DISEASES OF SWINE

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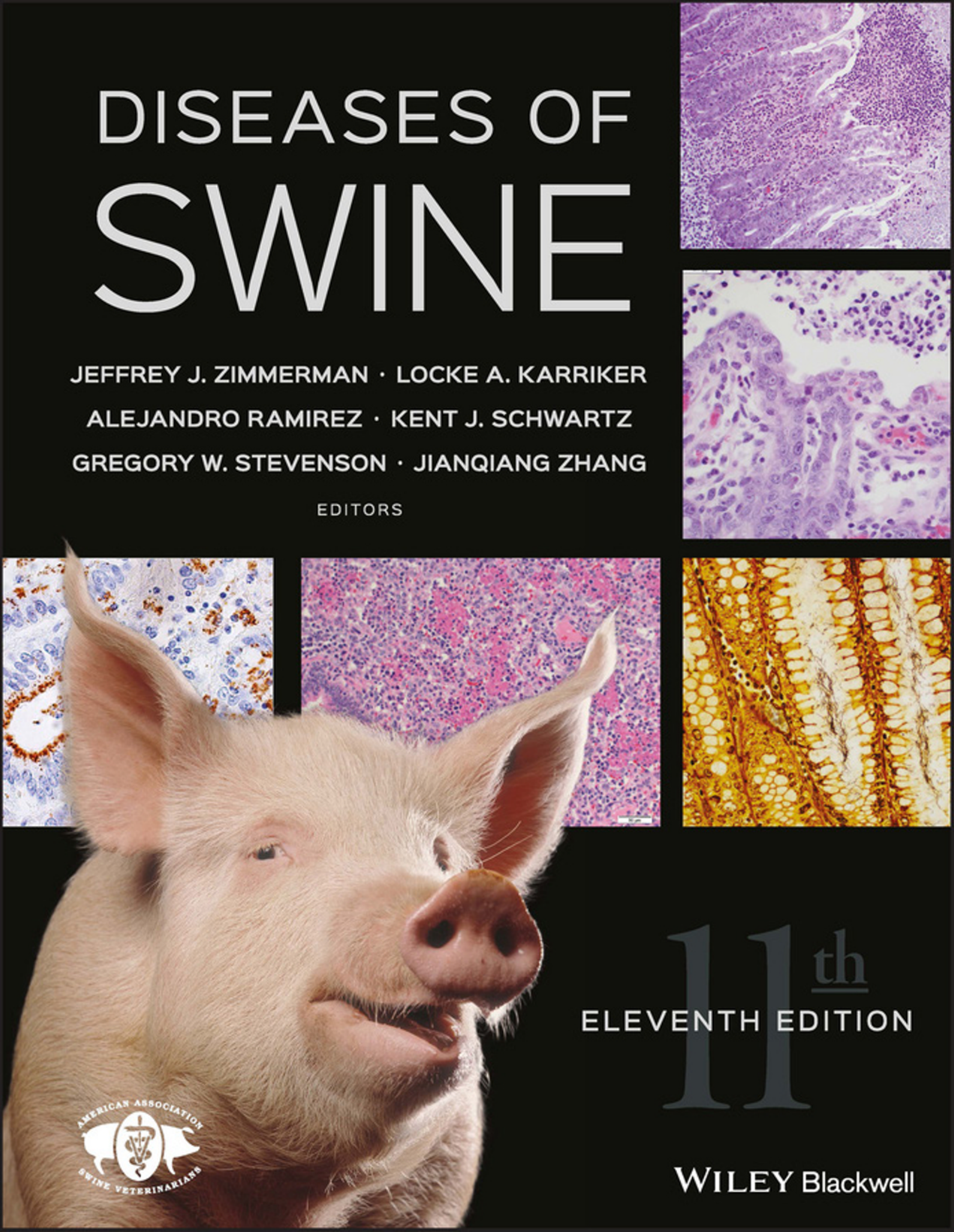

11th
ELEVENTH EDITION

WILEY Blackwell

Diseases of Swine

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Edited By

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Eleventh Edition

WILEY Blackwell

This edition first published 2019

Edition History
Iowa State Press (1e, 1958; 2e, 1964; 3e, 1970; 4e, 1975; 5e, 1981, 6e, 1986; 7e, 1992; 8e, 1999). Blackwell Publishing (9e, 2006). John Wiley and Sons, Inc. (10e, 2012).

Registered Office
John Wiley & Sons, Inc., 111 River Street, Hoboken, NJ 07030, USA

Editorial Office
111 River Street, Hoboken, NJ 07030, USA

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Library of Congress Cataloging-in-Publication Data

Names: Zimmerman, Jeffrey J., editor.
Title: Diseases of swine / edited by Jeffrey J. Zimmerman, Locke A. Karriker, Alejandro Ramirez, Kent J. Schwartz, Gregory W. Stevenson, Jianqiang Zhang.
Description: 11th edition. | Hoboken, NJ : Wiley-Blackwell, 2019. | Includes bibliographical references and index. |
Identifiers: LCCN 2018042674 (print) | LCCN 2018043009 (ebook) | ISBN 9781119350903 (Adobe PDF) | ISBN 9781119350897 (ePub) | ISBN 9781119350859 (hardcover)
Subjects: LCSH: Swine–Diseases. | MESH: Swine Diseases
Classification: LCC SF971 (ebook) | LCC SF971 .D57 2019 (print) | NLM SF 971 | DDC 636.4/0896–dc23
LC record available at https://lccn.loc.gov/2018042674

Cover image: © ChristianLohman / iStock.com,© Dr. Eric R. Burrough, © Dr. Darin Madson,© Dr. Gregory W. Stevenson, © Dr. Michael
Cover design by Wiley

Set in 10/12pt Warnock by SPi Global, Pondicherry, India

10 9 8 7 6 5 4 3 2 1

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Editors' Note

Dr. Howard Dunne and Iowa State University Press released the first edition of *Diseases of Swine* in 1958. Our goal for the 11th edition is to provide swine health specialists the knowledge needed for effective responses to pig diseases on farms and at local, regional, and global levels. In this we have endeavored to follow the standards of excellence initially established by Dr. Dunne.

