed local erythema, exfoliation, exudation, and crusting, while those inoculated with non-toxin-producing strains developed only local erythema that disappeared within 48 hours (Tanabe et al. 1996).

Prevalence studies indicate that toxigenic \textit{S. hyicus} is found more commonly from EE-affected pigs than healthy pigs (Andresen 2005; Kanbar et al. 2008). However, there was no clear pattern as to which toxin is most common, and regional differences apparently exist. Certain strains of staphylococcal species other than \textit{S. hyicus} that harbor exfoliative toxin genes appear capable of causing EE in pigs. A strain of \textit{S. sciuri} carrying ExhC gene was reportedly isolated from a diseased piglet with EE (Chen et al. 2007). Additionally, a toxin similar to ExhB was identified in a strain of \textit{S. chromogenes}, and this strain was able to induce clinical signs of EE in experimentally inoculated pigs (Andresen et al. 2005). Whether these findings represent emerging EE pathogens or isolated instances of non-\textit{S. hyicus}-induced EE has yet to be determined.

\section*{Clinical Signs}

Piglets as young as 3–4 days may develop severe, acute EE. Listlessness, depression, and anorexia are early signs and may involve part or all of a litter. Body temperatures are seldom elevated. Although the skin is reddened, there is no pruritis. Initial lesions may occur in the axilla or groin but often go unnoticed. Brownish spots, 1–2 cm in diameter and covered by serum and exudate, appear on the skin of the face or head. The lesions later enlarge as brown crusts. The lesions enlarge and coalesce, spread from the head posteriorly, and may generalize within 24–48 hours. Although initial lesions usually affect haired areas, ulcers may develop on the tongue or in the mouth (Andrews 1979). Young pigs can die within 24 hours. Others will survive longer, but usually die in 3–10 days. Overall morbidity and mortality are usually high in younger pigs. Weanling pigs may develop lesions on the feet, which can spread up the legs onto the rest of the body. Pigs older than about 6 weeks may have only a few localized lesions largely confined to the head. Tips of tails and ears may be affected secondary to bite wounds and may lead to necrosis of these areas. Adult swine occasionally have a few brown exudative lesions on the back and flanks and are often unrelated to known out-breaks in younger pigs. Recovery takes at least 10 days, and piglets that survive acute EE may remain chronically affected and stunted. Pigs moderately affected as weanlings or growers will often survive but tend to gain weight more slowly than nonaffected pigs. Other reported outcomes of \textit{S. hyicus} infection include polyarthritis (Hill et al. 1996) and abortion (Duncan and Smith 1992).

\section*{Lesions}

Typically, gross lesions begin as focal areas of reddened skin with clear exudate. These occur most commonly in the axilla and groin but often go unnoticed. Early lesions may also form around the eyes, mouth, ears, and areas of skin trauma. Exudates soon become thick and brownish and lesions coalesce. As bacteria and dirt accumulate, exudates become greasy and black, eventually forming a generalized odororous, crusty layer (Figure 61.1). Below the crusty layer, the skin is grossly thickened and wrinkled. At necropsy, dehydration and emaciation are evident. There may be linear streaks on renal papillae and accumulation of cellular debris in ureters and pelvis of the kidneys. Lymph nodes draining the skin are swollen and edematous.

Microscopically, a serocellular crust composed of neutrophils, fibrin, and proteinaceous material containing gram-positive bacteria overlies the epidermis. The epidermis may be ulcerated or hyperplastic with multifocal to coalescing intracorneal pustules and suppurative folliculitis. The dermis is congested and edematous with multifocal hemorrhage. Perivascular to interstitial infiltration by lymphocytes, plasma cells, and histiocytes is also found in the superficial dermis. The inflammation is more pronounced underlying areas of epidermal ulceration. Suppurative lymphadenitis is seen in enlarged lymph nodes. Renal involvement ranging from vacuolation and degeneration to sloughing of renal tubular epithelium may occur and is likely the result of dehydration (Blood and Jubb 1957).
**Diagnosis**
Clinical signs and lesions are characteristic if not pathognomonic for EE in young piglets. Diagnosis of EE in adults or animals with local lesions secondary to trauma is more difficult. Confirmation can be made by isolation of *S. hyicus* or by histopathology. If an outbreak is occurring, it is important to culture the organism and perform antimicrobial susceptibility testing. The organism can readily be recovered by swabbing moist, affected areas after removing the overlying crust. Enlarged superficial lymph nodes are also good specimens for bacterial isolation. *Staphylococcus hyicus* grows well on sheep or bovine blood agar; however, secondary organisms such as *Pseudomonas*, *Proteus*, and other *Staphylococcus* species may also be isolated from EE lesions. Selective media containing potassium thiocyanate (Devriese 1977) or less than 10% NaCl may aid isolation. *Staphylococcus hyicus* can be identified by using conventional biochemical tests or panel identification systems. Toxigenic and nontoxigenic strains can be isolated from the same lesion. A method of phage typing *S. hyicus* has been described, which aids in differentiating virulent from avirulent strains (Wegener 1993). However, this is not a simple method for routine testing. An indirect enzyme-linked immunosorbent assay (ELISA) test for the detection of toxins ExhA, ExhB, and ExhC was developed as an alternative to phage typing (Andersen 1999). Additionally, identification and toxin profiling with polymerase chain reaction is possible (Andersen and Ahrens 2004; Voytenko et al. 2006). However, these tests are not yet in widespread use in veterinary diagnostic laboratories.

Other skin diseases may look similar to EE. Swine pox (Chapter 30) has localized lesions and is rarely fatal. Mange (Chapter 65) is pruritic and mites can be demonstrated in skin scrapings. Ringworm (Chapter 17) has typical expanding superficial lesions in which dermatophytes can be demonstrated by culture or microscopic examination. Pityriasis rosea (Chapter 17) has expanding nongreasy erythematous circular lesions and is self-limiting and nonfatal. Zinc deficiency (Chapter 68) causes parakeratosis that appears as symmetrical dry lesions in 2- to 4-month-old pigs. Finally, dermatosis vegetans (Chapter 17) is a rare inherited disease in Landrace, which also causes a fatal pneumonitis.

**Treatment and Prevention**
Early treatment following onset of disease provides the best chance of success, but severely affected animals may not respond. Antimicrobial drugs are commonly used to treat EE. However, reports indicate that *S. hyicus* is resistant to many antimicrobial agents (Aarestrup and Jensen 2002; Teranishi et al. 1987; Wegener et al. 1994; Werckenthin et al. 2001). Although regional differences exist, resistance to penicillin, erythromycin, streptomycin, sulfonamides, and tetracycline is a frequent occurrence. Therefore, antimicrobial susceptibility testing of isolates recovered from EE lesions is recommended to provide evidence of the appropriate treatment. In the absence of drug susceptibility results, ceftiofur, enrofloxacin, or combination of trimethoprim/sulfadimethoxazole and lincomycin are the best choices. Injection of the antimicrobial is recommended, but it may be given orally in less severe cases. A Japanese study comparing antimicrobial susceptibility profiles of toxigenic and nontoxigenic *S. hyicus* isolates concluded that there was no significant correlation between toxin gene carriage and antimicrobial resistance (Futagawa-Saito et al. 2009). Other treatments include spraying the pigs several times with skin disinfectants such as novobiocin in mineral oil, chlorhexidine, or dilute tamed iodine. Care is needed to avoid chilling, particularly in the youngest piglets. Fluid and electrolyte replacement is important in severely affected piglets and may be given orally. Cross suckling of gilt piglets with older sows may provide some passive protection. Alternatively, autogenous vaccines using strains isolated from the affected herd may be helpful in protracted disease situations. Newly acquired sows or gilts vaccinated prior to farrowing will provide some colostral protection to offspring and help ward off severe forms of EE. Efforts should be made to minimize skin breaches by clipping needle teeth, removing abrasive surfaces, and treating for mange if present. Thorough cleaning and disinfection of facilities should be carried out between farrowings, and sows entering facilities should be washed and disinfected. Control of EE may depend largely on preventing trauma and improving the environment with better ventilation, cleaner and drier pens, controlled humidity, and reduced stocking density.

**STAPHYLOCOCCUS AUREUS**
*Staphylococcus aureus* is the only staphylococcal species apart from *S. hyicus* to be consistently isolated from lesions in pigs. Besides skin infections, *S. aureus* has been associated with septicemia, mastitis, vaginitis, metritis, osteomyelitis, and endocarditis. Although commonly found in the swine facilities and on the skin of normal pigs, *S. aureus* rarely causes disease and is not associated with herd outbreaks. Recently, there has been a public health concern regarding swine as a reservoir for methicillin-resistant *S. aureus* (MRSA). A unique MRSA sequence type (ST398) was first reported in Europe (Armand-Lefevre et al. 2005) and has since been found to be the predominant MRSA type that subclinically infects pigs in many parts of the world.

**Etiology**
Like all staphylococci, *S. aureus* is positive on Gram’s stain, and it forms white to golden, opaque colonies with a double zone of hemolysis on sheep blood agar.
The inner zone of complete hemolysis is caused by alpha-hemolysins, while the outer zone of incomplete hemolysis is caused by beta-hemolysins. *Staphylococcus aureus* grows best under aerobic conditions at 95–98.6°F (35–37°C). It tends to form grapelike clusters of coccoid organisms best observed from solid media. Besides hemolysins, the organism variably produces a number of substances that may be considered virulence factors. Among these are protein A, teichoic acids, coagulase, hemolysins, the organism variably produces a number of substances that may be considered virulence factors. Among these are protein A, teichoic acids, coagulase, alpha-hemolysins, while the outer zone of incomplete hemolysis is caused by beta-hemolysins. The inner zone of complete hemolysis is caused by beta-hemolysins.

### Public Health

*Staphylococcus aureus* in humans is a frequent cause of localized skin infections as well as life-threatening septicemia, pneumonia, endocarditis, and other soft tissue and bone infections (Falcone et al. 2009). MRSA strains emerged in the 1960s and are of great concern given their poor response to treatment and consequent higher mortality rates. Traditionally, most MRSA disease has been health care associated (HCA); that is, MRSA is the leading cause ofnosocomial infections and is also associated with patients treated by home health care personnel (Tiemersma et al. 2004). More recently, MRSA-associated disease has emerged in people in the community apart from HCA risk factors, so-called community-associated (CA) MRSA (Vandenesch et al. 2003). Specific sequence types of MRSA predominate in HCA and CA infections. Colonization by MRSA has been reported in livestock including pigs (van Loo et al. 2007), so-called livestock-associated (LA) MRSA. Studies in Europe, North America, and elsewhere have demonstrated that subclinical nasal infection in pigs is common and predominantly caused by the unique multilocus sequence type ST398 that is distinct from typical HCA and CA MRSA types (Smith et al. 2009; Smith and Pearson 2010). Animal caretakers, veterinarians, and others who spend considerable time in contact with infected pigs are at significantly increased risk for testing positive for MRSA from nasal swabs relative to the general population (Smith et al. 2009; Voss et al. 2005). In a study of 285 pigs and 25 farmworkers from 20 Canadian swine farms, 45% of farms, 25% of pigs, and 20% of people were MRSA positive (Khanna et al. 2008). In the same study, isolates from pigs and people from the same farm were indistinguishable, suggesting intertransmission. However, persistence of ST398 MRSA in humans is dependent on intensity of animal contact; that is, contamination of nasal mucus is probably more frequent than true colonization of nasal mucosa (Graveland et al. 2011). Transmission from ST398 MRSA-positive farmworkers to family members not in contact with swine has been reported, but spread into the general community seems to be infrequent (Cuny et al. 2009; Graveland et al. 2011). A limited number of reports implicate strain ST398 in a range of human disease conditions similar to other *S. aureus* strains, but it has been suggested that ST398 may not cause as much disease (relative to colonization) as other human MRSA strains (Cuny et al. 2009; Smith and Pearson 2010). Many unanswered questions remain about ST398 colonization in both humans and swine that need to be addressed prior to implementing a successful bacterial prevention or control strategy on farms or in the community.

### Pathogenesis

Damage to skin and mucosal surfaces can predispose to *S. aureus*-induced skin lesions similar to EE caused by *S. hyicus*. Some strains of *S. aureus* can produce exfoliative toxins, which are similar to those found in *S. hyicus*. *Staphylococcus aureus* may also invade causing bacteremia, which can evolve to life-threatening septicemia in neonates. More often, bacteremia leads to abscesses in the bones, joints, heart valves, liver, kidney, lymph nodes, and other internal organs. Abscesses may be observed at slaughter in otherwise normal pigs. Ascending infections may lead to mastitis, vaginitis, metritis, and umbilical abscesses.

### Clinical Signs and Lesions

Because *S. aureus* is associated with numerous diseases, clinical signs are not predictive of this organism as the causative agent. Most cases occur in individual animals, and animal-to-animal spread is rare. Neonatal septicemia may be fatal or results in stunted piglets at 7–10 days of age. Umbilical abscesses, polyarthritis, and vegetative endocarditis with cardiac enlargement may be found as well or animals may be found dead with no gross lesions. Chronic infections appear as abscesses of the skin, umbilicus, bones, joints, mammary glands, and internal organs. Osteomyelitis may lead to pathological fractures particularly in vertebrae. Bone and joint infections in the feet are most often due to extension of skin infections initiated by trauma. Abscesses typically contain creamy white or bloodstained pus and are often surrounded by thick fibrous capsules. Pus may also be in the peritoneal cavity, the pericardial sac, or the lumen of the uterus. A white, nonnodorous, pustular vaginal discharge can be seen with endometritis (Roberson et al. 2007). *Staphylococcus aureus* may cause sporadic abortions (Kohler and Wille 1980) and has also been rarely isolated from cases of enteritis where enterotoxins may be contributory (Taylor et al. 1982).

### Diagnosis and Treatment

Diagnosis of *S. aureus* is typically based on culture and isolation of the organism from suggestive lesions. Aerobic growth on sheep blood agar of small (1–2 mm diameter), yellow to white, opaque colonies with double-zone hemolytic pattern is highly indicative of *S. aureus*. Further identification is accomplished by Gram stain and biochemical testing. *Staphylococcus aureus* is positive for catalase, coagulase, mannitol, and
Voges–Proskauer reagent. These tests should differentiate *S. aureus* from other abscess-causing bacteria such as *Arcanobacterium pyogenes* and streptococci. In the past, further identification of *S. aureus* isolates for public health significance was accomplished by phage typing or plasmid profiling. These have largely been replaced by other methods such as multilocus sequence typing (MLST) or protein A gene sequence typing (*spa* typing).

Treatment of individual abscesses can be accomplished by surgical drainage and antimicrobial therapy. Prompt parenteral treatment is recommended to minimize development of extensive and fatal abscess development. Susceptibility testing for appropriate antimicrobial agent is recommended since many isolates are resistant to common drugs such as penicillin, ampicillin, chlorotetracycline, oxytetracycline, and spectinomycin. Ceftiofur, enrofloxacin, and spectinomycin. Ceftiofur, enrofloxacin, and trimethoprim/sulfadimethoxazole are good choices in the clinic, and spectinomycin. Ceftiofur, enrofloxacin, and spectinomycin. Ceftiofur, enrofloxacin, and trimethoprim/sulfadimethoxazole are good choices in the absence of individual isolate testing. Since *S. aureus* is somewhat resistant to disinfectants especially in heavily contaminated areas is prudent. *Staphylococcus aureus* is somewhat resistant to disinfectants especially when protected by organic materials, but it is sensitive to disinfectants, such as phenols, hypochlorites, iodine, and iodophors.

**REFERENCES**

INTRODUCTION

Several streptococcal species can be found in the tonsils, intestines, and genital tracts of clinically healthy pigs, and some of them are potential pathogens. The following are considered part of the intestinal microflora in swine: *Streptococcus hyointestinalis* (Devriese et al. 1988), *Streptococcus suis*, *Streptococcus alactolyticus* (*Streptococcus intestinalis*), and *Streptococcus bovis* (Devriese et al. 1994b). *Streptococcus suis*, *Streptococcus porcinus*, and *Streptococcus dysgalactiae* subsp. *equisimilis* (Vieira et al. 1998) are generally found in the tonsils (Devriese et al. 1994b). *Streptococcus orisuis* and streptococcal mutans-like strains are normal inhabitants of the oral cavities of pigs (Takada and Hirasawa 2007; Takada et al. 2008). Vaginal microflora may include some of the aforementioned species, as well as *Streptococcus hyovaginalis* and *Streptococcus thoraltensis* (Devriese et al. 1997). Members of the genus *Enterococcus* such as *Enterococcus faecalis*, *Enterococcus faecium*, *Enterococcus durans*, *Enterococcus hirae*, and *Enterococcus villorum* are also important members of the intestinal microflora.

In this chapter, diseases associated with streptococci and enterococci are discussed. Emphasis is given to *S. suis* owing to its importance in recent years, both in the swine industry and as a zoonotic infection. Less common conditions caused by other streptococci such as *S. porcinus* (Lancefield groups E, P, U, and V) and *S. dysgalactiae* subsp. *equisimilis* (Lancefield groups C, G, and L) as well as by enterococci are also discussed.

STREPTOCOCCUS SUIS

Etiology and Prevalence

*Streptococcus suis* is an encapsulated gram-positive coccus that possesses cell wall antigenic determinants somewhat related to Lancefield group D. Initial reports of *S. suis* infections were published by Jansen and Van Dorssen (1951) in The Netherlands and by Field et al. (1954) in England. Since then *S. suis* has been reported globally in both traditional and modern intensive swine operations.

There are 35 serotypes based on capsular polysaccharides (CPSs). Some confusion exists regarding early Lancefield groups R, S, and T, and their relationship with group D streptococci and the different *S. suis* capsular serotypes. The original “Lancefield groups R and S” were later identified as *S. suis* (Lancefield group D) and reclassified as serotype 1 (formerly group S), serotype 2 (formerly group R), and serotype 1/2 (formerly group R/S) (Windsor and Elliott 1975). Some years later, group T was also reclassified as serotype 15 (Gottschalk et al. 1989). The terminology of Lancefield groups R, S, RS, and T, sometimes used in papers describing human infections, should not be used to avoid confusion (Gottschalk et al. 2010b).

Between 1983 and 1995, 32 of the 35 recognized serotypes were described (Gottschalk et al. 1989, 1991; Higgins et al. 1995; Perch et al. 1983). Reference strains originated mostly from diseased pigs, although some originated from clinically healthy pigs, a diseased human, diseases calves, or a diseased lamb (Gottschalk et al. 1989; Higgins et al. 1995). *Streptococcus suis* was officially recognized as a new species in 1987 (Kilpper-Balz and Schleifer 1987) because it was genetically distinct and displayed no specific relationship with other streptococcal species examined. Subsequent comparative genomic analysis of serotype 2 strains demonstrated that approximately 40% of their 2-Mb genome is distinct from other *Streptococcus* species (Chen et al. 2007; Holden et al. 2009). Two serotypes (serotypes 32 and 34) may be excluded from *S. suis* and redesignated...
as *Streptococcus orisratti* (Hill et al. 2005), although results of studies conflict and there is currently no consensus. Strains within each serotype of *S. suis* are genetically diverse (Blume et al. 2009; Luey et al. 2007; Marois et al. 2006). This diversity needs consideration in diagnosis, surveillance, and control of the disease.

A majority of *S. suis* strains isolated from diseased pigs are serotypes 1–9 (Fittipaldi et al. 2009; Hogg et al. 1996; Messier et al. 2008; Reams et al. 1996; Vela et al. 2005). Although serotype 2 isolates predominate in most countries, other serotypes assume importance in certain locales including serotype 9 in some European countries (Vela et al. 2005; Wisselink et al. 2000), serotypes 1 and 14 in the United Kingdom (Heath and Hunt 2001) and serotype 7 in Scandinavian countries (Baums and Valentin-Weigand 2009). Untypeable isolates are uncommonly recovered mostly from sporadic occurrences of disease (Fittipaldi et al. 2009; Messier et al. 2008). Many of them are nonencapsulated, suggesting that they are members of known serotypes that lost CPS during in vitro culture (M. Gottschalk, unpublished observations). Serotype 2 is considered the most prevalent and virulent serotype in most Eurasian countries (Berthelot-Hérault et al. 2000; Wei et al. 2009; Wisselink et al. 2000). The situation seems to be different in North America. Although serotypes 2 and 3 are the most prevalent in diseased pigs, serotype 2 does not predominate and has a prevalence typically below 25% in Canada (Messier et al. 2008) and in the United States (Fittipaldi et al. 2009). It may be that Eurasian and North American serotype 2 strains of *S. suis* possess a different virulence potential (Gottschalk and Segura 2000; Gottschalk et al. 2010b).

**Public Health**

*Streptococcus suis* is an emerging zoonotic agent that has increased in importance in the last 5 years. Small skin wounds are the main route of entry in humans, although in some cases no wound is evident (Gottschalk et al. 2010b). Liquid soap inactivates *S. suis* type 2 in less than 1 minute at a dilution of 1 in 500, suggesting that soap and water washing would satisfactorily remove skin contamination (Clifton-Hadley and Enright 1984). *Streptococcus suis* can also colonize the nasopharynx and gastrointestinal tract in humans; diarrhea is often a prodromal clinical sign (Wertheim et al. 2009). The incubation period ranges from a few hours to 2 days. In humans, *S. suis* usually produces purulent meningitis, although endocarditis, cellulitis, peritonitis, rhabdomyolysis, arthritis, spondylodiscitis, pneumonia, uveitis, and endophthalmitis have also been reported (Wertheim et al. 2009). Mortality rates vary from less than 3% in most Western countries to 26% in some Asian countries (Gottschalk et al. 2010b). Deafness with vestibular dysfunction is the most striking sequel of *S. suis* meningitis in humans (Wertheim et al. 2009). Besides penicillin, ceftriaxone is used for the treatment of human *S. suis* meningitis worldwide (Gottschalk et al. 2010b; Wertheim et al. 2009).

In more than 20 Western countries where human infections are described, *S. suis* disease is considered a rare event. Most cases of human infection are related to employment in the swine industry including pig farmers, abattoir workers, people who transport pork, meat inspectors, butchers, and swine veterinarians (Gottschalk et al. 2010b). Handling diseased pigs increases the risk of human disease. However, colonization of human mucosa may also occur after handling infected clinically normal pigs. Many reports confirm isolation of *S. suis* from tonsils of healthy human carriers, mostly abattoir workers (Gottschalk et al. 2010b). In New Zealand and the United States, relatively high antibody titers against *S. suis* serotype 2 are reported in people with occupational contact with pigs (Robertson and Blackmore 1989; Smith et al. 2008). However, these findings should be interpreted cautiously since there are no standardized serological tests to detect *S. suis* antibodies in humans or pigs.

An outbreak in China in 2005 where 39 of 200 affected people died attracted worldwide attention and led to increased awareness of and research on human *S. suis* in subsequent years (Gottschalk et al. 2010b; Ye et al. 2006). A unique feature of the Chinese outbreak was high incidence of systemic disease with relatively low incidence of meningitis. The clinical presentation was characterized as streptococcal toxic shock-like syndrome (STSS) (Tang et al. 2006). There are many other reports in the literature indicating severe cases of *S. suis* septic shock that are similar to those described in the STSS (Gottschalk et al. 2010b). The fatality rate reached 63% among patients with septicemia and septic shock in the Chinese outbreak (Ye et al. 2006).

The situation of the human infection caused by *S. suis* in Asian countries differs greatly from Western countries. Human *S. suis* infection has recently been reported as the third most common culture-confirmed cause of community-acquired bacterial meningitis in Hong Kong and the most frequent cause of bacterial meningitis in adults in Vietnam (Gottschalk et al. 2010b). Differences in culture in Asian compared with Western countries likely impact the epidemiology of *S. suis* including lifestyle, common use of backyard systems of production, close contact of different animal species including humans with pigs, and the common practice of consuming raw pork (Gottschalk et al. 2010b). Consumption of raw or undercooked pork is the likely route of exposure in people not engaged in the swine industry in Asian countries (Gottschalk et al. 2010b). Since *S. suis* can survive in carcasses at 39°F (4°C) for 6 weeks, chilled or frozen meat could be a hazard long after being butchered. It has been shown that a high percentage of pork samples are positive for *S. suis* in markets in Asia (Cheung et al. 2008).
Other potential reasons that the prevalence of human *S. suis* disease seems to be very high in Asia, relatively high in Europe, and low in North America include misdiagnosis and greater prevalence of virulent strains for humans in some geographical areas (Gottschalk et al. 2010b). *Streptococcus suis* is a pathogen very well known by laboratories of human disease in Asia, relatively well known in Europe, and generally poorly known in America. Many diagnostic laboratories working in human diseases in Western countries (especially in North America) are unaware of this bacteria and often misidentify it as enterococci, *Streptococcus pneumoniae*, *S. bovis*, other streptococci, or even *Listeria* (Gottschalk et al. 2010b). Limited information suggests that strains differ in human virulence from different geographical regions. In addition to serotype 2, serotype 14 has been isolated from humans many times in Thailand and United Kingdom and uncommonly in France, Australia, and Canada (Gottschalk et al. 2010b). Sporadic cases due to other serotypes, such as serotype 4, serotype 1, and serotype 16, have also been reported (Gottschalk et al. 2010b). Some have suggested that a highly virulent strain for humans with unique pathogenicity is present in China; however, further studies are needed to confirm this (Gottschalk et al. 2010b). The relatively low prevalence of serotype 2 strains isolated from diseased pigs in North America compared with Europe and Asia might also explain the low number of human cases in North America. Because serotype 2 strains predominate in human cases, it may be that a lower prevalence of serotype 2 in pigs in North America translates to lower transmission to humans and lower prevalence of human disease.

**Epidemiology**

**Natural Habitat.** The natural habitat of *S. suis* is the upper respiratory tract, particularly the tonsils and nasal cavities, and the genital and alimentary tracts of pigs (Baele et al. 2001; Devriese et al. 1994b; Hogg et al. 1996; Luque et al. 2010). It is easily detected in pigs of all ages (Luque et al. 2010; Macllnnes et al. 2008). Serotype 2 strains can be carried subclinically in healthy pigs, but usually in a relatively low number of herds without clinical signs of infection and in a low number of piglets within these herds (Marois et al. 2007; Monter Flores et al. 1993). In herds with clinical signs, the number of *S. suis* serotype 2 carrier animals is usually high (Marois et al. 2007). Serotypes 9–34 are more likely to subclinically colonize nasal cavity and vagina than cause disease (Hogg et al. 1996). More than one serotype of *S. suis* often colonizes individual pigs. In one study, 31% of pigs had only one serotype of *S. suis* in their nasal cavities, 38% had two or three serotypes, and 6% had more than four serotypes (Monter Flores et al. 1993).

Wild boars are known to carry *S. suis* and may be an important reservoir in some countries (Baums et al. 2007). *Streptococcus suis* is also increasingly isolated from a wide range of animal species and birds (Devriese et al. 1994a; Higgins et al. 1990, 1995) that potentially could act as a reservoir and transmit virulent strains.

**Transmission.** Transmission of virulent strains between herds usually occurs by the movement of healthy carrier animals. The introduction of carrier pigs harboring virulent strains (breeding gilts, boars, weaners) into a noninfected recipient herd may result in the subsequent onset of disease in weaners and/or growing pigs. Sows infect their piglets during birthing via contamination from vaginal colonization and probably through the respiratory route (Amass et al. 1997; Clifton-Hadley et al. 1986b; Cloutier et al. 2003; Robertson et al. 1991). Although most weaned piglets carry *S. suis* strains, few carry virulent strains capable of inducing the disease after weaning (Cloutier et al. 2003; Marois et al. 2007). It has been reported for serotypes 2 and 5 that, even though different strains within the same serotype are present in the herd, a single strain usually causes disease (Cloutier et al. 2003; Marois et al. 2007). For other serotypes, such as serotype 1/2, strains isolated from diseased animals seem to be similar to those recovered from carrier animals in herds without clinical disease (Martinez et al. 2002). Horizontal transmission is important especially during outbreaks when diseased animals shed higher numbers of bacteria, increasing transmission by direct contact or aerosol (Cloutier et al. 2003).

Aerosol transmission without nose-to-nose contact has been confirmed for *S. suis* serotype 2 (Berthelot-Hérault et al. 2001). Serotype 1 and 2 strains were isolated from feed troughs of piglets and sows (Robertson et al. 1991), suggesting that environmental contamination may also be important in spread. *Streptococcus suis* may also be transmitted via fomites (Dee and Corey 1993; Robertson et al. 1991). Enright et al. (1987) demonstrated that flies can carry *S. suis* serotype 2 for at least 5 days and can contaminate materials on which they feed for at least 4 days. Thus, flies could spread infection within farms and between farms. The importance of other animal species (including birds) as reservoirs or vectors of the infection has still to be determined.

**Survival in the Environment.** The durability of *S. suis* in various environmental conditions has been studied using serotype 2 strains. Viability in water at 39°F (4°C) is retained for 1–2 weeks. In experimentally inoculated feces, *S. suis* survives at 32°F (0°C), 48°F (9°C), and 72–77°F (22–25°C) for 104, 10, and 8 days, respectively. In dust, survival at 32°F (0°C), 48°F (9°C), and 72–77°F (22–25°C) is for 54, 25, and 0 days. Thus, at a summer-time or nursery temperature of 72–77°F (22–25°C), the organism could survive about 8 days in feces but less
Virulence Factors and Pathogenesis of the Infection

Nearly all studies on virulence factors, pathogenesis, mechanisms of protection, and animal models of disease have used serotype 2 strains (Gottschalk et al. 2001). Extrapolation of findings to other serotypes should be done with caution. Determination of virulence factors for S. suis as well as protection studies have been hampered by variation in experimental design. There is a lack of uniformity in definition of virulence owing to different parameters being used to discriminate between virulent and avirulent strains (Gottschalk et al. 1999a). There is also much variation in animal models. Inoculation studies have been in pigs with different health status and different ages as well as in mice, guinea pigs, zebra fish and microbiologically defined Göttingen minipigs (Kay 1991; Madsen et al. 2001; Wu et al. 2010). Different routes of infection, variable bacterial doses, and animals pre-infected (or not) with other infectious agents or mucosal chemical irritants have also been used (Gottschalk et al. 1999a; Pallarés et al. 2003). As a consequence, important discrepancies exist in the literature regarding even the virulence of the same strain (Berthelot-Hérault et al. 2005). Nevertheless, it is clear that some serotype 2 strains are virulent while others are avirulent (Gottschalk and Segura 2000).

Virulence factors of S. suis are complicated and incompletely understood. Some described potential virulence factors are either not essential for virulence, are found in both virulent and avirulent strains, or could not be properly studied due to the unavailability of knockout mutants. For details, readers are invited to consult the excellent recent review of virulence factors of S. suis by Baums and Valentin-Weigand (2009).

The best validated virulence factor of S. suis is the CPS that is an important antiphagocytic factor. Its chemical structure has recently been reported as being D-Gal, 3; D-Glc, 1; D-GlcNAc, 1; D-Neu5Ac, 1; and L-Rha, 1 with terminal sialic acid (van Calsteren et al. 2010). Even though CPS is shown to be a major virulence factor, most avirulent strains are also encapsulated, indicating that multiple virulence factors are necessary for full virulence. Unencapsulated strains might also invade host tissue, though to a lesser extent than encapsulated strains (Baums and Valentin-Weigand 2009).

Bacterial cell wall components may be surface exposed even in encapsulated strains, inducing an exaggerated inflammatory response of the host. Peptidoglycan as well as teichoic and lipoteichoic acid components have been implicated as virulence factors, mainly involved in resistance to killing by phagocytic cells, adherence to host cells, resistance against cationic antimicrobial peptides, and/or induction of exaggerated inflammation (Fittipaldi et al. 2008a, b). In addition, several proteins with a C-terminal cell wall sorting signal including LPXTG or related motifs are sometimes important in virulence when compared with isogenic mutants lacking some of these factors (Baums and Valentin-Weigand 2009). Serotype 2 strains also possess a truncated homolog of Group B Streptococcus pilus island 2b, which includes the two genes encoding the ancillary and the major pilin subunits, named srtF cluster (Takamatsu et al. 2009). Pili are expressed from this cluster, although pili are formed by the major pilin subunit only due to nonsense mutations at the 5′ end of the gene coding for the minor pilus subunit, a putative adhesin (Fittipaldi et al. 2010; Holden et al. 2009). Other proteins including those lacking a known C-terminal cell wall sorting signal, hemagglutinins, lipoproteins, and enzymes have also been suggested as virulence factors of S. suis serotype 2 (Baums and Valentin-Weigand 2009). Factors secreted by S. suis are also suggested as virulence factors. Among these, the hemolysin (sulysin) is the best characterized, being toxic to epithelial, endothelial, and phagocytic cells (Gottschalk and Segura 2000). Additional potential virulence factors are recently described for the strain of S. suis type 2 purportedly highly pathogenic for humans that was involved in the 2005 human outbreak in China (Feng et al. 2010; Gottschalk et al. 2010b).

Although the bulk of literature may not confirm some putative virulence factors as significant, these factors may nonetheless still serve as virulence markers and/or be useful for phenotypic comparison of strains. Examples include the proteins muramidase-released protein (MRP) and extracellular factor (EF) (Smith et al. 1997; Vecht et al. 1991) as well as sulysin (Jacobs et al. 1994). Although isogenic mutants lacking either MRP and/or EF proteins or sulysin were shown to be as virulent to pigs as the respective parent strain (Allen et al. 2001; Smith et al. 1997), there is positive association between the presence of these proteins and strain viru-
Inflammation plays an important role in the pathogenesis of *S. suis*-induced septicemia and meningitis. Bacterial cell wall components are known to induce the production of proinflammatory cytokines by murine, human, and swine cells (Gottschalk and Segura 2000). In addition, hemoglobin that is released in vivo by the action of suilysin on red blood cells may also act in synergy with suilysin as an inducer. In a standardized mouse model of *S. suis* infection, Domínguez-Punaro et al. (2007) credited high levels of several proinflammatory cytokines and chemokines within 24 hours postinfection with the induction of septic shock and early death of some animals. Likewise, they also found that shortly after *S. suis* invasion of the CNS by *S. suis*, there is transcriptional activation of proinflammatory cytokines and chemokines and induction of inflammation that causes CNS clinical signs. Cases in humans and pigs with presence of shorter incubation time, more rapid disease progression, and a higher rate of mortality have been recently described (Feng et al. 2010; Gottschalk et al. 2010a,b) and may be due, in part, to enhanced induction of inflammation.

### Clinical Signs and Lesions

Even when the pig carrier rate is near 100%, the incidence of the disease varies from period to period and is usually less than 5% (Clifton-Hadley et al. 1986a). However, in the absence of treatment, mortality rates can reach 20% (Cloutier et al. 2003). In most cases, affected animals are generally between 5 and 10 weeks of age, but cases in pigs up to 32 weeks of age and a few hours old have also been described (Cloutier et al. 2003; Lapointe et al. 2002; MacInnes and Desrosiers 1999). Reams et al. (1996) indicated that in 75% of *S. suis* cases reported in the United States, pigs were 16 weeks or less of age. The earliest sign is usually a rise in rectal temperature to as high as 108.5°F (42.5°C). This may occur initially without any other obvious signs. It is accompanied by a detectable bacteremia or pronounced septicemia which, if untreated, may persist for up to 3 weeks. During this period, there is usually a fluctuating fever and variable degrees of poor appetite, depression, and shifting lameness (Clifton-Hadley et al. 1984; MacInnes and Desrosiers 1999).

In peracute cases, pigs may be found dead with no premonitory signs. A proportion of affected pigs typically develop nervous disease as a consequence of meningitis. Early nervous signs include incoordination and adoption of unusual stances, which soon progress to inability to stand, paddling, opisthotonus, convulsions, and nystagmus. The eyes are often staring, with reddening of mucous membranes. Less remarkable clinical signs include malaise and anorexia owing to septicemia and pneumonia as well as lameness caused by the bacterial invasion of the CNS (Segura et al. 2002; Tenenbaum et al. 2006, 2009).

A general overview of the pathogenesis of the infection is presented in Figure 62.1. Survival of the organism in the bloodstream may be facilitated by the CPS and cell wall components that efficiently hamper phagocytosis (Charland et al. 1998; Fittipaldi et al. 2008a,b; Smith et al. 1999). An early theory suggested that *S. suis* is phagocytosed by monocytes in the absence of specific antibodies, survives intracellularly, and transits the bloodstream as well as enters the central nervous system (CNS) in monocytes ("Trojan horse theory") (Williams and Blakemore 1990). Subsequent studies in numerous laboratories instead indicate that bacteria travel in the bloodstream extracellularly, either free in circulation or attached to the surface of monocytes ("modified Trojan horse theory") (Baums and Valentín-Weigand 2009; Gottschalk and Segura 2000; Segura and Gottschalk 2002). Furthermore, suilysin seems to contribute to the ability of encapsulated *S. suis* to resist killing by porcine phagocytes in the presence of complete serum lacking specific antibodies (Benga et al. 2008; Chabot-Roy et al. 2006).

The mechanisms by which *S. suis* is able to cross the blood–brain barrier (BBB) are only partially understood. Brain microvascular endothelial cells (BMECs) together with choroid plexus epithelial cells (CPECs) constitute the structural basis of the BBB. *Streptococcus suis* adheres to and invades BMEC with or without cytotoxicity (Benga et al. 2005; Vanier et al. 2004). *Streptococcus suis* also affects porcine CPEC barrier function and integrity through CPEC cell death that is predominantly due to necrosis, but apoptosis may also contribute. These and probably other mechanisms facilitate *S. suis* invasion of the CNS (Segura et al. 2002; Tenenbaum et al. 2006, 2009).
by arthritis. Less common clinical manifestations of *S. suis* are vegetative valvular endocarditis, rhinitis, abortion, and vaginitis (Sanford and Tilker 1982). In North America, *S. suis* is the infectious agent most frequently isolated from cases of endocarditis in pigs. Affected pigs may die suddenly or show various levels of dyspnea, cyanosis, and wasting.

In the United Kingdom, infections due to *S. suis* serotype 2 were primarily associated with septicemia and meningitis in weaned pigs (Windsor and Elliott 1982). In North America, *S. suis* is the infectious agent most frequently isolated from cases of endocarditis in pigs. Affected pigs may die suddenly or show various levels of dyspnea, cyanosis, and wasting.

### 62.1. Summary of proven and proposed steps in the pathogenesis of meningitis by *Streptococcus suis* serotype 2

Steps A and C show *S. suis* colonization of the epithelial cells of the upper respiratory tract and access to blood. Step A: Hem+ strains may use cell disruption (toxicity) and invasion to reach the bloodstream; mechanisms used by adhered Hem− strains are still uncertain. Step B: Bacteria may be directly taken up by Mo/Mφ and enter the bloodstream within circulating cells, but this is unlikely as *S. suis* is highly resistant to phagocytosis. Other routes of entry (humans) might include skin abrasions and oral contamination followed by intestinal tract translocation. Step C: In the bloodstream absent of specific antibodies, well-encapsulated *S. suis* resists complement-mediated phagocytosis and killing. It attaches to but is not ingested by professional phagocytic cells. Thus, *S. suis* transits in blood as free bacterial cells (step D), and also as Mo-associated, extracellular bound bacteria (step E). Steps F, G, H, I, and J describe the possible mechanisms used by *S. suis* to cross the BBB. Step F: Free Hem+ bacteria increase BBB permeability by necrosis of BBB cells through secretion of cytotoxic suilysin and possibly induction of apoptosis as well as by induction of inflammatory cytokines AA, PGE, and MMP by BBB-forming cells. Step G: Free Hem− bacteria may increase BBB permeability mainly via induction of apoptosis. Step H: Induction of inflammatory cytokines by both Hem+ and Hem− *S. suis* increase expression of CAMs and leukocyte migration that in turn “open the door” to free bacterial trafficking. Step I: Direct invasion and translocation of free Hem+ or Hem− bacteria across BBB cells have been reported. Step J: *S. suis* may cross the BBB inside macrophages ("Trojan horse," less probable) or adhered to the surface of macrophages ("modified Trojan horse," probable); mechanisms favored by release of PGE and MMP by activated phagocytes. Hem+, hemolysin(suilysin)positive strains; Hem−, hemolysin negative strains; Mo, monocytes; Mφ, macrophages; CAMs, cell adhesion molecules; BBB, blood–brain barrier; CNS, central nervous system; C′, complement; AA, arachidonic acid; MMP, matrix metalloproteinase; PGE, prostaglandin E.

lesions, in the absence of other pathogens, is still con-

Evidence of encephalitis, edema, and con-
sidered a lesion secondary to septicemia (Reams
et al. 1994, 1996). Strains considered for inclusion in autogenous vaccines should be isolated from systemic sites such as meninges, spleen, liver, and joints, and not from the lungs, nasal cavities, or tonsils.

Direct detection of \textit{S. suis} from infected tissues has also been studied (Boye et al. 2000), although its application is limited due to poor differentiation of serotypes. A colloidal gold-based immunochromatographic assay for the rapid detection of both \textit{S. suis} serotype 2 and 1/2 has recently been described, but the technique was validated with only cultured bacteria and its use with infected organs remains to be confirmed (Ju et al. 2010). Detection of some serotypes of \textit{S. suis} by polymerase chain reaction (PCR) has also been reported (Wisselink et al. 2002a). However, these methods are not used in routine diagnosis in veterinary medicine, although they have been used in humans (Gottschalk et al. 2010b).

After isolation, biochemical identification of \textit{S. suis} isolates is possible with a minimum of tests when serotyping is available (Higgins and Gottschalk 1990). Devriese et al. (1991) suggested the use of only two tests on pig isolates: amylase positive and Voges–Proskauer (acetoin) negative. However, this simplified identification schema can only be used for isolates recovered from diseased or dead pigs and from sites other than the upper respiratory tract.

Serotyping is still an important part of the routine diagnostic procedure. It can be carried out by different techniques, but many laboratories have adopted the coagglutination technique. Since the majority of type-
able isolates belong to serotypes 1–9 and 1/2, it is advisable for diagnostic laboratories to only use antisera corresponding to those serotypes and to send untype-
able isolates to a reference laboratory (Higgins and Gottschalk 1990; Hogg et al. 1996). Some isolates cross-react with more than one typing antisera in the

\begin{flushleft}
\textbf{Diagnosis}
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\textbf{Diseased Pigs.} Presumptive diagnosis of \textit{S. suis} infec-
tions is generally based on clinical signs, age of animals, and macroscopic lesions. Confirmation is achieved by the isolation of the infectious agent and observation of typical microscopic lesions in tissues. Collection of more than one alpha-hemolytic colony from different tissues of the same animal or from different animals in the same herd is recommended, because multiple serotypes and strains of \textit{S. suis} can be involved in a single outbreak (Higgins and Gottschalk 1990; Reams et al. 1996). Because strains causing disease in herds may change over time, Amass et al. (1997) suggested ongoing surveillance by periodic culture of CSF from pigs with meningitis. This is especially important to upgrade autogenous vaccines when used as a method of control. Strains considered for inclusion in autogenous vaccines
coagglutination and the capsular reaction tests. The most important cross-reactions are serotype 1/2 isolates with antisera against serotypes 1 and 2, and serotype 1 isolates with antiserum against serotype 14 (Gottschalk and Segura 2000; Gottschalk et al. 1989). However, atypical specific cross-reactions (mixed capsular antigens in the same isolate) can be sometimes observed with field strains (International Reference Laboratory for the serotyping of S. suis; M. Gottschalk, unpublished observations).

Genetic tools are useful in distinguishing isolates of S. suis, in determining the origin of infection in a given herd, in monitoring the kinetics of an outbreak, or in selecting strain(s) for inclusion in a vaccine. Restriction fragment length polymorphisms, randomly amplified polymorphic DNA, ribotyping, pulsed-field gel electrophoresis, and multiple-locus variable tandem repeat number analysis have been used (Li et al. 2010; Martinez et al. 2002; Tian et al. 2004). Genetic diversity exists among S. suis isolates within and between serotypes (Blume et al. 2009; Princivalle et al. 2009). Genomic fingerprinting allows the identification of isolate(s) involved in outbreaks within a herd (Cloutier et al. 2003). In some outbreaks, several distinct strains of S. suis serotype 2 may be isolated from systemic sites in the same or different animals (Marois et al. 2007). In herds affected with serotypes other than 2, such as serotype 1/2, clinical manifestations of the disease were more likely to be the result of inherent herd factors than the virulence of a specific isolate (Martinez et al. 2002). Overall, the great genetic heterogeneity of strains of S. suis, the isolation of different strains within the same herd, and the dominance or not of particular strains in clinical disease are evidence that infection by S. suis is a dynamic process and reinforce the idea that its epidemiology is very complex (Vela et al. 2003). Isolates of S. suis serotype 2 from different geographical origins may be genotypically and phenotypically different (Rehm et al. 2007).

For example, strains of S. suis serotype 2 from Europe and Asia (presenting an MRP+, EF+, suilysin+ phenotype) seem to be genotypically different from North American strains, which are mostly negative for these markers.

**Surveillance in Clinically Healthy Pigs.** Detection of S. suis from the tonsils or nasal cavities has no practical utility in the diagnosis of S. suis disease. *Streptococcus suis* is a normal inhabitant of the upper respiratory tract and is present in almost all herds, and mixtures of avirulent and virulent strains of different serotypes may reside in the same pig or herd (Baele et al. 2001; MacInnes et al. 2008). These sites are highly contaminated, and traditional bacterial isolation presents a low sensitivity (Gottschalk et al. 1999b). The presence of a specific serotype of S. suis in high numbers in nasal cavities and/or tonsils may reflect active transmission of the infection rather than a carrier state (Cloutier et al. 2003; Marois et al. 2007). The prevalence of a specific serotype may also be underestimated in nasal swabs or tonsils due to the lack of sensitivity of the isolation method. Selective isolation using immunomagnetic beads coated with serotype-2-specific antibodies or use of PCR significantly increases the detection rate from tonsils when compared with the standard isolation technique (Cloutier et al. 2003; Gottschalk et al. 1999b; Marois et al. 2007). However, the PCR for serotype 2 strains based on detection of the CPS gene will also detect serotype 1/2 strains (Marois et al. 2004; Wisselink et al. 2002a). PCR tests for direct detection of EF or MRP positive strains from tonsils have also been described (Swildens et al. 2005; Wisselink et al. 1999); however, virulent strains may be negative for both factors. Since many streptococcal species similar to S. suis are also normal inhabitants of the upper respiratory tract, untypeable isolates recovered from tonsils or nasal cavities should be confirmed as S. suis by genetic methods (Okwunabua et al. 2003). Biochemical identification alone may be misleading. Up to 50% of isolates recovered from tonsils of healthy pigs and biochemically identified as being S. suis may be negative using a species-specific PCR. Some of these non-suis streptococcal species even react with serotype-specific S. suis antisera, which further complicates identification (M. Gottschalk, unpublished observations).

Serological tests for the detection of antibodies against S. suis have been evaluated (del Campo Sepúlveda et al. 1996; Kataoka et al. 1996). However, these tests are generally not useful. A herd/strain-specific enzyme-linked immunosorbent assay (ELISA) test using a protein extract of the predominant virulent S. suis strain from the herd allowed the evaluation of maternal antibody levels to best time vaccination as well as monitoring of antibody levels after either natural infection or vaccination (Cloutier et al. 2003; Lapointe et al. 2002). Practically this approach has little utility as a routine test owing to the marked diversity of strains infecting herds and the impracticality of developing herd/strain-specific serology tests. Recently, a whole cell ELISA test has been reported (but not validated) to measure exposure to S. suis in humans (Smith et al. 2008).

**Treatment**

The choice of the best antibacterial agent against S. suis infections should be based on susceptibility of the organism, the type of infection, and the mode of administration. Determination of minimal inhibitory concentrations demonstrated a large number of isolates moderately susceptible to penicillin, but the sensitivity rate to amoxicillin and ampicillin was around 90% (Dee and Corey 1993; Shryock et al. 1992). Data from six European countries showed a high level of resis-
tance to tetracycline (48.0–92.0%) and erythromycin (29.1–75.0%) in all countries, whereas the level of resistance to ciprofloxacin and penicillin differed between the reporting countries (Hendriksen et al. 2008). No isolates from the United Kingdom, France, and The Netherlands were resistant to penicillin, whereas from Poland and Portugal, 8.1% and 13% of isolates were resistant to penicillin and 12.6% and 79% to ciprofloxacin. Another study encompassing seven countries showed no resistance to cefotiofur, florfenicol, enrofloxacin, and penicillin, whereas a low proportion of strains were resistant to gentamicin (1.3%), spectinomycin (3.6%), and trimethoprim/sulfamethoxazole (6.0%) and most were resistant to tetracycline (75.1%) (Wisselink et al. 2006). Serotype-specific patterns of susceptibility to antimicrobials are described and may differ between regions (Hendriksen et al. 2008; Marie et al. 2002; Vela et al. 2005). MacInnes and Desrosiers (1999) suggested that ampicillin, cefotiofur, gentamicin, tiamulin, and a combination of trimethoprim and a sulfonamide are the most useful antibacterial products for parenteral treatment. However, the choice of antimicrobials used for the treatment of diseased animals should preferably be based on the knowledge of the local pattern of resistance and, perhaps, on the serotype involved (Dee and Corey 1993).

Early recognition of nervous signs including holding back of ears, squinting, and dog sitting followed by immediate parenteral treatment with an appropriate antibiotic with or without an anti-inflammatory agent maximizes pig survival (Amass et al. 1997). During an outbreak, pigs should be checked two to three times daily and new cases should be treated. In segregated early-weaned pigs with acute *S. suis* meningitis, excellent results were obtained with injection of penicillin and dexamethasone (Clark 1995). In outbreaks where response to parenteral treatment of acute cases is poor, it is advisable to treat all the pigs in a pen when one is affected or found dead (MacInnes and Desrosiers 1999). Treatment can also be administrated via the drinking water or in medicated feed. However, treatment needs to be started very quickly. Whichever method of medication is to be used, treatment should be continued for at least 5 days (Denicourt and Le Coz 2000).

**Prevention**

**Reduction of Predisposing Factors.** *Streptococcus suis* emerged as an important pathogen with intensification of the swine industry. In addition to virulence of involved strains, other factors impact development of disease including immune status of the herd, mixing of infected and naive uninfected pigs, concurrent infections and immunosuppression, quality of the environment, and other management factors. Control of these factors help prevent disease in herds with virulent strains.

Overcrowding, poor ventilation, excessive temperature fluctuations, and mixing of pigs with an age spread of more than 2 weeks seem to be the most important stress factors involved in the development of *S. suis* infection in susceptible pigs (Dee and Corey 1993). Management practices such as all-in/all-out pig flow can help reduce the incidence of the disease. Dividing large buildings into smaller rooms can help minimize temperature fluctuations and the age spread between pigs. Cleaning each room between groups of pigs reduces buildup of microorganisms and improves health status, average daily gain, and feed conversion (Dee and Corey 1993).

Infection with some viruses is known to render pigs more susceptible to *S. suis* disease or potentiate lesions in pigs. Control of such viruses helps minimize the impact of *S. suis*. Acute infections with virulent North American strains of porcine reproductive and respiratory syndrome virus (PRRSV) significantly increase susceptibility to *S. suis* disease (Galina et al. 1994; Thanawongnuwech et al. 2000). Likewise, piglets infected with PRRSV acquired in utero are more susceptible to infection and disease following challenge by *S. suis* serotype 2 (Feng et al. 2001). Concurrent infection with pseudorabies (Aujeszky's) virus may enhance clinical disease caused by *S. suis* (Iglesias et al. 1992).

**Antimicrobial Preventive Medication.** Strategies for prophylactic antimicrobial medication for *S. suis* should reflect consideration of bioavailability, route of administration (feed or water), competition (feed and water availability), and serum concentration needed to kill *S. suis* (Amass et al. 1997; del Castillo et al. 1995). Oral procaine penicillin G results in measurable systemic concentrations; however, higher plasma concentrations are achieved with an equivalent dose of phenoxymethyl penicillin. Del Castillo et al. (1995) indicated that among penicillin preparations, only penicillin V in water given to fasted piglets could reach effective serum concentrations for *S. suis*. Even so, Byra et al. (2011) reported that potassium penicillin G administered in drinking water is effective in reducing mortality associated with *S. suis* infection. Amoxicillin is an antibiotic of choice since it rapidly achieves high plasma levels and diffuses well into the extracellular space, and most *S. suis* strains present low minimum inhibitory concentration (MIC) (Denicourt and Le Coz 2000). In other studies, use of ampicillin and penicillin G did not significantly reduce disease in animals exposed to a coinfection with *S. suis* and PRRSV Halbur et al. 2000; Schmitt et al. 2001). They found that treatment with ceftiofur was the only regimen that significantly reduced mortality, severity of gross lung lesions, and recovery of *S. suis* from tissues at necropsy.
Immunization. Vaccines used in the field to prevent \textit{S. suis} disease are either commercial or autogenous bacterins and results have been inconsistent (Halbur et al. 2000; Reams et al. 1996; Torremorell et al. 1997; Wisselink et al. 2001, 2002b). Possible reasons for failure include degradation of protective antigens or loss of antigenicity of the bacteria caused by heat or formalin processing (Holt et al. 1990a), production of antibodies to antigens not associated with virulence factors (Holt et al. 1988), and weak immunogenicity of the capsulated bacteria (del Campo Sepúlveda et al. 1996). In some cases, good homologous protection against challenge with serotype 2 could be obtained with bacterins (Baums et al. 2009); however, no protection could be obtained against a heterologous serotype 9 challenge (Baums et al. 2009). Most vaccination-challenge studies have been carried out with piglets. Vaccination of sows and gilts has also been somewhat effective (Amass et al. 2000; Swildens et al. 2007; Torremorell et al. 1997).

Because \textit{S. suis} disease is most often at 6–10 weeks of age and the first of two doses of bacterin need be given at approximately 3–4 weeks of age, interference by maternal antibodies must be considered. Lapointe et al. (2002) found better response to vaccination in 2- to 4-week-old pigs with lower levels of maternal antibodies to the serotype 1/2 bacterin strain as compared with cohorts with higher maternal titers. In addition, the adjuvant used seems to play an important role. Wisselink et al. (2001) showed that a bacterin with a water-in-oil emulsion adjuvant produced better results than with aluminum-hydroxide-based adjuvant.

Protection against \textit{S. suis} serotype 2 challenge can be passively transferred confirming the importance of humoral immunity in the prevention of \textit{S. suis} disease (Andresen and Tegtmeier 2001; Holt et al. 1988). Wisselink et al. (2002b) showed that antibodies against the CPSs and other bacterial components are essential for full protection against homologous challenge. Antigenicity of capsule and the role of anticapsular antibodies in protection are still controversial. Pigs experimentally or naturally infected with \textit{S. suis} type 2 only produced low levels of antibodies against the CPS (del Campo Sepúlveda et al. 1996). Similarly, Andresen and Tegtmeier (2001) obtained few antibodies against the CPS in one of two horses immunized for more than 40 weeks with whole cells of \textit{S. suis} serotype 2. Baums et al. (2009) showed that the serotype-specific protection obtained with a bacterin was due to the presence of opsonic antibodies directed to antigens different from the CPS (Baums et al. 2009).

Findings in the studies of subunit vaccines have been inconsistent. Early studies demonstrated good protection by vaccines of various serotype 2 proteins (Holt et al. 1990b). Likewise, a vaccine using MRP and EF proteins protected pigs against a challenge with virulent \textit{S. suis} serotype 2 strains (Wisselink et al. 2001). Different results were obtained by Kock et al. (2009) who showed that high levels of antibodies against MRP and EF did not confer protection against homologous and heterologous challenge. Confirming the latter results, a subunit vaccine containing, among other antigens, the MRP protein also failed to confer protection (Baums et al. 2009). Jacobs et al. (1996) reported that the suilysin, but not a protein concentrate rich in MRP and EF, was able to provide complete protection against \textit{S. suis} infection. In contrast, vaccination with a live avirulent suilysin-positive strain that generated suilysin neutralization titers did not protect against homologous or heterologous challenge relative to nonvaccinated controls (Kock et al. 2009). These inconsistent results may be due to differences in strains used in studies since not all virulent \textit{S. suis} strains contain MRP, EF, and/or suilysin (Berthelot-Hérault et al. 2000; Fittipaldi et al. 2009; Gottschalk et al. 1998).

Baums and Valentin-Weigand (2009) recently reviewed proteins tested as candidates for subunit vaccination. Conflicting results are frequent between studies for the same protein, for example, surface protein one (SAO) or alpha-enolase. Their review correctly asserts that conflicting results may be due to a lack of uniformity in experimental conditions used to test vaccine efficacy. Additional candidate subunit vaccines have been evaluated recently (Chen et al. 2010; Zhang et al. 2009). Results of vaccination-challenge studies need to be carefully scrutinized. Some studies used complete Freund’s adjuvant that is not allowed commercially. Others claim protection against potent challenges (10^9 CFU in mice) administered intravenously or intraperitoneally that are known to typically overwhelm otherwise protective immunity (Chen et al. 2010; Tan et al. 2009). Additional studies by additional research groups using different protocols and different strains are needed to confirm unambiguously that a particular subunit is protective against \textit{S. suis} disease. Due to the diversity of strains and serotypes, a mixture of proteins would probably be needed to achieve a respectable level of protection. So far, there is no single subunit vaccine in the market.

Since convalescent animals are protected against re-infection, administration of a low dose of virulent \textit{S. suis} was tested and shown to offer good protection, although some residual virulence was observed (Schmitt et al. 2001). Likewise, moderate protection was obtained in pigs following inoculation with live avirulent \textit{S. suis} serotype 2 strains (Busque et al. 1997; Holt et al. 1988). Because live avirulent strains induce protection similar to that of live virulent strains, it may be that the important immunogens are distinct from the virulence factors (Gottschalk and Segura 2000). It has been demonstrated that vaccination with a live acapsular mutant provided little or no protection (Fittipaldi et al.
2007; Wisselink et al. 2002b), probably because the bacteria were rapidly eliminated by phagocytes. Intranasal vaccination with a well-encapsulated avirulent mutant defective in the production of the serum opacity factor of \( S. \textit{suis} \) (OFS), known to be a critical virulence factor (Baums and Valentin-Weigand 2009), elicited specific antibodies against different \( S. \textit{suis} \) antigens such as MRP, EF, and sulysin, but failed to protect against systemic serotypes 2 and 9 challenges (Kock et al. 2009).

Because virulent systemic strains of \( S. \textit{suis} \) rarely colonize the upper respiratory tract of sows and gilts and few piglets are colonized with these strains by weaning, induction of nasal colonization in young pigs with the herd’s systemic strain was suggested as a means of disease prevention (Oliveira et al. 2001; Torremorell et al. 1999). These authors showed direct inoculation of 5-day-old piglets with the herd’s systemic strain of \( S. \textit{suis} \) tended to be more effective in reducing the morbidity and mortality than the colonization of piglets by nose-to-nose contact with inoculated sows (Oliveira et al. 2001).

**Eradication**

Attempts to eradicate \( S. \textit{suis} \) infection have focused only on serotype 2. Medicated early weaning (MEW) is ineffective since \( S. \textit{suis} \) is a very early colonizer. Cesarean section can be used to derive pigs free of \( S. \textit{suis} \) from infected dams. According to Clifton-Hadley et al. (1986b), only depopulation and restocking with clean pigs will ensure the eradication of the infection, and in most herds, this cannot be justified economically. Even if accomplished, strict biosecurity measures are needed that include eliminating rodents and flies in order to prevent reinfection (Amass et al. 1997). Mills (1996) described the procedures used to establish a purebred minimal-disease herd from gilts that were carriers of a virulent strain of \( S. \textit{suis} \) type 2. Amass et al. (1996) did not recommend such an approach, instead recommending optimization of management and environment of pigs coupled with strategic medication of clinically ill animals for control and prevention of mortality caused by streptococcus. \( \text{Streptococcus suis} \) can be purportedly eliminated from the tonsils of sows through vaccination with a bacterin combined with medication, resulting in progeny free of pathogenic \( S. \textit{suis} \) (Swildens et al. 2007). However, this approach must be confirmed before being recommended. Byra et al. (2011) showed that potassium penicillin G administered in drinking water was effective in reducing mortality associated with \( S. \textit{suis} \) infection as well as tonsillar carriage of \( S. \textit{suis} \).

Given the cost of eliminating \( S. \textit{suis} \), the risk of failure, the difficulty in maintaining a free herd, and the lack of tools to monitor herd status, it would appear reasonable to direct resources toward control measures rather than eradication.

**INFECTIONS CAUSED BY BETA-HEMOLYTIC STREPTOCOCCI**

**\( \text{Streptococcus porcinus} \)**

The name \( S. \textit{porcinus} \) was proposed in 1984 by Collins et al. (1984) to represent streptococci of serological groups E, P, U, and V, which formed a single DNA–DNA homology group. \( \text{Streptococcus porcinus} \) has a unique phenotypic profile in addition to serological differences that can be used to help identify the species. By rRNA sequencing, \( S. \textit{porcinus} \) is closely related to the other beta-hemolytic streptococci, such as groups A, B, and C (Facklam et al. 1995).

\( \text{Streptococcus porcinus} \) group E has been associated, particularly in the United States, with a contagious entity in growing pigs known as streptococcal lymphadenitis, jowl abscesses, or cervical abscesses. Transmission is by contact, drinking water, or ingestion of food contaminated by abscess discharge or infected feces. The organisms enter through the mucosa of the pharyngeal or tonsillar surfaces and are carried to the lymph nodes primarily of the head and neck regions where abscesses are formed (Wessman 1986). Losses due to this disease in the United States were important in the 1960s, but the incidence and importance has since declined. The disease is not recognized as an important economic entity in other countries, where the bacterium represents only a few percent of the microorganisms isolated from abscesses in swine (Wessman 1986). A report of an outbreak from Spain mentioned that 80% of 50 feeder pigs had mandibular and retropharyngeal purulent lymphadenitis (Real et al. 1992). Antibiotic treatment is not usually successful in abscessed swine or in elimination of carriers. Resistance to tetracycline has been reported (Facklam et al. 1995; Lämmler and Bahr 1996). Vaccination is possible but has not been widely used since the condition is not widespread.

\( \text{Streptococcus porcinus} \) can be isolated from the tonsils, pharynx, and nasal cavities of clinically healthy pigs. It is also occasionally found in the vaginal mucus of sows and in the semen and prepuce of boars. It is considered to be more of a secondary invader than a primary pathogen in conditions such as pneumonia, enteritis, encephalitis, and arthritis (Wessman 1986).

Other than group E, \( S. \textit{porcinus} \) groups P, U, and V were isolated by Hommez et al. (1991) from pig lungs, genital organs, and brains. However, no histological lesions could be associated with their presence. \( \text{Streptococcus porcinus} \) groups P and V were associated with abortions in pigs (Lämmler and Bahr 1996; Plagemann 1988). Finally, many strains of \( S. \textit{porcinus} \) were supposedly recovered from the human female genitourinary tract. However, it was later reported that these strains belong to a new species, \( \text{Streptococcus pseudoporcinus} \) sp. nov. (Bekal et al. 2006).
Katumi et al. (1997) found S. porcinus in 1.6% of slaughtered pigs with lesions of endocarditis in Japan. In 1998, the same authors reported that from a total of 170 beta-hemolytic streptococci isolated from lesions in slaughtered pigs, 22.4% were identified as S. porcinus. Of those, 3.0% belonged to group E, 3.0% to group P, 8.2% to group U, and 8.2% were ungroupable (Katumi et al. 1998).

*Streptococcus dysgalactiae subsp. equisimilis*

In 1984, Farrow and Collins, using DNA–DNA hybridization, DNA base composition, and biochemical tests, determined that S. dysgalactiae, S. equisimilis, and streptococci of Lancefield serological groups C, G, and L were a single species. In 1996, Vandamme et al. proposed that the name S. dysgalactiae subsp. dysgalactiae be used for strains of animal origin, and the name S. equisimilis subsp. equisimilis was used for human isolates. However, in 1998, Vieira et al., based on multilocus enzyme electrophoresis typing and genomic DNA relatedness, proposed a new classification. Alpha- and nonhemolytic streptococci of Lancefield group C are designated as S. dysgalactiae subsp. dysgalactiae, while beta-hemolytic streptococci belonging to groups C, G, or L are designated as S. dysgalactiae subsp. equisimilis.

In swine, members of the S. dysgalactiae subsp. equisimilis species are all beta-hemolytic streptococci. Although members of the normal flora, they are considered the most important beta-hemolytic streptococci involved in lesions in pigs (Hommez et al. 1991). *Streptococcus dysgalactiae* group C streptococci were reported to be common in nasal and throat secretions, tonsils, and vaginal and preputial secretions (Jones 1976). Vaginal secretions and milk from postparturient sows are the most likely sources of infection for the piglets (Woods and Ross 1977). Streptococci enter the bloodstream via skin wounds, the navel, and tonsils. A bacteremia or septicemia occurs, and the organisms then settle in one or more tissues, giving rise to arthritis, endocarditis, or meningitis. Insufficient consumption of colostrum or milk or inadequate levels of antibodies, especially in gilts, may predispose to disease (Windsor 1978).

Infection is usually first seen in pigs between 1 and 3 weeks of age. Joint swelling and lameness are the most obvious and persistent clinical signs. Elevated temperatures, lassitude, roughened hair coat, and inappetence may also be noted. Early lesions consist of periarticular edema, swollen hyperemic synovial membranes, and turbid synovial fluid. Necrosis of articular cartilage may be seen 15–30 days after onset and may become more severe. Fibrosis and multifocal abscessation of periarticular tissues and hypertrophy of synovial villi also occur. Hill et al. (1996) reported that in lame pigs up to 12 weeks of age the causative agents of arthritis were in decreasing order, S. dysgalactiae subsp. equisimilis (26.3%), *Staphylococcus hyicus* (24.6%), *Arcanobacterium pyogenes* (13.2%), *Staphylococcus aureus* (7.9%), and *Haemophilus parasuis* (7.9%), and most of the pigs culled for arthritis were under 6 weeks of age (Hill et al. 1996). Hommez et al. (1991) mentioned that S. dysgalactiae is frequently isolated from pigs with septicemia, arthritis, or valvular endocarditis. In 1997, Katumi et al. reported the isolation of S. dysgalactiae from lesions of endocarditis in 15.2% of slaughtered pigs, while S. suis was present in 25.7% of the animals. In 1998, Katumi et al. reported that during a 7-year period, 77.6% of beta-hemolytic streptococci isolated from slaughtered pigs in Japan belonged to the S. dysgalactiae species. Of those, 45.8% belonged to Lancefield group C, 25.3% to group L, and 6.5% to group G.

Since baby pigs are virtually assured of being exposed to S. dysgalactiae, effective preventive measures should be followed. Adequate intake of colostrum may ensure that the piglets receive protective antibodies (Zoric et al. 2004). Traumatic injuries to the feet and legs should be minimized by reducing the abrasiveness of the floor surface in the nursing area (Zoric et al. 2009). Beta-hemolytic streptococci are sensitive to beta-lactam antibiotics. Long-acting antibacterial agents may be beneficial, and treatment should be given before the inflammatory process is well advanced. There are no recent reports about vaccination against groups C or L streptococci. Autogenous bacterins have been used, and a reduction in incidence of arthritis has been reported when sows were vaccinated before farrowing (Woods and Ross 1977).

**OTHER STREPTOCOCCI**

*Streptococcus equi* subsp. *zooepidemicus* (*Streptococcus zooepidemicus*) is classified in Lancefield’s group C and causes respiratory disease in a variety of mammals. In Europe and the United States, *S. zooepidemicus* is the primary cause of equine respiratory tract infections in foals and infertility in mares. However, in China, *S. zooepidemicus* is the main pathogen causing disease in swine. In the summer of 1975, a pandemic swine streptococcosis outbreak occurred in western China, resulting in substantial economic losses. The pathogen was identified as *S. zooepidemicus* (Feng and Hu 1977). From the 1990s to the present, sporadic cases and regional epidemics are still impacting the pig industry in China (Mao et al. 2008).

Bacterial streptococcal strains were isolated in 2002 and 2003 from the lungs and kidney of two pigs with lesions associated with pneumonia and septicemia, respectively. The two isolates were recovered from different animals, in different farms located in different provinces of Spain and in different years. The isolates were classified as a new species, *Streptococcus plurextor -rum* sp. nov. (Vela et al. 2009). More recently, another new streptococcal species, *Streptococcus porci* sp. nov.,
has been associated with pneumonia and pericarditis (Vela et al. 2010). So far, there are no data about the habitat and/or the virulence properties of these two species.

**ENTERITIS ASSOCIATED WITH ENTEROCOCCI IN PIGLETS**

Enterococci are known as part of the intestinal flora, but some strains have the capacity to colonize the mucosal surface of the small intestine extensively. However, very few cases are described in the literature. Some enterococcal species that show typical adhesion to the apical surface of the enterocytes of the small intestine of young animals have been described as associated with diarrhea in different animal species (Vancanneyt et al. 2001). Cases have been reported in piglets between 2 and 20 days of age. Most cases were sporadic (Drole et al. 1990; Johnson et al. 1983), with only one outbreak reported (Cheon and Chae 1996). Taxonomic studies have shown that most of these enterococci are members of the *E. faecium* species group, mainly *E. durans* and *E. hirae*.

The pathogenesis of enteric disease associated with adherent enterococci is unclear. Adherence occurs with the help of fibrillar projections (Tzipori et al. 1984), and diarrhea is not associated with enterotoxin production or substantial mucosal injury (Cheon and Chae 1996). Because of the natural resistance of enterococci to some antibacterial agents, antimicrobial susceptibility testing is advised before the institution of a treatment. Due to the lack of knowledge about the clinical and epidemiological aspects of this infection, preventive measures are difficult to recommend.

**REFERENCES**

Tuberculosis

Charles O. Thoen

RELEVANCE

Tuberculosis continues to cause significant economic losses to swine producers throughout the world. Although tuberculosis due to *Mycobacterium bovis* has been nearly eradicated in many developed countries, lesions continue to be reported in the cervical and mesenteric lymph nodes of swine during meat inspection. The processing of tuberculous swine carcasses is costly and results in significant economic losses. Regulations of the meat and poultry inspection program of the United States Department of Agriculture (USDA) require that unaffected portions of swine carcasses with tuberculous lesions in more than one primary site, such as cervical and mesenteric lymph nodes, be cooked at 170°F (76.7°C) for 30 minutes before being approved for human food (National Archives and Records Services 1973). The value of a cooked carcass is only about 20–25% of the value of a carcass not cooked. In processing plants where facilities are not available for cooking, the carcass is condemned and there is no salvage value.

There has been no direct campaign to eradicate tuberculosis in swine. The campaign to eradicate bovine tuberculosis, which was started in 1917, led to a reduction of the disease in swine in the United States. The percentage of swine with suspect tuberculous lesions at slaughter peaked in 1922 and has progressively reduced since (Table 63.1).

ETIOLOGY

Swine are susceptible to infection with *Mycobacterium avium* complex (MAC), *Mycobacterium tuberculosis* complex, and some other mycobacterial species (Thoen et al. 1975). MAC serovars 1, 2, 4, and 8 are the most common isolates from tuberculous lesions in swine in the United States (Mitchell et al. 1975). At least 20 other MAC serovars have been isolated from swine in the United States (Thoen et al. 1975) as well as in other countries: Australia (Tammemagi and Simmons 1971), Brazil (Pestana de Castro et al. 1978), Denmark (Jorgensen 1978), France and Germany (Meissner et al. 1978), Hungary (Szabo et al. 1975), Japan (Nishimori et al. 1995; Yugi et al. 1972), South Africa (Kleeberg and Nel 1973), and the Czech Republic (Matlova et al. 2004). These reports indicate worldwide distribution of tuberculosis in swine due to MAC. The similarity of *M. avium* and the so-called *Mycobacterium intracellulare* has led to the proposal that the latter be considered serovars of MAC (*M. avium* subspecies *avium*, *M. avium* subspecies *hominisuis*, *M. avium* subspecies *intracellulare*) (Dvorska et al. 2002; Wolinsky and Schaefer 1973).

Molecular techniques including restriction fragment length polymorphism (RFLP) using insertion sequence IS 1245, polymerase chain reaction (PCR)-based mycobacterial interspersed repetitive unit-variable-number tandem repeat (MIRU-VNTR) typing and serotyping have been shown to be reliable for identifying MAC isolated from swine (Domingos et al. 2009; Komijn et al. 1999; Pavlik et al. 2000; Radomski et al. 2010; Thorensen and Saxegaard 1993; Van Soolingen et al. 1998). MAC serovars 1 and 2 occur mainly in birds but also in swine and humans, whereas serovars 4–6 and 8–11 are found in swine and humans. Molecular evidence supports a proposal to refer to human/porcine type of MAC as *M. avium* subsp. *hominisuis* (Braijnesteijn van Coppenraet et al. 2008; Mijs et al. 2002).

The decrease in prevalence of tuberculosis in swine in the United States is largely attributable to a lowering of the incidence of tuberculosis in poultry, which in

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disease, are not known. Chlorination of water delivery systems is known to provide a preferential environment for proliferation of MAC in biofilms, especially *M. avium* (Vaerewijck et al. 2005). To date, there are no data that implicate exposure to infected swine or consumption of pork as an increased risk for human infection.

**EPIDEMIOLOGY**

Because swine are not routinely tested with tuberculin, the only sources of information on the prevalence and geographical distribution of tuberculosis in this species are the data obtained from meat inspection records. On this basis, an increase in the rate of infection occurred in the United States until 1922 (Table 63.1). During 1922, 16.38% of all swine slaughtered under federal supervision had tuberculous lesions; in 0.2%, the disease was so extensive that the entire carcass was condemned. Since 1922 there has been a gradual decline; by 2004 and 2008, the prevalence had decreased to 0.0364% and 0.0178%, respectively, with only 0.001% and 0.0018% having evidence of generalized tuberculosis.

Data on the prevalence of tuberculosis in swine from meat inspection records may be misleading because diagnoses are made on the basis of the macroscopic appearance of lesions (Figure 63.1). A certain number of tuberculous infections escape detection because the lesions are not grossly visible.

**PUBLIC HEALTH**

The MAC bacteria, especially *M. avium*, cause disseminated disease or pneumonia in AIDS patients, less frequently in organ transplantees and in other immunocompromised individuals (Primm et al. 2004). They are also a cause of pneumonia in people with chronic obstructive pulmonary disease, cystic fibrosis, and other degenerative lung diseases (Parrish et al. 2008). In addition, *M. avium* has emerged in recent years as a cause of lymphadenitis in children (Bruijnesteijn van Coppenraet et al. 2008; Primm et al. 2004; Wolinsky 1995). Risk factors for infection in humans, apart from immunocompromised and chronic pulmonary

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**Table 63.1.** Prevalence of tuberculosis in swine in the United States as determined by inspection in abattoirs under federal supervision

<table>
<thead>
<tr>
<th>Year</th>
<th>Number Slaughtered</th>
<th>Percent Tuberculosis</th>
<th>Percent Condemned</th>
</tr>
</thead>
<tbody>
<tr>
<td>1912</td>
<td>34,966,378</td>
<td>4.69</td>
<td>0.12</td>
</tr>
<tr>
<td>1917</td>
<td>40,210,847</td>
<td>9.89</td>
<td>0.19</td>
</tr>
<tr>
<td>1922</td>
<td>34,416,439</td>
<td>16.38</td>
<td>0.20</td>
</tr>
<tr>
<td>1927</td>
<td>42,650,443</td>
<td>13.54</td>
<td>0.14</td>
</tr>
<tr>
<td>1932</td>
<td>45,832,422</td>
<td>11.38</td>
<td>0.08</td>
</tr>
<tr>
<td>1937</td>
<td>36,226,309</td>
<td>9.48</td>
<td>0.08</td>
</tr>
<tr>
<td>1942</td>
<td>50,133,871</td>
<td>7.96</td>
<td>0.026</td>
</tr>
<tr>
<td>1947</td>
<td>47,073,370</td>
<td>8.50</td>
<td>0.023</td>
</tr>
<tr>
<td>1952</td>
<td>63,823,263</td>
<td>4.40</td>
<td>0.015</td>
</tr>
<tr>
<td>1956</td>
<td>66,781,940</td>
<td>4.76</td>
<td>0.010</td>
</tr>
<tr>
<td>1962</td>
<td>67,109,539</td>
<td>2.25</td>
<td>0.008</td>
</tr>
<tr>
<td>1968</td>
<td>72,325,507</td>
<td>1.35</td>
<td>0.005</td>
</tr>
<tr>
<td>1972</td>
<td>83,126,396</td>
<td>0.85</td>
<td>0.007</td>
</tr>
<tr>
<td>1978</td>
<td>71,805,911</td>
<td>0.75</td>
<td>0.006</td>
</tr>
<tr>
<td>1983</td>
<td>79,992,743</td>
<td>0.41</td>
<td>0.003</td>
</tr>
<tr>
<td>1989</td>
<td>82,110,688</td>
<td>0.67</td>
<td>0.002</td>
</tr>
<tr>
<td>1995</td>
<td>94,490,329</td>
<td>0.21</td>
<td>0.003</td>
</tr>
<tr>
<td>2004</td>
<td>102,707,038</td>
<td>0.0364</td>
<td>0.001</td>
</tr>
<tr>
<td>2008</td>
<td>115,949,655</td>
<td>0.0178</td>
<td>0.0018</td>
</tr>
</tbody>
</table>


aIncludes all carcasses with evidence of tuberculosis, varying in extent from only small foci in cervical lymph nodes to generalized involvement.

bIncludes carcasses with evidence of generalized tuberculosis.

cFood Safety Inspection Service (FSIS) electronic Animal Disposition Reporting System (eADRS) USDA.
Where tuberculosis does occur in cattle, the infection may be transmitted to swine by the feeding of unpasteurized milk and dairy by-products. Feces of tuberculous cattle may contain viable tubercle bacilli, which provide an obvious hazard where swine and cattle are maintained in a common feedlot. The practice of feeding swine the offal from abattoirs or uncooked garbage is obviously unwise, because such material may contain tuberculous material from beef carcasses. Fichandler and Osborne (1966) described an epidemic of tuberculosis in a herd of swine in Connecticut that was fed improperly cooked offal from tuberculous cattle. A serious outbreak of avian tuberculosis in a swine-feeding establishment in Denmark was traced to the improper cooking of offal from poultry plants (Biering-Sorensen 1959).

Mycobacterium tuberculosis is occasionally isolated from tuberculous lesions in swine. No person known to have active tuberculosis should be permitted to have contact with swine or other animals. Uncooked garbage is a potential means of transmitting tuberculosis to swine. Feldman (1939) recorded that 75 (28.4%) of 264 garbage-fed swine were found to have tuberculous lesions at the time of slaughter. Of these, 47 contained tubercle bacilli, of which 35 were avian type and 12 were human type. It was concluded that garbage may contain the offal of tuberculous chickens and that material from tuberculous human patients is not properly disposed of.

Table 63.2. Summary of data compiled from reports in North America on the occurrence of tubercle bacilli in tuberculous lymph nodes of swine

<table>
<thead>
<tr>
<th>Reference</th>
<th>Date</th>
<th>Origin of Swine</th>
<th>Specimens</th>
<th>Avian Only</th>
<th>Mammalian Only</th>
<th>Mixed</th>
<th>None</th>
</tr>
</thead>
<tbody>
<tr>
<td>Van Es</td>
<td>1925b</td>
<td>Nebraska</td>
<td>248</td>
<td>74.6</td>
<td>4.4</td>
<td>5.6</td>
<td>15.4</td>
</tr>
<tr>
<td>Van Es and Martin</td>
<td>1925b</td>
<td>Michigan</td>
<td>14</td>
<td>92.9</td>
<td>None</td>
<td>7.1</td>
<td>None</td>
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<tr>
<td>Mitchell et al.</td>
<td>1934b</td>
<td>Canada</td>
<td>96</td>
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<td>Feldman</td>
<td>1938b</td>
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<td>30°</td>
<td>80.0</td>
<td>6.6 (bovine)</td>
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<td>1939b</td>
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<td>75°</td>
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<td>16.0 (human)</td>
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<td>Minnesota</td>
<td>89</td>
<td>61.8</td>
<td>None</td>
<td>None</td>
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<td>Pullin</td>
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<td>Eastern Canada</td>
<td>232</td>
<td>44.8</td>
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<td>Bankier</td>
<td>1946b</td>
<td>Alberta, Canada</td>
<td>102</td>
<td>88.0</td>
<td>1.0 (bovine)</td>
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<td>1971b</td>
<td>Minnesota</td>
<td>36</td>
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<td>Thoen et al.</td>
<td>1975b</td>
<td>United States</td>
<td>2036</td>
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<td>Pritchard et al.</td>
<td>1977b</td>
<td>Idaho</td>
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<td>Cole et al.</td>
<td>1978b</td>
<td>Georgia</td>
<td>112</td>
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<td>Margolis et al.</td>
<td>1994b</td>
<td>Pennsylvania</td>
<td>125</td>
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Note: Specimens obtained from abattoirs under federal supervision.

Sources of Infection

Swine are susceptible to infection with serovars of MAC, M. tuberculosis, and M. bovis. The occurrence of tuberculosis in swine, therefore, is in part related to the opportunity for direct or indirect contact with tuberculous cattle, humans, and fowl or organisms in the environment.

Mycobacterium bovis is not a frequent cause of tuberculosis in swine in localities where the disease in cattle is controlled by a campaign of eradication. For example, in the United States and Canada, M. bovis is rarely found in lesions of swine (Table 63.2). In Great Britain, during 1952–1955, the bovine type of tuberculosis in swine gradually declined concurrently with the eradication of the disease in cattle. The percentage of avian-type infection increased from 44% during the first 5 years of the study to 92% for the last 5 years (Lesslie et al. 1968). However, the occasional finding of M. bovis in swine is a reminder that the disease in cattle is a constant threat.

Where tuberculosis does occur in cattle, the infection may be transmitted to swine by the feeding of unpasteurized milk and dairy by-products. Feces of tuberculous cattle may contain viable tubercle bacilli, which provide an obvious hazard where swine and cattle are maintained in a common feedlot.

The practice of feeding swine the offal from abattoirs or uncooked garbage is obviously unwise, because such material may contain tuberculous material from beef carcasses. Fichandler and Osborne (1966) described an epidemic of tuberculosis in a herd of swine in Connecticut that was fed improperly cooked offal from tuberculous cattle. A serious outbreak of avian tuberculosis in a swine-feeding establishment in Denmark was traced to the improper cooking of offal from poultry plants (Biering-Sorensen 1959). Mycobacterium tuberculosis is occasionally isolated from tuberculous lesions in swine. No person known to have active tuberculosis should be permitted to have contact with swine or other animals.

Uncooked garbage is a potential means of transmitting tuberculosis to swine. Feldman (1939) recorded that 75 (28.4%) of 264 garbage-fed swine were found to have tuberculous lesions at the time of slaughter. Of these, 47 contained tubercle bacilli, of which 35 were avian type and 12 were human type. It was concluded that garbage may contain the offal of tuberculous chickens and that material from tuberculous human patients is not properly disposed of.

The frequent occurrence of MAC in lesions limited to the cervical and mesenteric lymph nodes in naturally infected swine indicates that infection usually
occurs by ingestion. Janetschke (1963) found that the primary complex involved the alimentary tract in 97.3% of 1000 carcasses with tuberculous lesions; a pulmonary route of infection was noted in only 2.7%, as indicated by involvement of the bronchial lymph nodes.

Schalk et al. (1935) found that swine contracted tuberculosis when placed on ground that had not been occupied by tuberculous chickens for the previous 2 years. Viable and pathogenic avian tubercle bacilli were found in the soil and litter of a chicken cage after 4 years. Schalk and coworkers concluded that soil contaminated by feces of tuberculous fowl is the most important source of infection for swine. No success was obtained in controlling the disease merely by use of the tuberculin test and elimination of reactors, because the soil remained contaminated. They recommended that an ideal program to control avian tuberculosis is to rear young birds on clean ground and to dispose regularly of all fowl more than 1 year old.

Schliesser and Weber (1973) studied the survival of MAC in sawdust. At 64–72°F (18–22°C), the survival time of two virulent strains was 153–160 days, and the survival time of two avirulent strains was 169–214 days. The survival times were greatly reduced when the contaminated sawdust was maintained at 98.6°F (37°C).

Wild birds may be incriminated as a source of M. avium infections in swine. Tuberculosis was found in starlings on a farm with a high incidence of tuberculosis in the swine but where no poultry had been kept for 8 years (Bickford et al. 1966). Tuberculosis due to M. avium has been found in various wild birds, some of which frequent feedlots (Thoen 1997).

The close contact of sows and slaughter pigs in yards and feeding pens provides opportunity for transmission of tuberculosis from animal to animal (Alfredsen and Skjerve 1993). The occurrence of intestinal lesions allows the spread of tubercle bacilli in feces. Feldman and Karlson (1940) and Pullar and Rushford (1954) demonstrated avian tubercle bacilli in the tonsils of pigs. The latter workers suggested that this may be a source of infection for other animals. Smith (1958) found M. avium in apparently normal lymph nodes of 7% of swine, 5% of sheep, and 5% of cattle but was unable to find them in adult normal chickens. He suggested that domestic mammals may contract M. avium from each other as well as from tuberculous fowl.

Pulmonary, uterine, and mammary tuberculous lesions in swine constitute sources of infection for other animals. Jorgensen et al. (1972) described an endemic (enzootic) of pulmonary tuberculosis resulting from M. avium in pigs. Lesslie and Birn (1967) found M. avium in the udder or milk of 18 cows and concluded that such animals may be a source of M. avium in pigs. Bille and Larsen (1973) reported congenital infection in swine caused by M. avium, suggesting that infected pregnant sows may have a role in the transmission of this infection. Recently, M. avium subspecies hominissuis has been associated with aborted fetuses in pigs (Wellenberg et al. 2010).

Where sawdust is used for bedding, serovars 4 and 8 of MAC have been isolated from lesions in swine as well as from the sawdust. Reactions to M. avium and to M. bovis tuberculin have been reported in boars exposed to sawdust from which M. avium or other nonphotochromogenic mycobacteria were isolated (Fodstad 1977). Schliesser and Weber (1973) found that M. avium would survive as long as 214 days in sawdust. In Hungary, Szabo et al. (1975) found that the incidence of tuberculous adenitis in swine was greater when sawdust was used as litter; when the use of sawdust was discontinued, the occurrence of such lesions decreased significantly. Dalchow and Nassal (1979) recorded that the same serovars of MAC as found in swine could be isolated from sawdust. These workers also reported that sawdust could contain infectious mycobacteria even after 4 years of storage. Songer et al. (1980) investigated herds of swine in Arizona and found in at least one herd that the source was sawdust and wood shavings.

Investigations conducted in the Czech Republic and Slovakia indicate that MAC serotype 8 may be spread by adult flies (Fischer et al. 2001).

**PATHOGENESIS**

The development of disease in swine depends on the ability of the tubercle bacillus to multiply within tissues of the host and to induce a host response. Although acid-fast bacilli initially encounter granulocytes and humoral components, activated mononuclear macrophages are considered to be more important in protection of the host against mycobacteria (Olsen et al. 2010).

The capacity of M. avium to produce progressive disease may be related to certain complex lipids present in the cell wall, such as the glycopeptidolipids (previously referred to as C-mycosides) localized in the exterior portion of the cell envelope (Rastogi and Barrow 1994). However, it appears that the effect of these components alone or together on phagolysosome fusion cannot account for virulence. Available information suggests that a combination of toxic lipids and factors released by virulent tubercle bacilli may cause disruption of the phagosome, interfere with phagolysosome formation, alter the release of hydrolytic enzymes from the attached lysosomes, and/or inactivate the lysosomal enzymes released into the cytoplasmic vacuole (Thoen and Barletta 2006). Certain serovars of M. avium are susceptible to bactericidal mechanisms of macrophages; however, the importance of reactive nitrogen intermediates and oxygen radicals in macrophages of animals exposed to virulent tubercle bacilli remains to be elucidated (Thoen et al. 2009). Although the mechanisms by which mycobacteria produce disease in swine...
have not been clearly defined, experimental studies in piglets revealed that nonspecific esterase activity was elevated in mononuclear macrophages of lymph nodes 7 days following inoculation of MAC serovar 8 (Momin et al. 1980). Granulomas of varying stages were observed in mesenteric and mandibular lymph nodes and intestinal mucosa at 14 days postinoculation. In other investigations, sensitized lymphocytes and detectable mycobacterial antibodies have been reported to occur at 14–28 days postexposure to M. avium or M. bovis (Muscoplat et al. 1975; Thoen et al. 1979).

CLINICAL SIGNS
Generally, the tuberculous lesions are limited to small foci in a few lymph nodes of the digestive tract without clinical signs. In extensive tuberculous infection, signs may be suggestive of an infectious disease, but are not sufficiently characteristic to suggest a diagnosis of tuberculosis.

LESIONS
Detailed discussions of the pathological anatomy of tuberculosis in swine may be found in Pallask (1931), Feldman (1938a), Francis (1958), and Kramer (1962). As seen in abattoirs, tuberculous lesions in swine are usually limited to lymph nodes of the cervical and the mesenteric regions. The lesions vary in appearance from small, yellowish white, caseous foci a few millimeters in diameter to diffuse enlargement of the entire node (Figure 63.1). The disease may be localized in one group of nodes or may involve a number of lymph nodes along the digestive tract.

Gross differentiation between tuberculous adenitis caused by MAC and that caused by M. tuberculosis complex is difficult, but in general, some features are characteristic of each. In an infection with M. avium, the lymph nodes may be enlarged and firm with no discrete purulent foci, or there may be one or more soft caseous areas with indistinct borders. Mineralization is seldom demonstrable. The cut surface of the lesion has a neoplastic appearance with a few caseous foci. Although there may be diffuse fibrosis, there is little encapsulation. Relatively large areas of caseation may be present and occasionally will involve the entire lymph node. The lesions due to tubercle bacilli of the avian type are generally not easily enucleated. In contrast, when the infection is due to M. bovis or M. tuberculosis, the lesions tend to be well encapsulated and are relatively easy to separate from the surrounding tissue. In addition, calcification is usually prominent in lesions. The individual foci appear to be discrete and caseous. These distinctions are by no means absolute, and there are many variations in the gross appearance of tuberculous lesions in the lymph nodes of swine.

Clapp (1956) examined, by bacteriological procedures, 420 lymph nodes (mostly submaxillary) designated as tuberculous upon meat inspection. There was some association between the gross appearance and the cause. Localized lesions that were not easily enucleated and large, dry calcareous processes involving an entire lymph node were usually due to M. avium. Indistinctly mottled and streaked lesions, large encapsulated purulent abscesses, and lesions that could be easily enucleated were usually not caused by tubercle bacilli. In the series of 420 specimens, only five were from swine with generalized tuberculosis, and all of these were associated with M. bovis and M. avium.

Microscopically, the changes induced in swine tissues are characterized by diffuse proliferation of epithelioid cells and giant cells. There may be some necrosis and calcification, especially in advanced lesions, but calcification is not usually prominent. Similar changes are observed in sows and slaughter pigs (Thoen et al. 1976b). Proliferation of connective tissue elements accompanies the process. Lesions caused by mammalian tubercle bacilli have a tendency to become encapsulated by a well-developed zone of connective tissue (Figure 63.2). In addition, there is often early caseation and marked calcification (Karlson and Thoen 1971). However, consistent histopathological differentiation between lesions caused by M. tuberculosis complex and MAC is not possible (Himes et al. 1983).

Generalized tuberculosis in swine is not commonly seen. In most instances, it is from infection with M. bovis, but it may also result from M. avium (Feldman 1938b; Jorgensen et al. 1972). The extent and character of generalized involvement vary from the occurrence of a few small foci in several organs to extensive nodules involving the liver, spleen, lungs, kidneys, and many lymph nodes. Generalized lesions from infection with MAC are usually diffuse. The cut surface is usually smooth, and there is no great tendency toward encapsulation by fibrosis. There may be foci of caseation, but calcification is not pronounced. Lesions resulting from infection with M. tuberculosis, however, are likely to be discrete, caseous, and well circumscribed by fibrosis. Calcification is prominent.

Bacteria Other Than Tubercle Bacilli
Various species of mycobacteria other than tubercle bacilli have been isolated from swine and other animals in different countries, but reports of such are few and usually concern only sporadic cases (Schliesser 1976). The significance of finding Mycobacterium kansasi, Mycobacterium xenopi, or Mycobacterium fortuitum is not clear. It may be important, however, to learn if animals and humans become infected from the same sources (Thoen and Williams 1994). Of potential importance is the recovery of Mycobacterium chelonei from swine because this bacterium has been isolated from
1 was *M. bovis*. Of the other 59, 38 contained a variably acid-fast “coccobacillus.” The acid-fastness, however, was not constant and was lost on subculture. The presence of this microorganism in localized tuberculosis-like lesions in swine was soon confirmed by other Scandinavian workers. Ottosen (1945) showed that *R. equi* occurs more frequently in the soil of hog pens than elsewhere. In Denmark, Plum (1946) studied a large number of tuberculous lymph nodes from swine and concluded that it is difficult for inspectors in abattoirs to differentiate between *M. tuberculosis complex* and *R. equi* infection. Barton and Hughes (1980) recorded 32 reports of *R. equi* infection in swine. *Rhodococcus equi* expressing a 20-kDa antigen has been observed in all pig isolates, and 2 of 5 plasmids from pig isolates were the same as those from human isolates, suggesting that the source of infection for humans may be pigs or the pig environment (Takai et al. 1996). There is also one report of the isolation of *Rhodococcus sputi* from granulomatous lesions in lymph nodes of swine (Tsukamura et al. 1988).

**DIAGNOSIS**

A clinical diagnosis of tuberculosis in swine is usually not possible since most cases will be asymptomatic or present with only nonspecific malaise. Although gross

63.2. Tuberculous changes in cervical lymph nodes in swine. (A) Mammalian tubercle bacillus infection. Peripheral fibrosis, necrosis, and calcification are typical of lesions due to bovine or human types of tubercle bacilli (hematoxylin and eosin [H&E] ×40). (B) Mycobacterium avium infection. Diffuse cellular infiltration with little necrosis (H&E ×95).
lesions of granulomatous lymphadenitis are sufficiently characteristic to include tuberculosis in a differential diagnosis, they are not specific. The great similarity between localized tuberculous lesions and those associated with *R. equi* and other bacteria has already been discussed. Also, chronic tuberculous granulomatous lesions may be difficult to differentiate grossly from parasitic nodules and neoplasms. Likewise, the mere demonstration of acid-fast bacilli in exudates or in lesions may be misleading. Some workers have recorded that *R. equi* is acid-fast in smears of necrotic material from lymph nodes of swine (Ottoesen 1945). Acid-fast microorganisms other than tubercle bacilli have been isolated from swine (Brandes 1961; Karlson and Feldman 1940). Characteristic gross and microscopic lesions with demonstration of acid-fast organisms allow a presumptive diagnosis. However, an unequivocal diagnosis also requires culture and identification of *Mycobacterium* spp. by biochemical and sero-agglutination tests and/or by molecular techniques (Thoen et al. 2009).

Enzyme-linked immunosorbent assay (ELISA) has been described for detecting antibodies in swine infected with MAC (Thoen et al. 1979; Wisselink et al. 2010). Positive ELISA reactions were observed in pigs experimentally infected and in those naturally infected. The ELISA is a rapid test that can be automated and may be of value in testing replacement breeding animals.

The tuberculin test for the diagnosis of tuberculosis in swine appears to be a useful procedure on a herd basis. The intradermal test, usually on the ear or vulva, may be employed. A number of investigators have found that some tuberculous swine may fail to react to the intradermal tuberculin test. Therefore, tests should be repeated in a herd in which animals with positive reactions have been identified and removed.

Because swine are susceptible to infection with *M. tuberculosis* complex and MAC, simultaneous tests must be made with biologically balanced *M. avium* and *M. bovis* purified protein derivative (PPD) tuberculins (Thoen and Karlson 1970). Fichandler and Osborne (1966) described an extensive outbreak of *M. bovis* in swine in which animals reacted to mammalian tuberculin by developing significant erythema and swelling of the ear, compared with slight reactions to the *M. avium* tuberculin.

Feldman (1938a) recommended the use of 0.2 mL 25% Old Tuberculin applied into the dermis on the dorsal surface of the ear, slightly anterior to the base. A positive reaction is indicated in 24 hours by a flat, reddish swelling up to 3 cm in diameter, which in 48 hours reaches its maximal intensity. At this time, the erythema and swelling are more pronounced; the central area becomes hemorrhagic, and ulceration may occur. McDiarmid (1956) described a means of testing swine in which restraint is not necessary. While the animals are feeding from a trough, 0.1 mL tuberculin is injected at a right angle into the skin at the junction of the ear and neck using a needle only 3.5 mm long.

With this short needle, most of the tuberculin is deposited in the skin. Reactions are recorded in 48 hours. A positive reaction varies from “puffy” edema to inflammation, with purple discoloration and necrosis. McDiarmid used Weybridge PPD, which, according to Paterson (1949), has 3 mg protein/mL for mammalian tuberculin and 0.8 mg protein/mL for *M. avium*.

Lesslie et al. (1968), using Weybridge PPD, tested 84 white pigs from a herd known to have tuberculosis. The *M. avium* tuberculin was given in injections of 0.1 mL, each containing 2500 tuberculin units (TU), and the mammalian tuberculin was given in injections of 0.1 mL, each containing 10,000 TU. The injections were made simultaneously, each at the base of an ear; in 48–72 hours, a positive reaction was recorded when the reaction consisted of edema and erythema. Swine experimentally infected with *M. avium* serovars 4 and 8 reacted well to the USDA avian Old Tuberculin and to PPD prepared from *M. avium* serovar 1 (Thoen et al. 1976a).

The intradermal injection of PPD tuberculin in the dorsal surface of the ear is the recommended procedure for applying the tuberculin test in swine. The injection site should be observed at 48 hours for induration and swelling.

**PREVENTION AND CONTROL**

Wood shavings, sawdust, and peat should be avoided for use as bedding. Feed and water should be protected from wild birds. Replacement sows and/or boars should be purchased from closed herds in which tuberculosis has not been diagnosed on slaughter examination or necropsy for 3 years or more. Animals originating from herds of unknown status should be held in isolation, and skin tests should be conducted using *M. avium* PPD tuberculin containing 5000TU at 2 and 6 months. Responders should be removed and necropsied; specimens (lymph nodes associated with the digestive tract) should be collected for mycobacteriological examination.

The control of tuberculosis in swine, as well as in other species, is dependent on the availability of economical and specific tests for detecting tuberculous animals. Tuberculin skin tests as described above should be conducted at 60- to 90-day intervals, and responders should be removed from the herd. A comparative tuberculin test using biologically balanced PPDs prepared from *M. avium* and from *M. bovis* is recommended for use in swine in herds in which the etiological agent may be *M. bovis* or *M. tuberculosis*. Serological tests (i.e., ELISA) are useful in identifying tuberculous in a herd, but are not of adequate sensitivity for identifying animals in the early stage of infection with MAC.
However, they may be of value in identifying some swine with advanced disease that fail to respond on the tuberculin skin test.

Adequate measures must be followed for cleaning and disinfection of premises using a tuberculocidal product such as One-Stroke Environ. MAC may persist in the soil, in buildings, or on equipment and will remain viable in the environment for long periods of time; therefore, it is necessary to repeat disinfection at 2–3 weeks. Depopulation may be the only approach to eliminate MAC infection in some instances where tuberculosis has persisted in the herd for extended periods of time and is widespread in the animal population.

REFERENCES


ACTINOBACULUM (EUBACTERIUM) SUIS

RELEVANCE, ETIOLOGY, AND PUBLIC HEALTH

*Actinobaculum suis* causes cystitis and pyelonephritis in individual sows or in small groups of sows and is carried by boars. It is not of public health concern. *Actinobaculum suis* was earlier classified as *Actinomyces suis* and *Eubacterium suis* (Lawson et al. 1997).

*Actinobaculum suis* is a gram-positive pleomorphic rod, 2–3 µm long, and 0.3–0.5 µm wide, which occurs as “Chinese letters” and palisades in smears of infected tissues. It is nonmotile and does not form spores. It grows on blood agar under anaerobic conditions. Colonies are 2–3 mm and nonhemolytic after 2 days, then they flatten and develop a characteristic dry, gray, opaque surface with a crenated edge attaining a size of 4–5 mm in 5–6 days. Growth is enhanced by the addition of urea to media with a final concentration of 1.2% (w/v). The organism produces urease.

EPIDEMIOLOGY AND PATHOGENESIS

*Actinobaculum suis* infection associated with urinary tract disease in sows has been reported from North and South America, Europe, Asia, and Australia. The pig is the main host of *A. suis* and most male pigs, aged 6 months or more, harbor *A. suis* in the preputial diverticulum. The organism may be found on the floors of pens occupied by male pigs, and uninfected males are readily infected when they are housed with carriers (Jones and Dagnall 1984). Carr and Walton (1990) isolated *A. suis* from footwear of handlers working with boars. It is rarely isolated from the vagina of healthy females.

Infection is by the ascending route. Larsen et al. (1986) demonstrated that some strains of *A. suis* are heavily fimbriated and adhere to epithelial cells of the porcine bladder where glycoconjugates are specific receptor sites for its attachment. Infection of the ureters and kidneys follows infection of the bladder. Most cases occur within 1–3 weeks of mating. Water restriction and the presence of crystalluria may predispose to infection (Wendt and Sobestiansky 1995). Cases may also occur at other times of the reproductive cycle, by recent infection of the urinary tract or by recrudescence of previously existing disease.

CLINICAL SIGNS, LESIONS, AND DIAGNOSIS

A small group of sows or gilts may die suddenly or be found ill, depressed, or thirsty with hunched backs and passing bloodstained, purulent urine with or without a vulval discharge. Hematuria is the main sign in the acute phase. As the disease progresses, there is loss of weight. Clinical signs may develop 2–3 weeks after service by a particular boar or may be delayed until farrowing. Some sows die suddenly from acute renal failure, and clinically affected sows frequently die rather than recover. Mild cases may occur in which inappetence and vulval discharge are the only obvious signs. Animals with pyelonephritis are typically uremic.

Lesions are limited to the urinary tract. The mucosa of the urethra, bladder, and ureters is inflamed,
catarrhal, fibrinopurulent, hemorrhagic, or necrotic. Affected kidneys often have irregular yellow areas of degeneration in the parenchyma that are visible on the surface. The renal pelvis may be dilated and contain mucoid fluid in which flakes of necrotic debris and altered blood are present. The medullary pyramids may contain dark foci of necrosis. The ureters are dilated and filled with reddish purulent urine.

Diagnosis is based on clinical signs and bacteriological examination of urine. The presence of hematuria and its occurrence 2–3 weeks after service suggests A. suis cystitis and pyelonephritis, rather than cystitis caused by Escherichia coli (Chapter 53). Bloodstained urine in the bladder and hemorrhagic lesions of the bladder mucosa and ureters are strongly suggestive of this condition. Actinobaculum suis is easily seen in Gram-stained films, often with other bacteria, notably streptococci. Anaerobic incubation for 4 days is essential for isolation from urine or affected tissue. A selective medium for the isolation of A. suis has been described (Dagnall and Jones 1982).

**PREVENTION AND CONTROL**

Actinobaculum suis is sensitive in vitro to several antibiotics including penicillin and tetracyclines. Antibiotics are frequently effective, but relapses occur and early slaughter of affected animals is recommended. Prolonged treatment for 20 days with ampicillin given at 20mg/kg may be used (Wendt and Sobestiansky 1995), and enrofloxacin given for 10 days at 10mg/kg may also be effective. Chronically affected animals in poor bodily condition and those that have not responded to treatment should be euthanized.

**Actinobaculum suis** may be transmitted from boars to sows at the time of mating. Culling of carrier boars, washing of the preputial diverticulum with a tetracycline solution, administration of antibiotics to sows immediately after service, or the use of artificial insemination may all help prevent disease.

**REFERENCES**


**ARCANOBACTERIUM PYOGENES**

**RELEVANCE, ETIOLOGY, AND PUBLIC HEALTH**

Arcanobacterium pyogenes is a common cause of suppurative lesions in pigs throughout the world. Clinical disease can result from the destruction of tissues. As a consequence of A. pyogenes infection, carcasses at slaughter may contain unsightly abscesses filled with creamy pus resulting in economic losses from trimming or condemnation. Arcanobacterium pyogenes is not considered important in public health.

Arcanobacterium pyogenes (Pascual Ramos et al. 1997) was previously named Corynebacterium pyogenes and Actinomyces pyogenes. It is a small, nonsporing, gram-positive pleomorphic rod that produces a hemolytic exotoxin, pyolysin, a thiol-activated cytolysin, with a molecular weight of 57.9kDa (Billington et al. 1997). It produces two neuraminidases, and a collagen-binding protein CbpA promotes adhesion to cells. Fimbriae are also expressed (Jost and Billington 2005).

Growth is enhanced by the addition of serum or blood. Arcanobacterium pyogenes is aerobic or facultatively anaerobic and grows best at 98.6°F (37°C). Colonies are translucent and small, taking 48 hours to achieve a diameter of 1mm. Colonies form narrow zones of complete hemolysis after 24 hours on blood agar. Arcanobacterium pyogenes is proteolytic, glucose is fermented but other carbohydrate reactions are variable, and biochemical identification is most easily carried out using API Coryne strips (BioMerieux, Durham, NC).

**EPIDEMIOLOGY AND PATHOGENESIS**

Infections with A. pyogenes are found worldwide in ruminants, pigs, and horses. It is part of the mucosal flora of the host species and can be isolated from feces in clinically normal swine and in discharges from the upper respiratory tract, udder, vulva, and feces in infected animals. Transmission may, therefore, be direct or indirect via drinkers or other fomites. The organism can survive freezing and drying when protected in organic matter such as discharge. Arcanobacterium pyogenes is susceptible to a range of disinfectants.

Infection is opportunistic, resulting from the invasion of skin or mucous membranes by resident A. pyogenes. Adhesion to tissues is aided by neuraminidases, fimbriae, and collagen-binding protein (CbpA), and tissue damage results from the production of the pyolysin. The organism can multiply locally on surfaces such as inflamed bronchiolar epithelium, the vaginal
and uterine mucosa, and in the urinary tract. It causes ascending or hematogenous infections in the mammary gland and spreads by bacteremia to colonize existing minor lesions in the joints, lungs, vertebral bodies, and parenchymatous organs. Their location and extent cause the clinical signs. Antibody develops to products of the organism, but it does not eliminate infection from abscesses.

**CLINICAL SIGNS, LESIONS, AND DIAGNOSIS**

The clinical signs are quite variable, since *A. pyogenes* is responsible for a range of pathological lesions. Endocarditis, bronchopneumonia and adhesive peritonitis, may be fatal and be associated with fever. Suppurative osteomyelitis generally affects the vertebral bodies, leading to pathological fractures, vertebral collapse, and compression of the spinal cord. Lameness results from polyarthritis or from cellulitis and periartitis. Purulent secretion from the teat is seen in mastitis, and abscesses are prominent in involuted glands. Creamy discharge may be seen on the vulva in endometritis, and the urine may be flecked with pus in cystitis and pyelonephritis. Subcutaneous or intramuscular abscesses are often clinically inapparent and are discovered only at postmortem or slaughter. Mortality occurs when damage to an organ becomes life-threatening as in pyelonephritis in sows. Frequently, abscesses do not cause clinical signs other than loss of condition.

Arcanobacterium pyogenes causes creamy, greenish mucoid pus on inflamed mucous surfaces and appears as flecks of pus in the urine in cases of cystitis and as ropes of pus in the kidney pelvis and ureters in pyelonephritis and in the uterus in metritis, especially around macerated piglets. The most noticeable and pathognomonic lesions are abscesses that may arise in almost every tissue in the body. Such abscesses vary from a few millimeters to several centimeters in size, usually have a thick fibrous capsule, and contain yellow-green pus of variable consistency. They may be in joints, over cracked ribs, in contaminated injection sites, and in parenchymatous organs. Mastitis may be confined to one gland or may involve several.

Arcanobacterium pyogenes infection is the major cause of purulent abscessation and purulent discharges of all types in pigs, and its presence should be strongly suspected in such cases. Other pyogenic bacteria such as staphylococci (Chapter 62) may also cause abscessation, endocarditis, mastitis, endometritis, and pyelonephritis (where *E. coli* [Chapter 53] and *A. suis* [see above] must also be considered), and may be involved in bronchopneumonia. Confirmation of *A. pyogenes* requires the demonstration of the organism in typical lesions by laboratory culture. Pinhead beta-hemolytic colonies on blood agar suggest its presence, and identification can be confirmed using API Coryne strips (BioMerieux). *Actinobaculum suis* may be present in early cases of cystitis and pyelonephritis and then be overgrown by *A. pyogenes*.

**PREVENTION AND CONTROL**

Arcanobacterium pyogenes is sensitive to a wide range of antimicrobial agents including penicillin, tetracycline, and erythromycin. Some strains have been shown to be resistant to sulfonamides and trimethoprim. Abscesses may be removed surgically where they can be identified in affected individuals. Effective vaccines for swine are not yet available. Prevention requires management to reduce or prevent conditions that predispose to the development of *A. pyogenes* lesions. The treatment of sows with antimicrobials prior to farrowing can eliminate infection and prevent endometritis.

**REFERENCES**


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**BACILLUS ANTHRACIS: ANTHRAX**

**RELEVANCE, ETIOLOGY, AND PUBLIC HEALTH**

Anthrax is rare in swine and may not be fatal. It is a zoonosis, and infections in swine represent a hazard to the farmworker and veterinarian, to the abattoir worker and to those preparing and eating contaminated pig products. Its importance is increased by the requirement to disinfect abattoirs and dispose of carcasses after the discovery of an infected animal. Meat processors are unwilling to slaughter pigs from infected farms, retailers are concerned about their duty of care to consumers, and the safe disposal of manure can be a major problem. Recent concern over the possible use of the organism in bioterrorism has resulted in new controls on the organism and on diagnostic reagents, adding a wider importance to the disease.

Anthrax is caused by *Bacillus anthracis*, a large gram-positive, aerobic, spore-forming, nonmotile rod. The individual bacilli are 1–5 µm in diameter and 3–8 µm...
long with square ends. *Bacillus anthracis* has a protective capsule of D-glutamic acid and a complex exotoxin. The exotoxin is composed of three fractions and is produced when bacteria reach $5 \times 10^6$ organisms per milliliter of blood (Davis et al. 1973). *Bacillus anthracis* grows well on common laboratory media. On blood agar plates, colonies can usually be detected within 12 hours. After 24 hours at 98.6°F (37°C), the colonies are nonhemolytic, have a “ground glass” appearance with irregular, wavy borders that give them the “medusa head” characteristic. *Bacillus anthracis* may be distinguished from other members of the genus by biochemical tests, and the use of a specific bacteriophage and polymerase chain reaction (PCR) techniques. Culture should not be attempted unless appropriate safety precautions are available. Personnel handling the organism should be vaccinated.

Humans are at risk to be exposed to swine anthrax unknowingly as the disease is rare and clinical signs are not always apparent. Human exposure occurs when animals that have died from the disease are examined postmortem without appropriate precautions or during carcass disposal. Exposure through open skin wounds causes focal cellulitis and lymphadenitis in humans that is often self-limiting. More serious is inhalation of spores that leads to flu-like symptoms and progresses to often fatal sepsis and toxemia. Because anthrax bacilli sporulate when exposed to oxygen, risk of inhalation of spores increases when infected carcasses are examined that have bloody exudates exuding from body orifices and contaminating the skin. Ingestion of vegetative cells from undercooked, contaminated meat can occur when infected animals or carcasses enter the food chain. Proliferation in the gastrointestinal tract leads to anorexia, bloody diarrhea, and abdominal pain that progress to often fatal sepsis and toxemia. The spores can contaminate the soil after disposal of infective material and human infection can result from exposure to this infected soil for up to 50 years.

**EPIDEMIOLOGY AND PATHOGENESIS**

Anthrax is present throughout the world and occurred in every continent during 2009. The incidence in swine remains low and sporadic. Some countries are free from infection. Anthrax is most severe and most easily recognized in ruminants, particularly cattle and sheep, but many mammalian species can be affected.

Anthrax in swine generally occurs following ingestion of feed that contains a large number of *B. anthracis* or viable spores, especially carcasses of animals dead of anthrax. The use of bonemeal or other animal products containing spores of *B. anthracis* in feed is the most common source of infection in swine (Ferguson 1986). The organism appears to spread in wet feed systems but rarely affects more than one to two animals in an infected herd. However, there are uncommon reports of outbreaks continuing for up to 14 weeks (Edginton 1990; Jackson 1967; Jackson and Taylor 1989).

Animal-to-animal spread is not common and infection can result from the ingestion of *B. anthracis* spores in soil or the environment in which they survive for many years. Biting flies (*Stomoxys calcitrans*) and mosquitoes (*Aedes aegypti* and *taeniorynchus*) transmit the disease experimentally 4 hours after feeding (Turell and Knudson 1987). Ticks (*Dermacentor marginatus*) may also harbor the organism.

Infection is oral and invasion occurs in the tonsils or mucosa of the pharynx. In some cases, the infection may remain localized in the lymph nodes of this region, and the disease is classified as pharyngeal. Primary invasion may also occur in the intestinal tract. When *B. anthracis* gains access to the general circulation, the septicemic form of the disease develops. The organism multiplies locally, resisting phagocytosis by means of the polyglutamic acid capsule, and toxin production results in the death of the animal by damaging the mitochondria.

**CLINICAL SIGNS AND LESIONS**

The incubation period ranges from 1 to 8 days when an increase in mortality may occur. Three forms of anthrax have been observed in swine: pharyngeal, intestinal, and septicemic. Cervical edema and dyspnea are commonly observed in pharyngeal anthrax with depression, inappetence, and vomiting. Fever with a temperature of 107°F (41.7°C) may occur. Death may follow within 24 hours of cervical edema, but swine may recover in the absence of treatment. Recovered animals may remain carriers of *B. anthracis*.

Intestinal anthrax may be severe with vomiting, complete loss of appetite, and diarrhea with bloody feces. Death follows in severely affected swine, but less severe cases are more common and may recover (Brennan 1953). When 50 pigs were infected in an experimental study (Redmond et al. 1997), 33 developed anorexia, lethargy, dullness, shivering, constipation, loose feces, blood in the feces, and ataxia at some point between 1 and 8 days after infection. Only two died. Fever did not exceed 107.4°F (41.9°C), peaking 48 hours after infection.