As a sojourner of a slower time, Dr. Dunne could not have foreseen either the extent or the accelerated pace at which innovations in engineering, genetics, management, molecular biology, and nutrition have revolutionized pig production. Biologically, economically, and ecologically, the successful application of new technologies to pig production has produced unprecedented advances that benefit society and provide healthful, wholesome pork products to the consumer.

Successes in disease control and pig health assurance are to be celebrated but tempered with the reality that control (much less elimination) of both emerging and historic swine health adversaries has faltered. Endemic viral and bacterial pathogens remain a pernicious burden on pig health. More sobering, the interconnectivity and interdependence of the contemporary world have accelerated the speed and inevitability with which emergent pathogens are dispersed to distant locations. Despite the considerable efforts of the animal health community, African swine fever virus, classical swine fever virus, foot-and-mouth disease virus, porcine circoviruses, porcine coronaviruses, porcine reproductive and respiratory syndrome viruses, and other major pathogens circulate widely in many parts of the world and threaten those that remain free.

Ideally, recognition of our shared vulnerabilities should spur the search for more effective solutions to animal and public health disease threats: there is much to be learned and applied. Thus, we respectfully dedicate this edition of *Diseases of Swine* to our readership as a tool in their search for solutions to swine and public health challenges.

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Acknowledgments

With our deepest appreciation, we thank Ella Akin, Cassandra Fitzgerald, Victoria Monahan, and Kristin J. Skoland for their excellent work on behalf of the 11th edition of *Diseases of Swine*. Without their dedication and herculean efforts, the outcome would have been in doubt.

Section I

Veterinary Practice

1

Herd Evaluation

Alejandro Ramirez and Locke A. Karriker

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 always limited. Consequently, swine veterinarians are highly motivated to be innovative. The use of modern technology, applied research, epidemiologic principles, 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. The successful veterinarian is not only the one that solves a problem but also creates opportunities and promotes the financial success of their 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 their 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 goal?"

Investigation of health or production issues is best approached by site visits – that is, inspection of pigs in their environment. As 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

If possible, 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 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's modern records allow for measuring slight differences in productivity (clinical manifestation),

Diseases of Swine, Eleventh Edition. Edited by Jeffrey J. Zimmerman, Locke A. Karriker, Alejandro Ramirez, Kent J. Schwartz, Gregory W. Stevenson, and Jianqiang Zhang.

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 benchmark information has 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 using 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 memorizing 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 and their impact on a breeding herd's wean 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 number of pigs weaned, issues like preweaning mortality are obvious, while 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 the owner wants to share with workers. In a larger corporate setting (corporate ownership or part of a producer cooperative),

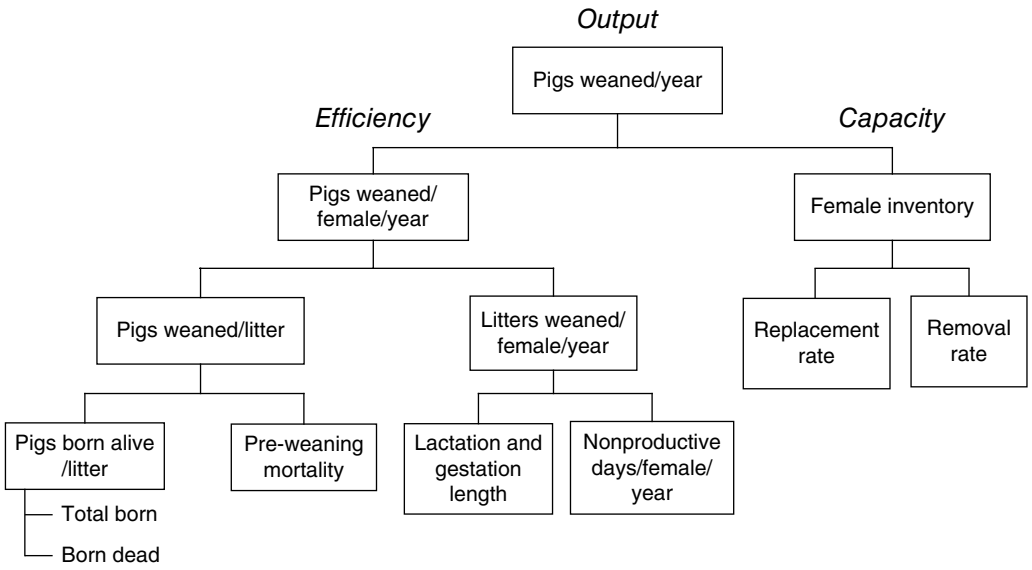

Figure 1.1 Weaned pig output productivity tree for investigating variables that impact the number of pigs weaned per year.
Source: Adapted from Gary Dial.

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/her 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. This topic is covered in greater detail in Chapter 9.

Protocols to prevent disease transmission into the farm, within the farm, or to neighboring farms are now commonplace. Swine veterinarians and personnel need to proactively follow proper biosecurity protocols to ensure the safety and security of our food supply. The key point when performing a herd examination is for the veterinarian to be fully aware, and fully comply, with all biosecurity guidelines for the operation. 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 meet the required downtime and 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 to minimize the opportunity for missing something important. Checklist may be helpful for specific routine evaluations, but often 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.

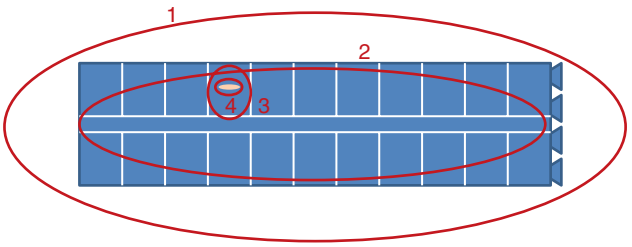

Figure 1.2 Diagram showing the concept of the four-circle approach to herd evaluation. (1) Complete circle evaluation of the "outside" of the building/site. (2) Complete circle evaluation of the "inside" of the building. (3) Complete circle evaluation of individual "pens." (4) Complete circle evaluation of individual "animals."