Septicemic anthrax is highly acute and frequently results in death without sickness being noticed by the owner. It is uncommon in swine. Young pigs may develop septicemia more frequently than older swine (Ferguson 1986).

Complete necropsy of anthrax cases is discouraged to reduce contamination with the spores. However, since pigs with anthrax may not be identified before
necrosis. Encapsulated bacilli may be observed in the capillaries of infected pigs (Redmond et al. 1997). Microscopically, abscesses may be present in the lymph nodes of recovered pigs with local petechiation. There may be marked petechiation and bloody discharge from the nose (Edgington 1990), and small ones may appear very pale and dehydrated. The cervical region may be edematous, with straw-colored, pink, or hemorrhagic fluid and the tissue has a gelatinous consistency. The tonsils are usually covered with a fibrinous exudate, and there may be extensive necrotic changes. The pharyngeal mucosa is frequently inflamed and swollen. Mandibular and suprathyroidal lymph nodes may be enlarged to several times their normal size. The cut surface of the affected node may vary in color from deep brick red to strawberry red. In more chronic cases, the color may be grayish yellow indicative of necrotic changes in the node. In the intestinal form, the copious pinkish peritoneal fluid may clot on exposure to air. The small intestine is usually inflamed with fibrinous adhesions on the serous surface. The mesenteric lymph nodes may be swollen, hemorrhagic, or necrotic, and edema of the mesentery is common. The intestinal mucosa is covered with a diphtheritic membrane and may be hemorrhagic. The intestinal wall may be grossly thickened. In the septicemic form, bloodstained fluid may be seen in the peritoneal cavity with local petechiation. There may be marked petechiation of the kidney or enlargement of the spleen. Small abscesses may be present in the lymph nodes of recovered pigs (Redmond et al. 1997). Microscopically, encapsulated bacilli may be observed in the capillaries and in the lymph nodes together with hemorrhage and necrosis.

**DIAGNOSIS**

The accurate diagnosis of anthrax is very important and depends on the isolation and identification of *B. anthracis* or its DNA. The officially recommended methods for the diagnosis of anthrax can be found in the *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals* (OIE 2010). Anthrax should be suspected when swine have cervical edema and dyspnea. Malignant edema from *Clostridium septicum* may also produce similar clinical signs (see Chapter 52). The presence of blood on the feces and fever may suggest intestinal anthrax, but it must be distinguished from swine dysentery (Chapter 50) and proliferative hemorrhagic enteropathy (Chapter 59) in which fever is unlikely.

When the carcass has been opened, edematous fluid in the pharyngeal region and enlarged cervical or mesenteric lymph nodes suggest anthrax. The presence of bloodstained fluid in the peritoneum, petechiation of the kidney or serosal surfaces, enlargement of the spleen and thickening and inflammation of the small intestine are features of anthrax. There is often a history of feeding animal protein products. Hog cholera/classical swine fever (Chapter 38) and African swine fever (Chapter 25) should be ruled out in septicemic cases.

*Bacillus anthracis* can be demonstrated in impression smears and cultures from the cut surfaces of the cervical lymph nodes, peritoneal fluid, spleen, mesenteric lymph nodes, intestinal mucosa, or kidney. Smears should be fixed and stained by polychrome methylene blue. The bacilli of anthrax appear as square-ended blue rods in a pinkish capsule. Other bacilli may be present and where antimicrobial treatment has been given, the bacilli may only be present as capsules. Slides and reagents used for diagnosis should be disposed of by incineration or formaldehyde fixation.

*Bacillus anthracis* is readily cultured and large, non-hemolytic colonies can be identified by their biochemical characters using API systems (BioMerieux). Final confirmation of pathogenic *B. anthracis* depends on susceptibility to the anthrax phage, animal inoculation, and 16S rRNA gene sequencing. All cultures and any experimental animals should be fixed in formaldehyde and incinerated. PCR tests are carried out on DNA extracted using commercially available kits and primers from the organism (Hutson et al. 1993). A competitive enzyme immunosorbent assay (EIA) has been described (Turnbull et al. 1986) to identify the presence of immunoglobulin G (IgG) antibody to the toxin.

**PREVENTION AND CONTROL**

Pigs with clinical anthrax can recover completely after penicillin treatment (Ferguson 1986). Anthrax antiserum in doses of 20–75 mL can also be given. Oxytetracycline is effective against *B. anthracis* and may be used parenterally in daily doses of 4.4–11.0 mg/kg body weight (Edgington 1990). Infection may persist for up to 21 days after infection in a population (Redmond et al. 1997), and this factor must be considered before animals are sent for human consumption.

Immunity develops in recovered animals and serum antibody to the toxin can be detected. Protective immunity can be stimulated by vaccines. Kaufmann et al. (1973) used the Sterne strain anthrax vaccine, an avirulent spore vaccine.

Control of the spread of anthrax differs significantly from control of most other animal diseases, because it depends on preventing access to viable spores of *B. anthracis* in soil, manures, or contaminated feeds such as bonemeal. Prevention of environmental contamination by the long-lived spores is essential. Few spores are formed in the unopened carcasses of animals dead of anthrax. The orifices and any cuts in a carcass should be covered with disinfectant-soaked cotton wool to prevent sporulation and spread of infection. Carcasses should be destroyed by incineration, preferably on the spot or by deep burial in at least 4 ft (1.25 m) of soil with the carcass covered with lime.
Disinfection can be achieved with 5% freshly prepared sodium hydroxide or, more controllably, with 10% formaldehyde (Edgington 1990). Only disinfectants capable of inactivating anthrax spores such as those containing glutaraldehyde and formaldehyde should be used. Disinfectants should be used prior to clearing up infected premises and contaminated articles should be burned. Exposed surfaces should be scrubbed or pressure washed with the disinfectant.

Human infection can be prevented by the safe disposal of all contaminated carcasses, articles, and fluids on the farm by the methods outlined above. Persons exposed to the infection can be given prophylactic antimicrobials such as penicillin and tetracyclines by qualified medical personnel and any cases can be treated. Vaccination can protect humans against longer-term exposure.

**BURKHOLDERIA PSEUDOMALLEI: MELIOIDOSIS**

**RELEVANCE, ETIOLOGY, AND PUBLIC HEALTH**

Melioidosis is a chronic bacterial infection of swine in tropical and subtropical regions. Humans can be infected, and swine may be a source of the infection in contaminated meat or from organisms shed in the feces. Both pigs and people may contract infection from a common source. *Burkholderia pseudomallei* is now considered a candidate agent for bioterrorists.

*B. pseudomallei* is a short, gram-negative rod, 0.8 by 1.5 µm, which does not form spores. It produces rough (wrinkled) or mucoid colonies on a wide variety of laboratory media at 98.6°F (37°C) and grows on MacConkey agar to give colorless colonies.

Melioidosis in humans can be fatal, presenting as septicemia, pneumonia, or as chronic suppurative lesions of skin, lymph nodes, or bone. Mortality in humans is 20–50% with or without treatment. It is rarely transmissible from person to person and is usually acquired by the ingestion of contaminated food and water. This contamination can be purely environmental or result from contamination of water and food with infected animal feces. Ingestion of improperly cooked meat from infected animals may also result in human infection.

**EPIDEMIOLOGY AND PATHOGENESIS**

*Burkholderia pseudomallei* is present in water and soil in tropical and subtropical areas and may infect pigs when water supplies are contaminated or when they consume infected animal or plant matter. It has been reported from Australia (Millan et al. 2007), Malaysia (Omar et al. 1962), and the Caribbean. Infection results from contamination of water or feed from environmental sources or by feces from an infected pig. In one recently reported incident (Millan et al. 2007), the source of infection for pigs was borehole water, which yielded *B. pseudomallei* of the same pulsed-field gel electrophoresis (PFGE) type as those isolated from the affected pigs.

Phenolic (2% Lysol), chlorine (0.1–0.5%), and oxidizing disinfectants (1% Virkon® [Dupont, Wilmington, DE], 3% hydrogen peroxide) or formaldehyde (4%) are effective against *B. pseudomallei*.

**CLINICAL SIGNS, LESIONS, AND DIAGNOSIS**

Infection is often clinically inapparent, but a raised rectal temperature of 104–108°F (40–42°C) may develop for up to 4 days. Unsteady gait, lameness, or weakness; slight nasal discharge; and subcutaneous swellings of the limbs may be seen. Deaths may occur but are rare in adults in which abortions and uterine discharges have been recorded (Laws and Hall 1964; Millan et al. 2007; Omar et al. 1962; Rogers and Andersen 1970).

Lesions are found in slaughter pigs in which clinical signs have not been seen and in those that have died from the disease. They consist of large abscesses filled with creamy or caseous yellow-green pus in the lungs, liver, spleen, kidneys, and mesenteric and subcutaneous lymph nodes. The organism can be isolated from them.

Melioidosis should be suspected in tropical environs on clinical grounds when prolonged raised rectal tem-
temperatures and unsteady gait are associated with subcutaneous swellings of the limbs. More frequently, presumptive diagnosis is based on the typical creamy abscesses found at slaughter (Ketterer et al. 1986). Diagnosis is confirmed by culture. A selective medium is used for environmental samples (Peacock et al. 2005). The identity of suspect colonies can be confirmed biochemically using API NE strips (BioMerieux). A hypersensitivity test resembling a tuberculin test (the melioidin test) and serum-agglutination and complement-fixation tests have been used to confirm diagnosis in the live pig. Antibody has been demonstrated within 7 days of experimental infection (Najdenski et al. 2004).

PREVENTION AND CONTROL

_Burkholderia pseudomallei_ is resistant in vitro to aminoglycosides but susceptible to some cephalosporins and to amoxicillin: clavulanic acid. The disease can be prevented by use of clean or chlorinated water supplies and preventing access to contaminated soil. As the disease is of public health importance, infected carcasses should be disposed of safely. Rigorous criteria for meat inspection of slaughter pigs will help to safeguard public health.

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CAMPYLOBACTER

RELEVANCE, ETIOLOGY, AND PUBLIC HEALTH

Campylobacters were first isolated from swine in the 1940s by Doyle (1948) who named his organism _Vibrio coli_ and considered them to be the cause of swine dysentery. Most information about their possible role in swine enteritis since that time has come from nonimmune pig Campylobacter jejuni infection models and from recording their presence in swine enteritis. Campylobacters are not associated with any generally recognized enteric syndrome in swine. In contrast, campylobacters, especially _C. jejuni_, are considered major causes of human food-borne enteric disease. _Campylobacter jejuni_ and _Campylobacter coli_ of swine origin can cause disease in humans.

Several species of _Campylobacter_ have been isolated from swine. The first to be isolated, _V. coli_ (Doyle 1948), has been reclassified as _C. coli_. _Campylobacter mucosalis_ was isolated from the pig intestine by Lawson et al. (1981). _Campylobacter hyointestinalis_ was isolated by Gebhart et al. (1985). _Campylobacter jejuni_, the most important cause of human campylobacteriosis, is also found in swine. The current nomenclature of the genus _Campylobacter_ was reviewed and confirmed by Vandamme et al. (1991). Other species and subspecies have been identified in swine and may reach high numbers and be associated with enteritis.

Campylobacters are gram-negative curved rods or short spiral organisms 2–3µm in length and 0.3µm in diameter with a single polar flagellum. They do not form spores. They are microaerobic and grow most readily on media containing blood or other sources of iron. Most commercial media for isolation also incorporate selective antimicrobials such as cefperazone and amphotericin B. Growth occurs readily at 98.6°F (37°C), but they are often cultivated at higher temperatures of 104–108°F (40–42°C). Colonies generally appear after 48 hours’ incubation. _Campylobacter coli_ forms sprawling watery colonies of blood agar after 48 hours, and _C. jejuni_ forms slightly smaller colonies 2–3mm in diameter. They can be identified presumptively to species using biochemical strips (API Campy, BioMerieux).

Campylobacters are the most common cause of food-borne bacterial enteric disease in humans. Poultry meat is the most common source of infection, but pig meat and offal may be contaminated with campylobacters and may lead to human infection. _Campylobacter coli_ is the most common contaminant of pig carcasses (Mafu et al. 1989) but is less common in human disease than _C. jejuni_. _Campylobacter jejuni_ has been identified on pig meat in livers in a number of countries. Chilling of the carcass, particularly blast freezing/chilling (Nesbakken et al. 2008), reduces the numbers of campylobacters on carcass meat and thus reducing consumer exposure. Contact with swine, their feces, or water contaminated with effluent from pig farms may also give rise to human infections.

EPIDEMIOLOGY AND PATHOGENESIS

The four species of _Campylobacter_ described here can be found in swine worldwide. _Campylobacter coli_ is most commonly found in the pig, but it also occurs in a wide...
range of hosts including cattle and poultry. *Campylobacter jejuni* is less frequently isolated from swine, but it is present in a wide range of mammalian and avian species where it may cause enteritis. *Campylobacter hyointestinalis* and *C. mucosalis* are also found in other mammalian species.

Infection takes place by the oral route and is usually direct from one infected pig to another. As the organism can survive in feces and contaminated water, indirect transmission may occur. Birds, rodents, and insects may contaminate feed and water and introduce infection to a herd. Piglets become infected from maternal feces (Soultos and Madden 2007), contaminated water or by horizontal transmission. Maternal immunity normally prevents clinical disease but does not prevent colonization. Infected animals remain carriers for long periods and pass $10^3-10^4$ organisms/g feces for months. The organism is carried principally on the ileal and large intestinal mucosa and shed in the feces.

Following infection, multiplication occurs in the small intestine, particularly the ileum and large intestine where the organism is closely associated with the mucosa. A brief bacteremic phase may occur, but the organism does not appear to penetrate the epithelium in any numbers. Recent studies suggest that it may produce a cytotoxin that could cause the inflammatory changes seen. Clinical signs begin after an incubation period of 1–3 days. These have only been reproduced repeatedly with nonimmune snatched or hysterectomy-derived, colostrum-deprived piglets (Vitovci et al. 1989). Experimental infections have demonstrated that infection with other enteric pathogens such as *Trichuris suis* results in increased shedding of *C. jejuni* (Mansfield and Gauthier 2004; Mansfield et al. 2003).

The capture of *Campylobacter* antigens by lympho-glandular complexes and the development of an IgA response in the large intestine have been described by Mansfield and Gauthier (2004) who used *C. jejuni* in experimental infections. Serum antibodies were detected by von Altrock et al. (2006).

**CLINICAL SIGNS, LESIONS, AND DIAGNOSIS**

Infection may be inapparent, but clinical signs may be identified in piglets, which may have a mild fever up to 105°F (40.6°C) for 2–3 days. A watery or creamy diarrhea containing mucus with occasional streaks of blood is present (Olubunmi and Taylor 1982). In weaned pigs, *C. coli* infection may be associated with chronic mucoid diarrhea in which no blood is seen. In both forms of the disease, loss of condition occurs but mortality is rare.

The common inclusion of antimicrobial growth promoters in pig rations prior to 2006 in Europe and their continued use in much of the rest of the world can affect numbers of campylobacters present and potentially prevent the development of clinical signs. Antimicrobial therapy for other diseases could also reduce the severity of any clinical signs.

In suckling pigs, gross changes are slight and are confined to the small intestine, which is slightly inflamed with thickening of the ileal wall, especially of the terminal portion. The mesenteric lymph nodes are prominent. The ileal mucosa is mildly inflamed and mucoid contents may be present and in the cecum. The mucosa of the large intestine is usually mildly inflamed or normal.

Histological changes are slight, the most prominent being stunting of the ileal villi and massive enlargement of the lymphoid tissue in the terminal ileum. Crypt abscesses may be present. In weaned pigs, there may be prominent thickening of the terminal small intestine. Large intestinal changes resembling chronic mild swine dysentery may be seen. *Campylobacter coli* or other campylobacters, including *C. jejuni*, may be isolated on selective medium from the small intestine in large numbers.

The diagnosis of uncomplicated campylobacteriosis in swine is currently by elimination of other causes of enteritis. *Campylobacter coli* or *C. jejuni* infections could contribute to or cause a mucoid diarrhea containing bloodstained mucus in piglets with little mortality and some loss of condition. *Campylobacter jejuni* can be isolated from some watery, mucoid diarrheas of weaned pigs and other campylobacters may contribute to enteritis generally. Other causes of diarrhea, such as rotavirus, epidemic diarrhea, *Clostridium perfringens*, *E. coli*, coccidia, and cryptosporidia should be eliminated to reach a diagnosis of primary campylobacteriosis in piglets. Proliferative enteropathy, salmonellosis, spirochetal diarrhea, and swine dysentery should be absent before a diagnosis of campylobacteriosis is made.

The involvement of campylobacters in an enteric syndrome should be suspected when there is lymphoid thickening of the mucosa of the distal ileum and enlargement of the mesenteric lymph nodes with no gross lesions of proliferative enteropathy. Confirmation of the presence of campylobacters in intestinal contents or feces is by culture. Since the organisms (especially *C. coli*) are common inhabitants of the porcine intestinal tract, they should only be considered significant when they are present in profuse culture or if *C. jejuni* is present in large numbers in weaned pigs.

Isolation has recently been supplemented by DNA probes, PCR, and real-time PCR for *C. jejuni* and *C. coli* (Jensen et al. 2005). Within species, restriction fragment length polymorphism (RFLP), PFGE, and multilocus sequence typing (MLST) have all been used to establish relationships between isolates of a species from different hosts, different locations, or with differing antimicrobial sensitivities. Antibody detection is not used in diagnosing infection, but a serum enzyme-linked immunosorbent assay (ELISA) test has been described by von Altrock et al. (2006).
PREVENTION AND CONTROL

Treatment is rarely carried out specifically for campylobacteriosis. Oral treatment with neomycin, other aminoglycosides, tetracyclines, macrolides, and fluoroquinolones may all eliminate or markedly reduce *Campylobacter* infection. Campylobacters are susceptible to most farm disinfectants.

Control has rarely been attempted in the pig. Low-level antimicrobial treatment would be an appropriate method, coupled with hygiene and chlorination of water supplies. It is possible to maintain pig units founded from primary hysterectomy-derived stock free from infection by strict isolation. An application of *Campylobacter* control in the field has been described by Weijtens et al. (2000).

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CHLAMYDIA

RELEVANCE, ETIOLOGY, AND PUBLIC HEALTH

Four species of two genera of *Chlamydia* are found in swine (*Chlamydia suis* and *Chlamydophila pecorum, abortus*, and *psittaci*). They have been identified in cases of conjunctivitis, enteritis, pneumonia, pleuritis, pericarditis, arthritis, orchitis, uterine infections, and abortion. They may be found alone or may form part of multiple infections. *Chlamydophila psittaci* of avian origin is a cause of fever and pneumonia in humans, and *C. abortus* of ruminant origin can cause abortion in humans; however, human infection with swine strains is not recorded.

The chlamydia were reclassified by Everett et al. (1999) into two genera: *Chlamydia* (containing *C. suis*) and *Chlamydophila* (containing *C. psittaci*, *C. pecorum*, and *C. abortus*). *Chlamydia* are gram-negative intracellular bacteria that can only multiply inside living cells and exist outside them as elementary bodies, inactive, trypsin-resistant, infectious particles 0.2–0.3 µm (200–300 nm) in diameter, which develop into reticulate bodies 1 µm (1000 nm) in diameter inside a cell. Reticulate bodies mature into elementary bodies and form chlamydial inclusions. Infected cells may lyse to release them or they may be budded from persistently infected cells. All *Chlamydia* can survive for considerable lengths of time as elementary bodies in the environment where they are resistant to drying.

*Chlamydia* can be grown in embryonated chicken eggs and in cell cultures, usually McCoy or L929 cells in which they grow readily. Sequences of the genes coding for outer membrane protein A (*ompA*) have been obtained (Anderson et al. 1996; Kaltenboeck and Storz 1992; Kaltenboeck et al. 1993) and are used in species classification and diagnosis. The main methods of differentiation between the different species and their biotypes are now antigenic, based on differences between the outer membrane proteins detected using monoclonal antibodies in immunoperoxidase, immunofluorescence, or ELISA methods and nucleic acid based, using genomic and *ompA* sequences for PCR (Anderson et al. 1996; Kaltenboeck and Storz 1992; Kaltenboeck et al. 1993).

EPIDEMIOLOGY AND PATHOGENESIS

Chlamydial infection in the pig has been reported from North America, Europe, and Asia. *Chlamydia suis* is found exclusively in pigs, but the others that infect swine also infect additional species: *C. psittaci* in birds and mammals; *C. pecorum* in ruminants; and *C. abortus* in cattle, horses, and sheep.

Infection is widespread in swine. Zahn et al. (1995) found evidence of infection in up to 67% of piglets, and Szeredi et al. (1996) found intestinal infection in 99% of finishing pigs.

Pigs are infected by inhalation, ingestion of contaminated feed, and by contact, particularly venereal. Vertical transmission occurs in the case of infections contracted in utero. Transmission by flies or dust is
involved in chlamydial conjunctivitis (Rogers et al. 1993).

*Chlamydia* can be shed from any infected site with access to the exterior of the body. Infection can occur in animals of every age group. Enteric infection occurs in piglets of less than 4 weeks of age (6.9%) and more commonly (41.8%) in piglets aged more than 4 weeks (Zahn et al. 1995). Conjunctival infection was recorded by Rogers et al. (1993) in pigs between 2 and 8 weeks of age. *Chlamydia* survive drying and can persist in dust in an infected environment but are susceptible to a wide range of disinfectants including oxidizing, phenolic, chlorine-based products; quaternary ammonium compounds; and some detergents.

Elementary bodies enter by the respiratory, oral, or genital routes, and multiply in epithelial cells or are taken up by macrophages and distributed to the lymph nodes. Infection may be local at the portal of entry and remain inapparent or latent, may cause local disease such as pneumonia, enteritis, or disturbances of reproduction, or may become generalized. Pneumonic lesions were produced in germfree pigs by Rogers et al. (1996) using a *Chlamydia trachomatis*-like isolate from a case of pneumonia. They identified mild multifocal rhinitis and diarrhea in addition to the pneumonic changes. The pathogenesis of enteric infection has been most recently described by Guscetti et al. (2009) using the type strain of *C. suis* in gnotobiotic piglets during which it was noted that peak multiplication of the organism in the villi occurred at 2–4 days postinfection. In genital infections, infected semen given to sows has resulted in the birth of weak piglets and continued shedding for up to 20 months. Serum antibody develops after infection or reinfection resulting in little or no further disease after 3–4 weeks.

**CLINICAL SIGNS, LESIONS, AND DIAGNOSIS**

Many chlamydial infections are inapparent, but respiratory tract and generalized infections result in inappetence and a rise in rectal temperature of 102–106°F (39–41°C). Dyspnea (Reinhold et al. 2005), pneumonia, and conjunctivitis may occur and may persist for 4–8 days. Pleuritis or pericarditis may occur, and lameness may result from synovitis and articular involvement in one or more joints. Other disturbances of gait include weakness in piglets and nervous signs in pigs of all age groups. Fatal infections are most commonly reported in younger animals. Respiratory tract and generalized infections develop after an incubation period of 3–11 days and pigment diarrhea after 2–5 days.

Experimental infection of gnotobiotic piglets with *C. suis* by Guscetti et al. (2009) resulted in moderate to severe diarrhea consisting of watery feces containing milk curd, transient anorexia, slight weakness, slight loss of condition, and weight loss between 2 and 3 days postinfection followed by weight gain. Many reports deal with genital tract infection and disturbances in reproduction. In the boar, infection is associated with orchitis, epididymitis, and urethritis, while infections in gilts and sows have resulted in late abortions and the birth of dead or weak piglets. Serological and isolation studies suggest that many genital tract infections are clinically inapparent.

Lesions in which *Chlamydia* are demonstrated often contain other agents. Lung lesions are distributed posteriorly in most cases (Harris et al. 1984). They are irregular and raised, of firm consistency, extending deep into the lung tissue, limited by lobular boundaries, and clearly demarcated from adjacent grossly normal tissue. Early lesions are pale red, becoming grayish as they age. Enlarged bronchial lymph nodes may be present. The microscopic findings include thickening of the alveolar septae by capillaries, septal edema, and neutrophils in peribronchial and subepithelial sites. Neutrophils and macrophages are common in the alveolar lumina, and in some areas, this exudate occludes terminal bronchioles. Edema and massive epithelial cell shedding have been reported in severely affected lung lobules (Martin et al. 1983). Antigen can be demonstrated in bronchial and broncholar epithelial cells and in pneumocytes in experimental (Rogers et al. 1996) and field cases (Done et al. 1992).

Pericarditis, pleuritis, hemorrhages of the kidneys and bladder, enlargement of the spleen, synovitis, arthritic changes, and interstitial edema and tubular degeneration in orchitis in boars have all been reported. Aborted piglets may be mummified, stillborn, or weak. Lesions in experimentally infected gnotobiotic piglets included watery colon contents with flakes of undigested curd, villous atrophy, lymphangitis, and multifocal necrosis of the apical portion of the villi (Guscetti et al. 2009; Rogers and Andersen 1996).

Presumptive diagnosis is difficult because the clinical signs of chlamydial infection are not distinctive and may include conjunctivitis, pneumonia, polyarthritis, enteritis (especially in piglets), late abortion, stillbirths, mummified piglets, and orchitis in boars. Differential diagnosis should include common causes of the clinical signs suspected of being produced by *Chlamydia*, and these other agents should be excluded by testing. *Chlamydia* may be detected in smears and in histological specimens after staining by Giemsa’s method or Koster’s stain. Specific immunofluorescence or immunoperoxidase tests may be used (Chasey et al. 1981). Commercial antigen ELISAs may be used to detect antigen in extracts of tissue (Guscetti et al. 2009), but PCR technology is currently preferred, as it allows the specific identification of the *Chlamydia* present. Primers include genomic DNA sequences, 16S rRNA gene sequences, *ompA* gene sequences, and plasmid sequences. A nested
PCR can be used to identify species in multiple infections (Schiller et al. 1997).

Isolation can also be carried using fertile chicken eggs or cell cultures. Handling chlamydias is dangerous, and severe human infections and death can result. Appropriate safety precautions should be observed. Antibody may be detected using ELISAs, but the absence of antibody does not confirm the absence of infection.

PREVENTION AND CONTROL

*Chlamydiophila psittaci* is most frequently treated using tetracyclines. Treatment for inadequate length of time may result in relapse. Complete elimination or suppression of infection to the latent state requires 21 days of treatment at a therapeutic level. Medication can be given in drinking water or feed.

Pigs should be prevented from coming into contact with infected pigs, other mammalian species, and with bird droppings. Any infected breeding stock should be used only after tetracycline treatment or kept in isolation until sufficient uninfected animals are available to replace them. Disinfection with phenols and formalin fumigation will eliminate elementary bodies from buildings.

**REFERENCE**


LISTERIA MONOCYTOGENES

**RELEVANCE, ETIOLOGY, AND PUBLIC HEALTH**

*Listeria monocytogenes* is commonly carried in the intestines of swine and is a rare cause of fatal disease in piglets, nervous signs in all age groups, and abortion in sows. The organism is a cause of serious but uncommon food-borne disease in humans and its carriage by slaughter swine is of concern to the food industry.

*Listeria monocytogenes* is one of a number of species of *Listeria* found in the swine environment, but it is the only one that causes clinical disease in swine and that is a potential cause of food-borne disease. *Listeria monocytogenes* is a gram-positive bacillus 1.2µm by 0.5µm and does not form spores. It carries the *hly* gene coding for listerolysin, the toxin responsible for pathogenicity. The organism grows at temperatures as low as 39°F (4°C) and up to 98.6°F (37°C) and can multiply in the presence of nutrients in refrigerated conditions or at room temperature. It is aerobic and produces 1-mm, grayish, and opaque colonies with a narrow zone of β-hemolysis on blood agar. It is capable of growth on a number of media.

*Listeria monocytogenes* is a cause of septicemia, abortion, and nervous signs in humans. Pregnant women, newborn infants, the elderly, and the immunosuppressed are most at risk. Although the organism is present in the environment, food, particularly meat products, is an important source. As the organism is present in the swine environment and swine are carriers, considerable effort has gone into monitoring the farm environment and the swine slaughter process for the organism. Thevenot et al. (2006) reviewed the contamination of pork and pork products with *L. monocytogenes*. Contamination increases from farm to end product because of cross-contamination and multiplication of the organism. Up to 30% of minced pork products may be contaminated.

**EPIDEMIOLOGY AND PATHOGENESIS**

*Listeria monocytogenes* occurs worldwide and is carried by most food animal species, and clinical disease occasionally develops. Carriage of *Listeria* sp. is common in pigs in the intestine and, to a lesser extent, in the tonsils. Studies in Denmark, Japan, and Yugoslavia suggest that about 10% of slaughter pigs are carriers. Carriage rate increases when liquid feed or silage are fed. Exposure in pigs is principally by ingestion.
Shedding is in the feces and in the products of abortion. The organism can be detected in manure for at least 55 days (Grewal et al. 2007) and is susceptible to most approved disinfectants. *Listeria monocytogenes* is destroyed by pasteurization, but food contamination can occur after heating.

The organism has two modes of entry. In nervous disease, it may travel up the nerves to the brain by retrograde movement, and in septicemia, it enters through the tonsil or gut to cause lymphadenitis and septicemic spread. Invasion of privileged sites such as the brain, joints, and uterus follows bacteremia/septicemia. Clinical signs are caused by the invasion and by production of listerolysin. Neonatal and pregnant animals are most likely to be clinically affected.

**CLINICAL SIGNS, LESIONS, AND DIAGNOSIS**

Infection rarely causes disease, but sudden death in piglets, septicemia, fever of 107.7°F (42°C), and nervous signs have all been recorded (Lopez and Bildfell 1989). Abortion, stillbirths, and the birth of weak piglets may all occur in sows. The incubation period is 24–48 hours in neonates. Clinical cases often die within 4 days of developing clinical signs, particularly if they have the nervous form of the disease. Listeriosis is uncommon in swine and the clinical signs of disease are not unique, so this disease would not normally be considered by a clinician. Perhaps the nervous signs are most likely to lead to suspicion on clinical grounds, and it should be distinguished from streptococcal meningitis in most countries.

Lesions in piglets may include small foci of necrosis in the liver and patchy lesions in the lungs and hydrothorax. Histological lesions include meningitis, perivascular cuffing, and microabscess formation in the brain. The bacteria may be seen in these. In the septicemic form of disease in piglets, small, gray translucent necrotic areas in the liver are suggestive of listeriosis but may also be observed in piglets with Aujeszky’s disease.

Demonstration of *L. monocytogenes* is required to confirm the diagnosis or prove carriage. The organism grows directly from clinical specimens to give the typical hemolytic colonies on blood agar, but for contaminated specimens, tonsillar material, cecal contents, feces, environmental samples, and foods, enrichment in the cold at 39°F (4°C) overnight in a simple enrichment medium or one supplemented with antimicrobials including amphotericin B and then subculture onto a selective medium including esculin gives good results.

PCR techniques using primers based on the *hly* gene sequence have been used for qualitative detection in conventional gel-based PCR and for quantitative demonstration of the organism using real-time PCR. The products of enrichment may be used in some of these tests. Microarrays are now available commercially and can cut the time for demonstration to 10 hours. Serum antibody may be present in infected pigs, especially in sows after abortion.

**PREVENTION AND CONTROL**

The organism is sensitive to a number of antimicrobials including penicillins and aminoglycosides and affected pigs may be treated if caught in time. Paralyzed pigs should be destroyed. Carriage of the organism may be reduced if dry feeding is practiced. Composting of manures at more than 131°F (55°C) or use of aerobic composting can reduce numbers of *Listeria* in wastes (Grewal et al. 2007).

**REFERENCES**

smooth, and mucoid. *Rhodococcus equi* is biochemically unreactive and is most easily identified using the API Coryne strip (BioMerieux).

*Rhodococcus equi* is of importance to public health as a cause of granulomatous lesions in the cervical lymph nodes of pigs. These must be distinguished from those of *Mycobacterium avium avium* and other mycobacteria, and are therefore of concern in meat inspection. *Rhodococcus equi* can infect humans and is becoming more common as a cause of necrotizing pneumonia and chronic illness in immunocompromised humans, particularly in those suffering from HIV infections. Infection results in high mortality, averaging 25%. Human isolates are similar to some pig strains.

**EPIDEMIOLOGY AND PATHOGENESIS**

Little is known of the epidemiology of the naturally occurring disease in swine, but *R. equi* has been reported worldwide and infects swine, cattle, deer, horses, sheep, goats, wild birds, and humans (Woolcock et al. 1979). *Rhodococcus equi* infection is likely to be acquired from the environment by ingestion in swine housed on pasture or in yards contaminated with *R. equi* (Barton and Hughes 1984). The bacterium is readily isolated from the feces of such pigs. Komun et al. (2007) examined 15,900 cervical lymph nodes for granulomatous lesions in The Netherlands and found them in 0.75% pigs. *Rhodococcus equi* was isolated from 44% of these. *Rhodococcus equi* is present in dust and even in cobwebs of farm buildings in areas where it occurs and is relatively resistant to chemical disinfectants.

The way in which *R. equi* causes granulomatous lymphadenitis of the head and neck in the pig is not clear. Infection is usually by the oral route, but Zink and Yager (1987) were able to produce pneumonia using aerosols.

**CLINICAL SIGNS, LESIONS, AND DIAGNOSIS**

*Rhodococcus equi* infection in swine is normally subclinical and has rarely been associated with serious clinical disease. The lesions of granulomatous lymphadenitis are detected only at slaughter. Affected submandibular and cervical nodes are enlarged and contain multiple yellow-tan foci that are often in a subcapsular location. Caseation and calcification of these foci sometimes occur.

Diagnosis is postmortem. *Rhodococcus equi* causes a high proportion of the granulomatous lesions found at postmortem meat inspection, but microbiological identification of *R. equi* and elimination of mycobacterial infection is necessary to confirm diagnosis. Selective media (Makrai et al. 2005; Woolcock et al. 1979) improve the results of culture as the creamy or pink domed colonies of *R. equi* require 48 hours to reach a size of 2–4 mm.

**PREVENTION AND CONTROL**

*Rhodococcus equi*-induced disease is not sufficiently important to necessitate antemortem diagnosis and treatment in swine or dedicated preventive measures.

**REFERENCES**


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**TREPONEMA PEDIS: CUTANEOUS SPIROCHETOSIS**

**RELEVANCE, ETIOLOGY, AND PUBLIC HEALTH**

*Treponema pedis*, a spirochete previously isolated from digital dermatitis in cattle (Evans et al. 2009), has now been isolated from ear necrosis and skin lesions in pigs (Pringle and Fellstrom 2010; Pringle et al. 2009). Other bacteria such as *Staphylococcus hyicus* and streptococci have been isolated from lesions of ear necrosis in the past, and *T. pedis* may not be the sole or initiating agent. The syndrome of ear necrosis in swine has not yet been reproduced experimentally. Past studies of affected pigs suggest that there are no statistically significant effects on mortality, growth rate, or lean meat content of carcasses. *Treponema pedis* has no known public health significance; hence, lesions in swine pose no risk to humans.

*Treponema pedis* is characterized by its morphology, cultural characteristics, 16S rRNA gene sequences, and flaB2 gene sequences. It is approximately 0.25 µm in diameter and 4–6 µm in length with a 3:6:3 flagellar pattern; does not form spores; is anaerobic; and produces esterases, lipases, and trypsin-like proteases. Cultivation is in strictly anaerobic conditions at 37°C on fastidious anaerobe agar (FAA) with 10% added horse blood. Colonies become obvious in 4–5 days.
CHAPTER 64 MISCELLANEOUS BACTERIAL INFECTIONS

EPIDEMIOLOGY AND PATHOGENESIS

Lesions of skin conditions, spirochetal granuloma, and ear necrosis have been reported in pigs from most parts of the world. Pigs appear to be the only species affected by these manifestations, but T. pedis is also found in digital dermatitis in cattle and sheep. Transmission in pigs may be through skin and ear biting as Pringle et al. (2009) found the organism in the gingiva of affected pigs. Persistence in the swine environment is not known, but infection persists in cattle slurry for some days.

The infection appears to be localized in the skin in infected lesions, possibly caused by biting or abrasion in the first instance. The production of esterases, lipases, and proteases may aid the development of the lesion. Initial infection and penetration into the tissue may be limited by the anaerobic nature of the organism.

CLINICAL SIGNS, LESIONS, AND DIAGNOSIS

Ear necrosis (see also Chapter 17) is a condition in which small inflamed areas on the margins of the ears develop after weaning, spread, intensify, and ultimately cause loss of the ear in severe cases. The lesions begin as small areas of damage on the edge of the ear and near its junction with the head, become scabby, extend, and become necrotic (Pringle et al. 2009). They can lead to loss of the entire pinna. On resolution, they heal leaving a scar. Similar lesions may occur on the flanks and upper parts of the hind limb, and Pringle and Fellstrom (2010) describe a shoulder ulcer from which they have obtained the organism. Morbidity is usually low, and affected pigs do not perform significantly differently from unaffected controls (Busch et al. 2010). An extensive literature associates this condition and its severity with behavioral changes attributed to housing and management (Smulders et al. 2008).

The lesion is a chronic ulcerative and pustular dermatitis. Its edges are covered by a thick crust of fibrin, exudate, and inflammatory cells with vasculitis with or without thrombosis in the underlying blood vessels. Bacteria can be demonstrated in the superficial layers, and spirochetes can be seen in silver-stained sections deeper in the tissue. Healed lesions may be seen as scars on older animals.

Ear necrosis and localized necrotic lesions elsewhere on the body are suggestive of infection by T. pedis, but Staphylococcus hyicus (Chapter 61) may be present in some lesions. Pig pox lesions must be ruled out (Chapter 30) but are typically localized and rarely lose their scabs. Bite wounds are much more difficult to differentiate, and behavior must be watched to confirm the relevant importance of simple biting in the present condition. The progressive nature of the gross lesions suggests spirochetal involvement, and the organisms can be demonstrated in the inflamed tissue. A preliminary identification can be made microscopically in tissue sections utilizing silver stains.

Isolation is by inoculation of fastidious agar broth enriched with fetal calf (25%) and rabbit (10%) serum with added rifampicin and enrofloxacin in anaerobic conditions. Growth can be purified by the inoculation of 0.22-µm pore size Millipore filters (Merck KGaA, Darmstadt, Germany) on FAA followed by streaking to produce the pinpoint grayish slightly hemolytic colonies (Pringle et al. 2009). The organisms can also be detected by using PCR for treponemes (Pringle et al. 2009).

PREVENTION AND CONTROL

Treatment of individual pigs is possible using parenteral antimicrobials but is rarely carried out as the condition does not warrant it. Topical lincomycin–spectinomycin combinations have been used in cattle to treat T. pedis. Local skin disinfection may also be of value. Improvement of environmental conditions and particularly the provision of manipulable materials may improve the environmental/social reasons for flank and ear biting. Formaldehyde and glutaraldehyde can reduce infection in the environment.

REFERENCES


YERSINIA SPP.

RELEVANCE, ETIOLOGY, AND PUBLIC HEALTH

Yersinia pseudotuberculosis and enterocolitica have been isolated from pigs with fever, enteritis, and diarrhea, and disease has been produced experimentally using both organisms. Antibody to Y. enterocolitica serotype O9 cross-reacts in serological tests for Brucella spp. in swine sera. Yersinia enterocolitica can also cause human food-borne disease.
**Epistemology and Pathogenesis**

*Yersinia enterocolitica* has been recorded from pigs in many countries, but *Y. pseudotuberculosis* is less commonly identified. *Yersinia enterocolitica* can be isolated from swine and a wide range of mammals including primates, while *Y. pseudotuberculosis* is most commonly found in rodents.

*Yersinia enterocolitica* persists in the tonsils of infected pigs for long periods and is shed in the feces for up to 30 weeks. Exposure may be from flies (Fukushima et al. 1979), infected feed, or from contaminated pens (Fukushima et al. 1983) in which infection can persist for 3 weeks. The organism may multiply at 20–22°C in suitable substrates. Yersinas are susceptible to disinfectants in the same way as coliforms, and all common farm disinfectants can be used.

*Yersinia enterocolitica* has been shown to infect pigs orally, to multiply and be found in the feces within 2–3 weeks of infection, disappearing from the feces within 30 weeks (Fukushima et al. 1984). Experimental studies by Nielsen et al. (1996) confirm infection of the feces between 5 and 21 days after infection and that tonsillar carriage persists for longer. Oral infection is followed by infection of the tonsils and by enteritis in the ileum and colitis in the large intestine (Schiemann 1999; Shu et al. 1995a, b, 1997). Serum antibody appears within 2 weeks of infection, peaks at 33 days postinfection, and has disappeared by 70 days postinfection (Nielsen et al. 1996).

**Clinical Signs, Lesions, and Diagnosis**

*Yersinia enterocolitica* is isolated in profuse culture from outbreaks of diarrhea in weaned pigs from which no other infectious agents can be recovered. Mild fever of 103°F (39.4°C) is present, and the diarrhea usually contains no blood or mucus and is blackish in color. The organism has been isolated from the rectal mucosa in cases of rectal stricture. Experimental infections in suckling piglets result in anorexia, vomiting, diarrhea, and reduction in weight gain (Shu et al. 1995a). *Yersinia enterocolitica* is present in many cases of “colitis,” a featureless diarrhea of weaned pigs often infected also with other enteric pathogens (Thomson et al. 2001). *Yersinia enterocolitica* has also been isolated from stillborn piglets, and infection of sows at 89 days of gestation reproduced the syndrome (Platt-Samoraj et al. 2009). Pigs with *Y. pseudotuberculosis* infection may be dull with inappetence; with bloodstained diarrhea; and with edema of the eyelids, lower face, and dependent parts of the abdomen (Neef and Lysons 1994).

Lesions in *Y. enterocolitica* infection include catarrhal enteritis in the small and large intestines. Microscopically, microcolonies of the organism are observed in areas of disrupted intestinal epithelium, and in pigs with rectal lesions, bacterial penetration and inflammation reach the muscularis mucosae (Shu et al. 1995a). Lesions observed in pigs with *Y. pseudotuberculosis* infection include miliary gray-white spots on the liver and spleen, swollen gray-white mesenteric lymph nodes, catarrhal and diphtheritic change in the colon and rectum, edema, and ascites (Morita et al. 1968; Neef and Lysons 1994). Microscopic lesions included necrotic foci containing masses of bacteria surrounded by a thin layer of granulation tissue in the lungs, liver, spleen, mesenteric lymph nodes, and lymphoid follicles of the large intestine.

Clinical diagnosis of yersiniosis is difficult because the clinical signs are not distinctive. Lesions may lead to differentials that include *Yersinia* spp., but diagnosis of infection depends on the isolation and identification of the organism. The indirect ELISA developed by Nielsen et al. (1996) for *Y. enterocolitica* O, 3 may be of value in the field. *Yersinia pseudotuberculosis* and *Y. enterocolitica* can readily be isolated at 37°C on blood and MacConkey agar from tissues with lesions. Most
isolation methods for *Yersinia* use cold enrichment techniques in which tissues or samples under investigation are enriched at 39°F (4°C), followed by subculture onto a selective medium. The selective medium may be MacConkey agar incubated at 86°F (30°C) or a specific medium for *Y. enterocolitica* (Catteau et al. 1983). Food microbiologists use a range of tests such as the immunomagnetic separation and PCR technique of Rasmussen et al. (1995).

**PREVENTION AND CONTROL**

*Yersinia* infections are rarely treated specifically. Isolates are often sensitive to oxytetracycline, furazolidone, neomycin, sulfonamides, and spectinomycin. Tetracyclines have been used in feed to eliminate infection and clinical signs. Since spread of *Y. enterocolitica* from pig to pig appears to occur from contact with feces, hygiene coupled with housing groups of pigs in separate drainage areas reduces infection. Control of flies and rodents and disinfection of pens before restocking will reduce transmission.

*Yersinia enterocolitica* contamination of pig meat is reduced by the removal of the tonsils at slaughter, but control may be required when serological cross-reaction in the brucellosis test occurs. Serological reactions peak at 33 days postinfection and have disappeared by 70 days postinfection (Nielsen et al. 1996), so pigs may be treated or managed at the time of infection to prevent the development of antibody or held and retested after antibody levels have declined. *Yersinia pseudotuberculosis* infection may be prevented by excluding birds and rodents (Morita et al. 1968).

**REFERENCES**

V Parasitic Diseases

65  External Parasites
66  Coccidia and Other Protozoa
67  Internal Parasites: Helminths
The importance of external parasites to pork production varies greatly among geographical regions because of differences in climate and differences in husbandry systems. Free-roaming pastured swine are exposed to more attacks by insects than confined swine. Furthermore, the controlled environment of confinement rearing reduces the impact that extremes of weather may have on the clinical expression of parasitic diseases. Mange mites, demodectic mites, lice, fleas, mosquitoes, flies, and ticks are all external parasites that can produce a range of clinical signs in swine, with rubbing and skin lesions being the most common. More difficult to appreciate are economic losses due to reduced growth rate, reduced feed efficiency, and loss of carcass value at slaughter. Indeed, skin blemishes from insect bites present at slaughter may lead to unnecessary trimming or even condemnation. In addition, products improperly used to treat ectoparasitism may produce residues in the tissues, causing contamination of the pork. Some external parasites are vectors of microbial pathogens.

**SARCOPTIC MANGE**

The most important ectoparasitism of swine worldwide is scabies (sarcoptic mange, sarcoptosis). Herds having scabies suffer reduced growth rates, reduced feed efficiency, and decreased fertility in breeding sows (Kessler et al. 2003). The real economic importance tends to be underestimated, because pork producers may not recognize the presence of scabies in their herds when it occurs. Two clinical forms of scabies are recognized: a chronic, hyperkeratotic form most commonly seen in sows, and a pruritic hypersensitive form most commonly seen in growing pigs. Scabies historically has a high prevalence in swine herds (40–90% is common), with prevalence within infested herds varying from about 20% to 95%. Modern production systems and seed-stock suppliers have been successful in the elimination of scabies making true estimates of herd prevalence difficult.

**Etiology and Life Cycle**

Scabies is caused by a burrowing mite, *Sarcoptes scabiei*, of the class *Arachnida*, order *Acarina* and family *Sarcoptidae*. The mite has a globular body approximately 0.5 mm in length, barely visible to the naked eye and more easily seen when viewed against a dark background. When observed with low-power magnification, the mite has four pairs of short, stumpy legs, some of which are provided with a long, unjointed pedicle that terminates in a sucker-like organ. These pedicles occur on legs I and II in the female mite and legs I, II, and IV in the male. The legs that do not have pedicles bear long bristles.

*Sarcoptes scabiei* is a permanent parasite of the skin, where eggs, larvae, nymphs, and adults develop. After mating on the surface, the females make tunnels into the upper two-thirds of the epidermis, laying a string of 40–50 eggs behind them as they progress forward. Burrowing is by extraoral digestion of the stratum corneum, stratum granulosum, and stratum spinosum. Burrows do not go deeper than the stratum spinosum. In about 30 days, the females die in the burrows. The larvae hatch in 3–5 days, molt to nymphs, and the nymphs molt to adults, all within the burrows. The adults reach the surface by means of side tunnels, and mating occurs in surface pockets, starting the cycle over. The time from egg to fertilized female takes 10–25 days, all of which is spent on the host pig.
Epidemiology

The main reservoir of scabies mites in a herd is sows, which transmit the mites by physical contact with other swine. Boars are less involved with the herd’s daily activities because of the increasing use of artificial insemination, but they can serve as another source of infestation. The usual infestation in sows and boars is characterized by hyperkeratotic lesions on the inner (scaphal) surface of the ears. A few adult swine may also have mites and hyperkeratotic lesions over the body. Susceptible pigs become infested while suckling infested sows or huddling with infested pigs. Transmission from pig to pig is fairly slow (Stegeman et al. 2000). It was estimated that the transmission rate among group-housed pigs is in the order of 0.06 new infestations per pig per day. The mite stage responsible for the transmission is considered to be newly fertilized females, which are on the skin surface instead of in the tunnels. Swine management practices that facilitate the spread of mites are group housing of sows, continuous-flow systems for growing pigs, and larger group sizes of growing pigs. The prevalence and severity of scabies increase in cool months and decrease in warm months (Davies et al. 1991).

Environmental contamination is not very important in the transmission of scabies mites; however, pigs may become infested when placed in pens immediately after infested pigs have been removed (Smith 1986). Although mites have been kept alive for 3 weeks under optimum laboratory conditions, under field conditions, the retention of infestability away from the host is limited. Viability is reduced by desiccation. Mites die within a few minutes in direct sunlight and after several hours at temperatures above 28°C. Even in colder climates mites do not survive more than 12 days (7–18°C, relative humidity of 65–75%) (Mikhalochkina 1975). Clinical evidence of scabies could not be detected when noninfested pigs were repeatedly exposed to contaminated bedding vacated 3 days previously in spring or autumn (Cargill and Dobson 1977), and laboratory experiments demonstrated that mites did not survive longer than 96 hours at temperatures less than 25°C, longer than 24 hours from 25 to 30°C, and survived less than 1 hour above 30°C.

The source of scabies mites seems to be limited to other swine. No reservoir species of animal other than swine has been implicated. Herd-to-herd transmission usually occurs when pigs with subclinical infestation are moved.

Economic Importance

The effects of sarcoptic mange on production have been reviewed by Davies (1995). Deaths are unlikely, unless concurrent disease is a complication. Field studies indicate that good control of scabies may increase lactation, reduce pig mortality due to overlying, and increase weaning weights (Hewitt and Heard 1982; Schultz 1986). Other economic effects include downgrading and trimming of carcasses at slaughter, as well as damage to pens and fixtures caused by rubbing pigs.

The most significant effect of sarcoptic mange is reduced growth rate and feed efficiency in growing pigs. The effect on growth rate has been shown by comparing experimentally infected pigs with noninfested controls (Davies 1995) or by comparing treated pigs with untreated pigs (Sheahan and Kelly 1974). Where growth rate was measured over a period of 12 weeks or more, or from less than 20 kg to more than 60 kg live weight, most studies show suppression of growth rate of 4.5–12%. Smets et al. (1999) also showed that breeding sows needed 5% less feed after eradication of scabies.

Clinical Signs and Pathogenesis

Pruritus is the most consistent clinical sign of scabies. Generalized pruritus occurs from 2 to 11 weeks after infestation. Following exposure, pigs go through several phases: nonresponsive phase, delayed-type hypersensitivity, delayed-and-immediate-type hypersensitivity, and finally immediate phase (Davis and Moon 1990). The onset of pruritus and the intensity of rubbing will depend on the number of mites in the initial exposure and the level of ongoing exposure. When pigs were exposed to low doses (100 mites) or high doses (1000 mites), the development of the delayed-type hypersensitivity (but not the development of the immediate-type) was found to be dose dependent. Desensitization has not been demonstrated experimentally, but field evidence suggests that it occurs.

After infestation, pigs develop encrusted lesions that are rich in mites, especially in the inner (scaphal) surface of the ears. These plaque-like lesions may coalesce to cover up to 70% of the surface of the pinnae, but they will regress with time as hypersensitivity develops. The epidermal changes and sequence of events have been well documented using electron microscopy (Morsy et al. 1989).

Focal erythematous skin papules associated with hypersensitivity occur in most animals as encrustations subside. The papules occur primarily on the rump, flank, and abdomen. Histologically, papules contain large numbers of eosinophils, mast cells, and lymphocytes. There is no evidence of mites. Immunoglobulin-secreting cells peak 2–5 weeks after infestation and then subside substantially after a few weeks (Morsy and Gaafar 1989). Reinfection results in only a small increase in immunoglobulin-secreting cells. The development of pruritus may result in connective tissue proliferation and hyperkeratinization, leading to alopecia and/or abrasions, especially over the flanks.

Hyperkeratotic mange, which is the prevalent form in mature swine, may occur also in growing pigs that
fail to develop the typical hypersensitivity response. The lesions are characterized by thick asbestos-like scabs and scurf that are loosely attached to the subjacent skin and by the presence of numerous mites. These lesions occur most frequently in the ears, but they may also spread over the back, neck, and other parts of the body.

An interrelationship among immunity, inadequate nutrition, poor management, and hyperkeratotic scabies has been noted. Hyperkeratotic mange is considered to be a disease of poor management and of poorly fed pigs. Diets low in protein and iron are associated with reduced hypersensitivity and a greater proportion of animals with hyperkeratotic mange (Sheahan 1974). The overall clinical picture is substantially influenced by the effectiveness of treatments and herd management practices.

**Diagnosis**

Scabies has potential to be present in most herds unless some special measures to eradicate the mites have been followed. Many seed-stock suppliers and production systems have eliminated mange, but reintroductions do occur. Scabies should be suspected when growing pigs with small red papules on the body are rubbing. Because nonspecific rubbing can occur in pigs, it is useful to calculate the amount of rubbing (rubbing index) to estimate the level of pruritus. The rubbing index is calculated by observing a group of 25–30 pigs for 15 minutes. The number of rubbing episodes is divided by the number of pigs in the group. A rubbing index greater than 0.1 suggests scabies is present (Pointon et al. 1995). Finding the mites by means of skin scrapings confirms the diagnosis, but often times the mites are difficult to find. The majority of pigs in an infested herd may be subclinically infested and may not manifest pruritus (Kessler et al. 2003).

The best method to find the mites is to use a flashlight to examine the inner surface of the ears of the breeding stock for encrustations. About 1–2 cm² of the lesion should be removed by means of a knife blade or chisel for examination for mites. The encrustations can be broken up onto a sheet of black paper and allowed to sit for a few minutes. When the crusts are carefully moved aside, mites may be seen with a magnifying glass against the dark background. A more sensitive technique is to macerate the scabs in 10% potassium or sodium hydroxide. Low heat may be used to accelerate the maceration. The chitinous exoskeleton of the mites is impervious to hydroxide, so the exoskeletons can be found under low magnification. A third technique is to place the ear scrapings in a petri dish and incubate under low heat overnight. Mites will emerge in great numbers or adhere to the bottom of the dish (Sheahan and Hatch 1975).

Only a small percentage of growing pigs will harbor significant populations of mites. The ear is more apt to be positive than skin sites (Bogatko 1974). Davies et al. (1996b) found that the number of pigs with positive skin scrapings was positively correlated with the level of papular dermatitis in the group, but the prevalence of positive skin scrapings ranged from 3% to 63%. In a group of pigs where 47% had hypersensitive mange and 5% had hyperkeratotic mange, mites were found in 33% of the hypersensitive pigs but in 81% of the hyperkeratotic pigs (Kambarage 1993).

It would appear that within a group of pigs, there are two populations. A smaller population of pigs harbors large numbers of mites but does not develop a severe form of hypersensitive mange. A large population of pigs harbors few mites but develops a marked hypersensitivity reaction (Davies et al. 1996a). In the latter population, the number of mites declines over time as the level of hypersensitivity increases (Cargill and Dobson 1979; Davis and Moon 1990). Continuing exposure over time from penmates maintains the allergic reaction and clinical signs in the hypersensitive pigs.

Examination of carcasses at necropsy or slaughter provides information on the scabies status of herds. Skin lesions are scored and categories defined according to the severity of the dermatitis. Minor spots must be disregarded because they may be caused by reactions to bedding or insect bites. However, the grading of dermatitis about the shoulders, underline, and rump is of interest in evaluating scabies (Cargill et al. 1997).

Several enzyme-linked immunosorbent assays (ELISAs) have been used to detect antibodies to *S. scabiei* in serum (Bornstein and Wallgren 1997; Bornstein et al. 2000; Deckert et al. 2000; Zalunardo et al. 2000). Individual sensitivity varies from 29% to 64%, but as a herd test sensitivity approaches 95%. Specificity in individual pigs varies from 78% to 97% (Smets and Vercruysse 2000). Specific antibodies are not detectable until 5–7 weeks after infestation, or approximately 3–4 weeks following the onset of clinical scabies (Bornstein and Zakrisson 1993). Furthermore, detectable antibodies may persist for 9–12 months (Smets and Vercruysse 2000). Although antibodies can persist several months in sows following treatment, the half-life of antibody levels appears to be less than 2 months (Bornstein and Wallgren 1997). ELISA may have some use in evaluating the efficacy of eradication programs (Cargill et al. 2004).

Differential diagnosis from other skin diseases is important. Conditions that can be confused with scabies include parakeratosis, exudative epidermitis, deficiencies of niacin and biotin, swinepox, dermatomycosis, sunburn, photosensitization, and insect bites. Occasionally, in scabies-free herds, ear scrapings may reveal mites and/or mite eggs. The mites may be pseudoparasites living in old straw bedding and the mites do not resemble *Sarcoptes* structurally.
**Treatment, Control, and Elimination**

Scabies may go unnoticed because farmers may consider rubbing by pigs to be a normal activity, but the recognition of the presence of scabies is essential to instituting the proper control measures. Several strategies are open to producers to reduce the economic effects of mange. Treatment and control of mange is an option on some farms, whereas elimination is often a preferred option.

Successful treatment of scabies is dependent on the correct use of acaricides. The large majority of registered products will keep scabies under control and some even eradicate it, provided the correct dosage and treatment schedules are used. Oil mixtures are more effective than aqueous ones because oil helps to soften the hard scab surrounding the mites. Today, oil mixtures continue to be used occasionally either by themselves or in conjunction with modern acaricides.

Older remedies (e.g., crankcase oil, diesel oil, and lime sulfur) are very limited in efficacy and not recommended. Likewise, chlorinated hydrocarbons (lindane, toxaphene) or organophosphates (malathion, trichlorfon, diazinon) were once used as acaricides in swine but, because of their toxicity, dwindling efficacy, or persistent tissue levels, are no longer recommended.

More modern acaricides (see Table 65.1) are safer, have higher efficacies, and are easier to administer. If the active ingredient is not ovicidal, it must be re-administered in 10 days in order to kill the emerging larvae. Instructions on dilutions, withholding periods, and other precautions by the manufacturer must be followed carefully. The availability of certain products

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Concentration</th>
<th>Parasites Affected</th>
<th>Directions for Use</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amitraz</td>
<td>0.1% solution</td>
<td>Mites</td>
<td>Spray pigs and surroundings, repeat in 7 days.</td>
</tr>
<tr>
<td>Clodrin</td>
<td>0.25%</td>
<td>Lice</td>
<td>Spray, repeat in 14 days.</td>
</tr>
<tr>
<td>Coumpos (Co-Ral)</td>
<td>0.06% solution</td>
<td>Lice, horn flies</td>
<td>Spray.</td>
</tr>
<tr>
<td></td>
<td>0.12% solution</td>
<td>Hard ticks</td>
<td>Treat wounds.</td>
</tr>
<tr>
<td></td>
<td>0.24% solution</td>
<td>Mites</td>
<td>Apply to pigs; simultaneously apply 20g/m² to fresh bedding.</td>
</tr>
<tr>
<td></td>
<td>1% dust</td>
<td>Screwworm, blowflies</td>
<td>Apply to ears and adjacent areas of head.</td>
</tr>
<tr>
<td>Diazanon</td>
<td>0.05% emulsion</td>
<td>Lice, mites</td>
<td>Spray three times at 10-day intervals.</td>
</tr>
<tr>
<td>Dioxathion (Delnav)</td>
<td>0.15% solution</td>
<td>Lice, hard ticks</td>
<td>Spray or dip. Do not treat sows within 2 weeks of farrowing or while lactating. Do not repeat treatment within a 2-week period.</td>
</tr>
<tr>
<td>Doramectin</td>
<td>IM injection</td>
<td>Lice, mites, fleas</td>
<td>300µg/kg body weight</td>
</tr>
<tr>
<td>Ivermectin</td>
<td>SC injection</td>
<td>Lice, mites, fleas</td>
<td>300µg/kg body weight</td>
</tr>
<tr>
<td>Lindane</td>
<td>0.06% emulsion</td>
<td>Lice, sarcoptic mites</td>
<td>Dip or spray. Do not use benzene hexachloride.</td>
</tr>
<tr>
<td></td>
<td>1% dust</td>
<td>Fleas</td>
<td>Dust head, neck, and back. Treat all wounds.</td>
</tr>
<tr>
<td></td>
<td>3% formulated in smear, paste, or pressurized aerosol</td>
<td>Fleas, screwworm, blowflies</td>
<td>Dust head, neck, and back. Treat all wounds.</td>
</tr>
<tr>
<td>Malathion</td>
<td>0.05% emulsion</td>
<td>Lice, ticks, mites</td>
<td>Spray.</td>
</tr>
<tr>
<td></td>
<td>6% dust</td>
<td>Fleas, lice</td>
<td>Dust thoroughly.</td>
</tr>
<tr>
<td></td>
<td>2.5% emulsion</td>
<td>Houseflies, stable flies, fleas</td>
<td>Spray environment.</td>
</tr>
<tr>
<td>Phosmet</td>
<td>SC injection, pour-on</td>
<td>Lice, mites, fleas</td>
<td>Pour 1 mL/10k body weight along back. Place some in each ear.</td>
</tr>
<tr>
<td>Polysulfide</td>
<td>2% solution</td>
<td>Sarcoptic mites</td>
<td>Spray.</td>
</tr>
<tr>
<td>Primiphos (Actelic 50 EC)</td>
<td>Powder</td>
<td>Control of fleas</td>
<td>Sprinkle through straw bedding (check withholding period).</td>
</tr>
<tr>
<td>Rabon</td>
<td>2% solution</td>
<td>Houseflies, stable flies, lice</td>
<td>Spray 4.5L/12-14g/m².</td>
</tr>
<tr>
<td>Ronnel (Korlan)</td>
<td>0.25% emulsion</td>
<td>Lice</td>
<td>Spray.</td>
</tr>
<tr>
<td></td>
<td>5% granules</td>
<td>Lice</td>
<td>Apply to bedding 25g/m².</td>
</tr>
<tr>
<td></td>
<td>5% pressurized aerosol</td>
<td>Screwworm, blowflies</td>
<td>Treat wounds.</td>
</tr>
<tr>
<td>Roteneone</td>
<td>1% powder</td>
<td>Fleas</td>
<td>Dust head, neck, and back.</td>
</tr>
<tr>
<td>Toxaphene</td>
<td>0.5% emulsion</td>
<td>Lice, hard ticks, mites</td>
<td>Spray.</td>
</tr>
<tr>
<td>Trichlorfon (Neguvon)</td>
<td>0.125% emulsion</td>
<td>Houseflies, stable flies, mites</td>
<td>Spray environs (avoid feed and water)</td>
</tr>
</tbody>
</table>

Source: Cargill and Davies (2006).

Note: It is important to follow the manufacturer’s recommendations and observe the withholding period specified for each chemical.

IM, intramuscular; SC, subcutaneous.
depends on the legislation of a particular country. Effective modern acaricides include phosmet, used as a pour-on; amitraz used as a spray; and the avermectins (ivermectin, doramectin, and moxidectin), which are given as injections or, in the case of ivermectin, can also be given orally in the feed. In the case of phosmet, it is recommended that a small amount of the product be placed in the inner aspect of each ear. The avermectins are broad-spectrum antiparasiticides effective against most internal parasites as well as lice and sarcoptic mange mites. Current products have varying levels of persistence. They are more efficient because of their systemic action and ease of administration.

Control
Ongoing control of scabies involves the identification of those animals that have chronic scabies, so that they can receive regular treatment to prevent transmission to the young pigs. Control programs target the breeding herd. Mercier et al. (2002) showed that a single dose of ivermectin (300µg/kg body weight) given to sows 8 days before farrowing was very effective in preventing transmission of the mites to the piglets. Any sows with extensive hyperkeratotic lesions should be culled and the rest of the sows should be treated before farrowing. The boars should be treated every 3–6 months to prevent transmission of mites during breeding. Pigs born to mite-free sows and housed in clean pens will remain free of mites unless they are exposed to infested pigs. If mange is present in both the breeding and growing pigs, the whole herd should be treated. However, successful elimination of mange from a two-site system can be achieved by treating animals at the breeding herd combined with hygiene and biosecurity measures at the growing facilities. All animals introduced into the herd must be treated before coming into the herd. Contaminated bedding should be removed, and the environment should be sprayed with an acaricide. Humans who handle infested pigs can act as mechanical vectors for the mites (Mock 1997), so it is important that workers with one herd must change clothes and shower before moving into another herd.

Elimination
The establishment and maintenance of mange-free herds and populations are facilitated by three important facts. First, piglets are born free of mites and become infested through contact with infested sows or older growing pigs. Second, the mites are highly host specific and survive poorly away from the pig. Third, modern acaricides are very effective.

Mange-free herds can be established with cesarean-derived pigs, by depopulation and repopulation from mange-free stock, by segregated rearing of treated pigs, and by eradication using avermectins and other products. Biosecurity measures that focus on careful scrutiny of minimal number of sources of incoming stock are usually adequate to prevent introduction of the parasite. In a number of countries, major breeding-stock suppliers maintain mange-free herds, and large populations of pigs in integrated production systems have been kept free of the parasite for many years. This should be the goal of most farms.

Eradication of sarcoptic mange is possible with a variety of programs developed around the use of effective acaricides, provided the processes and procedures are properly executed. Documented successes and failures of eradication plans usually relate to the diligence of animal caretakers in following treatment and biosecurity protocols. Eradication programs involve several key points. If the whole herd is to be treated, all marketable pigs can be sold before each treatment to reduce the cost, and the withholding period for the chemical must be observed. All pigs are treated twice at the recommended interval for the acaricide used. If only the sow herd is to be treated, two options are available. All sows and boars can be treated at the recommended interval, or individual sows can be treated prefarrowing and moved to clean pens or crates. If the latter approach is taken, boars should be treated every 3 months, and the progeny of treated sows must be isolated from the progeny of untreated sows. Eradication is made easier in growing pigs by changing from a continuous-flow management system to an all-in/all-out system, such as an age-segregated-rearing or a multiple-site production. However, changing the management system must be combined with effective prefarrowing treatment of sows. All of these approaches can be effective and are justified economically.

DEMODECTIC MANGE
Demodectic mange (demodosis, follicular mange) is relatively unimportant in swine, although it is quite common in the subclinical form. It is reported wherever swine are raised.

The causative agent is the mite Demodex phylloides, an alligator-looking mite that spends its entire life inside hair follicles, alongside the hair shafts. To adapt to its narrow living space, the mite has stumpy legs and a fusiform shape. It is assumed that the life cycle of D. phylloides resembles that of other demodicid mites. Ovigerous females live in the hair follicles and oviposit there. A series of larval and nymphal stages ensue, all in the same hair follicles. The next generation of adults is mature in about 2 weeks, and the life span of the adult is 1–2 months. The rate of cycling slows down as the pig ages, so that eventually a small population composed almost entirely of adults is in the follicles. Neighboring follicles are colonized by mites crawling out of one follicle and into another through the follicular orifice.

Transmission of the mites probably occurs by direct contact with neonates while nursing or huddling. The
mites can survive for several days in moist environments and up to 21 days under experimental conditions in pieces of skin kept cool and moist (Nutting 1976). However the mites can survive only 1 or 2 days if removed from the hosts' skin and are killed by desiccation in as little as 1 hour at 20°C on the skin surface.

The sites on the body that are most commonly affected are the snout, eyelids, jowls, underside of the neck, mammary area, and inside surface of the thighs (Walton 1967). Early lesions are red pinpoint foci, but older lesions are hyperkeratotic and nodular. The nodules are distended hair follicles that contain stages of the mite cycle, hyperkeratotic debris, and mild inflammation. When incised, the nodules ooze a thick, white, caseous material laden with mites. Such lesions may be confused with swinepox.

**Diagnosis**

The diagnosis of demodicosis is by finding numerous mites in deep skin scrapings. Because the mites are present in nearly all swine, it is not enough just to find a mite or two. A correlation between presence of the mites, indications of an actively growing population of mites (i.e., large number of immature stages in the follicles), and visible lesions must be seen to justify the diagnosis of clinical demodicosis.

**Treatment**

There are no reports of successful treatment of swine demodicosis with any acaricide, either topical or systemic. However, ivermectin or amitraz is successful in canine demodicosis, so they may be tried. Severely affected swine should be culled from the herd. Because there is definite evidence in canine demodicosis that the tendency to get clinical follicular mange is genetic, the same may be true for swine.

**LICE (PEDICULOSIS)**

Swine lice (*Haematopinus suis*) are among the largest of all lice, so they are easily observed. Herds treated routinely to control scabies seldom carry significant louse populations, because lice and mites are susceptible to the same therapeutic agents. The swine louse is almost worldwide in distribution, and *H. suis* is the only louse on swine.

*Haematopinus suis* is an anoplurid (i.e., sucking) louse. It is grayish brown with black markings. The females are about 6mm long, and the males are slightly smaller. The head is long and narrow and contains the stylets used for piercing and sucking the host's blood. They are strictly host specific, and their introduction to a swine herd does not involve wild birds, rodents, or other vermin—only other swine.

The entire life cycle of *H. suis* takes place on the swine. The adult female lays three to four nits (eggs) each day, totaling up to 90 nits over a 25-day period. Each nit is 1–2mm long and is attached by cementum to a hair. Nymphs hatch in 12–20 days from the nits. The nymphs develop through three instars, all of which suck blood that is attainable through the thin skin found on the ears and other sites. The third instar molts into an adult 23–30 days after the nit was laid. Lice are permanent parasites, living only on the host, and cannot survive for more than 2–3 days away from the host.

Lice are found on all parts of the body but are particularly numerous on the neck, jowls, flanks, and inner surfaces of the legs. They also seek shelter inside the ears, where they may be found in clusters and should be differentiated from ear ticks. The spread of lice is by direct physical contact.

The economic importance of lice has not been critically evaluated to the same extent as that for scabies. However, it is known that severe pediculosis results in anemia in young pigs and may affect growth rates and feed efficiency. One estimate of reduced growth rate was 50g/day (Hiepe and Ribbeck 1975), although others failed to demonstrate this effect. Lice have been considered to be vectors of swinepox, and hides from pediculotic swine may be unsuitable for high-grade leather manufacturers (Hiepe and Ribbeck 1975).

**Diagnosis**

Lice should always be considered in the differential diagnosis of pruritus in pigs. Infestation can be confirmed by identifying lice and/or nits. Nits will be cemented to the proximal (lower) part of the hair shafts, which helps differentiate them from blowfly eggs, which are more diffuse in distribution.

**Treatment and Control**

The treatment and control of pediculosis is aided by the fact that all stages of the parasite are localized on the swine. Therapeutic agents may be administered to the swine in the form of sprays, pour-ons, or dusts (see Table 65.1). Control can be assisted by sprinkling insecticide granules in the bedding. Pour-ons and dusts have the advantage that they can be used in cold weather, when spraying may not be indicated. If the active ingredient is not ovicidal, treatment should be repeated in 10 days.

Control and eradication strategies mentioned for scabies are also effective for pediculosis. These include special attention to the ears, boars in the treatment schedule, multiple treatment of sows prior to farrowing, segregation of clean from untreated livestock, and treatment of all introduced animals.

**FLEAS**

Fleas (*Siphonaptera*) show a low level of host specificity; each species of flea may parasitize a range of host species of mammals or birds. With their ability to trans-
fer from one host species to another, fleas gain considerable veterinary importance when considering disease transmission. Fleas may parasitize phylogenetically related species or they may parasitize species that are related to one another by common habitat. All fleas have the same basic structure. They are wingless, flattened laterally, have hind legs suited for jumping, and are mahogany brown in color.

The four fleas most often associated with swine are *Pulex irritans* (the human flea), *Echidnophaga gallinacea* (the sticktight flea), *Tunga penetrans* (the sand flea or jigger, not to be confused with chigger), and *Ctenocephalides felis* (the cat flea).

The life cycles are similar for all fleas. The adult flea feeds only on blood from the host and is the only stage found on the host. After mating, the female oviposits eggs that drop off the host into the environment. The larvae that hatch in 2–16 days are hairy, wormlike creatures that feed on detritus and on the adult flea’s blood-laden feces. Flea larvae require high humidity and warm temperature, allowing them to mature in 1–2 weeks. When ready to pupate, the larvae spin a cocoon, to which is stuck small particles from the environs, thus camouflaging the finished cocoon. Depending on environmental conditions, the cycle may be completed in 18 days or protracted to more than 1 year. Various stimuli (vibrations, carbon dioxide gradient, body heat, body odor) are cues to the flea that a host is approaching and causes the flea to jump. Fleas can survive for many months awaiting the arrival of a host. How long these fleas can lie in wait depends on the environmental conditions, but with optimum humidity and temperature, survivals of 1–2 years or more are known. This is a major factor in the survival of the flea population in the absence of the host. Another factor is delayed emergence from the pupa.

**Diagnosis**

The diagnosis of flea infestation is difficult unless the adult fleas can be found on the host. The eggs, larvae, and pupae are in the environment, not on the host, and therefore are especially difficult to find. Adults, however, remain on the host but are somewhat elusive to observation. Flea bites are not particularly distinguishable from those of other insects. Because fleas are not strictly host specific, humans in the same environment may also be attacked. Noticing attack by fleas on human observers may be the first indication of flea infestation.

The adults of most flea species are found roaming on the host’s skin, and bite lesions occur where fleas have fed. Bite lesions can occur anywhere, but they are most prevalent on the underline and inside surfaces of the legs. Allergic dermatitis (flea-bite dermatitis) similar to that seen in dogs has been described also in swine, and this may resemble scabies (Nesbitt and Schmitz 1978).

A special case is *T. penetrans*, the sand flea or jigger. This flea is found in Africa, the Caribbean, and tropical South America. The female of this tiny flea (ca. 1 mm) burrows into the dermis of the host and remains at that site for life. In the process of becoming ovigerous, she swells up to the size of a pea, causing a severe inflammation and ulceration. Sites affected are the feet, snout, underline, and scrotum. Eggs are released through the ulcerated surface, and the immature stages develop in the environment. An unusual case involved the mammary gland, where the fleas obstructed the ducts and caused agalactia (Verhulst 1976).

**Treatment and Control**

Treatment of fleas on the swine is readily accomplished by any of several products also used for other ectoparasites (see Table 65.1). However, flea stages in the bedding are much more difficult to eradicate. Environmental control may be attained by the removal and burning of litter, dirt, and manure. Spraying of the environment with chlorpyrifos, primiphos, or malathion may be effective, but animal exposure and withholding periods must be considered.

**MOSQUITOES**

While usually thought of as pests of humans, mosquitoes also attack swine and other livestock. The economic losses caused by these attacks have not been well documented, but in some cases, it must be clinically important. Irritation, discomfort, and sometimes viral transmissions are reportedly caused by several genera and species of mosquito. Attacks by mosquitoes may be significant even in well-managed confinement operations.

All species of mosquitoes require some type of water to complete their cycle. Eggs are laid in water and the larval and nymphal stages occur there. The type of water suitable for oviposition varies according to each species. It may be salty, brackish, or fresh; stagnant or flowing; sunlit or dark; open or impounded in secluded niches; and so on. Species of *Aedes* lay eggs in depressions (wheel ruts, hoof prints, tire carcasses) that are dry at the moment but will trap water at the next rain. Therefore, mosquito control measures must take into account which species are present, because treatment of the offending water is essential for control. One method will not be effective against all species. The active ingredient must be applied where the mosquitoes are and when the mosquitoes are present. Anything less will be ineffective. Screened-in enclosures may provide relief, but may be impractical. Effective mosquito control involves the treatment of large regions, because mosquitoes can fly in from surrounding untreated areas.

Reports of mosquitoes attacking swine in large numbers exist (Becker and Gross 1987; Dobson 1973).
The transmission of Japanese encephalitis virus, especially in rice-growing areas, was reported by Wada and Smith (1988). Also, the mechanical transmission of porcine reproductive and respiratory syndrome virus (PRRSV) was documented (Otake et al. 2003). The virus survived in the mosquito's gut for up to 6 hours, but it did not survive on the exoskeleton. Mosquitoes can also transmit *Mycoplasma suis* between pigs (Prullage et al. 1993).

**FLIES**

Flies are very important in swine production for several reasons. First, houseflies tend to be used as a measure of general sanitation by public health officials. Second, some flies and gnats bite swine, causing discomfort and disease. Furthermore, some flies cause myiasis, which leads to significant disease, even death. Flies express host specificity only a little, and so the same flies that are discussed here will also be pests of other livestock, wildlife, and humans.

**Housefly**

The most common fly in swine operations is the housefly, *Musca domestica*. It produces a brood of maggots in almost any moist organic matter (feces, carrion, garbage). The maggots crawl out of their larval environment and pupate in dry ground. Adult flies emerge from pupal cases and crawl to the surface in about 2 weeks, but that time is temperature dependent.

*Musca domestica* is nonbiting, but its crawling over an animal's skin is bothersome and it can transmit several organisms, such as *Salmonella*, anthrax, *Escherichia coli*, hog cholera virus, hemolytic streptococci, and nematode eggs. Dissemination of these and other pathogens occurs via the fly's exoskeleton (hairs, legs, proboscis), regurgitated from the fly's crop while dapping its proboscis on food, or in the fly's feces. Removal of breeding sites has primal importance in housefly control. This is augmented by applying residual insecticide sprays and fogs to building surfaces where flies rest (floors, ceilings, partitions, etc.). Manure removal at least weekly and spread thinly on the ground interrupts the fly cycle. Electric-light traps usually attract more flies than they kill.

**Stable Fly**

The second most common fly in swine operations is the stable fly, *Stomoxys calcitrans*. This fly is a pesky biter. Although preferring sunlit areas, it will enter buildings. The adult flies strongly resemble houseflies, but the distinguishing porrect, stiff mouth parts separate them. The presence of stable flies is associated with annoyance and thus subtly reduces feed conversion. The stable fly can vector hog cholera and *M. suis*. Stable flies breed in moist, decaying vegetable matter, such as stacks of straw and hay. The maggots crawl out of the larval brood site and burrow into the neighboring ground, whence adults emerge in about 2 weeks. Control of stable flies is the same as for houseflies.

**Tabanids**

Several other flies are of lesser importance to swine production but worth mentioning. Tabanids (horseflies, gadflies, mango flies, breeze flies, deerflies, etc.) are large, robust flies with fierce biting mouth parts. The bite is painful and the flies are fidgety feeders, so uneaten blood often trickles from the wounds. Water is necessary in the life cycle. Oviposition typically occurs on leaves of vegetation that hang over water. Hatched larvae drop into the water and feed on aquatic insects. Pupation occurs in dry ground and adults emerge in 1–3 weeks. They are capable of transmitting hog cholera and perhaps other pathogens that occur in the host's circulating blood. Control of tabanids is problematic, as insecticides and repellents work poorly. Screening of buildings or removal of swine to locations more remote from the fly's breeding site may be considered.

**GNATS**

Simuliids (buffalo gnats, blackflies, turkey gnats) are small, stout-bodied, hump-backed biting flies that occur throughout the world. They breed in streams, and the next generation of adults emerges from those streams in swarms. Most simuliids are ornithophilic, but many feed on a wide variety of mammals. Only the female bites, and the bite and swarming behavior are very worrisome to livestock. Left undisturbed, females feed to repletion in about 4–5 minutes. Control is similar to that for tabanids.

**Myiasis**

Screwworm flies cause primary myiasis. *Cochliomyia hominivorax* in South America (it has been eradicated from North America) and *Chrysomyia bezziana* in Africa and southern Asia are the major causes. These flies oviposit on fresh wounds (fresh umbilical cords, surgical incisions, tabanid bites, nail scratches, fight wounds, etc.), and the larvae (called screwworms) penetrate into living tissue, causing great excavation that may be fatal. Ovipositing females attack any species of mammal, laying about 150–500 eggs at the edge of the wound, and the larvae spend 3–6 days in the wound. The larvae crawl out of the host and pupate in the ground, reaching adulthood in 3 days to several weeks. Clearing the environment of snags that may cause wounds, immediate attention to newly farrowed pigs, and similar husbandry methods to reduce oviposition sites are important. Repellent wound dressing may be applied
before oviposition has occurred. If wounds are already infested, pressurized larvicial aerosols may be used if the larvae are still superficial.

Blowflies (Phaenicia, Calliphora, Phormia, etc.) cause nonspecific myiasis in a wide range of host species. These differ from screwworm myiasis in that oviposition occurs in necrotic, foul, or putrid sites. Some species of blowfly oviposit into wounds that are only slightly foul, whereas others are not attracted until the wound becomes very putrid, and still others are not attracted until the animal dies. Thus, there is a succession of different blowflies during the development of the wound. This type of myiasis is much more common than the screwworm type, but it is less dangerous to the affected host, because the maggots keep within the bounds of necrotic tissue and do not wander deeper. It can be prevented by the same procedure used against screwworm myiasis.

TICKS

Domestic swine are susceptible to infestation by ticks but do not commonly come into contact with them. Certainly swine raised in modern confinement units are protected from contact with ticks. Two kinds of ticks infest swine: ixodid (hard) ticks and argasid (soft) ticks. Ixodid ticks have a hardened shield covering the entire dorsum of males and a small part of the anterior dorsum of females. Argasid ticks lack this shield altogether. Species of tick are adapted to particular geographical ranges and climatic ranges, so one should seek local assistance in identifying species of interest. In the United States, ixodids occurring on swine are species of Dermacentor, Ixodes, and Amblyomma, and argasids are Ornithodorus and Otobius.

The life cycle of ixodids includes egg, larva (“seed tick”), nymph, and adult. In some species of ticks, all stages occur on the same individual host (one-host ticks); in some, the larva and nymph occur on one individual, the nymph drops off to molt, and the adult seeks another host (two-host ticks); in some, each stage in succession drops off the host and subsequently, the next stage seeks a new host (three-host ticks). A single, large mass of eggs is laid by the ixodid female after it drops off. In argasids, a small clutch of eggs is laid after each blood meal, and the adults are in the host’s lair, not on the host. Argasids seek a host several times in their lives (plural-host ticks).

The main economic importance of ticks is their ability to transmit pathogens, such as protozoa, rickettsiae, and viruses. The recovery of African swine fever virus from Ornithodoros moubata nearly a year after experimental infection (Greig 1972) speaks to the tick’s importance as a vector and to the role ticks can play in transmitting agents from wild swine to domestic.

The diagnosis of tick infestation is based on the known geographical distribution of ticks and the access of pigs to these areas. Hard ticks are readily seen by visual inspection. Although they may occur on any portion of the host, ticks are more commonly attached to the ears, neck, and flanks. Soft ticks seldom occur on the body—they are in the lair. Spinose ear ticks, Otobius megnini, are an exception, as they are found in the ear canal.

If only a few ticks are present, they can be removed manually, and the pigs must be removed from the offending pasture. Many products are effective acaricides.

REFERENCES

Coccidia are obligatory intracellular protozoan parasites. *Eimeria, Isospora, Cryptosporidium, Toxoplasma,* and *Sarcocystis* are important genera of protozoal parasites of mammals and birds. Domestic animals may be infected with several species of coccidia, but usually, only a few species are pathogenic for a given host.

The number of valid species of intestinal coccidia that infect swine is unknown because most are known only from the sporulated oocyst stage. Levine and Ivens (1986) listed 13 named species of *Eimeria* and 3 species of *Isospora* from swine. *Isospora suis, Isospora almatensis,* and *I. neyrai* are the species of *Isospora* described from swine. *Isospora almatensis* and *I. neyrai* are known only from oocysts in the feces and have not been observed in the United States; they are probably not valid species. Neonatal coccidiosis caused by *I. suis* is the most important protozoal disease of swine. Although the causative agent, *I. suis,* was described from pigs in 1934 (Biester and Murray 1934), it was not until the middle 1970s that clinical coccidiosis was recognized as a disease problem in nursing pigs (Sangster et al. 1976). In 1978, it was demonstrated that *I. suis* was the cause of piglet coccidiosis in natural cases, and coccidiosis was experimentally reproduced in nursing pigs (Stuart et al. 1978). Neonatal piglet coccidiosis has a cosmopolitan distribution and is found anywhere pigs are raised in confinement.

**Life Cycle of *Isospora suis***

Coccidial life cycles are divided into three phases: sporogony, excystation, and endogenous development (Figure 66.1). Each coccidial phase is unique for each species, and knowledge of life cycle phases is important in the diagnosis, treatment, prevention, and control of coccidiosis.

Sporogony is the process by which the oocyst (environmentally resistant stage) develops from the unsporulated noninfectious stage passed in the feces to the infective stage (Figure 66.2). Proper temperature and moisture must be present for sporulation to take place. The oocysts of *I. suis* sporulate rapidly at temperatures between 20 and 37°C (Lindsay et al. 1982). The supplemental heat between 32 and 35°C provided by producers for newborn piglets favors rapid development (within 12 hours) of *I. suis* oocysts in the farrowing crate. Oocysts are most sensitive to killing when in the unsporulated state and during sporulation. Once the oocysts are sporulated, they are resistant to most disinfectants. When fully sporulated, the oocysts of *I. suis* and all other *Isospora* species contain two sporocysts, each with four sporozoites.

Excystation is the phase of the life cycle that occurs immediately after the infectious oocysts are ingested. Passage through the stomach alters the oocyst wall and allows bile salts and digestive enzymes to activate the sporozoites. The activated sporozoites leave the sporocyst and oocyst and are freed into the intestinal lumen. The sporozoites then penetrate the enterocytes and begin the endogenous phase of parasite multiplication.

The endogenous stages of the life cycle of *I. suis* occur in the cytoplasm of enterocytes throughout the small intestine, with most stages being present in the jejunum and ileum. Occasionally, in heavy infections, parasites can be found in the cecum and colon as well. Coccidial stages are usually located on the distal portions of the villi and are in a parasitophorous vacuole.
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and an oocyst is formed. These sexual stages may also be seen 4 days postinfection (DPI), whereas oocysts are first seen in the feces 5 DPI (rarely 4 days).

Immunity to *Isospora suis*

Pigs that have been infected with *I. suis* and recover are resistant to challenge infection. These challenged pigs

below the host-cell nucleus (Lindsay et al. 1980). In severe clinical or experimental cases, stages may also be located in crypt enterocytes. There are two distinct types of asexual stages in the endogenous life cycle of *I. suis*. Sexual stages consist of microgamonts, which produce microgametes, and uninucleate macrogamonts. The microgametocytes fertilize the macrogamonts, and an oocyst is formed. These sexual stages may also be seen 4 days postinfection (DPI), whereas oocysts are first seen in the feces 5 DPI (rarely 4 days).

Immunity to *Isospora suis*

Pigs that have been infected with *I. suis* and recover are resistant to challenge infection. These challenged pigs

66.1. Life cycle of *Isospora suis*.

66.2. Oocysts of *Isospora suis* in fecal flotation. Bar = 10 µm. (A) Freshly excreted unsporulated oocyst. Note the hazy bodies (arrows) and the sporont (SP). (B) Oocysts several hours after excretion. Note that two sporoblasts (SB) are present. (C) Sporulated oocyst approximately 1 day after excretion. Note the sporozoites (S) and residual body (arrow) in the sporocysts (modified from Lindsay et al. 1982).
excrete no or very few oocysts (in contrast to initial infection) and do not develop clinical signs. Administration of corticosteroids (methylprednisolone acetate) does not cause these previously exposed pigs to reexcrete oocysts, suggesting good immunity has developed. Colostral antibodies against *I. suis* do not protect piglets from developing clinical coccidiosis. Serum antibodies against *I. suis* do not recognize sporozoites of *Eimeria* from pigs in an indirect fluorescent antibody test.

Pigs have age-related differences in susceptibility to experimental infection and disease (Koudela and Kucerova 1999). Pigs that are 1–2 days old develop much more severe disease than do pigs inoculated with an identical number of oocysts at 2 or 4 weeks of age.

**Clinical Signs of *Isospora suis***

Signs of disease occur in formerly healthy nursing pigs between 7 and 11 days of age (Stuart et al. 1978). Yellowish to grayish diarrhea is the major clinical sign. The feces are initially loose or pasty and become more fluid as the infection progresses. Piglets become covered with the liquid feces, causing them to stay damp and have a rancid odor of sour milk. The piglets usually continue to nurse, develop a rough hair coat, become dehydrated, and have depressed weight gains (Lindsay et al. 1985). Litters within the farrowing house vary in the degree to which they have clinical signs, and not all piglets within a litter are equally affected. Morbidity is usually high, but mortalities are usually moderate. Concurrent bacterial, viral, or other parasitic infections may lead to extreme mortalities and complicate diagnosis.

Occasionally, *I. suis* oocysts are present in the feces of recently weaned pigs, some of which may have diarrhea. Diarrhea caused by *I. suis* can occur in 5- to 6-week-old pigs. Diarrhea begins 4–7 days after the piglets are weaned and exposed to environmental oocysts. Morbidity is high, but mortalities are rare. As with neonatal piglets, other causes of diarrhea besides *I. suis* need to be ruled out. *I. suis* infections do not cause disease in finishing pigs or in breeding stock.

**Pathological Changes with *Isospora suis***

The degree of disease is dependent on the number of sporulated *I. suis* oocysts that a piglet ingests (Stuart et al. 1980). Necropsy examination may demonstrate gross lesions of neonatal coccidiosis characterized by a fibrinonecrotic membrane in the jejunum and ileum, but this is seen only in severely infected piglets. Hemorrhage is not seen even in extreme cases of natural infections or in experimental infections where large numbers of oocysts are given.

Microscopic lesions consist of villous atrophy, villous fusion, crypt hyperplasia, and necrotic enteritis (Stuart et al. 1980). The enterocytes at the tips of the villi may be destroyed, exposing the underlying lamina propria, or they may be replaced by flattened immature enterocytes. The functional ability for absorption is diminished in this altered epithelium, resulting in fluid loss and diarrhea. Lesions develop about 4 DPI and are associated with the presence of the asexual stages. In most natural cases, few parasites are present in the sections and most of these parasites are asexual stages. In severe cases, piglets may succumb to coccidiosis before the sexual stages are produced.

**Diagnosis of *Isospora suis***

Diarrhea in nursing pigs 7–14 days of age that does not respond to antibiotic treatment is suggestive of neonatal *I. suis* infection. Other agents such as enteropathogenic *Escherichia coli*, transmissible gastroenteritis (TGE) virus, rotavirus, *Clostridium perfringens* type C, and *Strongyloides ransomi* should be considered in the differential diagnosis.

Diagnosis of *I. suis* can be achieved by finding *I. suis* oocysts in the feces of clinically affected piglets (Figure 66.2A,B). This is the quickest method available for diagnosis. Fecal smears or fecal flotations should be made from several litters within the farrowing house that have been showing clinical signs for 2–3 days, because diarrhea starts about a day before oocysts are passed and peak oocyst production occurs about 2–3 days after clinical signs develop. Piglets excrete oocysts in several phases and may be negative during these phases. Pasty fecal samples are likely to contain more oocysts than are liquid samples. The oocysts of *I. suis* have characteristic structures called “hazy bodies” between the oocyst wall and the sporont (Figure 66.2A). These are diagnostic for *I. suis* because none of the oocysts of the swine *Eimeria* species have this structure (Lindsay et al. 1982). Additionally, some of the oocysts may be in the two-celled sporoblast stage (Figure 66.2B), which is also diagnostic for *I. suis*. Fecal fat may make identification of oocysts in flotation preparations difficult. A solution of saturated sodium chloride and glucose (500 g of glucose in 1000 mL saturated sodium chloride solution) has been recommended as an alternative flotation medium (Henriksen and Christensen 1992). Polymerase chain reaction (PCR) and autofluorescence of oocysts are other methods that can be used to demonstrate *I. suis* infection but are limited to use by diagnostic laboratories using specialized equipment.

Demonstration of developmental stages (Figure 66.3A–D) in mucosal smears can be used in the diagnosis of *I. suis* infection (Lindsay et al. 1983). The intestinal mucosa should be scraped with a scalpel or coverslip using just enough pressure to dislodge villi, and the scrapings should be prepared as a smear on a glass microscope slide. The smears are then stained with any of a number of routine blood stains.

The presence of paired type 1 merozoites (Figure 66.3D) is diagnostic. Other asexual stages (such as binucleated type 1 meronts or type 2 meronts and...
merozoites) and sexual stages (microgamonts and macrogamonts) will probably be present also, but their identification is more difficult and not needed for diagnosis. Histological diagnosis of *I. suis* in tissue sections is possible (Lindsay et al. 1983). As with mucosal smears, demonstration of paired type 1 merozoites is diagnostic (Figure 66.3E–H). The multinucleated type 2 meronts of *I. suis* are elongated and are often found in the same host cell. Finally, the macrogamonts of *I. suis* lack the characteristic prominent eosinophilic wall-forming bodies seen in *Eimeria* species.

**EPIDEMIOLOGY: EIMERIA SPECIES**

Eight species of *Eimeria* occur in swine in the United States. Reports of coccidiosis in swine caused by *Eimeria* species are rare (Hill et al. 1985), but age-segregated rearing and changes in housing over the last two decades have increased the risk for outbreaks. Experimental studies have demonstrated that inoculation of 3-day-old, 4-week-old, and 2- to 3-month-old pigs with 4–10 million oocysts of *Eimeria debliecki*, one of the most common coccidian of swine, does not cause clinical disease (Lindsay et al. 1987). Reports of natural cases of *Eimeria spinosa*-associated disease in weaned pigs suggest that this species can cause disease under appropriate conditions in the field (Lindsay et al. 2002; Yaeger et al. 2003). Clinical coccidiosis can occur in finishing animals exposed to contaminated facilities and can occur in breeding stock that are born and reared in confinement and then exposed as breeding stock (Caudie et al. 2004; Henry and Tokach 2008).

**Epidemiology: Isospora suis**

Once *I. suis* coccidiosis was recognized as a problem in nursing pigs, most veterinarians and researchers assumed that piglets were infected by ingesting *I. suis* oocysts from the sow’s feces. However, studies have failed to confirm this assumption. Surveys of the swine population in the United States indicate that *Eimeria* infections are common (60–95%) in animals raised in lots or in the wild, but less than 3% of the animals sampled excrete oocysts of *I. suis* (Lindsay et al. 1984). Another study examined the species of oocysts excreted by sows on farms with and without a history of *I. suis* infections in nursing pigs, and reported that 82% of

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66.3. **Diagnostic stages of Isospora suis in Wright’s Giemsa-stained intestinal smears (A–D: bar = 5 μm) or hematoxylin-and-eosin-stained histological sections (E–H: bar = 10 μm).** (A) Zoite with a single nucleus (n). (B) Type 1 meront, which has two nuclei (n). (C) Dividing type 1 meront. Note the nuclei (n). (D) Paired type 1 merozoites. Note that each has a single nucleus (n). (E) Zoite in a parasitophorous vacuole (arrow). (F) Type 1 meront. Note that two nuclei (arrows) are present. (G) Paired type 1 merozoites. (H) Host cell with a type 1 meront (arrows label nuclei) and a type 1 merozoite (modified from Lindsay et al. 1980).
the sows on farms with a history of coccidiosis had *Eimeria* infections but no detectable *I. suis* infections, whereas sows from farms with no history of neonatal coccidiosis had an infection rate of 95% with *Eimeria* species and less than 1% with *I. suis* (Lindsay et al. 1984).

In the United States, Stuart and Lindsay (1986) examined the transmission of *I. suis* on two farms in Georgia. Daily fecal samples were collected rectally from sows 1 week prior to farrowing, the day of farrowing, and for about 1 week after farrowing. Colostrum and placentas from several sows were examined microscopically for parasites. A coccidiostat (amprolium HCl, Amprol 25% feed grade) was given to half of the sows on each farm. Improved attention to sanitation has been the most successful method for reducing losses due to neonatal coccidiosis in pigs (Stuart and Lindsay 1986). A good sanitation program entails thorough cleaning of the crates to remove organic debris, disinfection, and steam cleaning. In extreme cases, sealing or painting solid surfaces within farrowing crates can help break the cycle of reinfection by the hardy oocysts. Producers should limit access to farrowing crates by workers to avoid crate-to-crate contamination with oocysts carried on boots or clothing. Likewise, pets should be prevented from entering the farrowing house and spreading oocysts from crate to crate on their paws. Rodent populations should be controlled to prevent these animals from mechanically transmitting oocysts.

Facilities need to be sanitized after every farrowing. Producers should be made aware that even though clinical disease is under control, the potential for future outbreaks is still present.

**TOXOPLASMOSIS (TOXOPLASMA GONDII)**

Toxoplasmosis is caused by infection with *Toxoplasma gondii*, a protozoan parasite related to the coccidia. Infections are common in humans and animals. Postnatally, animals or humans become infected by ingesting food and water contaminated with sporulated *T. gondii* oocysts or by consuming meat containing tissue cysts. Cats (and other felines) are the only animals that can excrete resistant oocysts in their feces and are important in the transmission of *T. gondii* to pigs and other animals (Figure 66.4). Tissue cysts are found in many edible tissues of infected animals, and they contain bradyzoites, which are slowly multiplying stages (Figure 66.5B). Tissue cysts remain viable in the tissues for many years and probably the life of the animal. After ingestion, oocysts or bradyzoites can survive passage through the stomach. Once in the intestine of the host, sporozoites or bradyzoites change into a fast-multiplying stage called tachyzoites (Figure 66.5A). Tachyzoites multiply in the lamina propria of the intestine and eventually spread throughout the body. Prenatal infection may occur if the mother is infected during pregnancy. Tachyzoites from the mother’s blood may cross to the fetus via the placenta. Tachyzoites cause tissue damage and eventually develop into the bradyzoite stage and form tissue cysts. Toxoplasmosis is a zoonosis, and pork is considered a source of *T. gondii* infection for humans in many countries (Dubey 2009; Dubey et al. 2005). Although the prevalence of *T. gondii* in feeder pigs in the United States is declining, there are still small pig establishments with *T. gondii* infection (Dubey et al. 2008).

**Clinical Signs**

Most infections in swine are subclinical (Dubey 1986). Abortions due to *T. gondii*, although uncommon, may
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Occur in sows infected during pregnancy. Transplacentally infected pigs may be born premature, dead, or weak, or they may die soon after birth. Pigs that live may develop diarrhea, incoordination, tremors, or cough. Few reports exist on clinical disease in pigs that acquire infection postnatally, but epidemics of clinical toxoplasmosis have been observed in both young and adult pigs. Experimental studies indicate that ingestion of *T. gondii* oocysts by pigs is more likely to produce clinical disease than ingestion of tissue cysts (Dubey 1986). Severity of disease is dependent on the number of oocysts ingested. Older animals are less likely to develop clinical disease.

**Pathological Changes**

Pathological changes are associated with necrosis of the host tissue caused by the rapidly multiplying tachyzoites. Enteritis, lymphadenitis, splenitis, hepatitis, pneumonitis, and less frequently myositis and encephalitis are seen in naturally infected pigs (Dubey 1986, 2009).

**Diagnosis**

Several serological tests are available for determining antibodies to *T. gondii* in pigs. The modified direct agglutination test is the most sensitive and specific for the detection of latent *T. gondii* infection in swine (Dubey et al. 1995a). Although finding *T. gondii* antibody in adult pigs only means exposure to *T. gondii*, finding antibody in a fetus indicates congenital infection because maternal antibodies are not transferred to the fetus in pigs. Histological examination of tissues may be utilized for a presumptive diagnosis based on lesion characteristics and parasite structure (Figure 66.5A,B) following routine histological staining of tissue sections.

**Epidemiology**

The prevalence of congenital *T. gondii* infection in pigs is less than 0.01%. Prevalence of *T. gondii* antibodies in feeder pigs (younger than 6 months) is lower (<1%) than in sows (15–20%) based on large-scale surveys (Dubey and Jones 2008; Dubey et al. 1995b; Hill et al. 2010; Patton 2001; Weigel et al. 1995). The presence of *T. gondii*-infected cats and infected rodents were identified as the main sources of *T. gondii* infection for pigs (Weigel et al. 1995). Cats become infected with *T. gondii* by ingesting infected animals (rodents, birds) soon after weaning. Therefore, infected juvenile cats are considered to be the main source of *T. gondii* for farm pigs.

66.4. Life cycle of *Toxoplasma gondii*.
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To prevent oocyst-induced infections, cats should never be allowed in buildings where pigs are housed or where feed is stored. Rodenticides should be used to control rodents and eliminate this possible source of tissue cysts. Any pigs that die should be removed promptly to prevent cannibalism. Uncooked garbage should never be fed to pigs. Feed should be kept covered to prevent cats from defecating in it.

**Treatment and Control**

Because porcine toxoplasmosis is usually subclinical, little is known about the treatment of the disease. In general, drugs used to treat toxoplasmosis in humans have been effective. These include pyrimethamine or trimethoprim in combination with a systemically active sulfonamide.

Control of *T. gondii* infection in pigs is important because of public health concerns over human infections. Toxoplasmosis causes mental retardation and loss of vision in congenitally infected children. Following experimental infection, viable tissue cysts of *T. gondii* can be found in most commercial cuts of pork, and studies have shown that the tissue cysts will be viable for at least 2.5 years (Dubey 1988). Cooking and freezing pork will inactivate tissue cysts.

Prevention of *T. gondii* infection in pigs can be achieved by practicing good husbandry. There is no vaccine. To prevent oocyst-induced infections, cats should never be allowed in buildings where pigs are housed or where feed is stored. Rodenticides should be used to control rodents and eliminate this possible source of tissue cysts. Any pigs that die should be removed promptly to prevent cannibalism. Uncooked garbage should never be fed to pigs. Feed should be kept covered to prevent cats from defecating in it.

**SARCOCYSTIS**

*Sarcocystis* spp. are coccidia parasites that have a two-host life cycle. Three species use pigs as the intermediate host and form tissue cysts (sarcocysts) in the pig muscles. *Sarcocystis miescheriana* has a pig–dog life cycle and is the only species found in the United States. Dogs excrete infective stages (sporocysts) in their feces. The other species are *Sarcocystis suihominis*, which uses the

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66.5. Toxoplasma gondii stages in smears of tissues of animals. Bar: 10µm. (A) Tachyzoites from the lung (arrows), Giemsa stain. (B) Tissue cyst from the brain, unstained. Note the hundreds of bradyzoites enclosed in a thin cyst wall (arrow). (C) Unsporulated oocyst (arrow) from cat feces, unstained. (D) Sporulated oocyst from cat feces, unstained. Note the two sporocysts (arrows). The sporozoites are barely visible (arrowheads).
human as the definitive host, and *Sarcocystis porcifelis*, which uses the cat as the definitive host (Dubey et al. 1989). Surveys indicate that 3–18% of commercial breeding sows and 32% of wild swine examined in the United States have *Sarcocystis* infection (Dubey and Powell 1994). There are no reports of naturally occurring clinical disease due to *Sarcocystis* infection in swine (Dubey et al. 1989). *Sarcocystis* infection in swine can be prevented by eliminating their exposure to canine feces (Figure 66.6).

**CRYPTOSPORIDIUM**

*Cryptosporidium* species are prevalent and ubiquitous worldwide in humans and animals. There are over 25 named species plus nearly twice that number of genotypes and the identification of new species and genotypes continue to increase rapidly. These obligate intracellular protozoan parasites of vertebrates range from host-restricted species to species capable of infecting a wide range of hosts (Fayer 2010). In pigs, the most frequently found species is *Cryptosporidium suis* followed by *Cryptosporidium* pig genotype II and occasionally *Cryptosporidium parvum*, the well-known widespread zoonotic pathogen highly prevalent in young cattle.

**Life Cycle**

Infection begins with ingestion of the environmentally hardy oocyst stage after direct contact with feces or from contaminated food or water (Figure 66.7). Four sporozoites are released from each oocyst (diameter ∼5µm) in the intestinal lumen where they enter cells lining the small intestine. All endogenous stages are intracellular but extracytoplasmic, appearing to be on the luminal surface of the mucosal epithelial cells. There are two or more asexual generations, each generation producing merozoites that invade additional gut epithelial cells. Merozoites eventually give rise to male and female stages. Fertilized female stages develop into oocysts that mature internally and are infectious when passed in the feces.

Generally, the prepatent period for infection with *C. suis* ranges from 2 to 9 days with oocysts excreted for 9–15 days.

**Cryptosporidia: Clinical Signs**

A wide range of clinical features has been reported for cryptosporidiosis in pigs, possibly reflecting differences between gnotobiotic and conventionally raised piglets as well as differences among the *Cryptosporidium* isolates that caused infection in pigs (Santin and Trout 2008). Clinical signs, including inappetence, depression, vomiting, and/or diarrhea, were observed after experimental infection of piglets with oocysts derived from calves (most likely *C. parvum*). However, cryptosporidiosis in pigs does not always result in clinical signs. In the absence of molecular analysis, it is not possible to determine which *Cryptosporidium* species or...
4.5 to 5.5 µm in diameter, and have no distinguishing features, microscopy can be used only to determine the presence of oocysts. Definitive identification of species and genotypes requires the use of molecular techniques such as gene sequencing or PCR-restriction fragment length polymorphism (RFLP). Pigs can harbor multiple species and genotypes, including *C. parvum* and *C. suis*, which are infectious for humans. Therefore, molecular analysis is needed to evaluate the risk of human infection (Table 66.1).

Developing stages of *Cryptosporidium* have been reported throughout the intestinal tract, with lesions of varying degrees of villous atrophy, villous fusion, cellular infiltration of the lamina propria and infrequently, sloughing of epithelial cells. All species examined appear to cause similar types of damage, but lesions from *C. parvum* seemed most severe, reflected by the more significant clinical signs. Extraintestinal infections have been reported in the gall bladder of naturally infected piglets and in the gall bladder, bile ducts, and pancreatic ducts of experimentally immunosuppressed piglets. Experimental inoculation of immunologically normal piglets produced infections in the trachea and conjunctiva. The significance of infection at these extraintestinal sites is unknown.

**Detection and Diagnosis**

Microscopy, fecal antigen enzyme-linked immunosorbent assay (ELISA), and PCR, with or without recovery and concentration of oocysts, have been used. However, recovery of oocysts from pig feces has been problematic with consistently lower recovery than from feces of other animals. Sucrose, cesium chloride, and other flotation media are effective in concentrating oocysts by density gradient centrifugation and reducing fecal debris. Because most oocysts of *Cryptosporidium* species and genotypes are nearly spherical, range in size from 4.5 to 5.5 µm in diameter, and have no distinguishing features, microscopy can be used only to determine the presence of oocysts. Definitive identification of species and genotypes requires the use of molecular techniques such as gene sequencing or PCR-restriction fragment length polymorphism (RFLP). Pigs can harbor multiple species and genotypes, including *C. parvum* and *C. suis*, which are infectious for humans. Therefore, molecular analysis is needed to evaluate the risk of human infection (Table 66.1).

![Life cycle of Cryptosporidium](image-url)
Table 66.1. Species and genotypes of Cryptosporidium, Giardia, and microsporidia found in swine and their zoonotic potential

<table>
<thead>
<tr>
<th>Species</th>
<th>Genotype/Assemblage</th>
<th>Zoonotic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cryptosporidium suis</td>
<td>II</td>
<td>Yes</td>
</tr>
<tr>
<td>Cryptosporidium sp.</td>
<td>II</td>
<td>No</td>
</tr>
<tr>
<td>Cryptosporidium parvum</td>
<td>II</td>
<td>Yes</td>
</tr>
<tr>
<td>Giardia intestinalis</td>
<td>A</td>
<td>Yes</td>
</tr>
<tr>
<td>Giardia intestinalis</td>
<td>E</td>
<td>No</td>
</tr>
<tr>
<td>Encephalitozoon cuniculi</td>
<td>III</td>
<td>Yes</td>
</tr>
<tr>
<td>Enterocytozoon bieneusi</td>
<td>D</td>
<td>Yes</td>
</tr>
<tr>
<td>Infects many mammals</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Enterocytozoon bieneusi</td>
<td>EbpC</td>
<td>Yes</td>
</tr>
<tr>
<td>Infects only pigs, eight genotypes</td>
<td>PigEBITS</td>
<td>No</td>
</tr>
<tr>
<td>Enterocytozoon bieneusi</td>
<td>EbpB, D, G, H, O</td>
<td>No</td>
</tr>
<tr>
<td>Infects only pigs, five genotypes</td>
<td>EbpA</td>
<td>No</td>
</tr>
<tr>
<td>Enterocytozoon bieneusi</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Infects pigs and cattle</td>
<td></td>
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**Epidemiology**

Point prevalence studies have found that most piglets are infected at 1–6 months of age with few infected under 1 month of age or as adults; several studies have found no *Cryptosporidium* in these two age groups (Santín and Trout 2008). In the farm environment, pigs become infected predominantly with *C. suis* followed by *Cryptosporidium* pig genotype II. Occasionally, *C. parvum* has been found. Pigs have been experimentally infected with *C. hominis* and *Cryptosporidium meleagridis*. Molecular methods were used to analyze individual fecal samples from pigs on farms in Australia, Canada, China, Czech Republic, Denmark, Ireland, Norway, Spain, and Switzerland but not in the United States. In most cases, *C. suis* was predominant followed by pig genotype II.

Regarding zoonotic potential, *C. parvum* is not highly prevalent in pigs, and although sporadic cases of *C. suis* have been reported in humans, the risk that pigs pose as a source for human infections is apparently limited.

**Treatment**

Disinfection of the environment is difficult because oocysts are numerous, can remain infectious for months, and are extremely resistant to a broad range of chemicals. Heat, drying, and sunlight are most effective.

Chemotherapeutic agents for the treatment of cryptosporidiosis have been thoroughly reviewed by Stockdale et al. (2008). Hundreds of compounds have been found ineffective. Paromomycin, once the first-line agent for the treatment of cryptosporidiosis in humans and animals, has been problematic. Nitazoxanide is the only drug approved for use in humans.

**OTHER PROTOZOA OF MINOR IMPORTANCE OR POTENTIALLY TRANSMISSIBLE TO HUMANS**

**Giardia**

*Giardia duodenalis* (also called *Giardia lamblia* and *Giardia intestinalis*) is possibly the most common intestinal parasite of humans and livestock worldwide. It is a species complex consisting of seven assemblages based on genetic analysis, whose members are morphologically indistinguishable. Assemblages A and B infect humans and a wide range of mammals. Others primarily infect specific groups of animals: C and D infect canids, E infects livestock, F infects felids, and G infects rodents.

Trophozoites are flagellated, pyriform-shaped protozoa that attach to the brush border of cells lining the small intestine where they absorb nutrients and multiply by binary fission (Figure 66.8). In response to unknown stimuli, trophozoites encyst in the small or large intestine and pass in the feces as environmentally resistant cysts. Freshly excreted cysts are immediately infectious and can remain infectious under moist and cool conditions for weeks. Trophozoites excreted in feces do not survive outside the body. Cysts are transmitted by the fecal–oral route by direct contact or by ingestion of cyst-contaminated food or water.
In the United States, no association was found between *Giardia* infections and clinical illness in pigs (Xiao et al. 1994). Likewise, on European farms, no clinical signs were associated with the presence of *Giardia* in pigs. In contrast, soft stools and diarrhea were associated with the presence of assemblage E in pigs in Australia, but because viruses and bacteria were not screened, it is possible that *Giardia* was not the cause of diarrhea (Armsom et al. 2009).

Detection of motile trophozoites (10–20µm in length) can be seen in saline suspensions of loose or diarrheic feces. Ovoid to ellipsoid cysts (9–15µm in length) are best detected after concentration in feces using zinc sulfate flotation (specific gravity 1.18). Sucrose and sodium chloride flotation media are effective but hypertonic, distorting cysts if examination is not made quickly. Because cysts might be excreted intermittently, multiple fecal samples should be collected and examined at intervals within a week. To identify the assemblages, the most commonly employed genetic marker is the beta-giardin gene, but portions of the small subunit ribosomal DNA, glutamate dehydrogenase, and the triose phosphate isomerase gene have been used also.

Epidemiology to determine the role of swine in the epidemiology of human infection and to trace sources of infection requires molecular analysis (Table 66.1). The role of domestic animals as sources for *G. duodenalis* in human infection is unclear. *Giardia* infections have been reported in pigs in all age groups from nursing piglets to boars and sows in the United States, Canada, Europe, Australia, and Asia with the prevalence ranging between 0.1% and 20% (Armsom et al. 2009). In Ohio, *Giardia* was detected in 8.3% of pre-weaned pigs, 2.6% of postweaned pigs, and 1.5% of sows (Xiao et al. 1994). In California, 7.6% of feral pigs were found infected with *Giardia* (Atwill et al. 1997), suggesting a possible health risk to humans and a source of infection for domestic pigs. In Canada, *Giardia* was documented in 70% of 50 farms and in 8.5% of fecal samples. Cysts were identified in 3.8% of piglets, 9.8% of weaned, 0.8% of growers, 15% of finishers, 5.7% of boars, and 4.1% of sows (Guselle and Olson 1999). In Denmark, the age-specific herd prevalence for infection was 22%, 84%, and 18% in piglets, weaned, and sows, respectively (Maddox-Hyttel et al. 2006). Isolates of zoonotic assemblage A was found in both weaned pigs and piglets in Denmark. In Australia, 17% and 41% of pre- and postweaned pigs, respectively, were infected with *Giardia*; assemblage E was predominant but A was also found (Armsom et al. 2009).

Three *G. duodenalis* subsassemblies A predominantly and preferentially cycle within defined hosts: AI in livestock, AII in humans, and AIII in wildlife. In North, Central, and South America and Europe, most livestock are infected with subsassemblage AI and a minority with AII, whereas the opposite is true in humans most being infected with subassemblage AII, and a minority with AI (Sprong et al. 2009).

There are no vaccines available to prevent giardiasis in large animals, and no drugs are approved for treating giardiasis in animals. However, fenbendazole and albendazole have been used to treat animals. Cysts are infectious when passed in the feces but can be rendered noninfectious by desiccation, many quaternary ammonium compounds, laundry bleach, and boiling water. After cleaning and treating contaminated spaces, thorough drying is recommended. Cysts in hog liquid manure holding tanks were found degraded, suggesting it is unlikely that distribution of liquid manure poses a serious threat for giardiasis from contamination of surface water (Guselle and Olson 1999).

**Microsporidia**

There are approximately 1200 named species of microsporidia, a diverse group of obligate intracellular parasites once regarded as protozoa but now recognized as fungi. Most infect invertebrates and fish, but 14 species in eight genera infect humans (Didier and Weiss 2006) and four of these, *Enterocytozoon bieneusi* and the *Encephalitozoon* species (*Enterocytozoon cuniculi, Encephalitozoon intestinalis, and Encephalitozoon hellem*), infect humans and other animals. Swine can serve as a potential source for humans. *Enterocytozoon bieneusi* is the most frequently diagnosed species in humans. It has been identified in swine in many countries, but its prevalence in pigs in the United States is not well studied. Surveys have demonstrated that pigs can serve as hosts for *E. bieneusi* genotypes that are identical to strains found in humans (Table 66.1). *Encephalitozoon cuniculi* genotype III (dog genotype) has been reported in swine and is the most common genotype reported in humans in the United States.