Farm-specific checklists or checklists for particular aspects of an 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 outside of the building/site

The first circle involves walking around the outside of buildings to assess the overall site. This first circle is especially important when visiting a new site. Evaluation of the outside of a building has value both clinically for the pigs and 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, insufficient staffing, or tight budgets? 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 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 1 or 2 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 general 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.

Table 1.1 Recommended space per pig by phase of production.

	Indoor		
	Solid	**Slatted**	**Outdoor**
Phase		**Area per pig in m^2 (ft^2)**	
Gilts	1.86 (20)	1.49 (16)	2.32 (25)
Sows	2.2 (24)	1.86 (20)	2.32 (25)
Farrow pen	8 (88)	NA	NA
Farrow crate	4.4 (48)	4.4 (48)	NA
Boars	NA	1.86 (20)	NA
Nursing	NA	2.0 (22)	NA
Nursery 20 kg	0.37 (4)	0.28 (3)	0.74 (8)
Nursery 40 kg	0.37 (4)	0.40 (4.4)	0.74 (8)
Grower 60 kg	0.56 (6)	0.53 (5.8)	1.86 (20)
Finisher 80 kg	0.74 (8)	0.67 (7.2)	1.86 (20)
Finisher 110 kg	0.75 (8)	0.75 (8)	1.86 (20)

Source: Dewey and Straw (2006). Adapted from English et al. (1982), Baxter (1984a,b,c), Patience and Thacker (1989a,b), and Gonyou and Stricklin (1998).
NA, not applicable.

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 4 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

Table 1.2 Weights and daily gain by age and relative growth rate.

	Slow				Moderate				Ideal			
Age	Weight		Daily gain in the previous 20 days		Weight		Daily gain in the previous 20 days		Weight		Daily gain in the previous 20 days	
Days	lb	kg	lb	g	lb	kg	lb	g	lb	kg	lb	g
20	8–10	3.6–4.5			10–12	4.5–5.5			12–14	5.5–6.4		
40	18–22	8.2–10.0	0.50–0.60	227–273	22–26	10.0–11.8	0.60–0.70	273–318	26–30	11.8–13.6	0.70–0.80	318–364
60	33–40	15.0–18.2	0.75–0.90	341–409	40–47	18.2–21.4	0.90–1.05	409–477	47–54	21.4–24.5	1.05–1.20	477–545
80	54–64	24.5–29.1	1.05–1.20	477–545	64–74	29.1–33.6	1.20–1.35	545–614	74–84	33.6–38.2	1.35–1.50	614–682
100	82–95	37.3–43.2	1.40–1.55	636–705	95–108	43.2–49.1	1.55–1.70	705–773	108–122	49.1–55.5	1.70–1.90	773–864
120	110–126	50.0–57.3	1.40–1.55	636–705	126–142	57.3–64.5	1.55–1.70	705–773	142–160	64.5–72.7	1.70–1.90	773–864
140	138–157	62.7–71.4	1.40–1.55	636–705	157–176	71.4–80.0	1.55–1.70	705–773	176–198	80.0–90.0	1.70–1.90	773–864
160	165–187	75.0–85.0	1.35–1.50	614–682	187–209	85.0–95.0	1.50–1.65	682–750	209–235	95.0–106.8	1.65–1.85	750–841
180	191–216	86.8–98.2	1.30–1.45	591–659	216–241	98.2–109.5	1.45–1.60	659–727	241–271	109.5–123.2	1.60–1.80	727–818
20–60			0.63–0.75	284–341			0.75–0.88	341–398			0.88–1.00	398–455
60–180			1.32–1.47	598–667			1.47–1.62	667–735			1.62–1.81	735–822
0–180			1.06–1.20	482–545			1.20–1.34	545–609			1.34–151	609–684

Source: Dewey and Straw (2006). Reproduced with permission of John Wiley and Sons.

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

	Water requirements		Feeder space/pig
	l/day	l/minute	mm (in.)
Restricted feed			
Gestating sows	12–25	2	457–610 (18–24)
Lactating sow	10–30	2	
Boar	20	2	
Nursing	1	0.3	
Nursery	2.8	1	254 (10)
Grower	7–20	1.4	260 (10)
Finisher	10–20	1.7	330 (13)
Ad libitum			
Nursery	2.8	1	60 (2.3)
Grower	7–20	1.4	65 (2.5)
Finisher	10–20	1.7	76 (3)

Source: 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).

stage of infection (e.g. fever tends to suggest an acute infection). 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). 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 BCS. 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

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

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

Source: Dewey and Straw (2006). Reproduced with permission of John Wiley and Sons.

Table 1.5 Sow body condition scoring.

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

Source: Adapted from Ken Stalder.

(also see Chapter 7), 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 pigs.
2) Animals must be selected that 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 are 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 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, 1 or 2 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 benefiting 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 or oral fluids), 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 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 heat detection and artificial insemination.

As a site visit is performed, it is also important to examine storage and utility areas and 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 pre-farrowing and yet has no vaccine on-site may need further evaluation and discussion to ascertain vaccine management and handling procedures. A second example may be a protocol describing a temperature to store semen but no thermometer in the semen storage unit.

On-site records

Production sites should have treatment and mortality records on-site. The minimum requirement for treatment records include date, animal ID, product name, dose, route, person administering, and product withdrawal time. Mortality 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 that 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 using the barn's electronic control system in order to validate the proper function of the controller. Finally, these records can be used to confirm each group of pigs is being checked at least daily.

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 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 to 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 is usually summarized based on time or by cohort. In a time-based report, data is 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.

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 it 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 has been collected, it is then necessary to interpret all the findings in the context of the 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.