The life cycle stages of microsporidia are depicted using *E. bieneusi* as an example (Figure 66.9). All are intracellular, in direct contact with the host cell cytoplasm, and consist of binucleate cells, sporogonial plasmodia, uninucleate sporoblasts, and spores. Spores (1.5 × 0.5µm) are the infective stage and are excreted in the feces from the infected host. Internally, spores have a polar tube with five to six coils, a single nucleus, and an anterior attachment complex extending to a polaroplast. After a spore is ingested, the coiled polar tube discharges, injecting the sporoplasm and nucleus into the host cell. Young proliferative stages become elongate and undergo nuclear division. Proliferative plasmodial cells contain multiple elongated nuclei. Later, sporogonial plasmodium develops disks, some in stacks or arcs in stages of polar tube formation. Individual nuclei with polar tube complexes segregate and mature into separate sporoblast cells that development into mature spores. Spores released from host cells are excreted with feces into the environment where they can be ingested by the next host.
The role of microsporidia in clinical disease is unclear.

Enterocytozoon bieneusi was detected in feces from four pigs suffering severe diarrhea and stunted growth (Rinder et al. 2000) and also in 28 healthy pigs (Breitenmoser et al. 1999). Thirty-eight piglets with diarrhea and 29 without diarrhea tested positive for E. bieneusi (Jeong et al. 2007). Whether certain genotypes are pathogenic whereas others are not is unknown.

Detection of microsporidial infection is difficult because the spores are very small (1.5 × 0.5 µm). Serological tests such as ELISA and indirect immunofluorescent antibody (IFA) microscopy can be used to detect antibodies to E. cuniculi. Spores can be detected by microscopic techniques used in specialized laboratories. However, no microscopic methods are sufficient to determine the species/genotype identity of microsporidia. Specific and sensitive molecular methods are now used to identify E. bieneusi and E. cuniculi genotypes. Although these tests are not routinely used in clinical diagnostic laboratories, they are widely used in research and some government public health laboratories. Because the ITS region of the rRNA gene has a high degree of diversity, among isolates it has become the present standard for detection and identification of E. bieneusi genotypes (Santín and Fayer 2009).

Epidemiology, too, is unclear since few cases of microsporidiosis in pigs have been reported. Reservoirs and the routes of transmission are still unknown. However, potential sources are beginning to be identified for both humans and a wide range of animals by comparison of genotypes from different hosts.

Treatment using the anthelmintic albendazole is effective for E. cuniculi, E. intestinalis, and E. hellem but has not been effective for treatment of E. bieneusi. The number of reported human cases has greatly decreased as the use of highly active antiretroviral therapy for HIV has increased.

Balantidium coli
Balantidium coli is a ciliate found in pigs and in humans. It is transmitted by cysts that are excreted in the host’s feces. The cysts are 50–70 µm in diameter and contain a macronucleus and a micronucleus. No division occurs in the cyst. Trophozoites are covered in short cilia, are up to 100 µm in length, and also contain a macronucleus and a micronucleus. Trophozoites are usually found in the lumen of the large intestine.

Most infections in swine and humans are subclinical. Estimates of the prevalence in swine raised in the United States under modern production conditions are not available. However, these organisms are frequently observed microscopically in exudates of colitis. A primary role in disease is unlikely, but other causes of colitis should be investigated.

Studies conducted in the 1980s found that infection rates increased with pig age and that animals raised on pasture or dirt lots had higher infection rates than did
swine raised in confinement. Human infections with *B. coli* are more prevalent in areas where swine are present (Schuster and Ramirez-Avila 2008). *Balantidium suis* is similar to *B. coli* and found in humans and swine. It is smaller than *B. coli* and its validity as a true separate organism from *B. coli* is questioned (Schuster and Ramirez-Avila 2008).

**Entamoeba Species**
Amoeba in the genera *Entamoeba* has been reported in pigs in various parts of the world. Swine are not a significant reservoir for *Entamoeba histolytica*. They are a proven reservoir for *Entamoeba polecki*, a nonpathogenic amoeba found in pigs and humans. The prevalence of these parasites in the U.S. swine population is not known.

**REFERENCES**


INTRODUCTION

Internal parasites are very common in swine worldwide, sometimes compromising production and occasionally the cause of clinical disease. Controlled trials with nematode infections consistently demonstrate a reduction in average daily gain (ADG) and increase in feed-to-gain (F/G) ratio of infected pigs compared with their controls. In addition, internal parasites can generally compromise vigor and may act synergistically with other endemic potential pathogens. More often, parasites are the cause of subtle but significant subclinical disease.

Subtle changes in pig performance or behavior may go unnoticed by the pork producers, because the damage occurs in small daily amounts. These “nickel-and-dime” losses become significant in the long run. The extent of these losses depends on the quality of nutrient intake, type of housing, climate in the geographical area concerned, genetics of the swine, veterinary costs associated with these infections, and similar factors. Attempts to prevent such losses usually entail administration of anthelmintics coupled with strategic management systems designed to interrupt the transmission of the parasites.

Historically, most schemes for parasite control have been aimed at reducing condemnation of livers caused by *Ascaris suum* or *Stephanurus dentatus*. The “McLean County” system developed in midwestern United States (Raffensperger and Connelly 1927), the “Profit” program in North Carolina (Behlow and Batte 1974), and the “Gilt-Only” system developed in Georgia (Stewart et al. 1964) incorporated sanitation, anthelmintics, and management, singly or in combination, to reduce condemnation and production losses. All anthelmintics introduced for swine since the 1950s have been highly efficacious against *A. suum*, yet it is still the most prevalent swine worm parasite in the world. Anthelmintics used alone will never suffice. Integrating knowledge of parasite life cycles, ecology, and risk factors can couple good management practices with strategic use of anthelmintics for more effective control or elimination.

Biological systems of control have been sought. For example, daily doses of chlamydospores of the microfungus *Duddingtonia flagrans* fed in feed over a 2-month period to pastured pigs infected with a known number of *Oesophagostomum dentatum* and *Hyostrongylus rubidus* resulted in lower herbage numbers of infective larvae of both species compared with the control pigs not receiving the microfungus (Murrell et al. 1996; Nansen et al. 1996).

Inasmuch as protozoan parasites are discussed elsewhere (Chapter 66), we are concerned here with helminths (nematodes, cestodes, and trematodes). They will be dealt with according to the host’s organ system affected.

DIGESTIVE SYSTEM

The digestive system offers ready and easy entry into and exit from the host. Therefore, a myriad of parasites have evolved using this system as their niche. The mouth is a chaotic niche, however, so this level of the system is not often parasitized. All other levels of the digestive tract harbor parasites.

Esophagus

*Gongylonema pulchrum*, the esophageal worm, is a spiruroid nematode occurring in tunnels burrowed into
the epithelium covering the esophagus and tongue. The tunnels are usually aligned with the longitudinal axis of the esophagus, but those that occur in the glossal mucosa are haphazard. The tunnels are formed into a uniform sine wave, with about 8–10 waves along the length. The males are 60 mm long, and the females are about 90 mm long when removed from their tunnels. The thick-shelled, broadly oval (55–65 × 30–35 μm), transparent, fully embryonated eggs pass in the host’s feces. Infective larvae develop when the eggs are ingested by cockroaches or certain coprophagous beetles, and transmission occurs when swine eat the insects.

Gongylonema cause minor irritation as they glide back and forth in their tunnels. Their main importance is at slaughter, where tissue is trimmed if the lesion is found. Ruminants and humans are also susceptible to G. pulchrum, but they must ingest the intermediate host insect to become infected, so trimming of affected tissue at slaughter is for appearance’s sake.

Stomach

Five nematode genera occur within the stomach. One, *Hyostrongylus*, is moderately common, but the other four (Ascarops, Physcephalus, Gnathostoma, and Simondia) are less common and limited geographically.

**Hyostrongylus.** *Hyostrongylus rubidus*, the red stomach worm, is a trichostrongylid nematode occurring unattached on the mucosa of the lesser curvature of the stomach. They are hairlike in width and less than 10 mm in length. The eggs have typical strongyle structure (ovoid, thin-shelled, transparent, 60–76 × 30–38 μm) developed to the 16- to 32-cell stage when laid. They resemble the eggs of *Oesophagostomum* spp., which occur in the large intestine. Their differentiation requires harvest of infective ensheathed larvae from incubated fecal cultures (Honer 1967). Eggs of the hookworm, *Globocephalus*, are also similar but smaller (52–56 × 25–35 μm).

The life cycle is direct, not requiring an intermediate host. Eggs pass in the feces and develop therein to infective larvae in about 7 days. The infective larvae migrate away from the feces and onto grass, where they are ingested by swine. Thus, *hyster.gylosis* is a disease of pastured swine. The ingested larvae enter the gastric glands, where they undergo two molts, and then reenter the gastric lumen. Some larvae may remain in the gastric glands in a state of hypobiosis, causing nodular distention of the affected glands. These hypo-biotic larvae enter the gastric lumen later and resume their delayed development to adults.

Little is known about their pathogenicity. They suck a small amount of blood and cause catarrhal gastritis, which may lead to mucosal erosion. It is debated whether or not they are a factor in the pathogenesis of gastric ulcerations of glandular stomach. These changes in the gastric mucosa may negatively impact feed conversion and weight gains (Stewart et al. 1985).

**Spiruroid Stomach Worms.** The other stomach worms, Physcephalus sexalatus, Ascarops strongylina, Gnathostoma spinigerum, and Simondia paradoxa, are spiruroid nematodes. They are considerably larger than *Hyostrongylus*, being stouter and attaining about 20 mm in length. The adults are attached by the mouth to the mucosa, but this attachment causes no visible damage, except for excessive mucus production. Female Simondia have their anterior ends enter the gastric glands, so that only their bulbous posterior ends are visible.

The life cycles of these four spiruroids, so far as is known, are all similar to one another. The typical spiruroid eggs (thick-shelled, transparent, ovoid, embryonated) pass in the feces, where they are ingested by coprophagous beetles. The eggs resemble, but are slightly smaller than, the eggs of Gongylonema. Whereas Gongylonema eggs measure 55–65 × 30–35 μm, these eggs are in the 30–40 × 15–20 μm range. Infective larvae develop in the beetles, which are then ingested by pastured swine. Prepatent periods are in the 4- to 6-week range.

**Small Intestine**

**Strongloides.** *Strongloides ransomi*, the minute threadworm, is a rhabditid nematode that is ubiquitous and common. In suckling pigs, it is particularly pathogenic, especially in tropical and subtropical climates, but in older swine, it has less importance.

**Life Cycle.** The minute (3–5 mm long), hairlike nematodes are embedded in the epithelium of the small intestine. The parasite female is parthenogenetic. She lays thin-shelled, transparent, embryonated eggs in the burrow in the mucosa. The eggs slough and appear in the feces. Rhabditiform larvae hatch in a few hours and may progress either to infective third-stage filariform larvae in about 3 days (homogonic cycle) or to free-living rhabditiform males and females (heterogonic cycle). The free-living cycle eventually produces infective larvae after a few generations. As a result, the heterogonic cycle produces an exponential increase in the population, resulting in a corresponding increase in the yield of infective larvae. Factors that govern which cycle, homogonic or heterogonic, prevails are probably environmental, such as moisture and availability of suitable substrate, with the favorable environmental factors favoring the heterogonic cycle.

Once infective larvae are produced, they can enter the next host by any of several routes: percutaneous, oral, transcolostral, or prenatal. Percutaneous penetration is common, and larvae entering by this route enter the bloodstream and are carried to the lungs, from where they are coughed up and swallowed (tracheal...
mation). This migration results in patency in 6–10 days. Larvae entering by the oral route must penetrate into the buccal mucosa and get carried to the lungs, because gastric juices are lethal to them.

The principal route of infection for neonates is transcolostral (Moncol 1975). Larvae acquired by the sow accumulate in the mammary fat and become mobilized and enter the mammary alveoli to be included in the colostrum. Once ingested by the nursing neonate, the colostrum-derived larvae develop directly into adult female nematodes without undergoing a tracheal migration (the tracheal migration has previously occurred in the sow, so it is not repeated in the neonates).

Prenatal infection occurs when the pregnant sow acquires infective larvae during the latter period of gestation. During larval migration, they enter the placental circulation and begin to accumulate in fetal tissues. Migration to the small intestines ensues at farrowing, resulting in patent infections in as little time as 2–3 days.

Pathology. Lesions are dependent on the number of infective larvae acquired and on the host’s resistance. It is common to find a small number of Strongyloides without associated lesions. In heavy infections, poor weight gains, diarrhea, and death may result. In neonates, heavy infection may result in death before the pigs are 10–14 days old. Even in these cases, pathognomonic lesions are lacking. Resistance develops due to previous exposure to infective larvae and due to age of the host.

Diagnosis. If the infections are patent, finding the typical embryonated eggs in fecal flotation is diagnostic. At necropsy, the female parasites may be found in mucosal scrapings. Because adult Strongyloides are tiny (3–5 mm), they may be confused with larvae of other nematodes. However, the finding of eggs within the parasite’s uterus or elsewhere within the scraping eliminates larvae (larvae do not have eggs) from consideration. Confusion with other causes of diarrhea and poor weight gain, such as coccidiosis or colibacillosis, is possible (Figure 67.1).

Ascarids. Ascaris suum, the large roundworm, is the most common and cosmopolitan nematode in swine, despite decades of aggressive pharmaceutical interventions. At one time, it was thought to be a variant of the human ascarid, Ascaris lumbricoides, but now it is recognized as a species by itself.

Ascaris suum is a robust-bodied large nematode. The female is up to 40 cm long, and the male is up to 25 cm. It lies in the lumen of the jejunum, unattached and swimming against peristalsis. Its eggs are thick-shelled, barrel-shaped to ovoid, and large (50–80 by 40–60 µm).

The colorless thick shell is coated with a sticky, brownish, mammillated proteinaceous layer. Inside is a single large cell. Each female lays many hundreds of thousands of eggs per day for a life span of about 6 months. The eggs are resilient and long-lived; so, the environment is apt to be heavily contaminated wherever swine exist.

Life Cycle. The life cycle is direct. Eggs passing in the feces develop to infectivity in about 4 weeks. The infective larva remains inside the egg until the egg is ingested. Because of this fact, larvae are protected against many environmental factors that might otherwise be lethal to them. After ingestion, the infective larvae hatch and penetrate the jejunal wall. Most are carried by the portal circulation to the liver. A few larvae are scattered in the mesenteric lymph nodes, peritoneal cavity, and elsewhere. These wayward larvae probably do not continue in the cycle. Larvae reach the liver by 1 or 2 days after infection, and they are then carried by the circulation to the lungs by the fourth to seventh day. After molting and spending a few days in the lungs, the larvae leave the pulmonary capillaries and enter the bronchioles, from where they are coughed up, carried up the tracheal escalator by ciliary action, and swallowed. They reach the small intestine by days 10–15 after infection and mature to adults. Oviposition begins on about day 43 after infection. The eggs are very resistant to temperature extremes and may remain infective on pasture for more than 10 years. Most chemicals have no effect on the eggs, but steam and direct sunlight destroy their viability. Because of the sticky outer layer on the egg, eggs are easily transported by boots, insects, and other mechanical means.

Senescent ascarids are expelled in the feces, but swine may continue to carry a light infection for several months. Ascarids typically occur in young pigs. By the time swine are 5–6 months old, they are resistant by reason of previous exposure to migrating larvae and/or by reason of age resistance. Resistance causes the larvae to be arrested in the small intestinal wall by an intense inflammatory reaction as they attempt to penetrate. A. suum larvae are aggressive migrants and, if found in an improper host, can cause visceral larva migrans. Such larvae seldom reach adulthood in improper hosts, but occasionally they do so in humans. Migrating larvae may cause serious verminous interstitial pneumonia due to their delayed and prolonged migration and death in the lungs of improper hosts, such as cattle.

Pathology. Adult ascarids compete with the host for nutrients, and they browse on the tips of the villi, causing interference with the absorption of nutrients. In addition, adult ascarids are strong swimmers with wanderlust, frequently occluding the bile duct and sometimes wandering up the bile duct for a
67.1. (A) Strongyloides egg, thin-shelled, lacking one of three layers, and embryonated; (B) Ascarops egg, embryonated, and similar morphologically to those of Physocephalus and Gongylonema; (C) the Ascaris egg has an outer proteinaceous layer, often missing; (D) Metastrongylus egg; (E) Oesophagostomum egg; (F) Hysterospilus egg; (G) Globocephalus egg; (H) Stephanurus dentatus egg passed in the urine; (I) Trichuris egg; (J) Macracanthorhynchus egg (all eggs photographed and printed at the same magnification).
considerable distance to approach the liver capsule. Ascarid occlusion of the bile duct is one of the major causes of icterus in swine.

Larval ascarids cause considerable damage to the liver and lungs during their migration. Repeated waves of larvae migrating through the liver cause increasingly severe eosinophilic infiltration into the portal areas and marked fibrosis of the interlobular connective tissue, seen grossly as “milk spots.” Early lesions in the liver become visible 7–10 days after infection and persist and expand if continued infection occurs. Larval antigens are present in these lesions, possibly coming from larvae killed by the intense reaction. “Milk spots” regress within 25 days if the liver does not receive additional larvae. Severe, ongoing infections result in diffuse liver fibrosis. Clinical signs attributable to the liver migration phase are subtle and consist mainly of elevated liver enzymes.

The larval migration through the lungs is associated with petechial hemorrhages into the alveoli caused by the larvae breaking out from the capillaries. At the same time, interstitial pneumonia develops in the areas of offending larvae, as well as bronchiolitis and alveolar edema. These changes are associated clinically with a characteristic heaving expirational cough known as “thumps.” If severe enough, these pulmonary changes are life-threatening. Lungs compromised by these lesions become more susceptible to other conditions, such as mycoplasmal pneumonia. The ingestion of infective eggs often occurs in a trickle, so lesions of larval migration will occur simultaneously with lesions caused by adults. This will continue until resistance factors come into effect.

Contact with ascarid eggs normally starts when neonates first come into contact with the ground (Eriksen 1982). If for some reason pigs are about 20kg or so before they first contact ascarid eggs, particularly if egg exposure is high, the reaction to the pulmonary larvae may be extreme. The lassitude, high fever, and dyspnea resemble the clinical signs of respiratory disease complex.

**Economic Importance.** Numerous studies have detailed one aspect or another of the economic importance of ascarosis in swine. The results of experimental infections show subtle changes, even at low levels of infection, in ADG and feed efficiency (Hale et al. 1985). Metabolic studies show a significant effect on nitrogen metabolism at the time of rapid growth of immature adults in the small intestine (about 1 month postinfection). There are losses due to condemnation or trimming of livers at slaughter, and severely icteric carcasses are tanked. The monetary value of these depredations is enormous, though difficult to quantify, but in 1980, they were estimated to be nearly $400 million in the United States alone (Stewart and Hale 1988).

**Diagnosis.** The diagnosis of ascarosis is straightforward when dealing with patent infections. The prodigious oviposition rate of the female ascarids makes eggs easy to find by standard flotation methods. If swine are reared with contact with the ground, one can assume they are infected with Ascaris because of the ubiquitous nature of the nematodes and the extreme longevity, several years, of the eggs on the ground. Pork producers often assume that their pigs are infected and administer prophylactic or therapeutic procedures accordingly, even without specific proof of infection.

At necropsy, the presence of “milk spots” in the liver is evidence that the animal ingested ascarid eggs within the past month and those eggs are likely to be A. suum eggs. Early, punctate lesions gradually expand in size before resolving. In areas where S. dentatus, the swine kidney worm, is present, “milk spots” due to ascarid migration must be differentiated from the scars of kidney worm migration. Scars in the case of the latter are more extensive, and similar scars occur in other organs, such as the lungs and perirenal fat. “Milk spots” may also be caused by the migration of larval Toxocara canis (Helwigh et al. 1999) and perhaps other parasites undergoing visceral larva migrans in swine.

Ascaris suum adults are so large that they are visible or palpable through the wall of the jejunum. The presence of A. suum in other levels of the gastrointestinal tract at necropsy is a result of postmortem wandering by these robust nematodes.

Sometimes morbidity or mortality may occur while the infection is still in the prepatent phase. Therefore, fecal flotation for diagnosis is of no value. Besides the presence of abundant small “milk spots” and pneumonia by gross examination, one looks for small, immature ascarids in the jejunum and migrating larvae in the lungs. Larvae can be collected by floating snips of lung on water in a funnel. Under incubation, larvae will migrate into the water and sink into the funnel stem, from where they can be drained off into a petri dish and found microscopically.

**Trichinellosis.** Trichinellosis occurs in most species of mammals, including humans (Despommier 1990; Kazacos 1986). At one time, it was thought that all cases in swine were caused by Trichinella spiralis, but DNA studies have uncovered a cluster of species with nomenclature uncertain (McLean et al. 1989), but includes T. spiralis in North America and Europe, Trichinella nativa in polar regions, Trichinella britovi in Eurasia, and Trichinella netsoni in equatorial Africa. Regulation of garbage feeding to swine, public health programs, and recently improved trichinoscopic and serodiagnostic techniques have reduced the incidence of this infection.
**Life Cycle.** Adult *Trichinella* spp. are tiny (2–4 mm long) nematodes that are short-lived and therefore not often encountered. The adults live in the intracellular tunnels in the epithelium of the villi. Within 5 days after mating, the female larviposits into the lamina propria and continues to do so for her life (2–3 weeks). These larvae are picked up by the circulation and are distributed throughout the body. When they penetrate the sarcolemma of the skeletal muscle cells, they continue to mature to infectivity in about 14 days. In the process, the myocyte transforms into a “nurse cell,” supporting the maintenance of the encysted larvae for years. Usually, there is one larva per cyst. The cyst is fusiform, with its long axis corresponding to the long axis of the myocyte. Circulating larvae that do not enter myocytes die in granulomas. After several months, the muscle cysts may begin to calcify, starting at the poles, but the larvae within may live for some time. When muscle cysts are ingested, the larvae excyst and rapidly develop to adults in less than 48 hours. Examples of transmission within swine herds include tail biting, scavenging carcasses (rats, raccoons, etc.), and eating of garbage that contains trichinous meat scraps.

**Pathology.** The intestinal phase is associated with subclinical enteritis. Lesions associated with the muscle phase are centered on the nurse-cell cyst. While the nurse cell is forming, there is malaise, pyrexia, and myalgia accompanied by eosinophilia. There may be a temporary reduction in growth rate, but often the disease runs a silent clinical course. The nurse cell is formed by dissolution of the myofibrils and hypertrophy of the muscle nuclei. The changes are local, and the affected section of the myocyte is walled off by collagen, with the larva coiled spirally within the cyst about 2.5 turns. Once the development of the nurse cell is complete, signs begin to regress, and normal growth rate resumes.

**Diagnosis.** Traditional methods of diagnosis of trichinellosis are aimed at finding the muscle cysts. These cysts are not distributed homogeneously throughout the musculature but instead are somewhat concentrated in the more active muscles (e.g., diaphragm, extrinsic muscles of the eye, and muscles of posture), possibly because those muscles receive more capillary flow. There are two main methods for finding muscle cysts at necropsy; both are labor-intensive and plagued by false negatives. One is to take several snips of muscle, compress them between glass plates, and examine microscopically for cysts (trichinoscopy). The other is to digest several grams of muscle in artificial gastric juice (1% pepsin, 1% hydrochloric acid at 37°C) (“stomacher”) and examine the sediment microscopically. A more efficient and sensitive method is the enzyme-linked immunosorbent assay (ELISA) test using larval excretory antigen (Murrell et al. 1986) tested against sera from individual swine or pooled sera from a herd. False-negative ELISA tests are mostly due to very low populations of larvae (less than 5 per gram of muscle).

**Public Health.** *Trichinella* spp. are zoonotic, infecting humans as well as other mammals. The source of *Trichinella* varies, but pork products are often suspected. Pork sausage is particularly of concern because one trichinous carcass finds its way into numerous sausages. However, in recent years, more cases of human trichinellosis in the United States have been caused by eating undercooked trichinous bear meat or home-slaughtered pork, both of which are outside the purview of the meat inspection process. The association of trichinellosis with venison sausage or beef sausage is due to adulteration with trichinous pork. In northern Europe, the long-term use of trichinoscopy has practically eliminated swine trichinellosis. The incidence of swine trichinellosis in the United States has varied between 0.1% and 0.3% since the mid-20th century. Modern husbandry methods (prohibition of feeding raw garbage, raising pigs under sanitary confinement, docking of tails, feeding high-level nutrition, etc.) have greatly reduced the incidence of swine trichinellosis. A program incorporating these methods to ensure safe pork (National Trichinae Certification Program) is new and voluntary in the United States.

The United States Department of Agriculture (USDA) recently recommended that fresh pork be cooked to an internal temperature of 63°C and allowing it to rest for 3 minutes between cooking and cutting. This temperature includes a safety factor that allows for variation in cooking methods (e.g., microwave ovens do not heat evenly). Freezing of fresh pork products less than 15 mm thick at −15°C for 20 days or at −29°C for 6 days will kill most larvae (Campbell 1988), but not the ones adapted to cold (*T. nativa* mostly, but *T. nativa* infections are not a major problem to the pork industry). Salt curing does not reliably kill larval *Trichinella*, so cured products should be cooked.

As a result of all the foregoing, the number of cases of trichinellosis in humans in the United States has dropped from about 450 cases per year in 1947 to about 12 cases per year 50 years later (Kennedy et al. 2009), and it is likely that similar results have happened elsewhere. Trichinellosis is still present at a low level in swine, so its lingering presence is justification for continued vigilance.

**Hookworm.** *Globocephalus urosubulatus* is widely distributed geographically, but in North America, it is limited to swine pastured in southern states. Adults attach by their mouth to the jejunal mucosa. It is not very pathogenic, but otherwise it is a typical hookworm. Young pigs are more likely to become anemic...
than older, more resistant swine. Adult *Globocephalus* are about 7 mm long and lay stronglyoid eggs that are 52–56 by 25–35 \( \mu m \). Infective larvae develop on the ground and enter another pig by ingestion or skin penetration. It is likely that its geographical distribution is limited to areas that do not freeze.

**Acanthocephala.** The acanthocephalan ("thorny head") of swine is *Macracanthorhynchus hirudinaceus*, a very large helminth, sometimes exceeding 40 cm. In the fresh state, it has a dusty coral pink color, and the anterior end has a visible spiny proboscis with which it attaches to the jejunal wall. Depending on its hydration state, its body may be turgid or flattened and wrinkled, so it may be mistaken for either an ascarid or a cestode. However, ascarids have no proboscis and do not attach, and cestodes have true segments (not mere wrinkles) and have a scolex (not a proboscis).

**Life Cycle.** Adult thorny heads lay eggs (70–110 by 40–65 \( \mu m \)) in the host's feces. These eggs have a three-layered shell and an almond shape, are brown, and contain a larva (acanthor). The acanthor has an ellipsoidal shape and needlelike hooks on one end. When an egg is ingested by grubs of certain beetles (May beetles, dung beetles, or certain water beetles), an infectious larva (cystacanth) develops in them in about 3 months. The cystacanth begins its development in the beetle larva (white grub), but it survives through metamorphosis to appear in the adult beetle. Pigs acquire the cystacanth from either stage while rooting. Once in the pig, the cystacanth matures to an adult. The prepatent period is 2–3 months.

**Pathology.** The thorny head inserts its proboscis into the jejunal wall. Because the proboscis is longer than the intestinal wall is thick, perforation may occur. Usually, the host produces a fibrous connective tissue encapsulation of the proboscis, sealing off any perforation. These nodules are large and easily seen at necropsy. There are more nodules than adult thorny-heads, suggesting that release and reattachment may occur. Nodules regress in about 1 month after the thorny head leaves. Seldom are there clinical signs, but when intestinal perforation occurs there may be abdominal pain, diarrhea, and emaciation.

A few scattered reports of human infections with *Macracanthorhynchus* exist in cultures that eat raw beetles. Because the life cycle is indirect, there is no danger in handling its eggs or tissues with the adults.

**Large Intestine**

Two kinds of nematodes occur in the cecum and colon of swine: nodular worms and whipworms. Both are very common, and both can lead to important clinical disease.

**Whipworm.** *Trichuris suis*, the swine whipworm, occurs primarily in the cecum, but in heavy infections, it can spread into the colon. *Trichuris* adult females are 60 mm long. About two-thirds of their length is the filamentous esophageal portion of the body, which is stitched into the mucosa and not readily noticed. What is evident upon casual inspection is only the thick posterior portion of the body, lying out on the cecal mucosa. Intact nematodes are extremely difficult to collect at necropsy, because the delicate, long esophageal portion of the nematode breaks. The eggs are thick-shelled and barrel-shaped, with a clear plug filling an opening at each pole of the egg. The egg shell is smooth and brown, and the eggs are 50–68 \( \times \) 21–35 \( \mu m \). Inside is a single large cell.

**Life Cycle.** The life cycle of *Trichuris* is direct. Eggs pass with the feces, and once outside the body, the larva begins to form inside the shell. In 3–4 weeks, it has reached infectivity. The infective first-stage larva remains within the egg until ingested by a swine. The egg can remain viable for several years on the ground. After ingestion, the polar lungs dissolve, releasing the first-stage larva, which penetrates into the lamina propria of the lower small intestine and cecum. Histoergic migration lasts for about 2 weeks, during which the larva undergoes four molts. The posterior end of the whipworm's body begins to extend out into the lumen. Oviposition begins 6–7 weeks after infection, and the life span is 4–5 months (Beer 1973).

**Pathology.** Light populations of adult *Trichuris* in the cecum cause minimal lesions. They may offer an entry point for agents of the swine dysentery complex. Heavy infections with *Trichuris* are associated with ulceration of the mucosa, mucosal edema, hemorrhage/dysentery, and a fibrinonecrotic membrane. Much of this tissue damage is caused by the histotrophic larvae, and no adults may be present.

**Diagnosis.** The diagnosis of the common case of trichurosis is finding the characteristic eggs in fecal floatations. *Trichuris* eggs are among the heaviest of eggs, so correct technique must be employed. Furthermore, egg laying by the female is somewhat sporadic, and the cecum empties irregularly, so false negatives occur. Also, the worst cases of trichurosis are caused by heavy larval migration, in which case, there are no eggs in the feces. Cases of dysentery are presumptive, and confirmation of trichurosis can be secured at necropsy by finding stages of the life cycle of *Trichuris*. Often, these forms are tiny and must be sought by examining mucosal scrapings or by histopathology. Whipworms can be recognized by their unusual esophageal structure known as a stichosome, a row of very large glandular cells running a single file down the length of the long esophageal portion of the body.
Nodular Worms. Nodular worms (Oesophagostomum spp.) are common and cosmopolitan (Stewart and Gasbarre 1989). These strongyles inhabit the mucosal surface of the cecum and colon. Adults range from 8 to 15 mm. There are several species, but all are quite similar. Eggs are typical strongyle eggs (70 × 40 µm, thin-shelled, transparent, in the morula stage).

The life cycle is direct. Eggs passing in the feces produce larvae, which develop to infectivity within the fecal matter in about 1 week. Infective larvae crawl away from the feces and onto vegetation, from where they are ingested by swine. Infective larvae retain their last molted skin as a sheath, so they are moderately resistant to environmental conditions and survive about 1 year. Ingested larvae exsheathe and enter the mucosal glands of the ileum, cecum, and colon. They penetrate into the lamina propria to molt and remain for about 2 weeks. They emerge into the intestinal lumen to become adults, and eggs begin to appear 3–6 weeks after infection.

Adults cause minimal damage to the mucosa and not much in the way of clinical signs. The migration and molting by the larvae in the lamina propria cause the characteristic nodule, which is usually a small (about 2 mm) abscess, but may be much larger. Often, the larva stays within the abscess for weeks before escaping to the lumen. Swine of all ages harbor nodular worms, so no effective resistance develops (Hass et al. 1972).

Antemortem diagnosis of oesophagostomosis depends on finding the eggs in fecal flotations. However, this is complicated by the fact that the eggs of Hyastronylus and Globocephalus resemble them. At necropsy, finding of the nodules in the cecum and colon is helpful, but the nodules must be differentiated from those of diverticulitis caused by inflamed lymphoglandular complexes due to chronic inflammatory insults.

RESPIRATORY SYSTEM

Lungworms

Metastrongylus apri, Metastrongylus pudendotectus, and Metastrongylus salmi are worldwide in distribution. The most common is M. apri, but mixed infections with two or three species are usual. The worms occur in the bronchi and bronchioles, usually in the diaphragmatic lobes.

The adults are slender, whitish, and long (40–50 mm). Masses of entwined worms covered with mucus occlude or nearly occlude the peripheral airways. The eggs are thick-shelled, rough-coated, colorless, and embryonated, and they measure 50–60 × 35–40 µm.

The life cycle is indirect. Eggs are coughed up, swallowed, and passed in the feces. Certain earthworms, notably Eisenia spp. and Allolobophora spp., ingest the eggs. Larvae hatch and invade the earthworms’ tissues (calciferous glands, heart, dorsal blood vessel, and crop). Infective larvae are found after about 8–10 days. When swine eat the earthworms, the lungworm larvae migrate to the lungs via the lymphatic system. Patency occurs in 4–5 weeks.

Lungs with metastrongylosis have wedge-shaped areas of emphysema (if the airways are only partially occluded) or atelectasis (if the airways are occluded). These lesions usually occur at the tips and about midway along the length of the diaphragmatic lobes, where major bronchi approach the lobe periphery. The right lung is more affected than the left. Associated with the mass of nematodes in the lumen is hypertrophy of bronchial muscle and hyperplasia of the epithelium, with nodular lymphoid hyperplasia. Surrounding alveoli often show pneumatic changes, which are caused by eggs falling into them instead of being coughed up, as well as agents such as Mycoplasma hyopneumoniae interacting in the compromised lung tissue. Adult male worms may be present alone in tiny bronchioles adjacent to parasitized bronchi. Clinical signs are not pronounced, but heavy infections and infections complicated with bacterial infections cause coughing and “thumping.”

Clinical diagnosis of metastrongylosis is accomplished by finding the characteristic eggs on flotation, but the eggs do not float well. Postmortem diagnosis is accomplished by trimming a 1-cm strip off the edge of the diaphragmatic lobe and squeezing. This will express adults from the bronchi.

Lung Flukes

Paragonimus kellicotti is a fluke that occurs in bronchial cysts of a wide range of hosts, including swine, in North America. Paragonimus westermani is a similar species occurring in southeastern Asia and South America. Paragonimus species are a large, fleshy, brownish fluke that measures 8–12 mm long, 4–6 mm wide, and 3–4 mm thick. They often occur in pairs. The cysts and related inflammatory reaction are about 2 cm in diameter and are easily seen and palpated at necropsy. Usually, there are no clinical signs, because a few cysts do not compromise lung function that much. However, heavy infections produce a productive cough, especially after exercise.

The life cycle includes two intermediate hosts. The first is a snail, and the second is a crayfish (for P. kellicotti) or either crayfish or crab (for P. westermani). When eaten by a swine, the metacercariae in the crustacean excyst, penetrate the intestine, burrow through the diaphragm, and enter the pulmonary parenchyma. They settle in pairs in a bronchus, mature, and begin oviposition in about 60 days.

The lesion in the lungs is pathognomonic at necropsy. Clinically, the presence of the very characteristic eggs is diagnostic. The eggs are relatively heavy, but they float in sugar solution with centrifugation.
The eggs are brown, vase-shaped, large (80–110 × 50–60 µm), with a thickening at the juncture of the operculum and shell proper. The number of eggs appearing at any one time is erratic, because their presence in the feces depends on their being coughed up and swallowed.

Some migrating excysted metacercariae may not reach the lungs, so encysted adults may occur ectopically in lymph nodes, liver, and so on.

**LIVER AND PANCREAS**

Aside from being the site of larval migration (e.g., *Ascaris* and *Stephanurus*), the liver and pancreas are the niches for only a few helminths.

**Liver Flukes (Hepatic Distomosis)**

*Fasciola hepatica* is a cosmopolitan fluke that has a broad host range, including swine. The adult fluke is large (30 × 10 mm), leaf-shaped, with a conical anterior end. The eggs are large (130–150 × 65–90 µm), oval, yellowish brown, operculated, and stuffed with granular cells (vitelline cells).

Eggs passed in the feces develop in water. A ciliated miracidium hatches and penetrates a lymnaeid snail, from which emerge hundreds of cercariae. Each cercaria encysts on grass blades or other surfaces to form the infective metacercaria. Metacercariae remain infective for a long period in a moist state, but die soon after desiccation. Ingested by the final host, the metacercaria penetrates the intestinal wall and the liver capsule, and migrates through the parenchyma for 6 or more weeks. It eventually reaches the biliary duct system and migrates to the larger ducts and the gall bladder. The prepatent period is 10–12 weeks.

Migrating adolescent flukes cause hemorrhagic necrotic tracts in the liver parenchyma. These regress once the flukes have entered the bile ducts. The sojourn in the bile ducts lasts months to years. The characteristic lesion of this stage is called “pipestem liver” because of the marked fibrosis, often with mineralization, of the bile ducts, making the ducts resemble the stems of clay pipes of yesteryear. Adult flukes and brownish exudate will be found if affected bile ducts are incised. Clinical signs associated with fasciolosis are anemia (from liver damage and from hemophagia by the adults) and weight loss/poor gains, but often it is clinically silent. The liver is condemned at slaughter.

A similar fluke, *Fasciola gigantica*, also infects swine in Africa and southern Asia.

**ECHINOCOCCUS (HYDATID DISEASE)**

Adult *Echinococcus* spp. cestodes occur worldwide in carnivores, and their larval cysts (hydatids) occur in various herbivores and omnivores, including humans and swine. The adults are very small (3–6 mm) and typically occur by the hundreds in the definitive host’s intestine. Egg-laden proglottids pass with the feces, contaminating the environment. The eggs are identical to those of other taenids (*Taenia, Multiceps*, etc.). When ingested by a pig, the larva (oncosphere) hatches in the small intestine, penetrates the intestinal wall, and enters the circulation. The liver stops about 70% of the oncospheres, where they nidate and develop into hydatids. By the fifth month, the cyst is 1 cm in diameter, and its wall has differentiated into an outer cuticle and an inner germinal membrane. Fully mature hydatids range from 1 cm to over 7 cm in size. The inner germinal membrane gives rise to many thin-walled brood capsules. Each brood capsule develops a germinal membrane that produces dozens of infective protoscolices. These brood capsules, plus individualized protoscolices, are collectively called “hydatid sand” and settle to the lowest points in the fluid-filled hydatid. The other main site for hydatids to develop is the lungs, but almost any organ is suitable. The end result is that thousands of infective protoscolices develop from each oncosphere. Some hydatids that develop in swine are “sterile”; that is, there are no protoscolices in the cysts.

Hydatid disease is not common in the United States. It occurs in those societies where husbandry methods allow for free-roaming boars and sows. There are no clinical signs that suggest hydatidosis, and diagnosis is done at necropsy.

**URINARY SYSTEM**

**Swine Kidney Worm**

*Stephanurus dentatus* adults occur in perirenal cysts that open by a fistula into the ureters. It is common to find ectopic cysts in other organs, such as the spleen, stomach, urinary bladder, and spinal cord. Renal parenchyma per se is seldom involved. Adult *S. dentatus* are large (20–40 × 2 mm) strongyle nematodes with a thin body wall, so that their internal organs are visible, giving them a mottled harlequin appearance. Their eggs are typical for strongyles and measure 120 × 70 µm. The infection is limited geographically to areas that do not experience severe winters (Smith and Hawkes 1978).

**Life Cycle.** Those eggs that reach the ureters are passed with the urine. The larva that develops inside hatches in 1–2 days and reaches infectivity in about 4–5 days. If the weather is moist and warm, the larvae may survive on pasture for several months. Infective larvae that are ingested or that actively penetrate the skin migrate to the mesenteric lymph nodes, where they molt before moving to the liver via the portal veins. Larvae spend several weeks in the liver, growing to about 5–6 mm, before they leave the liver and migrate to perirenal fat. An inflammatory cyst forms around...
clusters of developing adults, and a fistula opening into the ureter is formed. The prepatent period is long (9–12 months), and eggs pass with the urine for about 3 years. The migrating larvae are aggressive, and many end up in far-flung ectopic sites. Because much of the migration is along vascular channels, some go into the uterine vessels, from which they can infect pigs in utero.

Pathology. Migrating larvae produce hemorrhagic concretes wherever they go. Considerable abscessation, eosinophilia, portal phlebitis and thrombosis, and scarring occur in the liver. These are coarser and more distinct than the “milk spots” associated with Ascaris larvae, which tend to have lacy peripheries and no abscessation. The presence of thrombophlebitis is particularly characteristic. Lesions similar to those in the liver also occur in other tissues where larvae had migrated.

Economic Importance. In areas where stephanurosis occurs, it is a major cause of trimming at slaughter, due to scarring and abscessation. Reduction in growth rate and feed efficiency is another cause of loss (Hale and Marti 1983). Because of the unusually long prepatent period (9–12 months), incorporating a gilt-only breeding system is effective in reducing or eliminating the infection from a premise. After a gilt farrows, she is sent to slaughter before patency develops.

MUSCULOSKELETAL SYSTEM

The body musculature harbors immature stages of only a few helminths. Already mentioned above are the larvae of Trichinella spp. in “nurse cells” and aberrantly migrating larvae of S. dentatus. To be added to this list is the cysticercus of Taenia solium, which lives in the fibrous connective tissue in the muscles.

Cysticercosis (“Pork Measles”)

Taenia solium larvae (cysticercus) occur in the skeletal muscles and myocardium of swine and are known as Cysticercus cellulosae. The mature stage of this cestode occurs in the intestines of humans. The infection in humans was once cosmopolitan, but currently, it is limited by modern hygienic methods to societies that eat raw or undercooked pork. It continues to be a major public health issue in Latin America, for instance.

Life Cycle. Humans infected with adult T. solium shed proglottids in the feces. The egg-laden proglottids release eggs through a rent in the uterus during the sluggish movements by the proglottids. The eggs, which remain viable for many weeks on pasture, are ingested by swine. The oncospheres hatch from the eggs, penetrate the small intestine, and are carried around the body by the circulation. They nidate in skeletal muscles and myocardium and, in 2–3 months, become infective cysticerci. This condition is sometimes called “pork measles” by packers. The term “measle” refers to the spot that is the cysticercus. Cysticerci are subspherical, whitish, fluid-filled cysts about 1 cm in diameter. Visible through the surface is a white, 1–2 mm spot that is the inverted scolex. Cysts remain infective in muscle for up to 2 years, but eventually, they die and undergo caseation and mineralization. Cysticercus cellulosae develop in any muscle in the body, but active muscles including the heart, psoas, masseter, tongue, and those of limbs are most often involved.

Diagnosis. There are no clinical signs associated with cysticercosis. Factors in the history that are strongly associated with cysticercosis are free-roaming pigs fed domestic waste and with routine access to human feces (insufficient latrines). The presence of C. cellulosae is usually uncovered at necropsy/slaughter, where incisions into muscles reveal the cysts. Palpation of the tongue may also reveal deeply seated cysts. Squashing the white spot (scolex) reveals typical taenial hooklets under magnification. These hooklets never disintegrate, so they are demonstrable even in caseated inflammatory debris.

Immunoassay methods are being studied for their value in the antemortem diagnosis of cysticercosis. These tests are currently less accurate in field use, but show promise.

Besides C. cellulosae, the only other cysticercus found in swine is the long-necked bladderworm (Cysticercus tenuicollis, the larva of Taenia hydatigena of dogs). This cyst is easily distinguished from C. cellulosae by its much larger size (8 cm), its location in the body (omentum and mesentery), and the presence of a long, armlike extension (neck) that holds the inverted scolex.

Public Health. Viable C. cellulosae are infective for humans, so meat inspection procedures to search for them are used routinely. If inspection reveals only a few cysts, they may be excised and discarded. Finding several cysts requires that the entire carcass be cooked (170°C for 30 minutes), which kills the cysts, before the pork can be released for consumption. Finding an excessive infection with C. cellulosae is cause for condemnation of the whole carcass. Freezing cuts of pork is another method of killing cysticerci. The temperature and time held at that temperature depends upon the size and thickness of the piece of pork.

Besides acting as the definitive host for T. solium, humans can harbor the cysticerci after ingesting the eggs. In humans, the cysticerci have a predilection for the central nervous system, which makes this a very serious condition.
PREVENTION

Parasite control methods may be broadly categorized as either preventive or therapeutic. Those parasites requiring an intermediate host can be successfully prevented by removing pigs from contact with the intermediate hosts (e.g., dung beetles and earthworms). Therefore, maintaining pigs on concrete will prevent infection with the spiruroids, acanthocephalans, and metastrongyloids. An added benefit would also be the reduction or prevention of infection by other parasites, such as *Hyostrongylus*, *Globocephalus*, and *Trichostrongylus*, that require pasture conditions for transmission.

Good sanitation is a critical element in controlling parasitic infections. The major mode of transmission of internal parasites is through contamination of food, soil, or bedding with feces or urine. Since parasite eggs need moisture and warmth to develop and survive, direct sunlight or dry conditions shorten egg and larval survival. Thorough cleaning of buildings, pens, and equipment with detergent and steam is the best way to control parasite eggs and larvae. Steam penetrates cracks and crevices and kills the tiny eggs and larvae. The common disinfectants used on the farm do not kill cracks and crevices and kills the tiny eggs and larvae. The common disinfectants used on the farm do not kill the eggs of parasites such as ascarids.

Since parasites compete with the host for available nutrients, adequate nutrition aids in reducing the adverse effects on feed efficiency and ADG. It has been shown that increasing the protein and vitamins in the feed affects the performance of parasitized pigs by increasing the ADG and feed efficiency (Stewart et al. 1964).

A management system in which only gilts are used as breeders has been shown to be effective in eradicating kidney worms. This is possible because of the lengthy prepatent period of 6 months or longer and the fact that only animals 2 years old or older pass kidney worm eggs in significant numbers. Selling breeders as soon as first litters are weaned and maintaining boars separately or replacing them with young stock will prevent contamination of the premises. Eradication can be achieved in 2 years or less by using the “gilt-only” system (Stewart et al. 1964) (Table 67.1).

TREATMENT

Therapeutic treatment of pigs with anthelmintics may present only a temporary solution, unless the conditions under which the parasites were acquired are altered. No drug is effective against all parasites, and the tissue damage prior to patency results in slower growth rates and increased nutrient requirements. A good management system will incorporate practices, such as sanitation, genetic selection, and nutrition, to prevent infections and will not use treatment as the sole method of controlling parasites. The choice of anthelmintic is dependent on the parasite species present and the relative cost of the products. Routine surveillance and strategic treatment for parasites is advisable on farms with a previous history of clinical disease. Treatment reduces the number of egg-producing parasites and keeps further premise contamination to a minimum.

The use of chemical agents such as pyrantel tartrate fed for several weeks can be helpful under lot or pasture conditions to control infections and reduce lesions from ascarids and nodular worms. Prophylactic use of pyrantel or repetitive treatments with fenbendazole (FBZ) reduces worm populations and also appears to

| Table 67.1. Gastrointestinal helminths of lesser importance |
|-----------------------------|-----------------------------|
| **Locale**                  | **Description**             | **Comments**                           |
| *Gastrodiscus aegyptius*    | Small and large intestine  | Fleshy paramphistome flukes 5 × 14 mm; |
| *Gastrodiscoides hominis*   | in Africa and southern Asia | operculated eggs 150 × 70 µm            |
| *Fasciolopsis buski*        | Small intestine in         | Large fluke 20–75 mm; operculated     |
| *Gnathostoma spp.*         | southern Asia and India    | eggs 135 × 85 µm                       |
| *Ascarops strongylina*      | In nodules in gastric wall | 20–40 mm long, spinose cuticle; eggs   |
| *Physcocephalus sexalata*  | in Eurasia and Africa      | with one polar plug, 70 × 40 µm, brown |
| *Simondsia paradoxa*       | Mucosal surface of the     | pitted shell                           |
| *Eurytrema pancreaticum*   | stomach                    | 15–20 mm; eggs thick-shelled, transparent, |
| *Clonorchis sinensis*      | Pancreatic ducts           | embryonated, ellipsoidal 30–40 × 15–20 µm|
|                            | Bile ducts                 | Infected humans; infection acquired by |
|                            |                            | ingesting metacercariae on vegetation |
|                            |                            | Infects humans; cycle similar to that of *Fasciola hepatica* |
|                            |                            | Second intermediate host is small vertebrate (reptile, bird, etc.); prepatent period about 3 months |
|                            |                            | Intermediate hosts are coprophagous beetles; prepatent period 4–6 weeks |
|                            |                            | Cycle similar to that of *Ascarops* |
|                            |                            | Cycle similar to that of *Ascarops* |
|                            |                            | Cycle similar to that of *Ascarops* |
|                            |                            | Second intermediate host is grasshopper |
|                            |                            | Second intermediate hosts are freshwater fishes |
stimulate immunity against *A. suum* (Southern et al. 1989; Stankiewicz and Jeska 1990).

Treatment of sows 10–14 days before farrowing with ivermectin has been shown to be effective in preventing transmission of *S. ransomi* from sows to nursing pigs. Such treatment of sows is also beneficial in preventing transmission of *Sarcoptes scabiei*.

**Anthelmintics**

From 1960 to 1996, several new classes of anthelmintic compounds were developed for use in swine and marketed in formulations for administration in water or feed and by injection. The spectrum of activity varies by compound, with some effective against a few target species and others broader in effect.

**Macrocyclic Lactones.** Avermectins are derived from fermentation products of *Streptomyces avermitilis*. The mode of action is to stimulate the release of the inhibitory neurotransmitter gamma-aminobutyric acid (GABA) in target organisms. This inhibits neuromuscular transmission, leading to paralysis and death. The spectrum of activity includes many of the important internal and external parasites; a notable exception may be *T. suis*.

Avermectins are available as injectable formulations (e.g., ivermectin, doramectin) and as a feed additive (e.g., ivermectin). Avermectins generally control internal and external parasites; a notable exception is ivermectin, which results in an inability of the parasite to produce glucose uptake from luminal fluid of the nematode gut, which results in death. The recommended dosage is 275–440 mg/kg body weight.

**Organophosphate Compounds.** Dichlorvos is an organophosphate compound, the first broad-spectrum compound for use in swine, with good efficacy against *Ascaris*, *Oesophagostomum*, *Trichuris*, and *Hyostrongylus* with slightly lower efficacy against *Strongyloides* (Marti et al. 1978). Dichlorvos can be incorporated into polyvinyl chloride pellets that allow for slow release during passage along the intestinal tract. Slow release allows for continued effect in the cecum, producing the desired removal of whipworms. Dichlorvos is administered in the feed, mixed with one-third the regular ration. The recommended dose is 11.2–21.6 mg/kg body weight.

**Piperazine Salts.** An older generation of antiparasitic drugs includes piperazine salts that are still widely used. As purge dewormers, they are efficacious in the removal of adult ascarids and nodular worms. Because larval stages are unaffected, a second treatment is recommended 6–8 weeks later. Piperazine salts are administered in the feed or water and should be consumed in an 8- to 12-hour period; therefore, withholding feed or water the previous night is beneficial. The recommended dosage is 275–440 mg/kg body weight. It is approved for the control of *A. suum* and *Oesophagostomum* spp.

**REFERENCES**

References to publications prior to 1975 cited in the text are not included below but can be found in the 8th edition of *Diseases of Swine* (Straw BE et al., editors).


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VI Noninfectious Diseases

68 Nutrient Deficiencies and Excesses
69 Mycotoxins in Grains and Feeds
70 Toxic Minerals, Chemicals, Plants, and Gases
Proper nutrition is the foundation for sustained economic and environmental viability of any pork-producing operation. Pigs perform best and excrete fewer nutrients when they consume diets that contain the correct amount and balance of essential nutrients. Occasionally, pigs consume diets that contain insufficient or excessive amounts of one or more nutrients. The effects range from mild (slight, unnoticed reductions in weight gain and feed efficiency) to marked (obvious clinical and subclinical signs, including death).

The relationship between a nutrient deficiency and excess varies among nutrients (Figure 68.1). The absolute difference (the range of tolerance) between the nutrient intake associated with overcoming a deficiency (i.e., requirement) and the intake associated with a toxicity may be large (e.g., water-soluble vitamins) or small (e.g., selenium). This observation is critical inasmuch as it represents the window of safety for a particular nutrient.

This chapter presents clinical signs (outwardly apparent) and subclinical signs (evident only by necropsy or clinical methods) of nutrient deficiencies and excesses that have occurred in pigs. Although it is rare today to observe signs of a single nutrient deficiency or excess, the recognition of inadequate nutrition should be part of an overall approach to solving problems in swine operations.

The information presented herein was derived from research results with pigs and, in most instances, obtained by varying the intake of one nutrient at a time. However, nutrient interactions occur, and it is unclear if the information would apply when multiple deficiencies or excesses of nutrients exist (e.g., when a vitamin and trace mineral mix is not added to the diet properly).

On farms today, vitamin and mineral deficiencies are less likely to occur than excesses. This is because diets are routinely fortified with vitamins and minerals. Also, it is important to consider that many of the nutrient deficiency signs reported here were observed in pigs fed purified or semipurified diets. This was necessary in order to provide diets that contained a very low concentration of the nutrient of interest so deficiency signs could be observed. Ingredients used in practical swine diets contain a variety of essential nutrients (NRC 1998) that, even without recommended nutrient fortifications, would prevent the appearance of some signs.

CAUSES OF NUTRIENT DEFICIENCIES

Reduced Feed Intake
Pigs should consume a specific quantity of essential nutrients daily for optimum performance. Because diets are often formulated on a concentration basis, lower-than-expected feed intake by pigs could result in a nutrient deficiency. This situation is most commonly observed in gestating sows and breeding boars when feed intake is restricted to control weight gain, and in lactating sows, because they may consume less feed than needed for optimum performance.

Low Nutrient Bioavailability in Ingredients
Nutrients present in ingredients are not fully available to the pig for maintenance, growth, and/or lactation. The portion of the nutrient in the ingredient that is absorbed in a form utilisable by the pig is said to be bioavailable. The amount of that nutrient that is
bioavailable depends primarily on the nutrient source. For example, the phosphorus in monocalcium phosphate is more bioavailable than the phosphorus in grain and plant protein supplements. In these ingredients, the phosphorus is bound in a phytate complex and is not fully released during digestion. Thus, phosphorus deficiency could occur if bioavailability is not considered in diet formulation. However, some feed ingredients contain nutrients that have a low bioavailability but that still release sufficient amounts of nutrients to meet the animal's requirement. It should be noted that estimates of bioavailability are relative to a reference ingredient or ingredients; therefore, one should closely examine the nature of the reference ingredients.

**Variability in Nutrient Content of Ingredients**

Deviations in the nutrient content of ingredients from expected or “book” values can result in nutrient deficiencies if not taken into account in diet formulations. For example, samples of corn from 15 states in the United States contained between 0.24% and 0.31% lysine; for soybean meal, the range was from 2.7% to 3.0% (NCR-42 Committee on Swine Nutrition 1992). Greater variability has been reported for vitamins, and this has been attributed to agronomic, harvest, storage, and processing conditions (Roche 1991).

**Diet Formulation and Preparation Errors**

There are a number of possible mistakes in diet formulation and preparation that can result in nutrient deficiencies. These include using the incorrect quantity or the wrong ingredients in the diet and not cleaning the mixer properly between batches. In addition, nutrient deficiencies can occur when commercial feed products that are not designed to complement each other are used to prepare the diet. Haphazard additions of an extra package of minerals to a diet already adequately fortified may cause certain minerals to interfere with the utilization of others, resulting in deficiencies. Furthermore, according to Traylor et al. (1994), cutting short the time the feed mixer runs can cause significant variation in the nutrient content of the final diet and reduce pig performance.

**Variation in Nutrient Requirements of Pigs**

Pigs have varying potentials for lean growth and reproduction and therefore have different nutrient requirements. For example, a given level of dietary lysine may be seemingly adequate for diseased, low-lean-gain pigs, but deficient for high-health, high-lean-gain pigs (Williams et al. 1997). Stahly et al. (1991) also demonstrated that increasing the dietary lysine density improved performance to a greater extent as the lean-growth capacity of different genotypes increased, indicating a genotype by lysine density interaction. Moreover, within a contemporary group of pigs, some may show deficiency signs while others do not (Cunha 1977), indicating the need to carefully observe individual pigs for signs of nutrient deficiencies.

**Nutrient Excesses**

All essential nutrients must be digested and utilized by the pig to avoid deficiencies. To facilitate this process, it is important to maintain a proper balance of nutrients in the diet. There are common absorption sites for many nutrients in the small intestine. Thus, a high dietary concentration of one nutrient can impair the passage of another nutrient via competition through the absorption sites and cause a deficiency. In addition, an excess of one nutrient can cause the formation of certain chemical complexes that are poorly absorbed. The more frequent nutrient interactions that can cause problems in practical situations are between calcium and phosphorus; calcium and zinc; and copper, iron, and zinc.