Soap

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 is 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 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 distillers 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

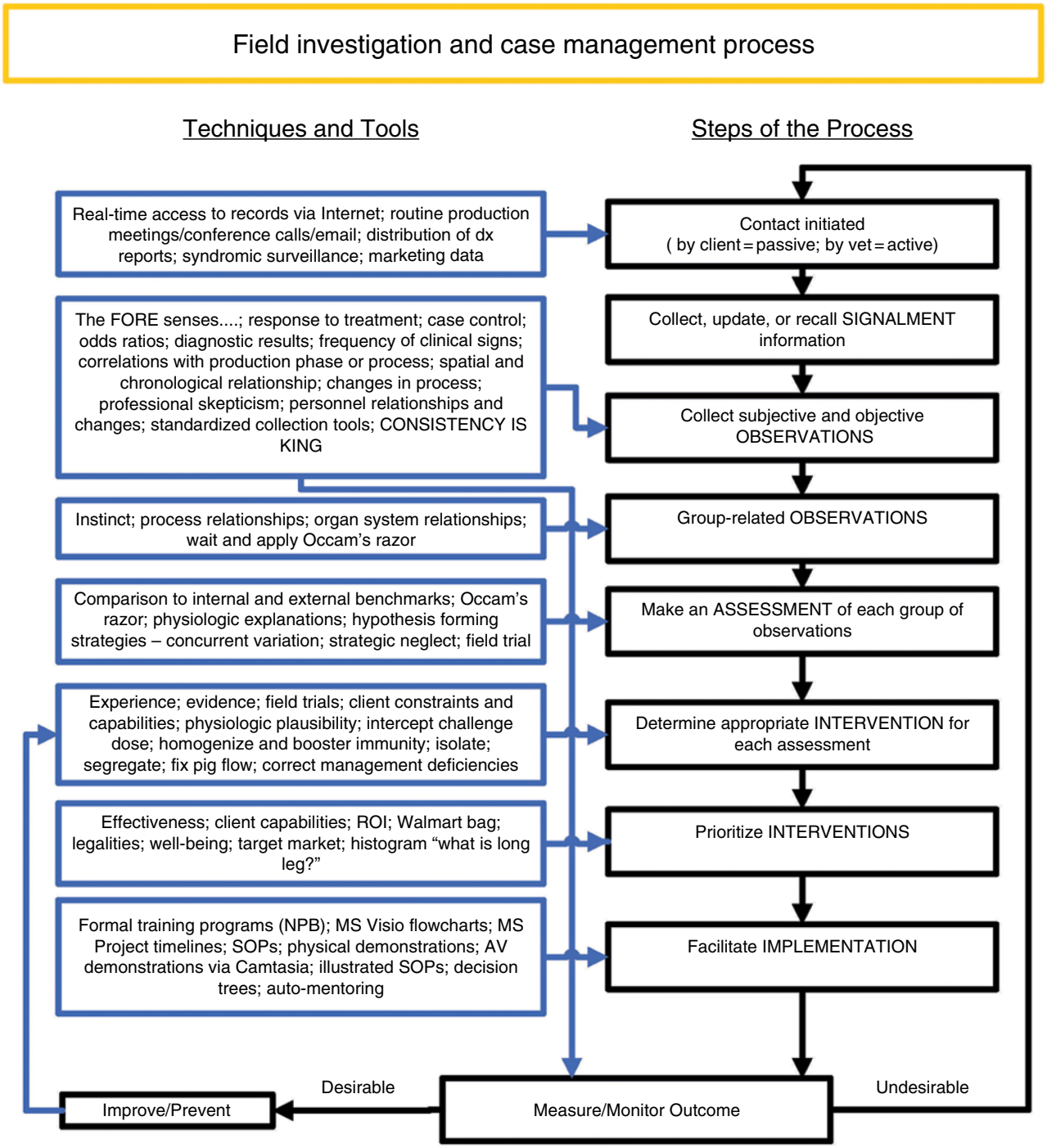

Figure 1.3 Chart depicting the flow of the field investigation and case management process. *Source:* Locke A. Karriker.

in Chapters 2 (behavior and welfare) and 4 (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 (Chapter 3) 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.

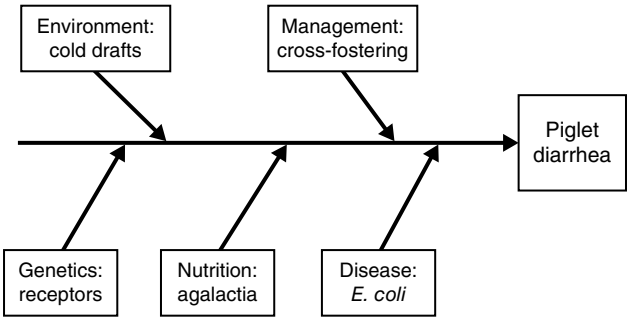

Figure 1.4 Fishbone diagram for piglet diarrhea incorporating the five production inputs model. *Source:* Kent Schwartz.

The five production inputs model works to integrate the interactions of different factors that may be working together at the same time and 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 place 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 to 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, oral fluids, etc.) will be discussed at the end of this chapter. Chapter 5 will cover some lists for differential diagnosis. Further general information on diagnostics and their interpretation are covered in Chapters 6–8.

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. 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 concise 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 client 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 (preferable up to 1 page long and definitely no more than 2 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 client's 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 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 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 non-visible, and thus a blind stick is performed. Mastery is achieved though practice. Much of this blood sampling information has been summarized from Dewey and Straw (2006).

Pig restraint

It is important to properly restrain pigs for safe sample collecting both from the perspective of the pig and from that of 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 neckline (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.

Figure 1.5 Method of restraining pigs weighing less than 20 kg for blood collection from the anterior vena cava (circle). Location of the cephalic vein is indicated by the dashed line. *Source:* Dewey and Straw (2006). Reproduced with permission of John Wiley and Sons.

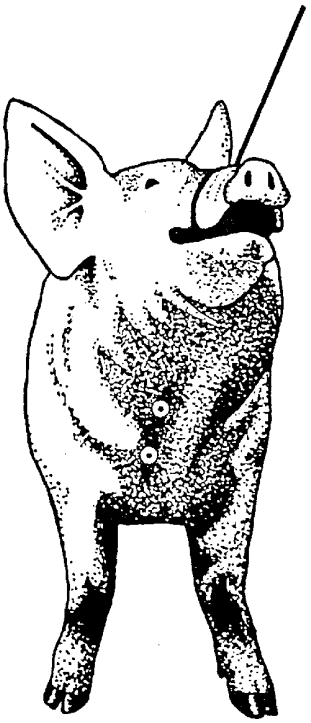

Figure 1.6 Pig restraint for blood sampling from a standing pig. The lower circle indicates the site for sampling from the anterior vena cava; the upper circle indicates the site for sampling from the jugular vein. *Source:* Dewey and Straw (2006). Reproduced with permission of John Wiley and Sons.

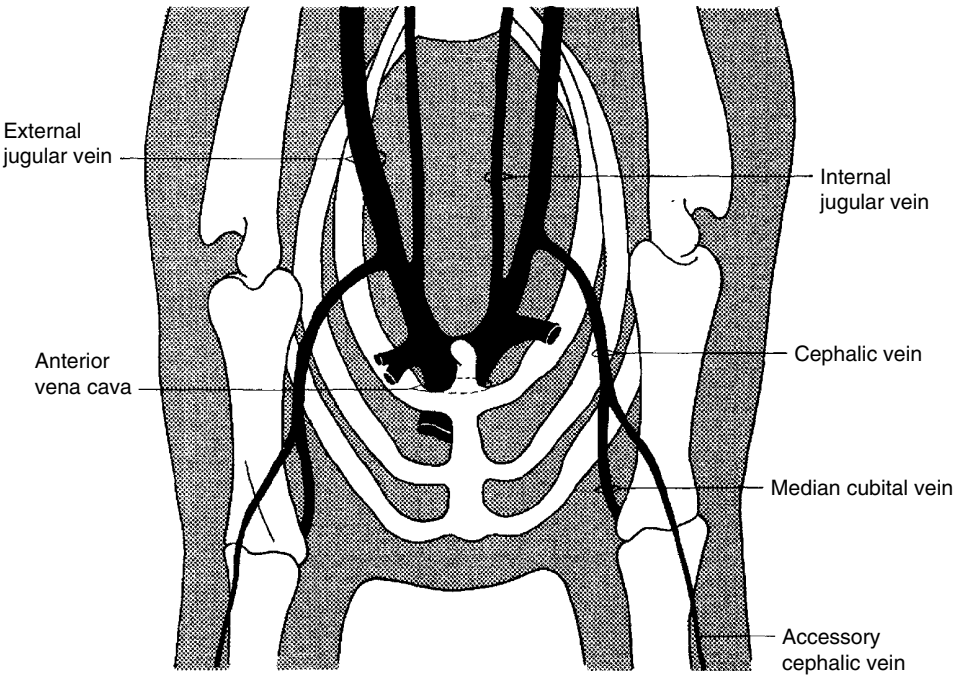

Figure 1.7 Location of some of the major veins in the pig in relation to the skeleton. *Source:* Dewey and Straw (2006). Reproduced with permission of John Wiley and Sons.

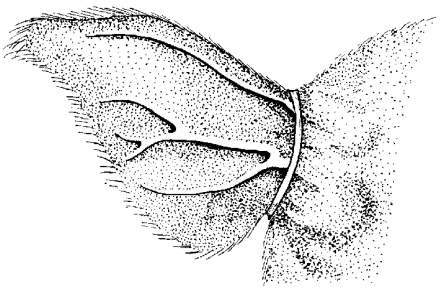

Figure 1.8 Ear veins of a pig raised by a rubber band placed on the base of the ear. *Source:* Dewey and Straw (2006). Reproduced with permission of John Wiley and Sons.

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 PCR testing a simple prick of an ear vein with the tip of a 20 g 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 venus sinus bleeding (Huhn et al. 1969) have all been described.

Oral fluid collection

Oral fluid collection for veterinary testing is becoming a more common practice in swine medicine. 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. Its use and diagnostic value is described in Chapters 7 and 8.

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. The variety of PCR and antibody assays validated for oral fluids continue to increase.

Acknowledgments

A special thank you goes to Dr. Anna Forseth for her input in improving this chapter.

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2

Behavior and Welfare

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Defining animal welfare and animal cruelty

Animal welfare

Understanding, maintaining, and promoting animal welfare are an integral component of all livestock production systems. Animal welfare is also a topic that in the past several decades has garnered significant attention from the public as the desire to know where and how food is raised intensifies. Over time, many animal welfare definitions have emerged within and between producers, researchers, veterinarians, consumers, packers, and retailers. However, all of them usually include some combination of the following areas of focus: biological function (immune function, growth, etc.), affective states (fear, pain, hunger, etc.), and living in an animal's natural environment (Duncan and Fraser 1997; Fraser et al. 1997).

Disagreements sometimes arise in what constitutes good animal welfare because stakeholders place different levels of importance on biological function, affective states, and natural living based on their personal values. Initially Tannenbaum (1991) and later Fraser (1995) have argued that there exists an "inextricable connection" between animal welfare and values, thus determining that animal welfare cannot be only assessed as a technical issue but must additionally include ethical consideration.

Some focus on the biological functioning of animals as a key welfare indicator, which includes parameters such as reproductive success, immune function, disease presence, and injury (Barnett et al. 1991; Broom 1986, 1991; Mormède 1990; Warnier and Zayan 1985). Assessing affective states such as fear, distress, and pain but also positive states such as pleasure is used in part to measure and understand an animal's welfare state. Duncan and Fraser (1997) has argued that using the term "welfare" in and of itself requires the inclusion of subjective feelings per its definition; if something impacts an animal's welfare, it must impact how an animal feels. The public usually voices concern regarding the inclusion of an animal's ability to act naturally as a significant impact to its welfare state. Although expressing natural behavior is important, some have argued that it actually may decrease overall animal welfare status to express all natural behaviors as some represent states of distress or fear, such as distress calls or behavioral responses to extreme temperatures (Hughes and Duncan 1988).