A wide calcium-to-phosphorus ratio will decrease the absorption of phosphorus, especially when phosphorus is fed near the requirement (NRC 1998) and reduce the effectiveness of exogenous phytase (Liu et al. 1998). To maximize the success with phytase and minimize problems associated with phosphorus deficiency (e.g., rickets), a calcium-to-phosphorus ratio of 1:1 is recommended (Patience and DeRouchey 2010).

**SYMPTOMS OF NUTRIENT DEFICIENCIES**

Clinical and subclinical deficiency signs for several nutrients are presented in Tables 68.1–68.3. There is variation in the amount of time that elapses before signs of nutrient deficiency begin to appear. For example, it takes 4–6 months for pigs fed a vitamin-D-deficient diet to develop signs of a deficiency (NRC 1998), whereas salt deficiency will be evident in a few days (Patience and Zijlstra 2001).
<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Clinical Signs</th>
<th>Subclinical Signs</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin A</td>
<td>Incoordination; lordosis; paralysis of the rear limbs; night blindness; congenital defects; reduced weight gain; respiratory dysfunction; roughness of the skin; tilting of the head; eye discharge; stillborn pigs; aborted fetuses with cleft palate, harelip, and deformed hind legs; impaired spermatogenesis; increased embryonic mortality</td>
<td>Retarded bone growth; increased cerebrospinal fluid pressure; degeneration of sciatic and femoral nerves; minimal visual purple; atrophy of epithelial layers of the genital tract; decreased plasma vitamin A</td>
<td>NRC (1979, 1998); Ullrey (1991); Darroch (2001)</td>
</tr>
<tr>
<td>Vitamin D</td>
<td>Rickets; osteomalacia; tetany; reduced weight gain; stiffness and lameness; posterior paralysis</td>
<td>Lack of bone calcification and proliferation of epiphyseal cartilage; rib and vertebra fracture; low plasma calcium, magnesium, and inorganic phosphorus; elevated serum alkaline phosphatase</td>
<td>NRC (1979); Cowka (1977); Crenshaw (2001)</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>Lactation failure; reduced litter size; extended parturition time; weak pigs at birth; sudden death (fast-growing pigs); postweaning diarrhea</td>
<td>Liver necrosis (hepatosis dietetica); cardiac muscle degeneration (mulberry heart); increased fluid in pericardial sac; gastric ulcers; anemia; yellow discoloration of fat tissue; skeletal degeneration; increased serum glutamic oxaloacetic transaminase and glutamic pyruvic transaminase; reduced prothrombin time, serum vitamin E, and immune response; testicular degeneration</td>
<td>NRC (1998); Mahan (1991, 2001)</td>
</tr>
<tr>
<td>Vitamin K (menadione)</td>
<td>Pale newborn pigs with loss of blood from the umbilical cord; massive subcutaneous hemorrhage; hematomas in ears; enlarged, blood-filled joints; sudden death following dicoumarin intake; blood in urine</td>
<td>Increased prothrombin and blood-clotting time; internal hemorrhage; anemia due to blood loss</td>
<td>NRC (1979, 1998); Fritschen et al. (1971); Crenshaw (2001)</td>
</tr>
<tr>
<td>Biotin</td>
<td>Excessive hair loss, skin ulcerations, and dermatitis; exudate around the eyes; inflammation of the mucous membranes of the mouth; transverse cracking of the hooves; cracking and bleeding of footpads; spasticity of the hind legs; diarrhea; reduced litter size</td>
<td>Reduced serum biotin</td>
<td>NRC (1998); Dove and Cook (2001)</td>
</tr>
<tr>
<td>Choline</td>
<td>Reduced weight gain, litter size, and farrowing rate; rough hair coat; unbalanced and staggering gait</td>
<td>Fatty infiltration of the liver and kidneys; reduced red blood cell count, hematocrit, and hemoglobin; increased plasma alkaline phosphatase</td>
<td>NRC (1998)</td>
</tr>
<tr>
<td>Folic acid</td>
<td>Reduced weight gain and litter size; fading hair color</td>
<td>Normocytic and macrocytic anemia; leukopenial thrombopenia; reduced hematocrit; bone marrow hyperplasia</td>
<td>NRC (1998); Dove and Cook (2001)</td>
</tr>
<tr>
<td>Niacin</td>
<td>Anorexia; reduced weight gain; rough hair coat; hair loss; severe diarrhea; dermatitis; vomiting</td>
<td>Buccal mucosa ulcerations, ulcerative gastritis; inflammation and necrosis of the cecum and colon; normocytic anemia</td>
<td>NRC (1998)</td>
</tr>
<tr>
<td>Pantothenic acid</td>
<td>Anorexia; reduced weight gain; dry skin; rough hair coat; hair loss; unusual gait (goose stepping); impaired sow reproductivity</td>
<td>Edema and necrosis of the intestinal mucosa; increased connective tissue invasion of the submucosa; loss of nerve myelin; degeneration of dorsal root ganglion, fatty liver; enlarged adrenal glands; atrophic ovaries; infantile uteri; reduced immune response; intramuscular hemorrhage</td>
<td>NRC (1998); Dove and Cook (2001)</td>
</tr>
<tr>
<td>Riboflavin</td>
<td>Reduced weight gain; cataracts; seborrhea; stiffness of gait; vomiting; hair loss; reduced farrowing rate; anestrous; higher piglet mortality</td>
<td>Increased blood neutrophil granulocytes; discolored liver and kidney tissue; fatty liver; collapsed follicles; degenerating ova; degenerating myelin of the sciatic and brachial nerves; elevated blood erythrocyte glutathione reductase activity coefficient; reduced immune response</td>
<td>NRC (1998); Dove and Cook (2001)</td>
</tr>
<tr>
<td>Vitamin B₃ (thiamine)</td>
<td>Anorexia; reduced weight gain; occasional vomiting; sudden death</td>
<td>Cardiac hypertrophy; bradycardia; increased plasma pyruvate; reduced body temperature; myocardial degeneration; flabby heart</td>
<td>NRC (1998); Dove and Cook (2001)</td>
</tr>
</tbody>
</table>

(Continued)
### Table 68.2. Signs of mineral deficiencies in swine

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Clinical Signs</th>
<th>Subclinical Signs</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Calcium</strong></td>
<td>Anorexia; reduced weight gain; convulsions; exudate development around the eyes; ataxia; vomiting; coma; death</td>
<td>Reduced bone breaking strength; low plasma calcium level; elevated serum phosphorus and alkaline phosphatase; reduced bone mineralization</td>
<td>NRC (1979); Peo (1991); Crenshaw (2001)</td>
</tr>
<tr>
<td><strong>Chromium</strong></td>
<td>None documented</td>
<td>None documented</td>
<td>Hill and Spears (2001)</td>
</tr>
<tr>
<td><strong>Copper</strong></td>
<td>Anorexia; reduced weight gain; bowing of legs; spontaneous fractures; ataxia</td>
<td>Microcytic, hypochromic anemia; reduced serum ceruloplasmin; reduced erythrocyte life span; aortic rupture; cardiac hypertrophy</td>
<td>Miller (1991); NRC (1998); Hill and Spears (2001)</td>
</tr>
<tr>
<td><strong>Iron</strong></td>
<td>Reduced feed intake, weight gain, and feed efficiency; rough hair coat; pallor; wrinkled skin; labored breathing; death</td>
<td>Hypochromic, microcytic anemia; enlarged heart and spleen; enlarged fatty liver; ascites; reduced serum iron, percent transferrin saturation, and hemoglobin (&lt;7g/100mL); thin, watery blood; reduced disease resistance</td>
<td>NRC (1979, 1998); Miller (1991); Hill and Spears (2001)</td>
</tr>
<tr>
<td><strong>Magnesium</strong></td>
<td>Hyperirritability; muscular twitching; reluctance to stand; weak pasterns; loss of equilibrium; tetany; death</td>
<td>Low serum magnesium and calcium; reduced bone magnesium</td>
<td>NRC (1979, 1998)</td>
</tr>
<tr>
<td><strong>Manganese</strong></td>
<td>Lameness; enlarged hock joints with crooked and shortened legs; increased fat deposition; resorbed fetuses; small, weak pigs born; reduced milk production; irregular or absent estrous cycles; delayed postweaning estrus</td>
<td>Replacement of cancellous bone with fibrous tissue; early closure of distal epiphyseal plate; low serum manganese and alkaline phosphatase</td>
<td>NRC (1979, 1998); Hill and Spears (2001)</td>
</tr>
<tr>
<td><strong>Phosphorus</strong></td>
<td>Reduced weight gain and feed efficiency; rickets; osteomalacia; spontaneous fractures; posterior paralysis (downer sow syndrome)</td>
<td>Reduced bone breaking strength and mineralization; enlarged costochondral junction (beading); reduced serum inorganic phosphorus</td>
<td>NRC (1979); Koch and Mahan (1986); Hall et al. (1991); Crenshaw (2001)</td>
</tr>
<tr>
<td><strong>Potassium</strong></td>
<td>Anorexia; reduced weight gain; rough hair coat; emaciation; ataxia; inactivity</td>
<td>Reduced heart rate; increased PR, QRS, and QT intervals on electrocardiogram; multifocal myocardial necrosis</td>
<td>Van Vleet and Ferrans (1986); NRC (1998); Patience and Zijlstra (2001)</td>
</tr>
<tr>
<td><strong>Selenium</strong></td>
<td>Sudden death; reduced milk production; prolonged farrowing time; weak progeny; postweaning diarrhea; lower sperm production and motility; sperm tail abnormalities</td>
<td>Liver necrosis (hepatosis dietetica); cardiac muscle degeneration (mulberry heart); increased fluid in pericardial sac; gastric ulcers; skeletal degeneration; increased serum glutamic oxaloacetic transaminase and glutamic pyruvic transaminase; reduced prothrombin time and immune response; reduced serum and skeletal muscle selenium; reduced glutathione peroxidase activity</td>
<td>Ullrey (1987); NRC (1998); Mahan (2001)</td>
</tr>
</tbody>
</table>

*Synthesized from d-glucose and related compounds in pigs. No deficiency signs have been observed; however, reports of improved pig performance from vitamin C additions to practical diets exist.*
Table 68.2. (Continued)

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Clinical Signs</th>
<th>Subclinical Signs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium and chloride</td>
<td>Reduced feed intake, weight gain, and feed efficiency; low water intake; unthriftiness; reduced pig birth weight and litter size; extended weaning-to-estrus interval; increased attraction to blood and possibly tail biting</td>
<td>Reduced plasma sodium and chloride (sodium deficiency); elevated plasma potassium (sodium deficiency); elevated plasma urea nitrogen (sodium and chloride deficiency); elevated plasma total protein and albumin (sodium deficiency)</td>
</tr>
<tr>
<td>Zinc</td>
<td>Anorexia; reduced weight gain and feed efficiency; parakeratosis; extended parturition time; increased stillbirth rate; reduced litter size and pig birth weight; alopecia; poor wound healing</td>
<td>Reduced serum, tissue, and milk zinc; reduced serum albumin and alkaline phosphatase; reduced thymus weight; retarded testicle development; deleted fat depots; serous atrophy of fat; depletion of thymocytes; keratinization of the tongue and esophagus; cardia of the stomach; reduced immune response</td>
</tr>
</tbody>
</table>

*Supplemental chromium (from chromium tripicolinate, chromium propionate, or chromium-L-methionine) has improved growth performance, muscling, and litter size (Southern and Payne 2003).

Table 68.3. Deficiency signs for other nutrients and dietary components in swine

<table>
<thead>
<tr>
<th>Item</th>
<th>Clinical Signs</th>
<th>Subclinical Signs</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy</td>
<td>Weakness; low body temperature; reduced weight gain and loss of body weight; impaired sow reproductivity; coma; death</td>
<td>Hypoglycemia; loss of subcutaneous fat; elevated hematocrit and serum cholesterol; reduced blood glucose, calcium, and sodium</td>
<td>NRC (1979); Pettigrew and Tokach (1991)</td>
</tr>
<tr>
<td>Fat (linoleic acid)</td>
<td>Dermatitis; hair loss; necrosis of the skin</td>
<td>Small gallbladder; elevated triene/tetraene in tissue lipids</td>
<td>NRC (1979); ARC (1981)</td>
</tr>
<tr>
<td>Protein/ amino acids</td>
<td>Reduced feed intake, weight gain, and feed efficiency; unthriftiness; impaired sow and boar reproductivity; increased carcass back fat, feed wastage, and attraction to blood (possibly tail biting)</td>
<td>Kwashiorkor-like signs in baby pigs, including reduced serum protein and albumin, anemia, gross edema, and increased liver lipids; increased plasma urea; reduced resistance to bacterial infection</td>
<td>NRC (1979, 1998); Baker and Speer (1983); Pettigrew and Tokach (1991); Fraser et al. (1991); Lewis (1992)</td>
</tr>
<tr>
<td>Water</td>
<td>Reduced feed intake and weight gain; dehydration; possible salt poisoning; increased respiration; diarrhea in piglets; death</td>
<td>Elevated hematocrit and plasma electrolytes; loss of temperature regulation; tissue dehydration; crystalluria; proteinuria; bacteriuria; cystitis</td>
<td>NRC (1979); Madec et al. (1986); Thacker (2001)</td>
</tr>
</tbody>
</table>

Nutrient requirements for swine are available from the NRC (1998). These can serve as a guide in formulating swine diets to minimize the occurrence of nutrient deficiencies. Because several factors are known to affect nutrient requirements (NRC 1998), it is prudent to add a margin of safety to these requirements when formulating practical swine diets to ensure optimum animal performance (Reese et al. 2010). Nutrient recommendations that contain margins of safety are available from various sources.

CAUSES OF NUTRIENT EXCESSES

Excessive Feed Intake
Sometimes gestating females and breeding boars are given more feed than they need for optimum performance. Consequently, they consume too much energy and get excessively heavy and fat, which reduces reproductive performance and longevity.

Diet Preparation Errors
The same mistakes in diet preparation that can cause nutrient deficiencies can also cause nutrient excesses.

Poor Water Quality
Water may be a medium for consumption of excess minerals and undesirable minerals (NRC 2005). Few studies have been conducted to investigate nutrient excesses caused by consuming poor-quality water. However, it appears that pigs can tolerate water containing up to 5000 ppm total dissolved solids although mild temporary diarrhea and some refusal may be
observed in swine not accustomed to it. Water containing more than 7000 ppm total dissolved solids should be deemed unsafe for swine (Carson 2006).

Contaminated Mineral Supplements

Mineral supplements, such as monocalcium phosphate and defluorinated rock phosphate, are added to swine diets to correct the deficiencies in diets formulated with grains and protein supplements. Often, these mineral supplements contain elements other than those of primary interest. For example, defluorinated rock phosphate contains 3.27% sodium and 0.84% iron (NRC 1998). In addition, some phosphorus sources may contain high concentrations of aluminum and fluoride, and some sources of calcium contain large amounts of magnesium and iron. These “extra” elements will not normally pose a problem to the pig if their contribution to the diet is accounted for during formulation. The type and amount of the “other” elements the mineral supplement contains depend on the raw material from which the supplement was made and the type of processing (NRC 1980).

SYMPTOMS OF NUTRIENT EXCESSES AND TOLERANCE LEVELS

Symptoms of excessive nutrient intake and estimated tolerance levels for several nutrients are presented in Tables 68.4–68.6. Because many minerals interact with each other during the digestive and utilization processes, a high intake of one mineral (e.g., calcium) can result in a deficiency of another mineral (e.g., phosphorus or zinc). To solve this kind of problem, either reduce the dietary concentration of calcium, for example, or increase the concentration of phosphorus and/or zinc in the diet.

Copper (at 250 ppm as copper sulfate) and zinc (at 3000 ppm as zinc oxide) are unique in that they promote additional growth in young growing pigs at dietary concentrations that far exceed the nutritional requirement for these nutrients (Hill and Spears 2001). However, like other minerals, too much copper or zinc in the diet will produce deleterious effects (see Table 68.5).

According to Lewis (2001), the deleterious effects of consuming incorrect amounts of amino acids have been classified into at least two main types: toxicity and imbalance. Toxicities result from the consumption of a large excess of an individual amino acid. Imbalances are also caused by excessive intake(s) of (an) amino(s), but in this case, the excess exacerbates a deficiency of the most limiting amino acid in the diet. An imbalance is corrected by the appropriate addition of that amino acid. Under practical conditions, toxicities and imbalances would be caused only by mistakes in formulation or manufacturing of a diet with crystalline amino acids. The only amino acids currently available in feed-grade form are lysine, tryptophan, threonine, and methionine.

With the exception of the study by Wahlstrom and Libal (1974) where supplemental lysine and methionine were evaluated, the effects of excess concentrations of amino acids reported in Table 68.6 were observed when the amino acid of interest was added to a basal diet considered to be adequate in all essential amino acids. In other words, a constant level of soybean meal was maintained in the basal and amino-acid-supplemented diets.

Wahlstrom and Libal (1974) added dl-methionine (0.2%) to a diet that contained less soybean meal than the basal diet. They observed a reduction in pig performance at a lower level of added methionine than other researchers have reported because they exacerbated a lysine deficiency (i.e., caused an amino acid imbalance). Nevertheless, according to Baker (1977), pigs appear to be particularly sensitive to excess methionine in the diet. It is important to recognize that there is probably a greater tolerance level of feed-grade forms of amino acids when they are added to a diet, which contains an adequate quantity of the 10 essential amino acids than when they are added individually to a low-protein diet.

For some nutrients, no documentation was found to indicate a tolerance level, because no adverse effects from an excessive intake have been reported in swine. Thus, when “none documented” appears in the tables, it should not be assumed that the nutrient is completely safe for swine. There are reports of magnesium toxicity in other species (NRC 2005), as well as for niacin, vitamin K, vitamin E, and pyridoxine (NRC 1987). In general, the vitamins that have not been observed to cause adverse reactions in swine are relatively safe at dietary concentrations much greater than the requirement. This is especially true for the B vitamins (e.g., riboflavin, folic acid), which are not extensively stored in the body and excesses are readily excreted in the urine.

Estimated tolerance for a nutrient is defined as the dietary concentration that, when fed for a defined period of time, will not impair pig health and performance (NRC 2005). Although the tolerance level will vary with the age and physiological condition of the animal (NRC 2005), only one tolerance level is given in the tables for each nutrient, except where information was deemed sufficient to give more. The tolerance values listed may not represent the actual tolerance levels for production situations. Many of the experiments on which the tolerance levels were based were conducted for a limited time period using nutrient sources that may have different bioavailabilities from those used in practical swine diets. “Not determined” in the tables indicates insufficient data were available to suggest a tolerance level.
Feed Intake

Many feed-related problems on farms occur because pigs do not consume enough feed. For example, access to feed by lactating sows and growing pigs is too often restricted. Feed access is often restricted because of human error, feed bridging in the bins or feeders, or equipment failure. Other reasons for inadequate feed intake are water quantity or quality problems, overcrowding, and poor feeder space and design. Eliminate

INVESTIGATION OF A POSSIBLE FEED-RELATED DISORDER

Good production records combined with close, daily observation of animals are important in identifying problems caused by inadequate nutrition. Monitor gain, feed intake, and feed efficiency, because they are typically impacted by many feed-related disorders. After it is apparent that growth performance is impaired or several animals appear abnormal, it is important to consider what aspects of feeding and nutrition could be a problem. Generally, feed-related disorders are caused by inadequate feed intake and/or impaired feed quality.

Nutrient Deficiencies and Excesses

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<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Clinical Signs</th>
<th>Subclinical Signs</th>
<th>Estimated Dietary Tolerance Level</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium</td>
<td>Reduced feed intake, weight gain, and feed efficiency; parakeratosis&lt;sup&gt;g&lt;/sup&gt;</td>
<td>Elevated plasma calcium; increased prothrombin clotting time&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.0%</td>
<td>NRC (1980, 2005); Foley et al. (1990); Hall et al. (1991); Crenshaw (2001)</td>
</tr>
<tr>
<td>Chromium</td>
<td>Anorexia; diarrhea; depression; inactivity; labored breathing; tremors&lt;sup&gt;3&lt;/sup&gt;</td>
<td>None documented</td>
<td>3000 ppm (oxide)</td>
<td>NRC (2005); Vishnyakov et al. (1985); Hill and Spears (2001)</td>
</tr>
<tr>
<td>Cobalt&lt;sup&gt;3&lt;/sup&gt;</td>
<td>Anorexia; reduced weight gain; stiff-legged; humped back; incoordination; muscle tremors</td>
<td>Anemia</td>
<td>100 ppm</td>
<td>NRC (1980, 1998, 2005); Hill and Spears (2001)</td>
</tr>
<tr>
<td>Copper</td>
<td>Anorexia; reduced weight gain; bloody feces; jaundice; death</td>
<td>Anemia; yellow appearance to liver; internal hemorrhage; ulceration of esophageal zone of stomach; pulmonary edema; elevated liver and kidney copper; reduced hemoglobin</td>
<td>250 ppm&lt;sup&gt;i&lt;/sup&gt;</td>
<td>NRC (1980, 1998, 2005); Cromwell et al. (1983); Miller (1991); Hill and Spears (2001)</td>
</tr>
<tr>
<td>Iodine</td>
<td>Reduced feed intake and weight gain</td>
<td>Decreased blood hemoglobin and liver iron</td>
<td>400 ppm</td>
<td>NRC (1980, 2005); Miller (1991); Hill and Spears (2001)</td>
</tr>
<tr>
<td>Iron</td>
<td>Reduced feed intake, weight gain, and feed efficiency; diarrhea; incoordination; shivering; tetanic convulsions; labored breathing; coma; dyspnea; drowsiness; death; rickets&lt;sup&gt;4&lt;/sup&gt;</td>
<td>Edema of stomach wall; hyperemia; extensive mucosal necrosis; pallor of skeletal muscles; swollen kidneys; epicardial hemorrhage; hypopericardium; hydrothorax; severe degeneration of the muscle; nephrosis; necrosis of the liver</td>
<td>3000 ppm&lt;sup&gt;b&lt;/sup&gt;</td>
<td>NRC (1980, 1998, 2005); Miller (1991)</td>
</tr>
<tr>
<td>Magnesium</td>
<td>None documented</td>
<td>None documented</td>
<td>0.24%</td>
<td>NRC (1998, 2005)</td>
</tr>
<tr>
<td>Manganese</td>
<td>Reduced feed intake and weight gain; stiffness</td>
<td>Reduced hemoglobin</td>
<td>1000 ppm</td>
<td>NRC (1980, 1998, 2005)</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>Reduced weight gain and feed efficiency</td>
<td>Urinary calculi; osteodystrophia fibrosa; metastatic calcification in soft tissue</td>
<td>1.0%i</td>
<td>NRC (2005); Hall et al. (1991); Crenshaw (2001)</td>
</tr>
<tr>
<td>Potassium</td>
<td>Reduced weight gain and feed intake</td>
<td>Abnormal electrocardiogram</td>
<td>1%</td>
<td>NRC (2005); Patience and Zijlstra (2001)</td>
</tr>
<tr>
<td>Selenium&lt;sup&gt;1&lt;/sup&gt;</td>
<td>Anorexia; reduced feed intake and weight gain; hair loss; separation of hoof and skin at the coronary band; reduced conception rate and litter size; pigs small, weak, or dead at birth; labored breathing; vomiting; prostration; frothing at the mouth; abnormal staggering movement; muscle twitching; squeal when approached; spinal paralysis; death</td>
<td>Degenerative changes in the liver and kidneys; pulmonary edema; elevated serum selenium and glutamic oxaloacetic transaminase; high liver selenium; fatty infiltration of the liver</td>
<td>4 ppm</td>
<td>Mahan (1991, 2001); NRC (1998, 2005); Kim and Mahan (2001)</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>Anorexia; weight loss; edema; nervousness; weakness; staggering; diarrhea; epileptic seizures; paralysis; death</td>
<td></td>
<td>3%&lt;sup&gt;b&lt;/sup&gt;</td>
<td>NRC (1998, 2005); Pretzer (2000); Patience and Zijlstra (2001)</td>
</tr>
<tr>
<td>Sulfur</td>
<td>Reduced feed intake; possible osmotic diarrhea</td>
<td></td>
<td>0.4%</td>
<td>NRC (2005)</td>
</tr>
</tbody>
</table>
**Table 68.5. (Continued)**

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Clinical Signs</th>
<th>Subclinical Signs</th>
<th>Estimated Dietary Tolerance Levela</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zinc</td>
<td>Reduced weight gain, feed intake, feed efficiency, litter size, and pig weight at weaning; arthritis; lameness; depression</td>
<td>Hemorrhage in axillary spaces; gastritis; osteochondrosis in sows; increased liver zinc; decreased liver iron and copper</td>
<td>3000 ppm (weanling pigs)</td>
<td>Poulsen (1995); NRC (1998, 2005); Hill and Spears (2001)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1000 ppm (carbonate)</td>
<td></td>
</tr>
</tbody>
</table>

*aWhen higher dietary levels were provided, certain clinical and subclinical signs were observed under experimental conditions. These levels may not represent actual tolerance levels in production situations, and they generally apply to growing pigs allowed ad libitum access to feed.

*bWith limited dietary zinc.

cIn the absence of supplemental vitamin K in the diet.

dTrivalent chromium given via stomach tube (3 g/kg body weight) to 60-day-old pigs.

eSelenium, vitamin E, and cysteine have provided some protection against excessive levels of cobalt.

Two hundred fifty parts per million has resulted in signs of excess when fed throughout the starter, growing, and finishing phases and when dietary iron, zinc, and sulfur intake was limited. Nursery pigs can tolerate diets with 500 ppm as copper sulfate for 28 days.

*Increasing dietary phosphorus has alleviated the rickets.

bAs iron dextran administrated intramuscularly (IM) to pigs born from vitamin-E-deficient dams.

*The amount of calcium in the diet is important. A deficiency of calcium may lower the tolerance level.

*Chronic selenosis can be treated by supplementing the diet with 40 ppm arsenic or 50–100 ppm arsenilic acid (Osweiler et al. 1985).

*Assumes ample water supplies are available. Water restriction will lower the tolerance level.

lAs zinc oxide for a maximum of 35 days.

---

**Table 68.6. Signs of excess and estimated tolerance level for other nutrients and dietary components in swine**

<table>
<thead>
<tr>
<th>Item</th>
<th>Clinical Signs</th>
<th>Subclinical Signs</th>
<th>Estimated Dietary Tolerance Levela</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy</td>
<td>Reduced sow feed intake in lactationb; increased carcass back fat; reduced embryo survivalc</td>
<td>Impaired development of mammary secretory tissue; elevated plasma nonesterified fatty acid level; reduced plasma insulin</td>
<td>Variable</td>
<td>Kirkwood and Thacker (1988); Weldon et al. (1991, 1994a,b); Australian Agricultural Council (1987)</td>
</tr>
<tr>
<td>Fat</td>
<td>Increased carcass back fat</td>
<td>Soft carcass fatd</td>
<td>Not determined</td>
<td>Wood et al. (1994); Azain (2001)</td>
</tr>
<tr>
<td>Protein</td>
<td>Reduced weight gain, feed efficiency, and carcass back fat; mild diarrhea</td>
<td>Increased plasma urea</td>
<td>Not determined</td>
<td>Hansen and Lewis (1993); Dewey (1993); Chen et al. (1995); NRC (1998)</td>
</tr>
<tr>
<td>Lysine</td>
<td>Reduced weight gain and feed efficiencye</td>
<td>None documented</td>
<td>Not determinedf</td>
<td>Wahlstrom and Libal (1974); Edmonds et al. (1987); Goodband et al. (1989); Campbell and Combs (1990b)</td>
</tr>
<tr>
<td>Methionine</td>
<td>Reduced weight gain, feed intake, and feed efficiency</td>
<td>None documented</td>
<td>Variableg</td>
<td>Wahlstrom and Libal (1974); Edmonds et al. (1987); Edmonds and Baker (1987); Campbell and Combs (1990c); Van Heugten et al. (1994)</td>
</tr>
<tr>
<td>Threonine</td>
<td>Reduced feed intake and weight gain</td>
<td>None documented</td>
<td>1%</td>
<td>Edmonds et al. (1987); Edmonds and Baker (1987)</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>Reduced feed intake, weight gain, and feed efficiency; diarrhea</td>
<td>None documented</td>
<td>1% (100-kg pigs) 2% (10-kg pigs)</td>
<td>Edmonds et al. (1987); Edmonds and Baker (1987); Chung et al. (1991)</td>
</tr>
</tbody>
</table>

*aWhen higher dietary levels were provided, certain clinical and subclinical signs were observed under experimental conditions. These levels may not represent actual tolerance levels in production situations, and they generally apply to growing pigs allowed ad libitum access to feed. Variable indicates tolerance levels are generally known, but they are too situation-dependent to describe here.

*bResult of excessive energy intake during gestation.

*cResult of excessive energy intake during rearing, the estrous cycle, and early pregnancy.

*dOccurs when high iodine number (highly unsaturated) fat(s) is present in the diet.

*eWhen provided as L-lysine acetate (4% of the diet) to 28-day-old pigs for 16 days.

*fL-lysine ≅ HCL (0.7% of the diet) provided to 31-kg pigs for 85 days did not affect performance. In addition, L-lysine ≅ HCL (1.03% of the diet) provided to 61-kg pigs for 50 days did not affect performance.

*gTolerance level appears to be impacted by pig age and dietary amino acid concentration and ingredient composition. The tolerance level is less than 0.2% added dl-methionine in finishing pigs fed a lysine-deficient diet. However, no ill effects were observed in nursery pigs fed diets containing 1% or 1.08% dl-methionine.
the possibility that inadequate feed intake may be causing suspected feed-related disorders before investigating feed quality.

**Feed Quality**

If a feed-related disorder cannot be explained by inadequate feed intake, look for possible feed quality problems. Observe for evidence of foreign contamination such as dirt, stones, rodent droppings, and other indicators such as color and odor. A significant change in color may indicate a change in ingredients (which is not necessarily a problem) or it may suggest improper processing such as overheating. Beware of moldy or mycotoxin-contaminated feed. Also, feed quality may be impaired because it contains too little or too much of one or more nutrients.

Nutrient deficiencies and excesses are seldom severe enough on farms to cause clinical or subclinical signs in pigs; however, some have occurred under practical conditions. Selenium, vitamin E, amino acid, biotin (in sows), zinc, phosphorus, and salt deficiencies have been observed. Problems with excess amounts of selenium, vitamin A, vitamin D, copper, and zinc have been observed.

To facilitate the identification of the nutrient(s) to focus on when troubleshooting suspected feed-related problems, use Table 68.7 as an initial screen. In this table, the clinical signs presented in Tables 68.1–68.6 are arranged in alphabetical order. Locate the clinical sign observed and determine the nutrient(s) that may be involved. For example, if pig feed intake is impaired, find “feed intake, reduced” in Table 68.7 for a list of nutrients that may be involved in decreased feed intake. If additional clinical signs are observed, use them to help narrow the list to fewer nutrients. Subsequently refer to the subclinical signs in Tables 68.1–68.6 to help make a more definite diagnosis. It is important to remember that some of the clinical signs in Table 68.7 may be caused by factors other than faulty nutrition (i.e., environment and infectious disease). Finally, collect a sample of the feed and analyze it for the nutrient(s) suspected to be involved.

**Sampling Procedures**

Poor sampling technique will result in inaccurate and misleading test results. Sampling will be most accurate if the proper tools are used. Common tools used to sample dry materials include a grain probe and a Pelican sampler or a clean, 1-lb can. Use a Pelican sampler or can to sample materials from an unloading or transferring stream. Pelican samplers consist of a container about 18 in. long, 2 in. wide, and 6 in. deep attached to a handle. Pass the Pelican sampler or can across the width of a free-falling stream to obtain an accurate sample (NPPC 1996).

Below are guidelines for proper sampling of feed and ingredients. In each case, collect the samples in a clean 5-gal bucket or similar container in preparation for sample reduction.

**Feed in Feeders.** Sampling from feeders may give the best assessment of the overall feed preparation program (mixing and handling procedure, ingredient quality, etc.). If there are 10 or fewer feeders present, sample all feeders; if ≥11 feeders are present, sample 10 randomly selected feeders. A grain probe works well to sample from feeders (Reese and Thaler 2010).

**Bulk Feeds.** Request a feed sample from the supplier as the truck is loaded for each type of feed delivered to the farm (Reese and Thaler 2010).

**Bulk Ingredients.** Cut the stream with a Pelican sampler or 1-lb can at least 10 times at equal intervals during the delivery of the stream (AAFCO 2000; NPPC 1996) or before unloading, probe the truck at four to six evenly spaced locations to represent the entire load of ingredients (Reese and Thaler 2010).

**Bagged Ingredients.** Lay each bag horizontally and remove the core sample diagonally from end to end. If 10 or fewer bags are present in the lot, sample all bags; from a lot ≥11 bags, sample 10 randomly selected bags (AAFCO 2000; NPPC 1996). Samples from each bag should weigh about 0.23 kg (0.5 lb) (NPPC 1996).

Collect the samples in a large, clean container and mix thoroughly. Obtain two 0.45-kg (1 lb) samples and seal each in individual, clearly marked and dated containers. Heavy plastic bags, plastic containers with lids, and clean, widemouthed jars are excellent for storing samples. Submit one sample to the laboratory and keep the other in the freezer until the analysis is complete (Reese and Thaler 2010).

**Interpreting Laboratory Results**

Even if the feed sampled was made to perfection, there are errors in sampling and laboratory analyses that at best can only be minimized. These errors can cause differences in nutrient concentrations between what the laboratory reports and the producer expects. Generally, there is no need for concern as long as analyzed nutrient values are not significantly different from the calculated nutrient content of the diet. Comparing analyzed values to the calculated nutrient content of the diet is an essential step in interpreting laboratory results.

Calculate the nutrient content of the finished feed from the diet formula, appropriate feed labels, and nutrient contents of ingredients. Compare those values with the “as-fed,” “as-is,” or “as-received” values from the laboratory report.

How much difference can there be between the calculated and analyzed values before it is appropriate to conclude there is a feed quality problem? The expected
<table>
<thead>
<tr>
<th>Clinical Signs</th>
<th>Caused by a Deficiency of:</th>
<th>Caused by an Excess of:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aborted fetuses</td>
<td>Vitamin A</td>
<td>Vitamin A, vitamin D, chromium, cobalt, copper, iodine, iron, manganese, selenium, zinc, calcium, sodium chloride, sulfur, energy (in gestation sows), methionine, threonine, tryptophan</td>
</tr>
<tr>
<td>Anestrus</td>
<td>Riboflavin, manganese, energy, protein/amino acids</td>
<td>Vitamin A</td>
</tr>
<tr>
<td>Anorexia</td>
<td>Niacin, pantothenic acid, vitamin Bi, vitamin Bn, vitamin B12, copper, iron, potassium, sodium chloride, zinc, protein/amino acids, water</td>
<td>Vitamin A, vitamin D, iron, selenium, sodium chloride, copper</td>
</tr>
<tr>
<td>Ataxia</td>
<td>Vitamin Bn, copper, potassium</td>
<td>Vitamin A</td>
</tr>
<tr>
<td>Blood-filled joints</td>
<td>Vitamin K</td>
<td>Vitamin A</td>
</tr>
<tr>
<td>Blood, increased attraction to</td>
<td>Sodium chloride, protein/amino acids</td>
<td>Chromium, iron, selenium</td>
</tr>
<tr>
<td>Bloody feces</td>
<td>. . .</td>
<td>Vitamin A</td>
</tr>
<tr>
<td>Bloody urine and feces</td>
<td>. . .</td>
<td>Chromium, iron, selenium</td>
</tr>
<tr>
<td>Breathing, labored</td>
<td>Iron</td>
<td>Iron</td>
</tr>
<tr>
<td>Coma</td>
<td>Vitamin Bn, energy</td>
<td>Vitamin A</td>
</tr>
<tr>
<td>Congenital defects</td>
<td>Vitamin A</td>
<td>Vitamin A</td>
</tr>
<tr>
<td>Death</td>
<td>Vitamin Bn, magnesium, energy, water, iron</td>
<td>Vitamin A, vitamin D, iron, selenium, sodium chloride, copper</td>
</tr>
<tr>
<td>Death, sudden</td>
<td>Vitamin E, selenium, vitamin Bi</td>
<td>Vitamin A</td>
</tr>
<tr>
<td>Dehydration</td>
<td>Water</td>
<td>Chromium, zinc</td>
</tr>
<tr>
<td>Depression</td>
<td>. . .</td>
<td>Chromium, zinc</td>
</tr>
<tr>
<td>Dermatitis</td>
<td>Biotin, niacin, linoleic acid, vitamin B12, pantothenic acid</td>
<td>chromium</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>Niacin, biotin, selenium, vitamin E, water</td>
<td>Chromium, iron, protein, tryptophan, sodium chloride, sulfur</td>
</tr>
<tr>
<td>Embryo survival, reduced</td>
<td>Vitamin A</td>
<td>Energy</td>
</tr>
<tr>
<td>Epileptic seizures</td>
<td>Vitamin A, biotin, vitamin Bi</td>
<td>Sodium chloride</td>
</tr>
<tr>
<td>Eye discharge</td>
<td>. . .</td>
<td>Selenium</td>
</tr>
<tr>
<td>Farrowing rate, reduced</td>
<td>Choline, pantothenic acid, riboflavin, energy, protein/amino acids</td>
<td>Calcium, phosphorus, iron, zinc, vitamin D, protein, lysine, methionine, tryptophan</td>
</tr>
<tr>
<td>Feed efficiency, reduced</td>
<td>Iron, phosphorus, sodium chloride, zinc, protein/amino acids</td>
<td>Vitamin A, vitamin D, chromium, cobalt, copper, iodine, iron, manganese, selenium, zinc, calcium, sodium chloride, sulfur, energy (in gestation sows), methionine, threonine, tryptophan</td>
</tr>
<tr>
<td>Feed intake, reduced</td>
<td>Niacin, pantothenic acid, vitamin Bi, vitamin Bn, vitamin B12, copper, iron, potassium, sodium chloride, zinc, protein/amino acids, water</td>
<td>Vitamin A, vitamin D, chromium, cobalt, copper, iodine, iron, manganese, selenium, zinc, calcium, sodium chloride, sulfur, energy (in gestation sows), methionine, threonine, tryptophan</td>
</tr>
<tr>
<td>Fractures, spontaneous</td>
<td>Calcium, copper, phosphorus</td>
<td>Vitamin A</td>
</tr>
<tr>
<td>Frothing at the mouth</td>
<td>. . .</td>
<td>Vitamin A</td>
</tr>
<tr>
<td>Gait, goose stepping</td>
<td>Pantothentic acid</td>
<td>Cobalt, manganese</td>
</tr>
<tr>
<td>Gait, stiff and stilted</td>
<td>Riboflavin, calcium</td>
<td>Cobalt, manganese</td>
</tr>
<tr>
<td>Goiter</td>
<td>Iodine</td>
<td>Cobalt, manganese</td>
</tr>
<tr>
<td>Hair coat, rough</td>
<td>Choline, niacin, pantothenic acid, vitamin B12, iron, potassium</td>
<td>Vitamin A, vitamin D</td>
</tr>
<tr>
<td>Hair loss (alopecia)</td>
<td>Biotin, niacin, pantothenic acid, riboflavin, linoleic acid, zinc</td>
<td>Selenium</td>
</tr>
<tr>
<td>Hemorrhage, subcutaneous</td>
<td>Vitamin K</td>
<td>Vitamin A</td>
</tr>
<tr>
<td>Hoof cracks</td>
<td>Biotin</td>
<td>Vitamin A</td>
</tr>
<tr>
<td>Hoof, skin separate at the</td>
<td>. . .</td>
<td>Vitamin A</td>
</tr>
<tr>
<td>coronary band</td>
<td></td>
<td>Vitamin A</td>
</tr>
<tr>
<td>Humped back</td>
<td>Calcium</td>
<td>Cobalt, vitamin D</td>
</tr>
<tr>
<td>Hypersensitivity/irritability</td>
<td>Vitamin B12, magnesium</td>
<td>Vitamin A, sodium chloride</td>
</tr>
<tr>
<td>Inactivity</td>
<td>. . .</td>
<td>Potassium, chromium</td>
</tr>
<tr>
<td>Incoordination/staggering movement</td>
<td>Vitamin A, vitamin B12, choline</td>
<td>Vitamin A, cobalt, iron selenium, sodium chloride</td>
</tr>
<tr>
<td>Joints, enlarged</td>
<td>Calcium, manganese</td>
<td>Vitamin A</td>
</tr>
<tr>
<td>Lactation failure</td>
<td>Vitamin E, selenium</td>
<td>Vitamin A</td>
</tr>
<tr>
<td>Lameness</td>
<td>Vitamin D, manganese, calcium</td>
<td>Vitamin D, zinc</td>
</tr>
<tr>
<td>Legs, bowed</td>
<td>Copper, manganese</td>
<td>Selenium, zinc</td>
</tr>
<tr>
<td>Litter size, reduced</td>
<td>Vitamin A, vitamin E, biotin, choline, folic acid, pantothentic acid, vitamin B12, sodium chloride, selenium, zinc, manganese, energy, protein/amino acids</td>
<td>Selenium, zinc</td>
</tr>
<tr>
<td>Milk production, reduced</td>
<td>Manganese, selenium, energy, protein/amino acids</td>
<td>Vitamin A</td>
</tr>
<tr>
<td>Night blindness</td>
<td>Vitamin A</td>
<td>Vitamin D</td>
</tr>
<tr>
<td>Osteomalacia</td>
<td>Vitamin D, calcium, phosphorus</td>
<td>Vitamin D</td>
</tr>
</tbody>
</table>

(Continued)
amount of variation associated with laboratory analyses for some nutrients is shown in Table 68.8. From these values and the calculated nutrient content of the diet, an expected range for the amount of that nutrient in the diet can be estimated. For example, assume the calculated phosphorus content of a diet is 0.65%. To allow for normal laboratory variation, the acceptable range of phosphorus levels in the diet will be from 0.57% to 0.73%:

\[
0.65\% \times 0.13 = 0.08; 0.65\% - 0.08\% \\
= 0.57\%; 0.65\% + 0.08\% = 0.73\%
\]

If the analyzed value falls within the acceptable range (e.g., between 0.57% and 0.73%), a feed quality problem associated with that nutrient probably does not exist. However, if the level of all or any one of the nutrients falls outside the acceptable range and proper sampling procedures were used, submit a portion of the retained sample to the same or another laboratory for a repeat analysis. If the results from the second analysis also fall outside the normal range, a feed quality problem likely exists.

Review the causes of nutrient deficiencies and excesses presented earlier in this chapter to help determine an explanation for the quality problem. Again it is important to remember that a nutrient deficiency may be manifested by the antagonism or excess of another nutrient. Therefore, deficiency signs may be observed in the presence of seemingly adequate dietary nutrient concentrations. This situation is most likely to occur between zinc and copper or calcium and zinc.

**PREVENTING NUTRIENT DEFICIENCIES AND EXCESSES**

Emphasis should be placed on reducing the opportunity for pigs to consume diets with inadequate or excessive levels of nutrients. Proper nutrition ensures that

---

Table 68.8. Analytical variations*

<table>
<thead>
<tr>
<th>Item</th>
<th>Variation (%)</th>
<th>Calculated Level</th>
<th>Normal Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude protein</td>
<td>±3</td>
<td>16%</td>
<td>15.5–16.5%</td>
</tr>
<tr>
<td>Lysine</td>
<td>±20</td>
<td>0.70%</td>
<td>0.56–0.84%</td>
</tr>
<tr>
<td>Calcium</td>
<td>±26</td>
<td>0.70%</td>
<td>0.52–0.88%</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>±13</td>
<td>0.65%</td>
<td>0.57–0.73%</td>
</tr>
<tr>
<td>Copper</td>
<td>±25</td>
<td>250ppm</td>
<td>187–313ppm</td>
</tr>
<tr>
<td>Zinc</td>
<td>±20</td>
<td>100ppm</td>
<td>80–120ppm</td>
</tr>
<tr>
<td>Selenium</td>
<td>±25</td>
<td>0.3ppm</td>
<td>0.22–0.38ppm</td>
</tr>
<tr>
<td>Vitamin A</td>
<td>±30</td>
<td>5500IU/kg</td>
<td>3850–71501IU/kg</td>
</tr>
</tbody>
</table>

*Adapted from AAFCO (2009)
the goals of economy, performance, health, and environmental stewardship are realized.

Meet the Pigs’ Nutrient Requirements
Several factors, including gender, age, season, and genotype affect nutrient requirements of pigs. Therefore, it is important to monitor pig performance (e.g., rate of lean gain and feed intake) on individual farms and formulate diets based on observed production rather than using a general set of nutrient recommendations. In addition, as pigs grow, their nutrient requirements decrease when expressed as a percentage of the diet. Thus, as pigs approach market weight, they should be fed diets that contain a lower density of nutrients. This is commonly called “phase feeding.” In typical phase-feeding programs, pigs growing from 20 to 122 kg (45–270 lb) would be fed four or more different diets. Also, because barrows consume more feed than gilts during the growing-finishing period, amino acid requirements (percent of the diet) for barrows are lower. Thus, producers may want to separate barrows from gilts and feed them diets containing different amino acid densities.

Implement a Quality Control Program
Monitor the nutrient content of ingredients and finished feeds on a periodic basis to help prevent problems associated with inadequate nutrition. Collect samples carefully to ensure they are representative and submit them to a reputable laboratory for analysis. It is not practical to analyze ingredients and diets for all the nutrients pigs require. Instead, analyze for the major nutrients provided by an ingredient or contained in the diet. In general, these include crude protein, lysine (if protein is low), calcium, and phosphorus for complete diets. Analyze protein supplements for crude protein and calcium and by-product ingredients (e.g., distillers dried grains with solubles) for protein, lysine, fat, and fiber. Analyze for calcium, phosphorus, at least one trace mineral (e.g., zinc), and vitamin A or E in base mixes and premixes. Compare the analyzed values with the expected nutrient content of the ingredient or diet when interpreting the results.

In situations where animals are not performing as expected, it is sometimes appropriate to collect a water sample for chemical analysis. Some commercial laboratories offer a “livestock suitability” test, which includes an analysis for various minerals. Minerals from water should not substitute for quantities recommended in the diet. Furthermore, when water contains a higher-than-normal mineral content, always compare the pig's daily requirement for that mineral with that which would be consumed through the water. Then decide whether the mineral content of the diet should be adjusted to prevent a problem with mineral excess. Often the mineral contribution from water is minute compared with the pig's requirement, and thus, no adjustment in the mineral concentration of the diet is warranted.

Adopt Good Feed-Manufacturing Practices
These include using products according to the manufacturer's directions, operating the mixer properly, and using a reliable set of scales to weigh feed ingredients. The bulk density or test weight of ingredients is variable; thus, adding ingredients to the diet on a volume basis is not recommended. In addition, be sure all feed ingredients are clearly labeled and that the mill area is kept clean. Monitor ingredient purchases and usage to ensure feed is being prepared according to specifications. Also, use feed within 30 days of manufacture.

Maximize Nutrient Intake and Minimize Nutrient Excretion
Only a portion of the nutrient content of a feed ingredient and/or diet is available to the pig. The inefficiencies of digestion and metabolism are associated with nutrients excreted in the feces and urine, respectively. Therefore, to account for the variability of nutrient availability among feedstuffs (especially by-product feedstuffs), diets should be formulated based on the digestible or available nutrient content when possible. Relative bioavailability of nutrients from several ingredient sources is available in various literature.

Blend Adulterated Feed
Feed that contains higher-than-intended levels of a nutrient is sometimes identified before it is offered to pigs. Often, the adulterated feed can be handled as a new ingredient and used to manufacture other diets.

CONCLUSION
Pigs will exhibit certain signs when they are not provided optimum nutrition. The challenges for producers and their advisors are to ensure that pigs continually receive the correct balance and amount of all essential nutrients and effectively monitor for and recognize signs of inadequate nutrition.

REFERENCES

INTRODUCTION
Mycotoxins are secondary metabolites of mold growth in grains or forages. Mycotoxins affect many body systems with a wide variety of signs, lesions, and impaired productivity. Their annual cost to U.S. agriculture has been estimated at $1.4 billion (CAST 2003). Wu and Munkvold (2008) estimated that, for fumonisins alone, if complete penetration of distillers dried grains with soluble (DDGS) occurs in U.S. swine feed at 20% inclusion, losses of $147 million annually could occur from weight gain reduction due to fumonisins.

This chapter will feature six high-risk mycotoxins for swine: aflatoxin B1 (AFB1), ochratoxin A (OTA), deoxynivalenol (DON), ergot, fumonisin B1 (FB1), and zearalenone (ZEA). Most swine mycotoxin problems involve feed grains (e.g., corn, wheat, milo, cottonseed, barley, and other cereals). Fungal growth requires readily available carbohydrate (grains), moisture, oxygen, and appropriate temperatures, often 12–25°C (Wilson and Abramson 1992). Plant or fungal stressors (drought, high ambient temperatures, insect damage, mechanical harvest damage, and reduced plant vigor) predispose crops to toxigenic fungal infection with subsequent mycotoxin production (CAST 2003).

Simple visual or cultural examination of grain or feed will not predict safety for animals. Toxigenic fungi can occur in grains without production of mycotoxins, and there is little correlation between spore counts, degree of fungal growth, and presence of mycotoxins. Conversely, absence of molds does not assure freedom from mycotoxins, since high temperature and pressure during milling/pelleting may kill molds but the heat-tolerant mycotoxin persists (CAST 2003). Mycotoxins can be concentrated threefold in some distillers by-products. Control of mycotoxins and reduction of effects in animals depends primarily on crop management, storage conditions, and appropriate use of mycotoxin binders in the diet.

MYCOTOXIN FORMATION
Both field fungi and storage fungi grow in crops prior to harvest and are a source of common mycotoxins. Field fungi (e.g., Fusarium spp.) require high equilibrium relative humidity (>70%) or grain moisture (>22%) for growth. They cause death of ovules, shriveling of seeds, and weakening or death of embryos. They grow poorly in storage after harvest and growth, and toxin production does not occur readily if dry grain is remoistened (Christensen and Kaufmann 1965).

Storage fungi include Aspergillus spp. and Penicillium spp., which may produce mycotoxins even when grain moisture is 12–18% and temperatures are 10–50°C. However, Aspergillus flavus, considered a storage fungus, often produces aflatoxin in crops prior to harvest. Conditions that favor fungal growth and mycotoxin formation are summarized in Table 69.1.

Certain geographical regions are at high risk for specific mycotoxins (Pier 1981), but regional predilection is altered by local growth conditions (e.g., drought, insect damage, early frost), transport or blending of grains, and improper storage.

Environment and management influence mycotoxin production and animal exposure to mycotoxins. Mycotoxins are higher in damaged or broken grain (e.g., screenings or milled grain). Screenings fed on-farm or locally at harvest increase mycotoxin exposure. Grain above optimum moisture continues to respire in storage, producing water; alternating warm and cool
Combinations of mycotoxins may be additive or synergistic in their effects. Mycotoxins that occur most frequently together are AFB1 with FB1 and DON with ZEA (CAST 2003). Documented synergistic combinations are AFB1 with FB1 and DON with FB1 (Harvey et al. 1995a, 1996).

Some mycotoxins are reported to alter immune function under certain conditions, enhancing development of infectious diseases (Bondy and Pestka 2000; Panangala et al. 1986; Pier 1981). Aflatoxins, trichothecenes, and OTA have been reported immunosuppressive in swine, but substantial diversity occurs among reports in swine. See individual mycotoxins for details.

Because immune suppression is often expressed indirectly, mycotoxin-facilitated disease is difficult to recognize or confirm and may sometimes be diagnosed incorrectly.

**INTOXICATION BY MYCOTOXINS**

Mycotoxicosis results from the consumption of contaminated grain by a susceptible animal. Dosage is usually expressed as parts per million (ppm) or parts per billion (ppb) in the diet. Convert from ppm (diet) to mg/kg BW with the equation: mg/kg BW = ppm(feed) × %BW eaten. The percentage must be expressed as a ratio (e.g., 3% = 0.03).

Dietary deficiencies of protein, selenium, and vitamins may predispose to mycotoxicosis, and drugs that alter foreign-compound metabolism could change metabolic response to mycotoxins (Coppock and Christian 2007).

**CLINICAL MYCOTOXICOSES**

Clinical response of swine to mycotoxins may be acute, subacute, or chronic and is both dosage and time dependent. Response is most often subacute or chronic and the presenting signs may be subtle, vague, or expressed as alterations in reproduction, feed intake, growth, feed efficiency, or immunosuppression. Nevertheless, knowing the range of effects for specific mycotoxins is important in differential diagnosis and evaluation of clinical prognosis. Common mycotoxins affecting swine are summarized in Table 69.2, and relevant information is discussed below.

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**Table 69.1. Sources and conditions for selected mycotoxins important to swine**

<table>
<thead>
<tr>
<th>Mycotoxin Produced</th>
<th>Fungal Source</th>
<th>Grains Most Affected</th>
<th>Optimal Conditions</th>
<th>Agronomic Influences</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aflatoxin: AFB1, AFB2, AFG1, AFG2; AFB1 is most toxic</td>
<td><em>Aspergillus flavus</em>&lt;br&gt;<em>A. parasiticus</em></td>
<td>Corn, peanuts, cottonseed, milo</td>
<td>24–35°C&lt;br&gt;ERH 80–85%&lt;br&gt;EMC 17%</td>
<td>Drought, insect damage, day–night temperature &gt;21°C; may produce toxin in storage&lt;br&gt;Alternating warm and cool growing season; humid conditions; less likely increase in storage</td>
</tr>
<tr>
<td>Deoxynivalenol</td>
<td><em>Fusarium graminearum</em></td>
<td>Corn, wheat, barley, other cereal grains</td>
<td>26–28°C&lt;br&gt;ERH 88%&lt;br&gt;EMC 22%</td>
<td></td>
</tr>
<tr>
<td>Ergot alkaloids (ergotamine, ergovaline, others)</td>
<td><em>Claviceps purpurea</em></td>
<td>Rye, wheat, triticale, oats, barley</td>
<td>Moderate-cool when seed forms; moist humid weather&lt;br&gt;Likely &lt;25°C&lt;br&gt;EMC &gt; 20%</td>
<td>Warm humid conditions, wind, and insects favor spread of infection&lt;br&gt;Dry/hot growing conditions followed by moist autumn</td>
</tr>
<tr>
<td>Fumonisin: toxins B1, B2 most toxic; B1 most prevalent</td>
<td><em>Fusarium verticilloides</em></td>
<td>Corn; other commodities not reported</td>
<td>Likely &lt;25°C&lt;br&gt;EMC &gt; 20%</td>
<td></td>
</tr>
<tr>
<td>Ochratoxin; ochratoxin A (OTA) is toxic fraction; citrinin toxin</td>
<td><em>Aspergillus ochraceus</em>&lt;br&gt;<em>Penicillium viridicatum</em>&lt;br&gt;<em>Penicillium citrinum</em></td>
<td>Corn, wheat, barley, rye</td>
<td>12–25°C; may produce toxin down to 4°C.&lt;br&gt;ERH 85%&lt;br&gt;EMC 19–22%</td>
<td>Lower temperatures favor increased toxin yield; endemic in some parts of Europe; rare in the United States&lt;br&gt;Alternating cool and warm conditions; overwintered crops</td>
</tr>
<tr>
<td>T-2 toxin</td>
<td><em>Fusarium sporotrichioides</em>&lt;br&gt;<em>Fusarium poae</em></td>
<td>Corn, barley, milo, wheat</td>
<td>8–15°C&lt;br&gt;EMC 22–26%</td>
<td></td>
</tr>
<tr>
<td>Zearalenone</td>
<td><em>Fusarium graminearum</em></td>
<td>Corn, wheat</td>
<td>7–21°C&lt;br&gt;EMC 24%</td>
<td>Alternating high and low temperatures during maturation</td>
</tr>
</tbody>
</table>

ERH, equilibrium relative humidity; EMC, equilibrium moisture concentration.
AFLATOXINS

Formation and Metabolism

*Aspergillus flavus*, *Aspergillus parasiticus*, and *Aspergillus nomius* produce aflatoxins (AFB1, AFB2, AFG1, and AFG2) before harvest and in storage. AFB1 and AFB2 are more likely produced by *A. flavus* in corn and cottonseed, while *A. parasiticus* usually produces all four fractions in peanuts (Coppock and Christian 2007). AFB1 is the most abundant and toxic fraction from natural contamination. Conditions supporting aflatoxin formation often occur in the southeastern United States (Bennett and Klich 2003) or during drought and insect infestation elsewhere. Rustemeyer et al. (2010) reported risk of aflatoxin exposure in DDGS; aflatoxins are not destroyed during ethanol production but concentrated three- to fourfold in the DDGS fraction. Thus, contaminated corn sold to ethanol plants may increase mycotoxicosis risk from these products. Variation in occurrence in DDGS is expected, hence the need for consistent sampling and testing.

AFB1 is metabolized by liver microsomal mixed-function oxidases to seven metabolites (Coppock and Christian 2007). The major toxic metabolite is an 8, 9 epoxide that binds covalently to DNA, RNA, and proteins. The DNA N7 adduct is resistant to repair and classified a carcinogen of chronic pulmonary effects; some reports of reduced litter size or stillbirths due to feed refusal.

Table 69.2. Characteristics of common mycotoxins in swine

<table>
<thead>
<tr>
<th>Toxin</th>
<th>Commodities</th>
<th>Clinical Effects</th>
<th>Lesions, Diagnostics, Residues</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aflatoxins: AFB1, AFB2, AFG1, AFG2</td>
<td>Corn, cottonseed, wheat, peanuts, sorghum/milo</td>
<td>Reduced protein synthesis; hepatoxicosis, cholangiohepatitis, hemorrhage, coagulopathy; chronic slow growth, poor feed conversion, reduced milk production, immune system dysfunction; classified a carcinogen</td>
<td>Hepatic necrosis, increased serum bile acids, bile duct hyperplasia; aflatoxin in feed; aflatoxin M1 in the liver or urine; return to normal 1–2 weeks postexposure</td>
</tr>
<tr>
<td>Ochratoxin and/or citrinin</td>
<td>Corn, wheat, peanuts, rye, oats, barley</td>
<td>Nephrotoxicosis with polyuria, polydipsia; gastric ulcers; anorexia and weight loss; reduced immunocompetence—for both ochratoxin and citrinum</td>
<td>Gastric ulcers and renal tubular damage or fibrosis; ochratoxin metabolites in the kidneys; high protein excretion in the urine; residues may persist for weeks</td>
</tr>
<tr>
<td>Trichothecenes, macrocyclic (e.g., T-2 toxin; diacetoxyscirpenol); relatively rare in North America</td>
<td>Corn, barley, wheat, rye sorghum</td>
<td>Hematopoietic suppression, anemia, leukopenia, hemorrhage, diarrhea, dermal irritation/necrosis; reduced immunocompetence; self-limiting due to feed refusal</td>
<td>Test mycotoxin in feed; oral ulcers; lymphoid depletion; residues brief—1–3 days; not common at clinical levels in North America</td>
</tr>
<tr>
<td>Deoxynivalenol (DON, vomitoxin)</td>
<td>Corn, wheat, barley, sorghum; common trichothecene worldwide</td>
<td>Feed refusal, vomiting, diarrhea, depression; variable effects on immunocompetence; rare reports of reduced litter size or stillbirths</td>
<td>DON concentration &gt;0.5 ppm in feed—mild effect; clinical results 1–8 ppm; residues rapidly excreted (1–3 days); glucomannan binders variable effects on some aspects of toxicosis</td>
</tr>
<tr>
<td>Zearalenone</td>
<td>Corn, wheat, barley, sorghum</td>
<td>Estrogenic</td>
<td>Enlarged uteri/vulva (gilts), retained corpora lutea (sows); vaginal cornification; zearalenone &gt;1 ppm in feed; excreted in urine, less in milk after 1–5 days</td>
</tr>
<tr>
<td>Fumonisins</td>
<td>Corn</td>
<td>Acute, fatal pulmonary edema (high dosages); hepatotoxicosis with icterus and liver necrosis (subacute exposure); some reports of chronic pulmonary effects; classified a carcinogen</td>
<td>Histological lesions of massive pulmonary interlobular edema; liver apoptosis and bile retention; residues brief, mainly liver/kidney; serum increases in AST, GGT, bilirubin, and cholesterol are characteristic</td>
</tr>
<tr>
<td>Ergot alkaloids (ergotamine, ergocristine, ergonovine, ergocornine, ergovaline)</td>
<td>Cereal grains (barley, rye, triticale, wheat, oats) and grasses</td>
<td>Acute high doses: peripheral vascular necrosis with peripheral gangrene (feet, tail, ears); in late pregnancy, causes reduced prolactin release with agalactia and piglet starvation</td>
<td>Ergot bodies should be &lt;0.3% in feed; ergot alkaloids in urine 1–2 days postexposure; ergot alkaloids recommended &lt;100 ppb in feed; residues rapidly excreted; residues typically not a problem</td>
</tr>
</tbody>
</table>
inability to mobilize fats causes early lesions of hepatic fatty change and necrosis, as well as reduced growth. Protein-deficient diets enhance aflatoxin effects on depression of weight gain (Coffey et al. 1989; Harvey et al. 1989a).

**Toxicity**

Aflatoxin effects depend on dosage, dietary interactions, exposure time, animal species, and age of animal. The single acute oral dose expected to cause death of 50% of animals exposed (LD$_{50}$) for swine is 0.62 mg/kg body weight (equivalent to approximately 20 ppm in diet for 1 day); dietary levels of 2–4 ppm for extended time can cause fatal toxicosis; rations containing 260 and 280 ppb for several weeks cause reduced growth (Allcroft 1969; Marin et al. 2002). A diet containing 2.5 ppm AFB$_1$ fed to 17.5-kg barrows for 35 days decreased body weight, rate of gain, and feed consumption (Harvey et al. 1995a,b); increased serum gamma glutamyl transferase (GGT) and total iron concentration (TIC); and decreased blood urea nitrogen (BUN) and total iron-binding capacity (TIBC). Relative liver weight increased and the liver was pale, rubbery, and resistant to cutting. Microscopic lesions included hepatic necrosis and degeneration accompanied by bile duct proliferation.

Prolonged dietary aflatoxin affects productivity and immune function (Cook et al. 1989; Dilkin et al. 2003; Harvey et al. 1988, 1989c; Marin et al. 2002; Rustemeyer et al. 2010). Liver lesions are caused by dietary concentrations of 140 ppb for 12 weeks in 18- to 64-kg swine, and 690 ppb produced mild liver lesions in 64- to 91-kg finishing hogs (Allcroft 1969). AFB$_1$ fed at 280 ppb to weanling pigs for 28 days caused significantly decreased weight gain, but no effect on total erythrocyte count, differential leukocyte count, total globulin, albumins, or total protein concentration in serum. Lower concentration of AFB$_1$ (140 ppb) resulted in reduced average daily gain that was not statistically significant ($P < 0.05$). Rustemeyer et al. (2010) fed 0, 250, and 500 ppb to barrows for 7, 28, or 70 days. The 500 ppb AFB$_1$ diet depressed feed intake and average daily gain but 250 ppb did not. Serum aspartate amino transferase (AST) was higher in aflatoxin groups, but BUN was lower than controls. Lower BUN may be from reduced protein turnover and/or reduced liver function. Both 250 and 500 ppb produced adverse effects on some measures of performance and health. Taken together, most studies suggest that a threshold for moderate effects on performance of swine is slightly above 200 ppb.

**Clinical Effects**

Acute to subacute aflatoxicosis causes depression and anorexia, anemia, ascites, icterus, hemorrhagic diarrhea, and sometimes coagulopathy from hypopro-

thrombinemia (Coppock and Christian 2007; Osweiler et al. 1985). Hepatocellular enzymes are elevated, including AST, alanine aminotransferase (ALT), alkaline phosphatase (ALP), and GGT. Other serum clinical chemistry changes are decreased TIBC, total protein, albumin, cholesterol, BUN, and glucose (Harvey et al. 1989c). Total bilirubin, icterus index, sulfobromophthalein clearance, prothrombin time, and partial thromboplastin time are elevated (Panangala et al. 1986).

**Lesions**

Porcine aflatoxicosis causes pale tan or clay-colored liver with centrilobular hemorrhages, fatty change, subserosal petechial to ecchymotic hemorrhages, and intestinal and colonic hemorrhages. The liver becomes yellow and fibrosis develops, with a firm parenchyma and accentuated lobular pattern. Icterus occurs at scleral, serosal, and mucosal surfaces. Microscopic alterations useful for diagnostic purposes include hepatocyte vacuolization, necrosis, and lipidosis, which are all predominant around central veins. As disease progresses to subacute or chronic, hepatomegalocytosis, multiple nuclei, interlobular fibrosis, and biliary hyperplasia appear (Cook et al. 1989; Harvey et al. 1988, 1989b).

**Reproductive Effects**

Abortion is not expected. Sows fed aflatoxin have maintained normal reproduction through four successive gestations at dietary concentrations of 500 and 700 ppb. Piglets nursing these sows had reduced growth from aflatoxin excretion in milk (Armbrecht et al. 1972; McKnight et al. 1983). Mocchegiani et al. (1998) observed reduced piglet birth weight where sows were fed 800 ppb AFB$_1$ from day 60 of gestation through 28 days postfarrowing.

**Immunocompetence**

Aflatoxin affects cell-mediated immunity and phagocytic cell function (Bondy and Pestka 2000). Common diseases influenced by aflatoxin under experimental conditions include swine erysipelas, swine dysentery, and salmonellosis (CAST 2003). Immunosuppressive effects are most likely at AFB$_1$ concentrations causing subtle or chronic changes typical of the mycotoxin (Osweiler 2000). Immunocompetence was reduced in piglets nursing aflatoxin-exposed sows (Silvotti et al. 1997), where sows fed 800 or 400 ppb purified AFB$_1$ through gestation and lactation had AFB$_1$ and AFM$_1$ residues in milk 5 and 25 days after parturition. Residues were approximately 1000-fold lower than that in the feed, with increases during the 25 days after parturition. Lymphoproliferative response to mitogens was reduced, and monocyte-derived macrophages failed to efficiently produce superoxide anions after oxidative burst stimulation in vitro. The ability of macrophages
to phagocytose red blood cells was not compromised, but granulocytic cells had reduced chemotactic response in vitro. Marin et al. (2002) reported that 4 weeks feeding of 280 ppb aflatoxin reduced weight gain in swine while increasing leukocyte count and raising serum gamma globulin. Immune response to Mycoplasma agalactiae was reduced, and cytokine mRNA expression was associated with decrease of some proinflammatory factors (interleukin-1 [IL-1] beta and tumor necrosis factor-α [TNF-α]), but increased anti-inflammatory IL-10 cytokine expression.

Residues
The AFM₁ metabolite can occur in tissues, milk, and urine of swine at relatively low concentrations and is not persistent. Dietary concentrations of 400 ppb resulted in tissue residues of 0.05 ppb or less, which rapidly disappear when aflatoxin feeding stops (Truckles et al. 1982).

Diagnosis
Depression, hemorrhagic diarrhea, acute icterus, hemorrhages, and coagulopathy suggest acute aflatoxicosis. Chronic signs include slow growth, malnutrition, icterus, and persistent low-grade infections. A history of contaminated feed is important. Liver lesions, clinical chemistry changes, and chemical analysis of the ration and grain supply are important for confirmation. For feed, sometimes the responsible grain is no longer available. Any grain sampling should be representative (sampling section below). Examination of suspect grain samples for bright greenish-yellow fluorescence (BGYF) under ultraviolet light is suggestive but not definitive for AFB₁. Effective and economical enzyme-linked immunosorbent assay (ELISA) kits are used for the detection and initial quantification of suspect grains. Use only USDA-GIPSA-approved test procedures, and if positives are identified, a confirmatory chemical analysis can verify the result (CAST 2003).

Therapy
Aflatoxicosis is generally not amenable to individual animal treatment. Specific practical antidotes for affected animals are not available. Increased dietary selenium, high-quality protein, and vitamins A, D, E, K, and B complex have been recommended (Coffey et al. 1989; Coppock and Christian 2007). Supplemental therapy with choline and methionine has been recommended for aflatoxicosis in poultry, but not evaluated in swine (Cullen and Newberne 1994). Aflatoxin may compromise the immune system, and animals with concurrent infectious diseases should be aggressively treated with appropriate antimicrobial therapy and passive immunization if appropriate. However, lincomycin and tylosin added to aflatoxin-contaminated diets neither decreased nor enhanced the detrimental effects of aflatoxicosis in growing swine (Harvey et al. 1995b).

Prevention
No U.S. Food and Drug Administration (FDA)-approved preventive additives for aflatoxicosis are available in the United States. Availability of preventive feed ingredients varies in other countries. One or more common anticaking agents for feed may provide effective physical binding for aflatoxin. Hydrated sodium calcium aluminosilicates (HSCAs) at 0.5% in the diet have consistently provided substantial protection against loss of gain and occurrence of lesions from dietary aflatoxin in swine (Harvey et al. 1989c; Phillips et al. 2002). Sodium or calcium bentonite also has value as an adsorbent (Schell et al. 1993). Treatment of grain with anhydrous ammonia for 10–14 days has reduced aflatoxin concentration in grain, and swine accept ammoniated grain with growth comparable with controls. Presently, this method of treatment has not been approved by the FDA.

Recent work has shown some potential for dietary glucomannans derived from yeasts in the prevention of aflatoxicosis. Meissonier et al. (2009) fed up to 1912 ppb AFB₁ to weaning pigs for 28 days. Dietary glucomannan at 0.2% decreased the severity of liver lesions, protected phase I metabolic enzymes, and restored ovalbumin-immunized specific lymphocyte proliferation compared with inhibition caused by aflatoxin, suggesting a potential beneficial role against aflatoxin (Table 69.3).

OCHRATOXIN AND CITRININ
OTA is a fungal nephrotoxin. Citrinin is a related nephrotoxin. See Table 69.1 for sources and conditions of production. OTA is commonly found from eastern and northern Europe, Canada, and the northern United States (Juszkiwicz et al. 1992). OTA has been prevalent in Denmark and is associated with the feeding of barley and oats (Carlton and Krogh 1979). Toxicosis has been documented in swine fed contaminated corn in the United States (Cook et al. 1986).

Toxicity is related to binding of OTA in specific renal organic ion transporters (Huessner et al. 2002) resulting from inhibition of phenylalanine-metabolizing enzymes, adenosine triphosphate (ATP) production, and lipid peroxidation (Marquardt and Frohlich 1992). Ochratoxin is a genotoxic carcinogen via oxidative DNA lesions coupled with DNA adducts (Pfohl-Leszkowicz and Manderville 2007). Immunosuppressive effects are a combination of suppressed lymphocyte proliferation and interference with the complement system (Bondy and Pestka 2000).

In swine, principal effects are in proximal renal tubules. OTA at 1 mg/kg body weight (33.3 ppm dietary) is lethal in 5–6 days. Concentrations of 1 ppm in the
### Table 69.3. Selected approaches to inactivate mycotoxins in swine feeds

<table>
<thead>
<tr>
<th>Mycotoxin</th>
<th>Agent or Process</th>
<th>Details and Comment</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aflatoxins</td>
<td>Ammoniation via anhydrous ammonia infusion or jack bean meal (urease)</td>
<td>Effectively destroys aflatoxins and is accepted by swine; not currently approved by FDA for use in food animals. Commercial products for aflatoxin destruction generally not available.</td>
<td>CAST (2003)</td>
</tr>
<tr>
<td></td>
<td>Bentonite, zeolite</td>
<td>Effective in some studies, generally less effective than HSCAs (see below).</td>
<td>CAST (2003)</td>
</tr>
<tr>
<td></td>
<td>Hydrated sodium calcium aluminosilicates (HSCAs)</td>
<td>Effective in improving performance (weight gain, feed efficiency) and protection against liver damage at 10g/kg diet (range 5–20g/kg). Available commercially as an anticaking agent but not FDA approved for this use.</td>
<td>Phillips et al. (2002); CAST (2003)</td>
</tr>
<tr>
<td>Deoxynivalenol (DON)</td>
<td>HSCAs, bentonite, and zeolite</td>
<td>Generally ineffective for binding of trichothecenes including DON</td>
<td>CAST (2003)</td>
</tr>
<tr>
<td></td>
<td>Glucomannan polymer adsorbents (GMA)</td>
<td>Benefits are variable for improving feed intake or rate of gain when DON is prominent. Some benefit occurred in studies where DON and/or zearalenol were associated with reduced fertility or live-born pigs, or with increased serum ammonia.</td>
<td>Avantaggiato et al. (2004); Diaz-Llano and Smith (2006, 2007); Swamy et al. (2002); Swamy et al. (2003); Diaz-Llano et al. (2010)</td>
</tr>
<tr>
<td></td>
<td>Physical decontamination</td>
<td>Abrasive pearling procedure, removed 66% of DON with loss of only 15% of grain mass.</td>
<td>House et al. (2003)</td>
</tr>
<tr>
<td>Ergot</td>
<td>Physical methods of cleaning to remove ergot bodies</td>
<td>Chemical binders generally have not been tried or have not been effective in feeds.</td>
<td>CAST (2003)</td>
</tr>
<tr>
<td>Fumonisins</td>
<td>Binding of fumonisins with glucose or fructose</td>
<td>Chemical inactivation of fumonisins has been accomplished with glucose or fructose; this method, while effective, has not yet been developed for commercial use.</td>
<td>Fernández-Surumay et al. (2005)</td>
</tr>
<tr>
<td>Zearalenone</td>
<td>GMA binders</td>
<td>Some studies have shown benefits from GMA binders where zearalenone is present; more work is likely needed in this area.</td>
<td>See references for DON above.</td>
</tr>
<tr>
<td></td>
<td>Activated charcoal or alfalfa meal</td>
<td>Activated charcoal in rations or high levels (≥20%) of alfalfa meal have also been effective against zearalenone.</td>
<td>Avantaggiato et al. (2003); James and Smith (1982)</td>
</tr>
<tr>
<td>Mold growth</td>
<td>Prevention of mold growth</td>
<td>Keep storage conditions clean and moisture to recommended levels. For wet or damaged grains, organic acids (e.g., propionic acid) are available to control mold growth but do not destroy preformed mycotoxins.</td>
<td>CAST (2003)</td>
</tr>
</tbody>
</table>

Diet for 3 months cause polydipsia, polyuria, reduced growth, and lowered feed efficiency. Levels as low as 200 ppb for several weeks cause detectable renal lesions. Additional clinical signs are diarrhea, anorexia, and dehydration. Sometimes no clinical signs are noted, and the only effect observed is the appearance of pale, firm kidneys at slaughter, commonly associated with ochratoxin in endemic areas such as eastern Europe, Denmark, and Sweden.

Clinical pathology changes are increases in BUN, plasma protein, packed-cell volume, AST, and isocitric dehydrogenase (ICD), increased urinary glucose, and proteinuria. Riley and Petska (2005) identified a correlation between histological renal damage from ochratoxin and high-level excretion of urinary proteins. Citrinin, ochratoxin, and penicillic acid are synergistic and primarily produce nephrosis characterized by necrosis of the proximal convoluted tubules, followed by interstitial fibrosis. Liver damage, with fatty change and necrosis, may occur but is less severe than for other primary hepatoses. Gastric ulceration is a characteristic and consistent lesion in prolonged clinical cases (Carlton and Krogh 1979; Szczech et al. 1973). Boars given 20µg OTA per os for 6 weeks had reductions in ejaculation volume, and sperm viability and motility after 24 hours of storage were significantly reduced compared with controls (Biro et al. 2003).

OTA has caused spontaneous occurrence of dose-related clinical Salmonella choleraesuis infection in piglets fed 1 and 3 ppm dietary ochratoxin (Stoev et al. 2000). In further studies, Serpulina hydysenteriae and Campylobacter coli infections were concomitant with immunosuppression and delayed immunization response.

Diagnosis is confirmed by finding toxin and/or metabolites (ochratoxin alpha) in feed or fresh kidney combined with history and characteristic lesions. High performance liquid chromatography (HPLC) can detect...
from 0.3 to 3 ng OTA or OTB in clinical specimens and feedstuffs. The approximate half-life for OTA in swine tissue is 3–5 days, and little or no ochratoxin can be found in the kidneys 30 days after ochratoxin exposure ceases (Carlton and Krogh 1979). Mildly affected animals may recover if removed promptly from the contaminated feed, but if clinical course is prolonged, recovery is slow.

Countries with known or potential ochratoxin contamination have regulations controlling the presence of OTA in food and animal feeds because of concerns for food contamination and potential carcinogenicity. Currently, FDA has no guidelines for OTA in feed.

**TRICHOTHECENES**

The trichothecenes include at least 148 structurally related compounds. Those of known veterinary importance are produced by *Fusarium*, especially *Fusarium graminearum* and *Fusarium sporotrichioides* (Table 69.1). This group of sesquiterpene toxins has an epoxide group responsible for most toxic effects. Those receiving most attention worldwide are T-2 toxin, diacetoxyscirpenol (DAS), and DON (vomitoxin). Even though much work has been done with the macrocyclic trichothecenes (T-2 and DAS) in swine, they rarely occur at toxic concentrations in North American grains. DON, however, is a common contaminant with potentially multiple effects (Mostrom and Raisbeck 2007).

Trichothecenes are metabolized in two phases. Phase 1 oxidation and hydrolysis is followed by conjugation with glucuronic acid. In the gut, the epoxide ring is cleaved by gastrointestinal (GI) microflora (Bauer 1995). Serum, bile, urine, liver, kidneys, and muscle may contain DON metabolites (mainly de-epoxy DON) (Doll et al. 2003; Goyarts et al. 2007). The glucuronide is rapidly excreted in urine and appears not to accumulate in plasma (Eriksen et al. 2003).

T-2 toxin in large dosages can cause direct skin irritation and necrosis, profound lymphoid depletion, gastroenteritis, diarrhea, shock, cardiovascular failure, and death from experimental direct dosing. Chronic administration causes hematopoietic suppression and eventual pancytopenia, and T-2 and DAS are immunosuppressants. Although T-2 and DAS are potent toxins, they occur at relatively low concentrations, and their tendency to induce feed refusal and/or vomiting in swine makes them somewhat self-limiting as toxins, except as potential causes of reduced feed consumption.

DON is a common mycotoxin of corn, barley, and wheat and is an economic issue because of feed refusal or reduced feed intake in swine (Bergsjo et al. 1993; Rotter et al. 1996; Trenholm et al. 1984). In corn, DON occurs at low levels during some harvest seasons, with prevalence as high as 50%. Contamination in other grains and in other parts of the world also occurs (CAST 2003).

**Clinical Effects of DON**

Swine reduce voluntary feed consumption in a dose-related manner at DON concentrations of approximately 1 ppm or more, and total feed refusal may occur at concentrations in excess of 10 ppm (Bergsjo et al. 1992; Pollman et al. 1985; Rotter et al. 1996; Young et al. 1983). DON at four dietary levels <1 ppm caused no reduction of feed intake (Accensi et al. 2006). Prelusky (1997) demonstrated reduced feed consumption and weight gain after intraperitoneal infusion of DON.

Studies of hematology and clinical chemistry for DON toxicosis are limited. Opinions differ for whether DON causes primary effects on swine hematology and biochemical measures at low or moderate dosages. Accensi et al. (2006) found that pigs accepted DON feeds containing <1 ppm, and there were no changes in nine standard hematological variables or 18 routine biochemical variables (cations, glucose, urea, creatinine, bilirubin, cholesterol, triglycerides, and plasma enzyme activity). Low concentrations caused no changes in piglet immune responses for immunoglobulin (lg) subset concentration, lymphocyte proliferation, and cytokine production. Based on pair-fed control studies, altered clinical laboratory values appear due to differences in feed intake; thus, hematological and blood chemistry tests may be of limited value in diagnosing low-level dietary DON in swine (Accensi et al. 2006; Lun et al. 1985; Prelusky et al. 1994; Swamy et al. 2003).

Many controlled studies of DON in swine are in a dosage range of 2–8 ppm DON, a common level for naturally contaminated grains and for variable effects on feed refusal. This section reviews the effects of DON that cause reduced feed consumption along with related clinical laboratory or immunological changes. Generally, most experience shows that DON effects are transitory and any disturbed function is recovered shortly after DON is removed from the diet as compensatory or adaptation mechanisms are established (Rotter et al. 1994).

DON feeding studies with dietary levels from 2 to 8 ppm show linear reductions in feed intake and rate of gain, but variable results for feed efficiency (Dänicke et al. 2008; Doll et al. 2008). Signs include lethargy, restlessness, weight loss, cannibalism, and one report of increased skin temperature. Gross lesions are absent to variable but have included loss of body mass, empty GI tract, increased folding of the esophageal stomach, increased liver weights, and reduced thyroid size. The most consistent clinical laboratory changes have been decreased serum proteins, globulin, and alpha-globulin with increased albumin/globulin ratio and reduced serum urea. Variable or inconsistent laboratory values have included decreased hematocrit, segmented neutrophilia, hypocalcemia, hypophosphatemia, increased serum thyroxine (T4) and changes in serum cortisol, or no consistent changes reported (Bergsjo et al. 1993;...
Dänicke et al. 2008; Diaz-Llano and Smith 2007; Doll et al. 2007, 2008; Rotter et al. 1994).

Laboratory evidence of immune dysfunction is variable among swine studies. In general, DON appears to increase immunoglobulin A (IgA) concentration in blood, and nonspecific lymphocyte proliferation is both increased and decreased (Doll et al. 2009; Frankic et al. 2008; Pinton et al. 2008; Tiemann et al. 2006). Pinton et al. (2008) fed pigs 2.2–2.5 ppm DON for 9 weeks. Results included increases in ovalbumin-specific IgA and IgG. Lymph nodes from treated pigs had reduced expression of TGF-β and interferon-γ (IFN-γ) mRNA, interpreted by the authors that DON reduces vaccinal immune response. Doll et al. (2009) found in vitro changes that included the following: (1) DON and lipopolysaccharide (LPS) were synergistic for increased mRNA expression of TNF-α in hepatocytes, (2) DON stimulated a dose-dependent induction of IL-6 mRNA, (3) supernatant concentrations of LPS-induced IL-6 were significantly decreased, and (4) mRNA expression of the anti-inflammatory IL-10 was increased by DON. They concluded that DON has the potential to provoke and modulate immunological reactions of porcine liver cells.

Variable results from in vitro to in vivo studies and between animal trials are characteristic of DON response in other mammals as well. There is no clear dose-response relationship across available studies.

Recent DON mycotoxin studies clearly demonstrate progress and also focus on the need to refine and expand the clinical and laboratory knowledge of DON in swine.

Feed Refusal Mechanisms
DON in swine causes conditioned taste aversion, and flavoring agents will not correct that refusal (Osweiler et al. 1990). Low-level DON exposure (30 µg/kg intragastric) increases cerebrospinal fluid 5-hydroxy-indoleacetic acid (SHIAA). DON feeding sequentially elevates brain tryptophan, serotonin (5-hydroxytryptamine [5HT]), and SHIAA. Hypothalamic dopamine (DA) is decreased, and SHIAA:5HT ratios are elevated. Brain noradrenaline levels decrease as well. Serotonin (5HT) increases initially and then drops significantly at 8 hours (Prelusky 1993, 1996; Swamy et al. 2002, 2004). Fusaric acid (FA) is a less well-known mycotoxin that appears to interact with DON in the tryptophan–SHIAA-serotonin relationship. It is considered an interacting or potentiating mycotoxin with DON for feed refusal and is likely an important part of feed refusal mechanisms (Swamy et al. 2002, 2004).

Diagnosis
Mycotoxin-related feed refusal presents a difficult problem to the clinician. Other toxins, drugs, concurrent disease, inclement weather, and reduced water intake may contribute to feed refusal. There is no apparent detectable effect of DON on plasma levels of brain neurotransmitters at DON doses that cause vomiting in swine. Thus, sampling of peripheral blood would not predict the central serotoninergic effects associated with DON toxicosis (Prelusky 1994). Trichothecenes are rapidly metabolized, and diagnosis by analysis of tissue or blood samples is rarely practical. Variable blood and tissue values and rapid excretion of metabolites does not support diagnostic differentiation of animals fed low levels of DON (Doll et al. 2008). Fortunately, rapid metabolism and excretion reduces residue potential in edible swine tissues (Bauer 1995).

Often the DON concentration detected chemically in feed is insufficient to fully explain feed refusal. Feed concentrations are approximations, sampling is never entirely representative, and many factors in the herd and environment may not be apparent. Recently, the discovery of conjugated trichothecene mycotoxins, including conjugated DON, has shown that standard chemical methods may not detect all DON present in grains, but they may be released by hydrolysis in the GI tract. Analytical laboratories are currently working to detect this portion of mycotoxins in feed sources (Berthiller et al. 2005; Zhou et al. 2007).

Therapy
Antiemetics that are specific serotonin (5HT)-receptor antagonists (ICS 205-930, BRL 43694 A) prevent DON-induced vomiting in swine, and anticholinergic compounds were moderately effective at high dosages acting directly at the emetic center. However, antihistaminic and antidopaminergic antiemetics were not effective against DON (Prelusky and Trenholm 1993).

Prevention
Attempts to prevent DON effects have centered on feeding adsorbents or on chemical or physical means of detoxification. These have included calcium aluminosilicate, bentonite, and sodium bisulfite. None of these have been successful in an economic and practical way. Recently, Avantaggiato et al. (2004) developed an in vitro GI model for reduction in intestinal absorption of DON and nivalenol. With 2% activated carbon (charcoal), there was a reduction of absorption from 51% to 28% for DON and from 21% to 12% for nivalenol. Continued work is needed to confirm the in vitro model in feeding trials.

Recent studies have expanded knowledge of yeast-derived glucomannan polymers for reducing feed refusal effects of DON through binding of the toxin in the GI tract. Feeding of a polymeric glucomannan adsorbent (GMA) to starter pigs prevented some known effects of trichothecenes on brain neurochemistry and promoted increased serum Ig concentrations, but did not improve growth rate (Swamy et al. 2002). Swamy et al. (2003) also fed starter pigs diets contaminated...
with combinations of DON, FA, and ZEA, and tested the ability of GMA to control mycotoxin effects. They also included a pair-fed control group to account for effects of reduced feed intake. While GMA prevented some toxin-related changes in metabolism, growth depression was not corrected by GMA. Most of the adverse effects on laboratory values were caused by reduced feed intake. Diaz-Llano and Smith (2006) fed 5.5–5.7 ppm DON to pregnant gilts from gestation day 91 through farrowing. A comparable group fed DON was supplemented with 0.2% GMA. Gilts had reduced daily gain and a significant increase in stillborn piglets, but no effects on standard clinical chemistry values. Addition of 0.2% GMA significantly increased the percentage of pigs born live. Diaz-Llano and Smith (2007) continued with 5.5–5.7 ppm DON fed to first-parity sows from day 91 of gestation through weaning on day 21. DON diets reduced average daily feed intake (ADFI), body weight, and serum protein, but not milk composition or piglet body weight. Addition of 0.2% GMA did not prevent reduced feed intake, body weight loss, or a somewhat longer weaning to estrus interval. Based on published work, GMAs may have potential to improve some aspects of DON effects in swine, but continued work is needed to clarify the variability reported in recent work.

Physical decontamination of DON from barley was demonstrated by use of an abrasive pearling procedure, which removed 66% of DON with loss of only 15% of the grain mass (House et al. 2003). This method could provide a practical means for reducing DON contamination to a manageable level in years of widespread crop contamination. The FDA in 2010 updated their nonbinding guidance statement from 1993 on the use of DON in animal feeds. Current allowance is for 5 ppm in grain and grain products with maximum dietary inclusion of 20%, resulting in a 1 ppm maximum for finished feeds.

**ZEARALENONE (F-2 TOXIN)**

**Sources and Mechanism**

*Fusarium graminearum* (*Fusarium roseum*) produces ZEA, an estrogenic mycotoxin in corn, milo, and wheat. *Fusarium roseum* can produce either ZEA or DON (Diekman and Green 1992). High moisture (23–25%) is required for growth. Poorly dried ear corn and alternating high and low ambient temperatures favor ZEA production (Christensen and Kaufmann 1965). Often, it is produced in the field prior to harvest.

ZEA is a substituted resorcylic acid lactone similar in structure to the anabolic agent zearanol used in cattle. ZEA binds competitively to estrogen receptors of the uterus, mammary gland, liver, and hypothalamus. It causes hypertrophy of the uterus and cornification of vaginal epithelium. ZEA is rapidly absorbed from the intestine and is metabolized to alpha- and beta-zearalenol and then conjugated with glucuronic acid for excretion in bile and urine (Meyer et al. 2000).

**Clinical Signs**

Response to ZEA varies with dosage and age of swine exposed. In prepubertal gilts, concentrations as low as 1–5 ppm in the ration cause vulvovaginitis, which is characterized by turgescence and edema of the vulva and vagina and precocious mammary development. Tenesmus is common, occasionally with resultant rectal prolapse (Osweiler 2000). ZEA at clinically effective dosage in sexually immature gilts causes ovarian follicle atresia and apoptotic-like changes in granulosa cells. Intensified cell proliferation occurs in both uterus and oviduct (Obremski et al. 2003). Prepubertal gilts fed 2 ppm for up to 90 days attained normal sexual maturity with no adverse effects on subsequent reproductive function (Green et al. 1990; Rainey et al. 1990). Doll et al. (2003) fed contaminated corn to piglets up to a diet maximum of 4.3 ppm DON and 0.6 ppm ZEA. Body weight gain was significantly reduced, and uterine weights compared with body weights were increased by nearly 100%.

Reproductive effects of ZEA on mature cycling sows are quite different from the effects seen in prepubertal gilts. As with other estrogens, ZEA is luteotropic in swine, and dietary concentrations of 3–10 ppm can induce anestrus in sows if consumed during the middle portion of the estrous cycle. Since estrogens are luteotropic in swine, the probability of abortion in the last two trimesters of gestation appears very unlikely. Anestrus and elevated serum progesterone persist for several months, long after exposure to ZEA has stopped (Edwards et al. 1987).

Fewer pigs per litter are seen in sows fed high concentrations of ZEA. The susceptible period for reduced litter size appears to be in the preimplantation stage at 7–10 days postmating (Diekman and Long 1989; Long et al. 1983). ZEA fed at 1 mg ZEA/kg body weight (equivalent to approximately 30 ppm dietary ZEA) on days 7 through 10 after mating resulted in mild blastocyst degeneration by day 11 and advanced degeneration by day 13. Viability of individual embryos is apparently not maintained beyond 21 days. ZEA did not cause morphological changes in the endometrium associated with hyperestrogenism (height of the endometrial luminal epithelium and morphology of secretory vesicles in the endometrial glandular epithelium) (Long et al. 1992). ZEA at 22.1 ppm in the ration of breeding gilts caused a decrease in the number of corpora lutea, ovarian weight, and number of live embryos, but an increase in dead-born piglets and abortions (Kordic et al. 1992).

ZEA and its metabolites, alpha- and beta-zearalenol, are present in milk of exposed sows and may contribute to estrogenic effects in piglets, including enlarged external genitalia and uteri (Dacasto et al. 1995;
Palyusik et al. 1980). A perinatal hyperestrogenic syndrome reported in swine herds and by experimental verification included lower conception rate, increased numbers of repeat breeders, decreased litter size, and increased numbers of stillbirths. Clinical signs in neonatal gilts were swelling of the vulva and teats and edematous infiltration of the perineal region, ventral abdomen, and umbilicus, usually accompanied by exudative, crusted inflammation and necrosis of the teats. An increase in splayleg and trembling piglets has been reported. Lesions of hyperestrogenism included enlargement of the ovary and uterus, ovarian follicle maturation, glandular proliferation of the endometrium, and epithelial proliferation in the vagina (Vanyi et al. 1994). Swine diets containing 2 ppm ZEA from day 30 of gestation through weaning did not adversely affect reproduction in sows. Estrogenic effects on testes and on uterine and ovarian weights were observed in the piglets at 21 days of age, but subsequent breeding performance was not affected (Yang et al. 1995).

Preputial enlargement may occur in boars exposed to ZEA. Young boars may have reduced libido and decreased testicular size, but mature boars are unaffected by concentrations of ZEA as high as 200 ppm (Ruhr et al. 1983; Young and King 1983).

### Diagnosis

ZEA toxicosis may resemble effects of estrogenic feed additives and natural estrogens such as coumestrol in mature alfalfa. Suspect rations of corn should first be analyzed for the presence of ZEA, then for other estrogens. Feed samples at the time of anestrus or return to service may not represent the contaminated feed that initiated the problem. For a summary of ZEA effects in different classes of swine, see Table 69.4.

### Table 69.4. Exposure guide to mycotoxin effects in swine

<table>
<thead>
<tr>
<th>Toxin</th>
<th>Category of Swine</th>
<th>Dietary Level</th>
<th>Clinical Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aflatoxins</td>
<td>Growing–finishing</td>
<td>1) &lt;100 ppb</td>
<td>1) No clinical effect</td>
</tr>
<tr>
<td></td>
<td>Brood sows and gilts</td>
<td>2) 200–400 ppb</td>
<td>2) Reduced growth and feed efficiency; possible immunosupression; mild microscopic liver lesions</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3) 400–800 ppb</td>
<td>3) Microscopic liver lesions, cholangiohepatitis; elevated serum liver enzymes; immunosuppression</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4) 800–1200 ppb</td>
<td>4) Reduced growth; decreased feed consumption, rough hair coat; icterus, hyproproteinemia</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5) &gt;2000 ppb</td>
<td>5) Acute hepatitis and coagulopathy; deaths in 3–10 days</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6) 400–800 ppb</td>
<td>6) No effect on conception; deliver normal piglets that grow slowly due to aflatoxin in milk</td>
</tr>
<tr>
<td>Ochratoxin and citrinin</td>
<td>Finishing</td>
<td>1) 200 ppb</td>
<td>1) Mild renal lesions seen at slaughter; reduced weight gain</td>
</tr>
<tr>
<td></td>
<td>Sows and gilts</td>
<td>2) 1000 ppb</td>
<td>2) Polydipsia; reduced growth; azotemia and glycosuria</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3) 4000 ppb</td>
<td>3) Polydipsia and polyuria</td>
</tr>
<tr>
<td>Trichothecenes</td>
<td>Growing–finishing</td>
<td>1) 1 ppm</td>
<td>1) No effect</td>
</tr>
<tr>
<td>T-2 and DAS</td>
<td></td>
<td>2) 3 ppm</td>
<td>2) Decreased feed consumption</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3) 10 ppm</td>
<td>3) Decreased feed consumption; oral/dermal irritation; immunosuppression</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4) 20 ppm</td>
<td>4) Complete feed refusal, vomiting</td>
</tr>
<tr>
<td>Deoxynivalenol (DON, vomitoxin)</td>
<td>Growing–finishing</td>
<td>1) &lt;1 ppm</td>
<td>1) No clinical effect; minimal (10%) reduction in feed consumption at &gt;0.5 ppm</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2) 2–8 ppm</td>
<td>2) 25–50% reduction in feed consumption; taste aversion to the same diet. Limited and variable immunosuppression—humoral and cell mediated; occasional reports of stillbirths</td>
</tr>
<tr>
<td>Zearalenone</td>
<td>Prepuberal gilts</td>
<td>1) 1–3 ppm</td>
<td>1) Estrogenic; vulvovaginitis, prolapse in prepuberal gilts</td>
</tr>
<tr>
<td></td>
<td>Cycling sows and gilts</td>
<td>2) 3–10 ppm</td>
<td>2) Retained corpora lutea; anestrous; pseudopregnancy</td>
</tr>
<tr>
<td></td>
<td>Pregnant sows</td>
<td>3) &gt;30 ppm</td>
<td>3) Early embryonic death when fed 1–3 weeks postmating</td>
</tr>
<tr>
<td></td>
<td>Mature boars</td>
<td>4) 200 ppm</td>
<td>4) No effect on fertility</td>
</tr>
<tr>
<td>Ergot</td>
<td>All swine</td>
<td>1) 0.1%</td>
<td>1) Reduced weight gain</td>
</tr>
<tr>
<td></td>
<td>Sows last trimester</td>
<td>2) 0.3% or &gt;3 ppm</td>
<td>2) Decreased feed consumption; agalactia, reduced piglet birth weight; piglet starvation</td>
</tr>
<tr>
<td></td>
<td>ergot alkaloids</td>
<td></td>
<td>3) 1.0%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>3) Gangrene of ears, tail, feet</td>
</tr>
<tr>
<td>Fumonisins</td>
<td>All swine</td>
<td>1) 25 ppm</td>
<td>1) Minimal changes in clinical chemistry—increased AST, alkaline phosphatase (AP)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2) 50–75 ppm</td>
<td>2) Minimal reduction in feed intake; possible mild hepatitis</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3) 75–100 ppm</td>
<td>3) Reduced feed intake, reduced weight gain; hepatitis with icterus and increased bilirubin and GGT</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4) &gt;100 ppm</td>
<td>4) Acute pulmonary edema after 3–5 days consumption; survivors develop hepatitis</td>
</tr>
</tbody>
</table>
Treatment
Reversal of effects depends on the nature of the effect and the age and reproductive status of swine. Removal of the feed from prepubertal gilts will allow regression of signs within 3–7 days. Medical and surgical treatment of vaginal and rectal prolapse may be needed. For mature, nongravid sows with anestrus, administration of one 10-mg dose of prostaglandin F₂α (PGF₂α) or two 5-mg doses on successive days is useful in eliminating retained corpora lutea (B. N. Day, personal communication; Green et al. 1990).

Prevention
Dehydrated alfalfa has experimentally shown some protection from ZEA-induced enlargement of the uterus of gilts (James and Smith 1982), although the high concentrations needed (>20%) are not considered practical in swine diets. Activated charcoal or cholestyramine has been used at 2% in an in vitro GI model system to evaluate its binding effect on ZEA. Both activated charcoal and cholestyramine reduced the absorption of ZEA from 32% to 5% and 16%, respectively (Avantaggiato et al. 2004). The dramatic reduction caused by activated charcoal could be useful for contaminated grain if feeding trials are effective.

ERGOT
Ergot, *Claviceps purpurea*, is a parasitic fungus that affects cereal grains, especially rye, oats, and wheat. The fungus invades the grass ovary, forming a dark elongated body (sclerotium), which produces alkaloids that cause gangrene and reproductive interference. Major toxic alkaloids include ergotamine, ergotoxine, and ergometrine; total ergot alkaloid content commonly ranges from 0.2% to 0.6% of sclerotia weight. The United States Department of Agriculture has set a tolerance of 0.3% ergot in grain (Christensen and Kaufmann 1965).

Gangrenous ergotism is the result of a combination of vasoconstriction and endothelial damage, leading to prolonged ischemia and eventually gangrene of appendages. Because venous and lymphatic drainage remains intact, the gangrene is “dry” in nature. Signs occur over a period of days or weeks and include depression, reduced feed intake, rapid pulse and respiration, and general ill thrift. Lameness may occur, most commonly in rear limbs, and in advanced cases, necrosis and sloughing of the tail, ears, and hooves can occur. Signs may be exacerbated by cold weather. Reduced weight gain may be caused by as little as 0.1% ergots in the ration. Higher levels (3.0%) have been implicated in feed wastage and slow growth (Roers et al. 1974).

Ergot alkaloids from *C. purpurea* have consistently affected piglet productivity by stimulating D₂ DA receptors leading to prolactin suppression and agalactia in pregnant sows fed ergot sclerotia. Piglets are born healthy but starve because of agalactia in the sow (Whitacre and Threlfall 1981). Pregnant gilts fed either 0.3% or 1% sclerotia during gestation had low piglet birth weights, low piglet survival, and poor piglet weight gains. Agalactia occurred in 50% of gilts fed 0.3% sclerotia in the gestation and lactation rations (Nordskog and Clark 1945). Recently, Kopinski et al. (2008) expanded on earlier work, showing that *Claviceps africana* ergot sclerotia fed to sows up to 1.5% of the diet (equivalent to 7 ppm ergot alkaloids) 6–10 days prior to parturition caused agalactia and 87% of piglets died. Blood prolactin was reduced. The authors recommended no more than 0.3% ergot or 1 mg/kg dietary ergot alkaloids for multiparous sows and no more than 0.1% ergot for primiparous sows or avoid ergot completely in primiparous sows.

**Differential Diagnosis and Treatment**
Evaluation should include ZEA or other estrogenic factors, bacterial infections, and mastitis–metritis–agalactia syndrome. If the clinical signs suggest ergotism, grains should be examined for the presence of significant amounts of ergot sclerotia. In the case of ground or processed feeds, feed microscopy or chemical analysis for ergot alkaloids may be necessary to confirm the diagnosis.

Gangrenous areas should be cleaned and treated locally and secondary infections controlled with broad-spectrum antibiotics. Removal of the feed is followed by improvement within 2 weeks for gangrenous effects. When agalactia has occurred, milk production returns 3–7 days after feed is changed. In the interim, supplemental nutrition and milk replacers may be used to save the piglets. In equines, parenteral use of the D₂ DA antagonist domperidone is effective in the prevention of agalactia from ergot alkaloids, but information about this therapy in swine has not been found.

**FUMONISINS**
*Fusarium moniliforme* and *Fusarium proliferatum* fungi are ubiquitous in white and yellow corn worldwide (Bezuidenhoudt et al. 1988; Gelderblom et al. 1988). Recently, these fungi have been identified as the source of the fumonisins mycotoxins. Swine consuming fumonisins are affected by the disease generally known as porcine pulmonary edema (PPE). Fumonisins are produced when corn is stressed by moderate drought followed by persistent rainfall or high humidity late in development (P. Nelson, personal communication). Corn screenings are the most likely source of fumonisin toxicosis (Harrison et al. 1990; Osweiler et al. 1992; Ross et al. 1991, 1992).

Fumonisins commonly present in corn are primarily FB₁, FB₂, and FB₃. They are water-soluble, heat-stable,
and alkaline-resistant aliphatic hydrocarbons with a terminal amine group and two tricarboxylic acid side chains (Steyn 1995). FB₁ and FB₂ are of approximately equal toxicity (P. F. Ross, personal communication), while FB₃ is nearly nontoxic to swine (G. D. Osweiler, unpublished data).

Mechanism of Action and Toxicity
Fumonisins are poorly absorbed orally (3–6% of ingested dose) and, once absorbed, are excreted readily and rapidly in bile and urine (Prelusky et al. 1994). Fumonisins inhibit the enzyme-mediated conversion of sphinganine to sphingosine, raising the sphinganine/sphingosine (SA/SO) ratio and potentially interfering with cell cycle control and cell function (Vos et al. 2007). FB₁ affects cell signaling proteins including protein kinase C (PKC), a serine/threonine kinase involved in a number of signal transduction pathways that include cytokine induction, carcinogenesis, and apoptosis (Gopee and Sharma 2004). FB₁ also appears to inhibit ceramide synthase in the sphingolipid signaling pathway on the ascending aortic impedance spectrum of pigs. This is associated with inhibition of myocardial L-type calcium channels, a decrease in cardiac contractility and mean systemic arterial pressure, decreased heart rate, and increased pulmonary artery pressure leading to left heart failure, and massive pulmonary edema and hydrothorax (Constable et al. 2003; Smith et al. 2000). Zomborszy-Kovács et al. (2002) reported that very low concentrations of FB₁ fed for 8 weeks resulted in chronic pulmonary changes of connective tissue proliferation in subpleural and interlobular connective tissue of lungs and in peribronchial and peribronchiolar areas.

More than 120 ppm dietary fumonisins for 4–10 days produces acute PPE (Colvin et al. 1993; Haschek et al. 1992; Osweiler et al. 1992). Surviving pigs develop subacute hepatic toxicosis 7–10 days later. Hepatosis results after feeding dietary levels >50 ppm for 7–10 days. Although 25 ppm or less causes no apparent clinical effects, mild microscopic hepatic lesions are documented for dietary levels as low as 23 ppm. The serum SA/SO ratio has been altered experimentally by diets containing 5 ppm fumonisins, although the clinical relevance of this change is not known (Moetlin et al. 1994; Riley et al. 1993). Fumonisins have been evaluated for their potential interactions with aflatoxins and DON. Effects of aflatoxins and fumonisins were found to be additive when fed together, except for the variables cholinesterase and ALP, which showed a synergistic response to aflatoxins and FB₁ (Harvey et al. 1999a). For a combination of FB₁ and DON, the effect on most variables was additive. However, for body weight, weight gain, hepatic weight, and mean corpuscular hemoglobin concentration, the responses were interactive in a greater-than-additive manner (Harvey et al. 1996).

Clinical Signs and Lesions
Dietary fumonisins greater than 120 ppm are likely to cause acute interstitial pulmonary edema and hydrothorax, with attack rates up to 50% and case fatality rates of 50–90%. Initially, there is lethargy, restlessness, depression, and dermal hyperemia. Mild salivation, dyspnea, openmouthed breathing, posterior weakness, recumbence, and moist rales develop rapidly, followed by cyanosis, weakness, and death. Initial signs begin after 4–7 days of continuous fumonisin consumption (Colvin and Harrison 1992; Osweiler et al. 1992). Once signs appear, death usually occurs in 2–4 hours. Survivors may develop hepatic disease. Feeding fumonisins at concentrations from 75 to 100 ppm for 1–3 weeks, without development of pulmonary edema, causes hepatic disease characterized by icterus, anorexia, ill thrift, and weight loss (Osweiler et al. 1993).

Serum chemistry analyses include elevated concentrations of GGT, AST, ALP, lactate dehydrogenase (LDH), cholesterol, and bilirubin. Early increases in the serum enzymes and cholesterol are followed by increased GGT and serum bilirubin accompanied by clinical icterus (Colvin et al. 1993; Osweiler et al. 1992).

Based on current evidence, fumonisins are considered moderate immunosuppressants in swine. One study has shown transient reduction in lymphocyte blastogenesis and delayed titer response to pseudorabies vaccine (Osweiler et al. 1993). Others have reported decreased lymphoblastogenesis (Harvey et al. 1995a, 1996). Tornynos et al. (2003) fed pigs a high FB₁ dose (100 mg/animal/day for 8 days) or low dose (1, 5, and 10 ppm) for 3–4 months and then vaccinated against Aujeszky’s disease with inactivated vaccine. No changes were seen in a nonspecific lymphocyte stimulation test (LST) or humoral immune response (specific antibody titer by virus neutralization). They concluded that FB₁ had no significant effect on the humoral and cellular specific and nonspecific immune response.

Lesions of pulmonary edema and hydrothorax occur with 200–350 mL of clear, cell-free, straw-colored thoracic transudate. Lungs are heavy and wet with wide seams (3–10 mm) of interlobular edema. Bronchioles, bronchi, and trachea are relatively clear with little alveolar edema (Colvin et al. 1993; Haschek et al. 1992; Moetlin et al. 1994; Osweiler et al. 1992; Palyusik and Moran 1994). Acidophilic, fibrillar material is found in alveoli and interlobular lymphatics, and hylalinized alveolar capillary thrombi may be present. Increased numbers of pulmonary intravascular macrophages (PIMs) filled with osmiophilic material are reported by electron microscopy—possibly a result of phagocytosis of damaged cellular components. Pancreatic necrosis and hepatitis with disrupted hepatic architecture, increased mitotic figures in hepatocytes, apoptosis, and single-cell hepatic necrosis are seen with subacute fumonisin toxicosis (Haschek et al. 1992). Chronic exposure can include hepatic
hyperplastic nodules and medial hypertrophy of small pulmonary arteries.

Abortions 1–4 days after acute spontaneous toxicosis are presumably due to fetal anoxia caused by severe pulmonary edema in the dam (Becker et al. 1995; Osweiler et al. 1992). Concentrations of 100 ppm FB1 fed in the last 30 days of gestation caused no pulmonary edema and did not cause abortion, fetal abnormalities, or infertility in sows (G. D. Osweiler, unpublished data).

Diagnosis
Clinical signs of acute respiratory distress with high mortality and lesions of interstitial edema and hydrothorax suggest fumonisin toxicosis. A history of consumption of corn screenings or poor-quality corn is typical. Serum chemistry changes and elevated serum SA/SO ratios are expected, and the acute liver enzymes usually peak at 4–7 days after initial exposure, while bilirubin and GGT continue to increase for 1–2 weeks if sublethal exposure continues. Serum SA/SO ratio is the most sensitive indicator of fumonisin exposure and appears unique to the fumonisins (Moetlin et al. 1994; Riley et al. 1993). However, this assay is currently not widely available as a diagnostic test. Assay for fumonisins in feeds or corn is routine in many veterinary diagnostic and private laboratories. Chemical analyses to detect fumonisins in tissues are not readily available, and the rapid metabolism and excretion rate of fumonisins generally precludes this mode of diagnosis (Pre-lusky et al. 1994).

Treatment and Management
There is no antidote. The acute and massive changes of PPE do not allow for effective symptomatic and supportive therapy. Because clinical signs appear after days to weeks of exposure, oral detoxification is usually not useful. Liver damage from fumonisin toxicosis may be lessened by appropriate supportive care.

Recently, Fernández-Surumay et al. (2005) demonstrated that a process binding fumonisins with glucose effectively prevents the development of clinical signs, lesions, and clinical chemistry changes of fumonisins. This finding is currently being evaluated for potential to develop a commercial or industrial-scale detoxification of contaminated corn.

Analysis of corn or feeds for fumonisins can identify a source and help in estimating the risk from a specific feedstuffs. Analysis of corn or feeds for fumonisins is routine in many veterinary diagnostic and private laboratories. Chemical analyses to detect fumonisins in tissues are not readily available, and the rapid metabolism and excretion rate of fumonisins generally preclude this mode of diagnosis (Pre-lusky et al. 1994).

Prevention and Management of Mold and Mycotoxin Problems
When mycotoxicosis occurs or is suspected, corrective actions should include a change of the source of feed even when a specific mycotoxin is not identified and a thorough inspection of grain storage bins, mixing equipment, and feeders for caking, molding, or musty odors. Remove all contaminated feed, clean equipment, and sanitize storage areas with hypochlorite (laundry bleach) to reduce contaminating fungi.

Analyze representative samples of feeds or feedstuffs for known mycotoxins. Although spore counts or fungal cultures alone do not confirm a diagnosis, they may give some indication of the potential for mycotoxin production.

If storage conditions are adverse or grain moisture is high, use a mold inhibitor such as sodium or calcium propionate to reduce or delay mold growth. Remember, mold inhibitors do not destroy preformed toxins.

Dilution of contaminated grain with clean grain is commonly used to reduce mycotoxin effects; but care must be taken that wet or contaminated grain does not introduce new fungi and conditions of contamination.

Since mycotoxin effects can be delayed, a prudent practice is to save a representative sample from each diet mixed until swine are marketed or at least a month beyond when the feed was consumed.

Samples of feeds or grain should be representative, taken after feed is ground and mixed, then passing a cup through the moving auger stream at frequent intervals, mixing samples thoroughly, and saving a 4.5-kg (10-lb) sample for analysis (Davis et al. 1980).

High-moisture samples should be either dried to 12% moisture or stored frozen. Long-term storage is recommended in paper bags permanently marked with the date and source of the feed or grain, and samples should be held in a dry, clean location.

Mycotoxins present formidable challenges to the swine producer and veterinarian. Diagnosis is sometimes difficult and effective therapy is virtually lacking. A sound and practical preventive program should be a part of every swine management system.

REFERENCES
Although modern confinement facilities, accurately formulated rations, and improved management practices have reduced some risks of poisoning, cases of poisoning in swine still occur. The occurrence of swine toxicoses associated with the environment, feed, or management practices is frequent enough to warrant their inclusion in differential diagnostic considerations for swine health problems. The following discussion summarizes the impact of potentially toxic agents to which swine may be exposed.

**ESSENTIAL MINERALS**

Most swine formula feeds are properly fortified with trace elements. However, some trace minerals may deliberately be added in excess for various reasons, including copper (Cu), selenium (Se), and occasionally iron (Fe) and zinc (Zn). The existence of concentrated premixes of these minerals raises the risk of feed mixing errors resulting in the accidental feeding of high, potentially toxic levels of these elements.

**Copper**

Dietary requirements of 5–6 ppm Cu have been established for swine. A dietary level of 250 ppm is generally considered the maximum tolerable level (MTL) of Cu for swine. Ration levels ranging from 300 to 500 ppm cause reduced growth and anemia. The tolerance to Cu is related positively to dietary levels of Fe and Zn. For example, animals consuming feed containing 750 ppm Cu are essentially normal if also supplemented with 750 ppm Fe and 500 ppm Zn. Copper in both organic and inorganic forms has been added to growing swine diets at concentrations as high as 134 ppm with positive effects on growth and health. Additive effects of higher levels of dietary copper and zinc in diets for nursery pigs has been demonstrated. Copper sulfate at 250 ppm and zinc oxide at 0 or 3000 ppm from days 0 to 14 and 0 or 2000 from days 14 to 28 has been shown to increase average daily gain, average daily feed efficiency, and feed/gain.

Copper toxicity can cause icterus, anemia, hemoglobinuria, and nephropathy associated with a hemolytic crisis in swine, although not as commonly as in sheep. Diagnosis can be suggested by clinical signs and a history of feeding excess Cu. Liver and kidney Cu levels greater than 250 and 60 ppm, respectively, on a wet-weight basis are diagnostically supportive.

**Iron**

The recommended dietary levels of Fe range from 40 to 150 ppm, the highest requirements being in the youngest pigs. Many factors influence the risk of Fe toxicosis. Elemental Fe and iron oxides are relatively nontoxic, whereas iron salts are more toxic. Dietary phytate, phosphate, cobalt (Co), Zn, Cu, manganese (Mn), and disaccharides competitively depress Fe absorption. Ascorbic acid, sorbitol, fructose, and several amino acids improve Fe absorption, which is facilitated by being chelated with citric, lactic, pyruvic, and succinic acids; Fe chelated by desferrioxamine is poorly absorbed.

Pigs fed 1100 ppm Fe as a salt have shown reduced weight gains. Animals fed 5000 ppm have displayed depressed feed intake and rates of gain as well as rickets characterized by hypophosphatemia and reduced bone ash. The condition has not been prevented by providing 0.92% dietary phosphorus (P). High single doses of iron salts will cause gastroenteritis, followed by apparent recovery and then, frequently, collapse and death.
within 2 days. Diagnosis may be facilitated by consider-
ation of history, clinical signs, and necropsy changes. Feed and serum should be analyzed for Fe. Normal serum Fe levels are approximately 100 mg/dL and will increase during toxicosis. Iron toxicosis should be differ-
etiated from other forms of rickets.