In 1965 the Brambell Commission was formed to determine what components are essential to ensuring animal welfare in livestock species (Brambell Commission 1965), and this was a poignant event in the realm of livestock species animal welfare. Based on a review of the scientific literature available at the time, the commission proposed several conditions deemed necessary to ensure livestock welfare. In 1979, the Farm Animal Welfare Council revised the Brambell Commission recommendations and created the "five freedoms." The "five freedoms" serve as the basis for many of the livestock welfare educational, assessment, and third-party auditing programs and regulations globally. The five freedoms include the critical aspects of biological functioning (health and nutrition), nature-based measures (expression of normal behavior), and affective states (fear and distress) as discussed previously.

These freedoms are:

1) Freedom from hunger and thirst by ready access to freshwater 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.

Diseases of Swine, Eleventh Edition. Edited by Jeffrey J. Zimmerman, Locke A. Karriker, Alejandro Ramirez, Kent J. Schwartz, Gregory W. Stevenson, and Jianqiang Zhang.

4) Freedom to express normal behavior by providing sufficient space, proper facilities, and company of the animal's own kind.
5) Freedom from fear and distress by ensuring conditions and treatment, which avoid mental suffering.

A widely recognized animal welfare definition 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." More 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 provides 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 animal welfare discussions, including those regarding international trade. As retailers and food chains become more focused on animal welfare within their supply chain, many companies have been adopting the "five freedoms" as part of their animal welfare policies for suppliers.

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 is 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, could potentially be committed due to a lack of knowledge or ability of the owner and can be corrected through education and training. In the swine industry, animal abuse and neglect are defined as acts outside of normally accepted production practices that intentionally cause pain and suffering including but not limited to malicious hitting or beating an animal; applying electric prods to sensitive areas of the animal; driving pigs off high ledges, platforms, or steps while moving, loading, or unloading; dragging of conscious animals by any part of their body; purposeful dropping or throwing animals; causing physical damage to the snout or tusks of a boar as a means to reduce aggression (excludes nose ringing and tusk trimming); and purposeful failure to provide food, water, or minimal care that results in serious harm or death (NPB 2017).

While there are currently no federal laws in the United States that govern livestock care on farm, there are animal cruelty laws in all 50 states. The language, enforcement, and penalties of these laws vary 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 recordkeeping and documentation of these cases are essential. All of the major audit tools that have been created to monitor animal welfare within the livestock supply chain include some measure of animal abuse and neglect. The North American Meat Institute Animal Handling Audit (NAMI 2013) and the National Pork Board Common Swine Industry Audit (NPB 2017) include observation of animal abuse or neglect as an immediate audit failure. Additionally, pig farms are expected to have a zero-tolerance policy for animal abuse and neglect. All caretakers should be trained on the policy, understand how to report abuse and neglect, and understand the disciplinary steps that are associated with abuse and neglect (NPB 2017).

Comparisons between the domesticated and wild pig

When a veterinarian is assessing animal welfare, 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 pig may choose to engage in and how this may be relevant to the domesticated pig. Comparisons between a variety of species when domesticated and wild 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 1997). For example, the domesticated pig may perform the same behaviors as its wild ancestor but may not perform those behaviors as frequently, or more frequently. Stolba and Woodgush (1989) observed adult pig behavior in a semi-natural 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 stereotypies (behavior(s) performed repeatedly without an obvious function) can be indicative of impaired welfare. Pig stereotypical behaviors 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 animal welfare

Stress can be defined as the nonspecific response of the body to any demand (Selye 1973). In commercial swine production, stressors, defined as stress-producing factors (Selye 1973), can include handling by humans, novel environments (Gray 1979), disease prevalence, high or low temperature, and aggressive pig temperament (Black et al. 2001). While the stress response is essential for animal survival and biological function, it can antagonize swine production goals such as feed efficiency, growth, carcass quality, and welfare.

Stress can occur during both positive and negative situations. Moberg (2000) defines eustress as a nonthreatening stress response and distress as a stress response with deleterious effect on the individual's welfare. Stress is often closely related to pig welfare and hence is often measured. The short-acting stress response, also referred to as the "fight or flight" response (Cannon 1929), is controlled by the sympathetic–adrenal medullary system and is typically measured through epinephrine and norepinephrine. The longer-acting, sustained stress response is controlled by the hypothalamic–pituitary–adrenal axis and is typically measured through adrenocorticotropic hormone (ACTH) and cortisol. Other measures that are commonly used to evaluate the pigs' stress response include endorphin, lactate and glucose concentrations in the blood, heart rate, respiration rate, electroencephalography, and behavior.

Responses to stress influence key metabolic, immunological, and reproductive processes governing disease resistance and production performance. Therefore, health and production performance are also used as animal welfare indicators. Stress can have negative consequences on swine performance as it results in catabolism of body tissues through lipolysis, proteolysis, and glycogenolysis (Weissman 1990). Additionally, behavioral stress responses of decreased feed intake and altered activity level also alter swine performance (Elsasser et al. 2000). 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). Unpleasant handling, in comparison with sympathetic handling, resulted in pigs that were more fearful and 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.

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 "faring well" but a healthy pig with high productivity could be mentally compromised.

Affective states of animals

Affective states, also referred to as emotional or psychological states, are an integral component of an animal's overall welfare. 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). Thus, pigs are considered sentient.

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. It 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 weaning stress by studying different weaning methods (Colson et al. 2012). Additionally, caretakers try to ameliorate practices that cause stress and fear in pigs 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.

Modern animal welfare studies are shifting toward evaluating positive in addition to negative affective states. In a study evaluating pig behavior in anticipation of a reward, Reimert et al. (2013) identified play, playbark vocalizations, and tail movements to be indicators of positive affective states. 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.

Animal affective states are not only characterized by changes in behavior but also by changes in certain physiological parameters such as activation of the hypothalamic–pituitary–adrenal axis and the sympathetic–adrenal medullary system (i.e. a "stress response"). These changes occur to prepare the animal for the stressor with which they are confronted. 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 benchmarks. These benchmarks can then be used for decision-making regarding best management practices and provide a way for producers to demonstrate that their pigs are receiving 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 resourced-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" indicators, 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).

A robust animal welfare assessment program should include both animal-based measures to identify and fully understand the actual welfare of the animal and resource-based measures to identify potential causes of poor welfare. An animal's welfare state is dynamic and can be influenced by subtle changes in its health or the environment. Therefore, monitoring animal welfare must be an ongoing process.

Several science-based programs have been developed to assess on-farm swine welfare through a combination of first-, second-, and third-party evaluations. Through live observation, the observer evaluates the animals, caretakers, facilities, and records. The objective for first- and second-party evaluations is to benchmark performance and educate on good production practices. The objective of third-party evaluations is independent verification of compliance with a set standard of care. The value of these on-farm evaluations to an animal's welfare, regardless of the observer's relationship with the farm, is found in the feedback of strengths and opportunities for improvement. The producer can use this information to make informed decisions about production practices and procedures and ultimately protect and promote good animal welfare.

Recent technology advancements have introduced the concept of remote video auditing as a tool for animal welfare assessment and monitoring. Video auditing technology can help achieve good biosecurity because new people or materials are not entering the farm to conduct an audit. The technology also provides opportunity for continuous monitoring and spontaneous audits. However, remote video auditing may be difficult to implement on farm due to some facility designs. Video auditing protocols require further development to assure animal- and resource-based measures can be properly evaluated. Wearable video technology may hold merit to resolve this limitation.

Maternal behaviors

Pre-farrowing behaviors of the Sow

Gilts and sows exhibit a specific pattern of behaviors prior to farrowing (Widowski and Curtis 1989, 1990). In non-confined sows (i.e. outdoor arks, indoor huts, or pens), nest building occurs during the last 24 hours' pre-parturition 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 (Edwards 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 that sows that did not crush any of their piglets ("non-crushers") showed a more protective mothering style than those that crushed several piglets ("crushers"). Non-crushers 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 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 (Damn 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 for two possible reasons. First, persistent suckling attempts force them to stay close to the sow for long periods of time (Alonso-Spilsbury et al. 2007), and second, they have poor mobility due to decreased milk intake, and

they are often too weak to respond in a timely fashion to move out of the way of a sow changing postures (Marchant et al. 2001).

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). The cause of savaging in sows is poorly understood. 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 the 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 firstborn 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 could 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 and Morrison 1984), and remove the piglets from the sow until farrowing is complete. However, Chen et al. (2008) point 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." This is an important point, especially when discussing animal pain as they use auditory, physiological, and physical signs 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. 2008). Not only is pain complex from the standpoint of transmission, processing, and control, but it is also complex in that there are different types of pain that have been identified based on cause or pathophysiology, the most important of which are acute and chronic pain. Because of the complexity of pain, it is understandable that pain management and pain control are complicated and difficult.

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 physiologic 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. 2008). 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 anesthesia on commercial pig farms in the United States (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 (Marchant-Forde et al. 2009) to prevent tail biting (refer to tail biting in the "ORAL AND LOCOMOTOR 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 side cutting pliers caused an increase in cortisol concentrations compared with non-docked controls up to 60 minutes after docking (Sutherland et al. 2008, 2011). 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 a heated cautery iron did not affect ACTH, cortisol, or lactate concentrations in young pigs (Prunier et al. 2005; Sutherland et al. 2008), and cortisol concentrations were lower in pigs tail docked using a cautery iron 60 minutes after docking compared with pigs tail docked using side cutting pliers (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 anesthetic prior to tail docking or inducing general anesthesia using carbon dioxide gas reduced the percentage of stress vocalizations performed by pigs during tail docking (Herskin et al. 2016; Sutherland et al. 2011). In addition, pigs administered a nonsteroidal anti-inflammatory drug (NSAID) 30 minutes prior to tail docking were less likely to spend time isolated from other pigs than docked pigs given a placebo (Tenbergen et al. 2014). However, administering anesthetic locally or topically to the wound, inducing general anesthesia using carbon dioxide gas, or administering an NSAID did not reduce the cortisol response to tail docking in pigs (Sutherland et al. 2011; Tenbergen et al. 2014). 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 LOCOMOTOR 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 (Heinritzi et al. 1994). Maintaining the appropriate equipment and utilizing good technique also help 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 are 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 non-castrated 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 ineffective at reducing the behavioral response to surgical castration in pigs (McGlone et al. 1993). However, administering an NSAID (meloxicam) prior to castration reduced postsurgical pain-related behaviors (Hansson et al. 2011). General anesthetics including an injectable anesthetic consisting of xylazine, ketamine hydrochloride, and glyceryl guaiacolate administered intravenously (McGlone et al. 1993); ketamine, climazolam, 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), carbon dioxide gas (Gerritzen et al. 2008; Sutherland et al. 2012; Van Beirendonck et al. 2011), and nitrous oxide (Rault and Lay 2011) have been used to reduce the pain 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 inhaled anesthetic (i.e. ketamine, climazolam, 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 (Kluivers-Poodt et al. 2012; 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. However, topical administration of a short- or long-acting anesthetic to the castration wound was not effective in reducing the pain associated with surgical castration in pigs (Sutherland et al. 2010).

Alternatives to castration include slaughtering pigs before they reach sexual maturity, using immunocastration techniques, sperm sexing for selection of female offspring, and genetic selection for pigs with low levels of boar taint (Rault et al. 2011). 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 lbs), 5% of carcasses still exhibit boar taint (Bonneau 1998). Furthermore, the average weight of pigs at slaughter is increasing, and light carcasses are less profitable for commercial swine processors (EFSA 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 (EFSA 2004; von Borell et al. 2009).

Tusk trimming

Boars with tusks pose a potential risk to both human handlers and 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, either for boars in transit or when being 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 hacksaw 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 gum line and is innervated in both vascularized and non-vascularized 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.