Injections of Fe, usually as the dextran, have caused intoxications characterized by cardiovascular shock and death within hours after administration as well as staining at injection sites and in regional lymph nodes, liver, and kidneys. The incidence of this acute toxicosis appears to be decreasing.

There is no practical individual treatment for Fe toxicosis. Desferrioxamine (Desferal) may be used in selected cases. Dietary imbalances should obviously be corrected.

**Selenium**
The recommended dietary level of Se varies from 0.1 to 0.3 ppm. Selenium, as the selenate or selenite, is approved for addition to swine feeds at up to 0.3 ppm. Accidental oversupplementation of swine feeds with selenium premixes has been a sporadic problem, especially when mulberry heart disease has been diagnosed in a nursery.

Selenium levels of 5–8 ppm fed to growing swine have caused anorexia, alopecia, separation of hooves at the coronary band, and degenerative changes in the liver and kidneys. Liver lesions may look remarkably like those described for vitamin E–selenium deficiency (hepatosis dietetica). A level of 10 ppm fed to breeding sows has caused retarded conception and pigs dead or weak at birth. Misformulated feeds containing 10–27 ppm Se produced a paralytic disease in growing swine characterized by quadriplegic or posterior paralysis while the pigs remained mentally alert and continued to eat and drink. Focal symmetrical poliomyelomalacia was found in affected swine (Casteel et al. 1985; Harrison et al. 1983).

Several injectable products containing varying concentrations of Se are currently available for treatment or prevention of Se-responsive diseases. Death losses have approached 100% when Se overdose occurred from the mistaken use of a more concentrated product or from miscalculation of the recommended dosage. The minimum lethal dose of injectable Se is about 0.9 mg/kg body weight, with pigs that are Se deficient being the most susceptible to toxicosis (Van Vleet et al. 1974). Weakness and dyspnea progressing to irregular gasps and death occur within 24 hours of the parenteral overdose.

Diagnosis of Se toxicosis in swine can be made by consideration of a history of Se supplementation, clinical signs, necropsy findings, and chemical analysis of tissues and feeds. Liver and kidney Se concentrations greater than 3 ppm (wet weight) are expected with toxicosis.

**Zinc**
Recommended dietary levels of Zn for swine vary from 15 to 100 ppm based on age, sex, stage of production, and other ration components. A level of 2000 ppm Zn produced growth depression, arthritis, intramuscular hemorrhage, gastritis, and enteritis. The MTL is probably less than 300 ppm, possibly because zinc salts in large concentration are unpalatable. Zinc interacts competitively for absorption with Fe, Ca, and Cu. Pigs fed 268 ppm Zn developed arthritis, bone and cartilage deformities, and internal hemorrhages. However, feeding 3000 ppm Zn as zinc oxide for 14 days has shown increased weight gains and reduction of postweaning scours without adverse signs. Diagnostic considerations should include clinical signs, history, and chemical analyses of feed and tissues. Normal kidney and liver levels of Zn are 25–75 ppm (wet weight) and may increase during toxicosis. However, excretion is quite rapid.

**NONESSENTIAL MINERALS**

**Arsenic**
Inorganic arsenicals, which are distinctly different from the phenylarsionic feed additives discussed later, have been used in antiquated ant baits, herbicides, insecticides, and some animal medications. Pigs are relatively resistant to inorganic arsenic (As) poisoning with 100–200 mg/kg body weight of sodium arsenite being a lethal oral dose. This is equivalent to about 2000–4000 ppm in the feed. However, pigs have refused to consume 1000 ppm in the feed. Clinical signs of acute As poisoning are colic, vomiting, diarrhea, dehydration, collapse, convulsions, and death within hours to days. Prominent necropsy findings are dehydration and severe hemorrhagic gastritis and enteritis with sloughing of mucosa and edema. Diagnostic considerations should include history, clinical signs, lesions, and chemical analyses. Kidney and liver tissues levels of 10 ppm (wet weight) are significant. Prognosis is generally poor and depends on the degree of tissue damage and dehydration.

**Fluorine**
Fluorosis may be observed in animals consuming water or forages contaminated by nearby industrial plants or eating crops raised on soils high in fluorine (F). A common source is consumption of minerals high in F. Feed-grade phosphates by law must contain no more than one part F to 100 parts P. It is recommended that swine be fed feeds containing no more than 70 ppm F during their lifetime to prevent fluorosis. Sodium fluoride has been used as an ascaricide at levels of 500 ppm; higher levels have caused vomiting. Other signs of acute toxicosis are diarrhea, lameness, tetany, collapse, and death.
A tentative diagnosis of chronic fluorosis may be difficult, since the lameness may appear similar to rickets, mycoplasmosis, and erysipelas. Normal bone F levels in swine are 3000–4000 ppm. Higher levels are associated with fluorosis. Normal urine F levels are 5–15 ppm; higher levels are diagnostically significant. Necropsy findings may reveal exostoses on the long bones and tooth mottling. Treatment should be aimed at reducing dietary F and feeding aluminum or calcium (Ca) mineral supplements.

**Lead**
Swine are quite resistant to elevated lead (Pb) exposure. Consequently, field cases of lead poisoning in swine are extremely rare. Experimentally, pigs fed 35.2 mg lead (as the acetate)/kg body weight for 90 days did not die from lead poisoning despite blood lead concentrations of up to 290 µg/dL. (Lassen and Buck 1979). If toxicosis is suspected, a thorough diagnosis workup including kidney and liver analyses should be performed.

**Mercury**
Mercury (Hg) has been used in paints, batteries, paper, and fungicides, but most uses have now been restricted. All mercurial compounds are toxic, but organic forms are the most toxic to all animal species. Mercury is cumulative, and toxicity depends on form, dose, and duration. Swine have been poisoned after consuming seeds treated with organic mercurial fungicides.

Initially, signs of gastroenteritis may be evident, followed by uremia and central nervous system (CNS) disturbance, including ataxia, blindness, aimless wandering, paresis, coma, and death. Mercury toxicosis may be confused with erysipelas, cholera, or poisoning by pigweed or phenylarsonics. Clinical signs, history, necropsy findings, and chemistry should aid in the diagnosis. Kidney and liver normally contain less than 1 ppm Hg but will contain much higher levels following Hg toxicosis. Treatment is usually disappointing.

**FEED ADDITIVES**
Adverse effects of drug additives are rare except in cases of misuse or misformulation of rations (Lloyd 1978). Details of specific drug effects have been reviewed (Brown 1996).

**Phenylarsonic Compounds**
The phenylarsonic compounds, occasionally referred to as organic arsenicals, have at times been used as growth promotants and for treatment of swine dysentery or erythrocytosis. Arsanilic acid and roxarsone (3-nitro-4-hydroxyphenylarsonic acid) have been used in swine rations, and their sodium salts have been used in drinking water. Arsanilic acid use in complete swine rations is at levels ranging from 50 to 100 ppm (45–90 g/t). Currently, availability is very limited and toxicosis would not be expected unless old sources are used off-label.

Clinical signs of arsanilic acid toxicosis start within a few days at feed levels of 1000 ppm, 2 weeks at 400 ppm, and 3–6 weeks at 250 ppm. Clinical signs include ataxia, posterior paresis, blindness, and quadriplegia. Paralyzed animals will continue to live and grow if provided food and water. Swine that receive lower doses for extended periods are prone to develop hypermetria or “goose stepping” and total blindness from sciatic and optic nerve damage, respectively. Swine that receive very large doses, for example, 10,000 ppm in the ration, may exhibit a gastroenteritis resembling poisoning by inorganic arsenic compounds.

Roxarsone can be used continuously in swine rations at levels of 22.7–34.1 ppm or at 181.5 ppm for 5–6 days. Poisoning may result with feed roxarsone levels of 250 ppm or more from 3 to 10 days. Clinical signs include uncontrolled urination and defecation as well as muscle tremor and convulsive seizures, all of which are induced by physical stimulation. Ataxia may be observed, although not as severe as the “drunken sailor” incoordination of arsanilic acid toxicosis. In advanced stages, pigs show paraparesis and paraplegia, but will continue to eat and drink.

Clinical signs and a history of administration of arsenicals in feed or water may be the best basis for suspecting phenylarsonic toxicosis. Necropsy findings are generally unremarkable, but histopathological examination of peripheral nerves, especially the sciatic, may reveal demyelination.

Chemical analysis of tissues for the specific phenylarsonic compounds may not be helpful, since the compounds are excreted within a few days after withdrawal. However, analysis of kidney, liver, muscle, and feed for As may assist in the diagnosis. Elemental arsenic levels (wet weight) greater than 2 ppm in the kidneys and liver and 0.5 ppm in the muscle are illegal and indicative of excess As intake. Further analyses of feed for the specific phenylarsonic compound will provide more diagnostic evidence. Deficiencies of B-complex vitamins, especially pantothenic acid and pyridoxine, may cause a similar demyelination of peripheral nerves, as can the effects of a delayed organophosphate such as ortho-cresyl-phosphate. Chronic phenylarsonic toxicosis may also resemble rickets. Sodium ion toxicity, organic mercurial poisoning, and certain viral diseases that affect the CNS may be confused with phenylarsonic compound toxicosis. Toxicoses are reversible if arsenicals are promptly removed from the feed and water.

**Carbadox**
Carbadox (Mecadox), where available, is added to feed at 10–25 ppm as a growth promotant or at 50 ppm to control swine dysentery or bacterial enteritis. A feed
level of 100ppm has caused decreased feed consumption and growth retardation. Higher levels have caused feed refusal and emesis. Mild lesions in the glomerular zone of the adrenal cortex are reported with 50ppm carbadox in the feed for 10 weeks, while more extensive lesions are seen at feed levels of 100–150ppm after 5 weeks of consumption (Van der Molen 1988). When fed a ration containing 331–363ppm carbadox, recently weaned pigs refused to eat and showed poor weight gains; posterior paresis; the passing of hard, pelleted feces; and death in 7–9 days (Power et al. 1989).

Dimetridazole
Dimetridazole, listed as an anti-histomoniasis drug used in turkey rations, was once used for treatment and prevention of swine dysentery but is now illegal for use in swine in the United States. A level of 1500ppm has caused no toxicosis, but 17,000ppm have caused diarrhea in swine. Large overdoses of dimetridazole would cause ataxia, bradycardia, dyspnea, salivation, muscle spasms, prostration, and death. Death or recovery would be rapid.

Monensin and Lasalocid (Ionophore)
Monensin is marketed as Rumensin for use in cattle or as Coban as poultry coccidiostat. Use levels are up to 120ppm for poultry and 44ppm in cattle feeds; some premixes may contain up to 440ppm. Swine may be fed monensin by mistake, but the drug is not highly toxic to them. The greatest risk of poisoning from monensin in swine appears to be with the concurrent administration of tiamulin, an antibiotic commonly used in swine, which potentiates the effect of monensin (Van Vleet et al. 1987). Swine poisoned from this combination show acute massive necrotizing myositis of the skeletal muscles, myoglobinuria, and acute death.

Pigs fed monensin levels ranging from 11 to 120ppm in the feed for 112 days were not affected, nor was feed consumption or weight gain altered. Gilts fed 110–120ppm had a transient anorexia for 14 days; thereafter, only weight gains were depressed. The dose expected to be fatal for 50% of animals exposed (LD50) of monensin in swine is 16.8mg/kg. Pigs suffering from monensin toxicosis showed openmouthed breathing, frothing around the mouth, ataxia, lethargy, muscle weakness, and diarrhea. These signs were visible within 1 day of exposure and persisted for about 3 days. Myocardial and skeletal muscle necrosis was present in pigs receiving 40mg monensin/kg (Van Vleet et al. 1983).

Lasalocid is a polyether antibiotic marketed as Bovatec for feedlot cattle and Avatec for poultry to improve feed efficiency and weight gains. Swine fed lasalocid at 2.78 and 21mg/kg showed no adverse effects. However, transient muscle weakness occurred at a dose of 35mg/kg (equivalent to about 1000ppm of lasalocid in the feed), and death occurred at 58mg/kg when fed for 1 day. Additional ionophores used as feed additives include salinomycin marketed as Bio-Xox or Sacox and laidlomycin marketed as Cattlyst.

Sulfonamides
Overdoses of sulfonamides, which are antibacterials commonly used in swine medicine, will cause crystalluric nephroses. Pigs are not likely to be intoxicated from drinking water containing sulfonamides because of the lack of palatability, but overdosing in the feed, coupled with low water intake, may cause nephrosis and uremia. The only feed-grade sulfonamides labeled for swine in the United States are sulfathiazole and sulfamethazine. Nephrotic mycotoxicoses such as those caused by citrinin and ochratoxin will predispose sulfonamide toxicoses. Sulfonamide residues in pork are related to persistence of the drugs in feed and excreta and are not a toxicosis.

Urea and Ammonium Salts
Swine may be fed cattle feeds containing nonprotein nitrogen compounds such as urea and ammonium salts. Urea is relatively nontoxic for swine, a level of 2.5% causing only reduced feed intake and growth rate, elevated blood urea nitrogen (BUN), polydypsia, and polyuria. Higher levels of urea should not cause signs of acute toxicosis. Ammonia and ammonium salts are toxic for swine, however, with individual doses of 0.25–0.5g/kg body weight causing intoxication and doses of 0.54–1.5g/kg being lethal. Considering that growing swine consume feed equal to 5–10% of their body weights, the expected toxic and lethal levels of ammonium salts are 0.25–1% and 1.5–3%, respectively. Pigs poisoned with ammonia and ammonium salts would be expected to become depressed, have tonicclonic convulsions, and either die or recover within a few hours.

Ractopamine
Ractopamine is a beta-2 agonist used as a feed additive (4.5–9g/t of complete feed) during the last 6 weeks of finishing to stimulate muscle growth in swine. Beta-2 agonists have the potential for adverse effects, including tachycardia, hypotension, tremors due to overstimulation of skeletal muscle beta-2 receptors, behavioral changes of anxiety or restlessness, weakness or lethargy, and hypokalemia (Rosendale 2004).

PESTICIDES: INSECTICIDES
Concurrent production of both livestock and crops on the same premises may provide a unique opportunity for exposure of swine to agricultural chemicals. Among the chemicals presenting the greatest potential hazard of poisoning are the organophosphorus (OP), carbamate, and the older chlorinated hydrocarbon (CH) insecticides.
Poisoning may occur when insecticides are accidentally incorporated into swine feed. Discarded or unlabeled portions of granular insecticides can be mistaken for mineral mixes or dry feed ingredients and added to swine feeds. When farm equipment used for feed handling is also used for insecticide transportation, contamination of this equipment may result in insecticides being inadvertently mixed into animal feeds. In addition, swine may have accidental access to insecticides when they are stored or spilled on the farm premises. Improperly operating back rubbers and oilers may provide an additional source of these insecticides for livestock.

Miscalculation of insecticide concentrations in spraying, dipping, and pour-on procedures may also result in toxicosis. Retreating animals with OP or carbamate preparations within a few days' time may result in poisoning.

Organophosphorus and Carbamate Insecticides

The OP and carbamate insecticides are discussed together because of their similar mechanisms of action. Cholinergic nerves utilize acetylcholine as a neurotransmitter substance. Under normal conditions, acetylcholine released at the synapses of parasympathetic nerves and myoneural junctions is quickly hydrolyzed by cholinesterase enzymes. When the hydrolyzing enzymes are inhibited, the continued presence of acetylcholine maintains a state of nerve stimulation and accounts for the clinical signs observed with poisoning from these insecticides.

The clinical syndrome produced by OP and carbamate insecticides is characterized by a rapidly progressing overstimulation of the parasympathetic nervous system and skeletal muscles. Earliest clinical signs of acute poisoning frequently include mild to profuse salivation, defecation, urination, emesis, stiff-legged or “sawhorse” gait, and general uneasiness. As the toxicosis progress, signs observed include profuse salivation; gastrointestinal hypermotility resulting in severe colic and vomiting (especially common in swine); abdominal cramps; diarrhea; excessive lacrimation; miosis; dyspnea; cyanosis; urinary incontinence; muscle tremors of the face, eyelids, and general body musculature; and acute death. Hyperactivity of the skeletal muscles is generally followed by muscular paralysis, as the muscles are unable to respond to continued stimulation. Swine may exhibit increased CNS stimulation but rarely, if ever, convulsive seizures. More commonly, severe CNS depression occurs. Death usually results from hypoxia caused by excessive respiratory tract secretions; bronchoconstriction; and erratic, slowed heartbeat. The onset of clinical signs of acute poisoning may appear within a few minutes in severe cases to several hours in milder ones.

Lesions associated with acute OP or carbamate toxicosis are usually nonspecific but may include excessive fluids in the respiratory tract as well as pulmonary edema.

Diagnosis is usually by history of exposure to OP or carbamate insecticides associated with clinical signs of parasympathetic stimulation warrants a tentative diagnosis of poisoning with these compounds. Chemical analyses of animal tissues for the presence of insecticides are usually unrewarding because of the rapid degradation of OP and carbamate insecticides, resulting in low tissue residue levels. However, finding the insecticide in the stomach contents and the feed or suspect material can be quite valuable in establishing a diagnosis.

In addition, the degree of inhibition of cholinesterase enzyme activity in the whole blood and tissue of the suspected animal should be assessed. A reduction of whole-blood cholinesterase activity to less than 25% of normal is indicative of excessive exposure to these insecticides. The cholinesterase activity level in the brain tissue of animals dying from these insecticides will generally be less than 10% of the normal brain activity. Whole-blood and brain samples should be well-chilled but not frozen for best laboratory results. Samples of stomach contents as well as the suspect feed or material should be submitted to a laboratory for chemical analysis.

Treatment of animals poisoned by OP or carbamate insecticides should be considered an emergency because of the rapid progression of respiratory distress in the clinical syndrome. Initial treatment for poisoned swine should be the intramuscular use of atropine sulfate at approximately 0.5 mg/kg body weight. One-quarter of this dose may be given intravenously for a quick response in especially severe cases. Atropine does not counteract the insecticide–enzyme bond but blocks the effects of accumulated acetylcholine at the nerve endings. Although a dramatic cessation of parasympathetic signs is generally observed within a few minutes after administration of atropine, it will not affect the skeletal muscle tremors. More atropine at approximately one-half the initial dose may be required but should be used only to control recurring parasympathetic signs. Although the use of atropine alone is generally adequate, especially if vomiting has occurred, specific cases may warrant the use of pralidoxime chloride or activated charcoal.

Oral activated charcoal is recommended for treatment of any ingested insecticide to reduce continued absorption of the insecticide from the gut. Although a useful treatment, the need for activated charcoal in swine may be reduced when vomiting helps empty the gut and thereby reduces further absorption of the insecticide.

The use of the oximes (e.g., TMB-4, 2-PAM, pralidoxime chloride) in large animals, although efficacious,
may be economically unfeasible. If used, pralidoxime chloride is recommended at a dose of 20 mg/kg body weight. The oximes are of no benefit in treating carbamate toxicoses.

Dermally exposed animals should be washed with soap and water to prevent continued absorption of these compounds.

Morphine, succinylcholine, and phenothiazine tranquilizers should be avoided in treating OP poisoning.

**Chlorinated Hydrocarbons**
The CH insecticides (e.g., toxaphene, chlordane, aldrin, dieldrin, and lindane) produce toxicosis in swine by acting as diffuse but powerful stimulants of the CNS. Even though these are old products that have been restricted from the market for over 30 years, improperly discarded leftover products may become available to animals when older barns or storage areas are cleaned out.

Clinical signs often appear 12–24 hours after exposure. Initially, animals may appear apprehensive. A period of hyperexcitability and hyperesthesia characterized by exaggerated responses to stimuli and spontaneous muscle spasms is usually observed. The spontaneous tremors and fasciculation are usually in the facial region and involve the lips, muscle, eyelids, and ears, progressing caudally to involve the heavy muscles of the shoulder, back, and hindquarters. These spasms may progress into a tonicclonic convulsive seizure. Abnormal posturing, elevation of the head, and chewing movements may be observed. Varying degrees of respiratory paralysis occur during the seizures, with periods of depression and inactivity between successive seizures. The rapidity of onset and severity of clinical signs provide a poor index of the prognosis of the episode in individual animals. Occasionally, animals will die during seizures, while others may completely recover following several severe episodes.

Diagnosis is tentatively made by observation of clinical signs of hyperexcitability and tonicclonic convulsive seizures, along with a known exposure to CH insecticides. Specific lesions other than those from the physical trauma of the seizures are not observed. Demonstrating the presence of significant levels of CH insecticide in the liver, kidney, and brain tissue is essential for confirming a diagnosis. Samples of these tissues as well as stomach contents and suspect material such as feed or spray should be submitted to a laboratory. Avoid contamination of specimens with hair or gut contents to prevent erroneous analytic results. Laboratory tests are usually required to differentiate this toxicosis from pseudorabies, water deprivation, or edema disease.

Treatment is essentially symptomatic, since there is no specific antidote for the CH insecticides. Animals should be sedated with long-acting barbiturates to control convulsive seizures. Animals with dermal exposure should be washed with warm, soapy water to remove the chemical and prevent continued contact. If the chemical is orally ingested, activated charcoal in a water slurry may be used to prevent further absorption. Oil-based cathartics should be avoided, as they may hasten absorption of the chemicals. Intravenous fluids plus glucose may be needed in protracted cases.

Animals dying from CH insecticide toxicosis are a source of contamination for feed ingredients such as tankage, meat and bone meal, and fats because of the persistence of CH insecticides and their concentration in fat deposits of the carcasses. Therefore, proper disposal of contaminated carcasses is very important. Tissue residues of these chemicals in swine surviving an episode of insecticide exposure should be an important consideration in market animals. In some cases, the time required for excretion of these residues is too long to make decontamination economically feasible.

**Synthetic Pyrethroids**
Several synthetic pyrethroids (e.g., permethrin, fenvalerate) are commercially available for fly and external-parasite control. As a class, the synthetic pyrethroids are relatively nontoxic to mammals and are unlikely to produce poisoning in swine.

**Formamidines**
Amitraz is a formamidine pesticide with insecticidal and acaricidal properties. It is available in the United States as Taktic for control of lice and mange on swine. This compound has low mammalian toxicity and is unlikely to produce toxicosis in swine.

**Neonicotinoids**
The neonicotinoids were developed in the late 1970s and are used as insecticides. Imidacloprid is the most common insecticide in this class and has a wide safety margin. These insecticides act on the postsynaptic nicotinic receptors and unlikely to produce toxicosis in swine.

**Phenylpyrazoles**
Fipronil is a member of the phenylpyrazole class of pesticides. These compounds target the gamma-aminobutyric acid (GABA)-regulated chloride channels. These are very safe compounds and have LD₅₀’s of greater than 97 mg/kg in rats—unlikely to produce toxicosis in swine.

**FUNGICIDES**
Captan has been widely used as a seed treatment although some newer fungicides are now being employed. Field corn seed produced commercially in the United States has generally been treated with captan at a level of approximately 1000 ppm. Consumption of captan-treated seed corn represents little...
hazard of poisoning, as the acute lethal dose of captan for livestock is greater than 250 mg/kg body weight.

The organomercurials include phenyl mercuric chloride, phenyl mercuric acetate, various aliphatic compounds such as ethyl mercuric chloride, and complex aromatic derivatives like hydroxy mercuric cresol. The toxicoses associated with mercury-based seed treatments are discussed above, in the section on mercury.

Pentachlorophenol (PCP) has been employed for over 45 years as a wood preservative and fungicide. PCP- or “penta”-treated wood has found application in livestock handling and housing facilities, where wood is in contact with soil, manure, or moisture. Acute poisoning is not a major problem from PCP-treated wood, although toxicosis, including stillborn pigs, may occur when livestock have contact with surfaces that have been freshly treated with PCP preparations (Schipper 1961). A single oral dose of 80 mg/kg was not fatal to a weanling pig. If toxicosis occurs, depression, emesis, muscular weakness, accelerated respiratory rate, and posterior paralysis are clinical signs that may be observed. A problem of greater concern may be the recognition of blood and tissue PCP residues in swine that have been in contact with PCP-treated facilities. Finding from 10 to 1000 ppb PCP in whole blood is apparently unrelated to manifestations of toxicosis.

Chromated copper arsenate (CCA) is widely used as a wood preservative in consumer lumber intended for outdoor use. CCA-treated wood generally presents a negligible hazard to swine, as the metallic salts are bound in the wood fibers. However, the residual inorganic arsenic in the ashes of burned CCA wood can produce poisoning in swine. Effective December 31, 2003, no wood treater or manufacturer may treat wood with CCA for residential uses, with certain exceptions according to the U.S. Environmental Protection Agency (EPA).

**HERBICIDES**

Organic and selective herbicides are commonly used for control and elimination of noxious weeds. Toxicity from consumption of treated plants or overspray is rare; most are associated with human error or accidental ingestion of concentrates or sprays.

Chlorophenoxy herbicides (e.g., 2,4-D; 2,4,5-T; MCPA, Silvex, dicamba) are selective herbicides widely used in crop production and pasture and range management. Because the toxic dose of 2,4-D and 2,4,5-T is greater than 300 mg/kg body weight for several days, the hazard of poisoning by these compounds under normal conditions of use is low. When large doses have been administered experimentally, depression, anorexia, weight loss, muscular weakness, and incoordination have been observed.

Amide herbicides (e.g., thiomide, alilochlor, propa-nil) may cause anorexia, salivation, depression, and prostration. The toxic doses are quite high, making intoxication under typical field conditions quite rare. Other classes of herbicides (e.g., glyphosates, triazines, and benzoic acid derivatives) are relatively nontoxic.

Dipyridal herbicides (e.g., diquat, paraquat) are plant-desiccant types of herbicide and have found widespread application in no-till farming. Accidental, as well as malicious, poisoning of swine with paraquat has resulted in toxicosis. An approximate lethal dose of paraquat for swine is 75 mg/kg. Acute effects involving necrosis and erosion of the oral and gastric mucosa are attributed to the carrier solvent. The more classic effects, however, occur 7–10 days after ingestion and are characterized by pulmonary congestion and edema. The pulmonary lesions progress to a severe diffuse interstitial pulmonary fibrosis. Initial clinical signs include emesis and diarrhea, and the later stages are characterized by respiratory distress. Once clinical signs suggesting pulmonary involvement develop, therapeutic measures are usually futile.

**RODENTICIDES**

The rodenticides are used to control rat and mouse populations in or around farmsteads, feed storage areas, and swine production facilities. Accidental access to these compounds constitutes the usual route of exposure, although malicious poisoning of swine with rodenticides has also occurred.

**Anticoagulant Rodenticides**

The anticoagulant rodenticides (e.g., warfarin, diphacinone, chlorophacinone, bromadiolone, brodifocoum, pindone) compose the largest group of rodenticides available through retail outlets. Swine are quite susceptible to this class of compound, as evidenced by toxicosis occurring after a single oral dose of warfarin at 3 mg/kg body weight. Repeated oral doses of only 0.05 mg/kg/day for 7 days also produced toxicosis in swine (Osweiler 1978). These rodenticides produce lowered prothrombin levels by interfering with vitamin K utilization. The physiological result is increased blood-clotting time, which is manifested clinically as mild to severe hemorrhage. The clinical signs—including lameness, stiffness, lethargy, recumbency, anorexia, and dark tarry feces—are related directly to extravasation of blood. Observed lesions include hema-toma, articular swelling, epistaxis, intermuscular hemorrhage, anemia, and melena.

A diagnosis of anticoagulant rodenticide toxicosis should include demonstration of a defect in the clotting mechanism as evidenced by increases in clotting time, one-stage prothrombin time, or activated partial thromboplastin time. The chemical detection of the rodenticide in samples of blood, liver, or suspect baits is also helpful.
Injectable vitamin K and oral vitamin K supplements are included in a successful treatment regime. Whole-blood transfusions may be utilized successfully in special cases.

**Strychnine**

Strychnine, an indole alkaloid, is available commercially, often as either a green- or red-dyed pellet or grain or as a white powder. This alkaloid acts by selectively antagonizing certain types of special inhibitory neurons, thereby allowing uncontrolled and relatively diffuse reflex activity to proceed unchecked. The approximate oral lethal dose of strychnine for swine varies from 0.5 to 1 mg/kg body weight.

Clinical signs appear within 10 minutes to 2 hours after ingestion and are characterized by violent tetanic seizures that may occur spontaneously or in response to external stimuli such as touch, light, or sound. The intermittent seizures are usually separated by periods of relaxation. Death occurs from anoxia and exhaustion during the seizures, often in less than 1 hour.

Diagnosis is best confirmed by detection of the strychnine alkaloid in either the stomach contents or urine. Treatment consists of controlling seizures with long-acting barbiturates and other muscle relaxants.

**Cholecalciferol**

Rodenticides containing cholecalciferol (vitamin D₃) are commercially available as Rampage, Quintox, or Ortho Rat-B-Gone. Toxic doses of these products produce vitamin D poisoning with hypercalcemia, mineralization of soft tissues, and clinical signs of depression, weakness, nausea, anorexia, polyuria, and polydipsia.

**Bromethalin**

Bromethalin-based rodenticides, marketed as Assault, Vengeance, or Trounce, produce cerebral edema and signs of rear-leg ataxia and/or paresis and CNS depression. Hyperexcitability, muscle tremors, and seizures are characterized by hydropic degeneration and coagulative necrosis of both proximal and distal convoluted tubules. Glomeruli may be shrunken, with dilation of Bowman’s capsules. Proteinaceous casts are numerous in distal and collecting tubules.

As a consequence of severe renal disease, there are elevations in BUN, serum creatinine, and serum potassium. The electrocardiograph of affected swine is characteristic of hyperkalemic heart failure (Osweller et al. 1969). Immediate removal of affected pigs from the source of the weeds is the only definite therapeutic recommendation that can be made at this time.

**Xanthium Spp. (Cocklebur)**

Cockleburs, including *Xanthium strumarium* and other species, are annual herbs that reproduce only from seed. They may be found throughout the world in cultivated fields, fencerows, and ditches and may heavily infest pastures as a result of being washed in from adjacent cropland.

The greatest potential for cocklebur poisoning arises when the more toxic two-leaf seedling stage or ground seeds are ingested. The unpalatable more mature plant contains less of the toxic principle, carboxyatractyloside. Within 8–24 hours after ingestion, swine develop signs of depression, nausea, weakness, ataxia, and subnormal temperature. Spasms of the cervical muscles, vomiting, and dyspnea may occur. Death occurs within several hours after the onset of signs.

Lesions typically include ascites with large fibrin strands on the surface of the liver and other visceral congestion and centriflobular accentuation of the liver. Microscopically acute centrilobular hepatic necrosis is observed (Stuart et al. 1981). Treatment includes mineral oil per os to delay absorption of the carboxyatractyloside. Intramuscular injection of 5–30 mg physostigmine may produce a dramatic response in some cases (Link 1975).
**Solanum Nigrum (Black Nightshade)**

Even though black nightshade is easily recognized and found in the woods, permanent pastures, and fencerows, actual cases of poisoning are rare. The alkaloid solanine is found principally in the leaves and green berries, but the plant is generally not palatable and is usually consumed under conditions of its abundant growth and lack of other suitable forage.

Affected animals display anorexia, constipation, depression, and incoordination. Poisoned swine may vomit. Dilation of the pupils and muscular trembling are neurological signs observed. Animals may be seen lying on their sides and kicking with all feet, progressing then to coma and death. Necropsy may reveal some degree of gastrointestinal irritation. The toxic alkaloid is rapidly eliminated through the urine (Kingsbury 1964).

**Nitrates and Nitrites**

As monogastric animals, swine are relatively resistant to the effects of nitrate, especially when compared with cattle. Nitrate or nitrite toxicosis occurs most commonly when these ions accumulate in either plants or water sources. Some fertilizers, such as ammonium nitrate or potassium nitrate, may also be a source of nitrate for animals. Several different plants may accumulate nitrate, depending on varying climatic and soil fertility conditions. Nitrate may accumulate in the lower stalk and perhaps leaves of corn and other plants, but not in the fruit or grain.

The nitrates from both water (see the section on “Water Quality”) and plant sources are additive and should be evaluated together. The nitrate ion (NO₃⁻) itself is not particularly toxic and may produce no more than gastrointestinal irritation. However, nitrite (NO₂⁻), the reduced form of nitrate, is quite toxic. The nitrite ion oxidizes ferrous iron in hemoglobin to the ferric state, forming methemoglobin, which cannot accept and transport molecular oxygen. The result is tissue hypoxia from poorly oxygenated blood.

Pigs given single oral doses of greater than 10–20 mg nitrite-nitrogen (as potassium nitrite)/kg body weight developed clinical signs of poisoning but recovered, whereas those given doses greater than 20 mg nitrite-nitrogen/kg body weight died within 90–150 minutes after ingestion (London et al. 1967). Clinical signs became apparent when approximately 20% of the total hemoglobin was present as methemoglobin; death was associated with methemoglobin levels of approximately 80%.

Clinical signs observed with acute nitrite toxicosis include increased respiratory rate, salivation, miosis, polyuria, weakness, ataxia, and terminal anoxic convulsive seizures. The blood and tissues are a chocolate brown color from the methemoglobin. Treatment of acute nitrite toxicosis consists of intravenous injection of 10 mg methylene blue/kg body weight in a 4% solution (Link 1975).

**WATER QUALITY**

Water is one of the most important nutrients for swine. The availability of adequate quantities of good-quality water is essential for successful swine production. Even though it may be easy to incriminate water as the cause of poor performance and vague disease conditions in swine, water evaluation should be part of a thorough diagnostic investigation. A detailed history of the animals as well as the water source, careful clinical evaluation, and submission of representative animal and water specimens should be included in the investigation. Results of water tests should be evaluated in view of existing standards for livestock water quality. Some general guidelines for evaluating water quality parameters for livestock are presented in Table 70.1.

Information about the source of the water should be recorded. Ponds, wells, and regional rural water systems are the most common water sources, and each may influence the quality of the water supplied. The depth of wells may also be helpful, as deeper wells tend to have a higher mineral content, while shallower wells

<table>
<thead>
<tr>
<th>Item</th>
<th>Maximum Recommended Limit (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium</td>
<td>1000</td>
</tr>
<tr>
<td>Nitrate + nitrite</td>
<td>100</td>
</tr>
<tr>
<td>Nitrite alone</td>
<td>10</td>
</tr>
<tr>
<td>Sulfate</td>
<td>1000</td>
</tr>
<tr>
<td>Total dissolved solids</td>
<td>3000</td>
</tr>
<tr>
<td>Aluminum</td>
<td>5.0</td>
</tr>
<tr>
<td>Arsenic</td>
<td>0.5a</td>
</tr>
<tr>
<td>Beryllium</td>
<td>0.1b</td>
</tr>
<tr>
<td>Boron</td>
<td>5.0</td>
</tr>
<tr>
<td>Cadmium</td>
<td>0.02</td>
</tr>
<tr>
<td>Chromium</td>
<td>1.0</td>
</tr>
<tr>
<td>Cobalt</td>
<td>1.0</td>
</tr>
<tr>
<td>Copper (swine)</td>
<td>5.0</td>
</tr>
<tr>
<td>Fluoride</td>
<td>2.0c</td>
</tr>
<tr>
<td>Iron</td>
<td>No guideline</td>
</tr>
<tr>
<td>Lead</td>
<td>0.1</td>
</tr>
<tr>
<td>Manganese</td>
<td>No guideline</td>
</tr>
<tr>
<td>Mercury</td>
<td>0.003</td>
</tr>
<tr>
<td>Molybdenum</td>
<td>0.5</td>
</tr>
<tr>
<td>Nickel</td>
<td>1.0</td>
</tr>
<tr>
<td>Selenium</td>
<td>0.05</td>
</tr>
<tr>
<td>Uranium</td>
<td>0.2</td>
</tr>
<tr>
<td>Vanadium</td>
<td>0.1</td>
</tr>
<tr>
<td>Zinc</td>
<td>50.0</td>
</tr>
</tbody>
</table>

Source: Canadian Task Force on Water Quality (1987).

*a* 5.0 if not added to feed.

*b* Tentative guideline.

*c* 1.0 if fluoride is present in feed.
are more likely to have higher nitrate levels and coliform counts. Sometimes, the age of the well and type of pumping device may suggest certain mechanical problems, including a cracked casing or defective sanitary seal. Estimates of the amount of water consumed using water meters may also be helpful when investigating potential water problems.

Microbiological Standards
Microbiological examination of water samples determines the general sanitary quality of the sample and indicates the degree of contamination of the water with waste from human and animal sources. These examinations usually do not attempt to isolate pathogenic bacteria but rather detect the presence of indicator organisms. The coliform groups of bacteria are used to assess the degree of water pollution and thus the sanitary quality of the particular sample. The differentiation of fecal coliforms as a subgroup within the general category of coliforms is increasingly available. The U.S. Environmental Protection Agency (1973) proposed that acceptable levels for water to be used directly by livestock should not exceed 1000/100 mL. Since animals are allowed to range freely and drink surface waters, these proposed limits are unenforceable and of doubtful value. The standard plate count, which enumerates the number of bacteria multiplying at 35°C, is of doubtful significance in evaluating livestock water sources other than helping to judge the efficiency of various water treatment processes.

Salinity
Salinity, or total dissolved solids (TDSs), generally expressed in milligrams per liter, is an expression of the amount of soluble salts in a particular water sample and is one of the most important parameters used to evaluate water quality. The ions most commonly involved in saline waters are calcium, magnesium, and sodium in the bicarbonate, chloride, or sulfate form. Hardness is sometimes confused with salinity, but the two are not necessarily correlative. Hardness is expressed as the sum of calcium and magnesium reported in equivalent amounts of calcium carbonate. Although hardness of water may affect the mechanical function of valves and waterers because of the formation of mineral deposits, hardness itself has a minimal impact on animal performance.

Water containing less than 1000 mg soluble salts/L should present no serious hazard to any class of swine. Water containing between 1000 and 5000 mg soluble salts/L may cause mild temporary diarrhea or be refused at first by swine not accustomed to it, although health or performance should not be greatly affected (Anderson and Strothers 1978; National Research Council [NRC] 1974; Paterson et al. 1979). Water containing 5000–7000 mg soluble salts/L may present a health risk for pregnant, lactating, or stressed animals. Water containing more than 7000 mg soluble salts/L should be considered unsafe for swine.

In some regions, sulfates are a major portion of the TDS in water. Veenhuizen et al. (1992) demonstrated that except for an increase in fecal moisture content, water containing up to 1800 mg of sodium and/or magnesium sulfate per liter had no effect on nursery pig performance. An epidemiological study of water on swine farms did not find an association between sulfate concentrations and prevalence of diarrhea, although water sulfate levels increased with the depth of the wells tested (Veenhuizen 1993). A study of water quality on 173 Iowa swine farms found a mean TDS of 343 mg/L (range 100–2500), but measured no significant effects of elevated TDS on several performance parameters (Ensley 1998).

Nitrates and Nitrites
Nitrates and nitrites are water soluble and thus may be leached from the soil or soil surface into groundwater. Animal wastes, nitrogen fertilizers, decaying organic matter, silage juices, and soils high in nitrogen-fixing bacteria may be sources of contamination through surface-water runoff to adjacent poorly cased, shallow, or low-lying wells or reservoirs.

The upper limit for nitrate in human drinking water is 45 mg nitrate/L (U.S. Environmental Protection Agency [USEPA] 1975), established as preventive for the methemoglobinemia or “blue baby” syndrome in human infants who receive formulas made from high-nitrate waters. There is no evidence to suggest that neonatal swine have greater susceptibility to elevated nitrates. Emerick et al. (1965) concluded that 1-week-old pigs are no more susceptible to nitrite-induced methemoglobinemia than older growing swine. A review of water quality for livestock (National Research Council [NRC] 1974) proposed 440 mg nitrate/L as the maximum nitrate that could safely be allowed in livestock water.

Reports of experimental production of a chronic or low-level nitrate-poisoning syndrome in livestock have been extensively reviewed (Emerick 1974; Ridder and Oehme 1974; Turner and Kienholz 1972). The bulk of the evidence indicates that sublethal or chronic effects are extremely rare and difficult to verify. London et al. (1967) fed growing pigs up to 18.3 mg nitrite-nitrogen/kg body weight for 124 days without serious effects developing. No effect on the performance of growing-finishing swine or on reproductive performance of gilts was observed when the drinking water contained 1320 ppm nitrate (Seerley et al. 1965).

MISCELLANEOUS TOXICANTS
Sodium Ion Toxicosis
Sodium ion toxicosis, also called water deprivation or salt poisoning, is a common problem in swine. The occurrence of sodium ion toxicosis is inversely related
to water intake and is almost always related to water deprivation caused by inadequate water supply or to changes in husbandry. The likelihood of toxicosis will also increase with increased dietary salt, but the condition may occur when rations contain normal levels of added salt, for example, 0.25–1%. It has also been associated with the feeding of whey and other milk by-products. Sodium ion toxicosis may occur after water deprivation of only a few hours, but in most cases, the time exceeds 24 hours.

The initial clinical signs are thirst, anorexia, and constipation, followed by CNS involvement. Intermittent convulsions start within 1 to several days after water deprivation and may be exacerbated by rehydration. The frequency of the characteristic tonic-clonic convulsions with opisthotonos, often starting from a sitting position, increases with time. Affected animals are afebrile and may also wander aimlessly and appear to be blind and deaf. Moribund pigs become comatose, often lying on their sides with continuous paddling. Most affected animals die within a few days. Some pigs that appear to be unaffected may succumb later from polioencephalomalacia. Salt poisoning from eating excess salt or consuming brine usually will cause vomiting and diarrhea.

Diagnosis is best accomplished by establishing that water deprivation occurred, which may be difficult in some cases. Necropsy findings may reveal an absence of ingesta, gastritis, constipation, or very dry feces; rehydration-exacerbated deaths may have stomach distended with water. Chemical analysis of serum and cerebrospinal fluids may confirm a hypernatremia with levels of Na above 160 mEq/L (Osweiler and Hurd 1974). However, after rehydration, normal values of 140–145 mEq/L may exist. Brain sodium levels above 1800 ppm (wet weight) are consistent with a diagnosis of sodium ion toxicosis. Histological examination of brain tissue, especially cerebrum, often reveals a nearly pathognomonic eosinophilic meningoencephalitis characterized by cuffing of meningeal and cerebral vessels with eosinophils. However, when pigs live several days, the eosinophils may disappear or be replaced by mononuclear cells. Brains of pigs affected subacutely may have a laminar subcortical polioencephalomalacia. Analysis of feed for sodium is usually of limited value. Differential diagnosis should include viral encephalitic diseases such as pseudorabies and hog cholera, CH insecticide poisoning, and edema disease. In known cases of water deprivation, rehydration should be gradual, but the prognosis is poor.

**Coal Tar Pitch**

Coal tars are a mixture of condensable, volatile products formed during the destructive distillation of bituminous coal. The phenolic portions of these products have the greatest acute toxicity. Sources of these substances for swine are clay pigeons, lignite tar flooring slabs, tar paper, and tar used in waterproofing and sealing. Because of the rapid clinical course, sudden death is often the first sign observed. Weakness, depression, and increased respiratory rate can be observed in animals that may live for several hours or even days. Icterus and a secondary anemia may develop. Necropsy of pigs poisoned by coal tar pitch reveals a greatly enlarged friable liver. The hepatic lobules are very distinct grossly; some are darkened in color, and others are yellowish orange. Microscopically, this lesion is observed as severe centrilobular necrosis with subsequent intralobular hemorrhage. Ascites and large turgid kidneys may also be observed.

There is no specific treatment for this condition. Removal of animals from the source of the coal tar is important to prevent recurrence of poisoning.

**Ethylene Glycol**

Many permanent antifreeze/coolant mixtures for liquid-cooled engines contain approximately 95% ethylene glycol. A hazard of poisoning exists when animals have accidental access to antifreeze solutions during periods of engine maintenance or when these solutions are used in plumbing systems to prevent freezing. Swine may be poisoned by ingesting 4–5 mL ethylene glycol/kg body weight. Ethylene glycol toxicosis is exhibited in two clinical phases. Initially, the glycol can enter the cerebrospinal fluid, producing a narcotic or euphoric state of intoxication. Subsequent clinical signs of acidosis and renal failure are associated with the highly toxic metabolites of the glycol and formation of calcium oxalate crystals in the kidney tubules. Renal tubular blockage with development of uremia is observed 1–3 days after ingestion. Clinical signs generally include emesis, anorexia, dehydration, weakness, ataxia, convulsions, coma, and death. The entire course of illness may be as short as 12 hours following consumption of large quantities of ethylene glycol. Oxalate nephrosis can be demonstrated histopathologically and is characterized by finding pale yellow birefringent oxalate crystals in the tubules. Polarizing filters greatly aid in the detection of oxalate crystals in kidney sections or in impression smears of freshly incised kidney.

Once clinical signs of renal failure are evident, treatment is usually of no avail. If treated within the first 6–12 hours after ingestion, reasonable response has been achieved in ethylene-glycol-poisoned dogs by using 5.5 mL/kg body weight of 20% ethyl alcohol intravenously and 98 mL/kg body weight of 5% sodium bicarbonate intravenously.

**Gossypol**

Cottonseed meal (CSM), a by-product of the cotton fiber and cottonseed oil industries, is an important protein supplement for livestock rations in cotton-producing regions. Its use as a protein supplement for
swine, however, is limited by gossypol content, which varies with the strain of the cotton plant, its geographical location, climatic conditions, and the oil extraction procedure used. Gossypol, a polyphenolic binaphthalein, is a yellow pigment in glands of decorticated cottonseed. The toxic “free” gossypol becomes partially inactivated (bound) during the extraction and milling processes, as well as spontaneously in the prepared meal. Toxicity of gossypol depends on the species and age of the animal and on various components of the diet, particularly the protein, lysine, and iron concentrations (Eisele 1986).

Toxicosis only follows prolonged feeding (weeks to months) of CSM with a high content of free gossypol and may be manifested simply as ill-thrift or as an acute respiratory problem followed by death. The main pathological changes are cardiomyopathy, hepatic congestion and necrosis, skeletal muscle injury, and severe edematous changes throughout the animal. A decrease in hemoglobin total serum, protein concentration, and packed-cell volume is seen in pigs fed a diet containing 15–16% protein diet. Tolerance to gossypol can be induced by adding FeSO₄ (400 mg/kg) at a 1:1 weight ratio with free gossypol. Increasing the amount of crude protein or supplementing with lysine can also induce tolerance (Pond and Maner 1984).

VENTILATION FAILURE AND TOXIC GASES

Confinement of swine in closed structures increases the risk of potential gas toxicosis and other dynamics dependent on mechanical ventilation. Fortunately, even at relatively low ventilation rates used during cold weather, concentrations of ammonia and hydrogen sulfide, the two most potentially dangerous gases associated with manure decomposition, usually remain below toxic levels. However, accidents, poor design, power failure, mechanical failure, and improper operation may result in insufficient ventilation and increase the concentration of poisonous gases to toxic levels. It is useful to be reminded of some of the terminology used when investigating ventilation failures: hyperthermia (elevated body temperature), asphyxiation (displacement of oxygen by another gas such as CO₂), intoxication (toxic effect of gas such as hydrogen sulfide on structure or function), and suffocation (physical obstruction of air passages).

The most important gases released by the decomposition of urine and feces either in anaerobic underfloor waste pits or in deep litter or manure packs are ammonia and hydrogen sulfide. Carbon dioxide and methane are also produced but seldom reach significant concentrations. A number of vapors responsible for the odors of manure decomposition are also produced. These include organic acids, amines, amides, alcohols, carboxyls, skatoles, sulfides, and mercaptans. Concentrations of toxic gases are usually expressed as parts of the gas per million parts of air (ppm) by volume.

Much more common than the accumulation of toxic gases are swine deaths associated with failure of a mechanical ventilation system. The failure of ventilation may occur in whole confinement buildings and result in high death losses at any time of the year. Similar dynamics and death losses occur in tightly sealed insulated trucks used to transport pigs.

Hyperthermia

When ventilation fails because of storms, power outages, or mechanical failure, the dynamics of air, heat, and moisture in the confined space may quickly become critical. The retention of heat and moisture leads to high relative humidity and poor evaporative cooling that often and quickly leads to fatal hyperthermia in confined swine. Death losses may approach 95% under these circumstances; frequently, only the smallest pigs survive. Although there is no method to specifically confirm hyperthermia as a cause of death, a history of ventilation failure, rapid carcass decomposition, a pale “cooked” appearance to the muscles, and blood-tinged foam in the trachea are usually seen.

Ammonia

Ammonia (NH₃) is the toxic air pollutant most frequently found in high concentrations in animal facilities, and production is especially common where excrement can decompose on a solid floor. This gas has a characteristic pungent odor that humans can detect at approximately 10 ppm or even lower. The NH₃ concentration in enclosed animal facilities usually remains below 30 ppm even with low ventilation rates; however, it may frequently reach 50 ppm or higher during long periods of normal facility operation.

Ammonia is highly soluble in water, and as such will react with the moist mucous membranes of the eye and respiratory passages. Consequently, excessive tearing, shallow breathing, and clear or purulent nasal discharge are common signs of aerial NH₃ toxicosis.

At concentrations usually found in practical animal environments (<100 ppm), the primary impact of this gas is as a chronic stressor that can affect the course of infectious disease as well as directly influence the growth of healthy young pigs. The rate of gain in young pigs was reduced by 12% during exposure to aerial NH₃ at 50 ppm and by 30% at 100 or 150 ppm (Drummond et al. 1980). Aerial NH₃ at 50 or 75 ppm reduced the ability of healthy young pigs to clear bacteria from their lungs (Drummond et al. 1978). At 50 or 100 ppm, aerial NH₃ exacerbated nasal turbinate lesions in young pigs infected with Bordetella bronchiseptica but did not
add to the infection-induced reduction in the pigs’ growth rate (Drummond et al. 1981a). In another study, aerial NH₃ at 100 ppm reduced the rate of gain by 32% and ascarid infection by 28%; however, effects of the NH₃ and infection, when imposed on the pigs at the same time, were additive, and the rate of gain was reduced by 61% (Drummond et al. 1981b). More extensive reviews of aerial NH₃ and its effect on animal production are provided by Curtis (1983) and the National Research Council (1979a).

**Hydrogen Sulfide**

Hydrogen sulfide (H₂S) is a potentially lethal gas produced by anaerobic bacterial decomposition of protein and other sulfur-containing organic matter. The source of H₂S that presents the greatest hazard to swine is liquid manure holding pits. Most of the H₂S, which may be continuously produced, is retained within the liquid of the pit. However, agitation of waste slurry to may be continuously produced, is retained within the liquid manure holding pits. Most of the H₂S, which may be continuously produced, is retained within the liquid of the pit. However, agitation of waste slurry to resuspend solids prior to being pumped out causes the rapid release of much of the H₂S that may have been retained within it. Hydrogen sulfide is heavier than air and is consequently found in, and will move to, pits, tanks, and other low areas of a facility. The concentration of H₂S usually found in closed animal facilities (less than 10 ppm) is not toxic, but the release of gas upon agitation may produce concentrations of H₂S up to 1000 ppm or higher within the facility.

Acute H₂S poisoning is directly responsible for deaths of swine and humans in closed animal facilities, particularly during pit manure agitation and removal. Humans can detect the typical “rotten egg” odor of H₂S at very low concentrations (0.025 ppm) in air. Exposures to these low concentrations have little or no importance to human health, and thus the olfactory response is a useful warning signal of its presence. However, at higher concentrations (greater than 200 ppm), H₂S presents the distinct hazard of a paralyzing effect on the olfactory apparatus, thus effectively neutralizing the warning signal (National Research Council [NRC] 1979b).

Hydrogen sulfide is an irritant gas. Its direct action on tissues induces local inflammation of the moist membranes of the eye and respiratory tract. When inhaled, the action of H₂S is more or less uniform throughout the respiratory tract, although the deeper pulmonary structures suffer the greatest damage. Inflammation of the deep lung structures may appear as pulmonary edema. If inhaled at sufficiently high concentrations, H₂S can also be readily absorbed through the lungs and can produce fatal systemic intoxication (O’Donoghue 1961). At concentrations in air exceeding 500 ppm, H₂S must be considered a serious imminent threat to life; between 500 and 1000 ppm, it produces permanent effects on the nervous system. If spontaneous recovery does not occur and artificial respiration is not immediately provided, death results from asphyxia or from respiratory paralysis of intoxication.

Management is the most important part of preventing animal deaths from H₂S. When manure stored in a pit beneath a building is agitated, animals should be moved out of the building if at all possible. When movement of the animals is not possible, other steps should be taken to protect the animals during agitation. In mechanically ventilated buildings, the fans should run at full capacity, even during the winter; in naturally ventilated buildings, manure pits should not be agitated unless there is a brisk breeze blowing. Immediate rescue of affected swine should not be attempted for the rescuer may quickly become a victim of H₂S toxicosis.

**Carbon Dioxide**

Carbon dioxide (CO₂) is an odorless gas present in the atmosphere at 300 ppm. It is given off by swine as an end product of energy metabolism and by improperly vented, though properly adjusted, fuel-burning heaters. It is also the gas evolved in the greatest quantity by decomposing manure. Despite all this, CO₂ concentration in closed animal facilities rarely approaches levels that endanger animal health (Curtis 1983).

**Methane**

Methane (CH₄), a product of microbial degradation of carbonaceous materials, is not a poisonous gas. It is biologically rather inert and produces effects on animals only by displacing oxygen in a given atmosphere and thereby producing asphyxiation. Under ordinary pressures, a concentration of 87–90% CH₄ in a given atmosphere is required before irregularities of respiration and eventually respiratory arrest due to anoxia are produced. The chief danger inherent in this material is its explosive hazard as concentrations of 5–15% by volume in air are reached (Osweiler et al. 1985).

**Carbon Monoxide**

Carbon monoxide (CO), which is produced from the inefficient combustion of carbonaceous fuel, is also potentially lethal to swine. Poisoning occurs when improperly adjusted and improperly vented space heaters, furnaces, or equipment is operated in tight, poorly ventilated buildings such as farrowing houses. Ambient background levels of CO are 0.02 ppm in fresh air, 13 ppm in city streets, and 40 ppm in areas with high vehicular traffic.

Carbon monoxide acts by competing with oxygen for binding sites on a variety of proteins, particularly hemoglobin. The affinity of hemoglobin for CO is some 250 times that for oxygen. Therefore, CO is preferentially bonded to the heme group, which forms carboxyhemoglobin, thereby reducing the oxygen-carrying capacity of heme. Cellular and tissue hypoxia occurs but the carboxyhemoglobin will often cause
blood and tissues to appear “cherry red” by gross examination.

High concentrations of CO (>250ppm) in swine farrowing houses can produce an increased number of stillborn piglets. Stillbirths are often accompanied by a clinical history, which includes nonexistent ventilation; inadequate ventilation due to blocked apertures of natural systems or reduction to minimal winter rates for mechanical systems; use of unvented or improperly vented liquefied petroleum gas (LP)-burning space heaters; a high percentage of near-term sows delivering dead piglets within a few hours of being put in an artificially heated farrowing facility; sows that appear clinically normal but that produce whole litters born dead; and negative laboratory results for the detection of infectious causes of abortion (Carson 1990).

Exposure to high levels of CO can be confirmed by actually measuring the CO level in the air or by measuring the percentage of carboxyhemoglobin in the blood of the affected animals. In addition to these two parameters, carboxyhemoglobin concentration of greater than 2% in fetal thoracic fluid may be used as an aid in diagnosing CO-induced stillbirth in swine (Dominick and Carson 1983).

**Anhydrous Ammonia**

On occasion, swine may be exposed to anhydrous ammonia (gas-NH₃) used as an agricultural fertilizer nitrogen source. This gas presents a unique risk of exposure to both animals and people because of its presence on farms and the fact that it is stored, transported, and applied under high pressure. Poisoning with gas-NH₃ is associated with gas release from broken hoses, failure of valves, and errors in operating transport or application equipment. Once released, gas-NH₃ rapidly combines with water and forms caustic ammonium hydroxide. The cornea, mouth, and respiratory tract are high in moisture and especially susceptible to the resulting strong alkali burns. Acute death from laryngospasm and accumulation of fluid in the lungs can occur within a matter of minutes. Blindness from corneal opacity and sloughing epithelium in the respiratory tract may be seen in swine surviving initial exposure. Residual respiratory damage and secondary bacterial invasion may not allow affected animals to regain full productive status.

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17.1. (A) Exudative epidermitis (chronic); (B) ear necrosis (spirochetosis); (C) erysipelas; (D) swine pox; (E) ringworm (*Microsporum nanum*); (F) sarcoptic mange.
17.2. (A) Skin necrosis; (B) parakeratosis; (C) pityriasis rosea; (D) epitheliogenesis imperfecta; (E) porcine dermatitis/nephropathy syndrome; (F) porcine dermatitis/nephropathy syndrome.
21.1. Nasal turbinates from a normal pig. The nasal septum (NS) is straight and the turbinates (T) fill the nasal cavity.

21.4. Gross lesions of atrophic rhinitis in swine. The nasal septum (NS) is distorted and turbinates are shrunken and misshapen resulting in increased airspace (arrow) in the nasal cavity.