Pain management in the neonatal piglet during routine management: grading the quality of evidence and strength of recommendations

In 2014 global pain experts were invited by the National Pork Board to review the current published work on pain management in the neonatal piglet and were charged to provide recommendations related to pain mitigation. The Dzikamunhenga et al. (2014) systematic review's aim was to synthesize the existing primary scientific literature regarding the effectiveness of pain management interventions used for routine procedures on piglets. The review question was, "In piglets under twenty-eight days old, undergoing castration, tail docking, teeth clipping, and/or methods of identification that involve cutting of the ear tissue, what is the effect of pain mitigation compared with no pain mitigation on behavioral and non-behavioral outcomes that indicate procedural pain and post-procedural pain?" A review protocol was designed a priori. Data sources used were AGRICOLA (EBSCO), CAB Abstracts (Thomson Reuters), PubMed, Web of Science (Thomson Reuters), BIOSIS Previews (Thomson Reuters), and ProQuest Dissertations and Theses Full Text. No restrictions on year of publication or language were placed on the search. Eligible studies assessed an intervention designed to mitigate the pain of the procedures of interest and included a comparison group that did not receive an intervention. Eligible non-English studies were translated using a translation service. Two reviewers independently screened titles and abstracts for relevance using predefined questions. Data were extracted from relevant articles onto predefined forms. From the 2203 retrieved citations, forty publications containing 52 studies met the eligibility criteria. In 40 studies, piglets underwent castration only. In seven studies, piglets underwent tail docking only. In one study, piglets underwent teeth clipping only, and in one study piglets underwent ear notching only. Three studies used multiple procedures. Thirty-two trial arms assessed general anesthesia protocols, 30 trial arms assessed local anesthetic protocols, and 28 trial arms assessed NSAID protocols. Forty-one trial arms were controls where piglets received either placebo or no treatment. Forty-five outcomes were extracted from the studies; however only the results from studies that assessed cortisol (six studies), β-endorphins (one study), vocalizations (nine studies), and pain-related behaviors (nine studies) are reported. Other outcomes were reported in only one or two studies. The authors concluded that confident decision-making would likely be difficult based on this body of work because lack of comprehensive reporting precludes calculation of the magnitude of pain mitigation for most outcomes. In a companion paper, O'Connor et al.'s (2014) objectives were to develop recommendations for pain mitigation in 1- to 28-day-old piglets undergoing castration. Recommendation development followed a defined multistep process that included an evidence summary and estimates of the efficacies of interventions. Recommendations were developed for three interventions: CO_2/O_2 general anesthesia, NSAIDs, and lidocaine for use during castration. The ability to make strong recommendations was limited by low-quality evidence and strong uncertainty about variation in stakeholder values and preferences. The panel strongly recommended against the use of a CO_2/O_2 general anesthesia mixture, weakly recommended for the use of NSAIDs, and weakly recommended against the use of lidocaine for pain mitigation during castration of 1- to 28-day-old piglets.

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 diphyodont 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 ($2 \times$ incisors $^{3\ \text{upper}}/_{3\ \text{lower}}$, canines $^{1}/_{1}$, premolars $^{3}/_{3}$, molars $^{0}/_{0}$) with their permanent set increasing to 44 ($2 \times i^{3}/_{3}$, $c^{1}/_{1}$, $p^{4}/_{4}$, $m^{3}/_{3}$), the most comprehensive for any eutherian mammal (Tonge and McCance 1973). The majority of teeth used for masticating feed (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. 2010). 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 p^{3} and p_{4} erupted (i.e. the two premolars required for initial occlusion) and have higher weight gains in the following 3 weeks (Tucker et al. 2010). 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 (ADG) 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 (Widowski et al. 2008b). 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 (Fujii et al. 1990; Phillips et al. 2001). Between birth and 4 weeks, water intake increases as a function of age, but consumption per kg 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). Another creep feed attribute that improves feed intake following weaning is pellet diameter. van den Brand et al. (2014) found that piglets prefer larger versus small pellet diameter (12 mm vs. 2 mm) in the preweaning period, and this led to increased feed intake and body weight gain after weaning.

Ingestion of creep feed can help prepare the gastrointestinal system for the post weaning 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 in 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 locomotor behaviors

Tail biting

Tail biting behavior occurs when one pig takes the tail of another pig into its mouth and causes damage to the appendage (Schrøder-Petersen and Simonsen 2001). The behavior is often described as beginning with non-damaging exploratory behavior by one pig, termed "tail-in-mouth" behavior, which leaves no visible trauma but which then escalates to a damaging stage and the development of lesions (Fraser and Broom 1997; Schrøder-Petersen and Simonsen 2001). Once damage occurs, other pigs may quickly join in performing tail biting behavior, which can result in pen-wide or even barn-wide outbreaks (Fraser 1987). In addition to causing pain and distress, tail biting is associated with reduced feed intake and weight gain (Sutherland et al. 2009; Wallenbeck and Keeling 2013). The behavior can also lead to infection, spinal abscess, disease transmission, carcass damage, and, in some cases, cannibalism and death (Kritas and Morrison 2007; Schrøder-Petersen and Simonsen 2001).

Recommended as an animal-based measure for on-farm welfare audits (Goossens et al. 2008), 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 (Goossens et al. 2008; Schrøder-Petersen and Simonsen 2001), numerous management, environmental, and individual factors have been implicated (D'Eath et al. 2014; Sutherland et al. 2009; Wallenbeck and Keeling 2013). For example, a barren growing environment has been shown to result in an increased percentage of pigs with a bitten tail (Bolhuis et al. 2005; Moinard et al. 2003). 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 post weaning period that is most critical (Bovey et al. 2010; Smulders et al. 2008). Therefore, adequate space allowance in the nursery and grower barns can help reduce the behavior, as can the installation of chains or 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. 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. It has been proposed that longer tails are more easily damaged, as pigs are able to bite them with their check teeth (Paoli et al. 2016).

Not all pigs tail bite, and it appears that some pigs may be predisposed to performing injurious oral behaviors, while others are predisposed to receiving it (Brunberg et al. 2013). 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. There also appears to be a genetic component to tail biting, with the behavior correlated with lean tissue growth and backfat thickness (Breuer et al. 2003). In addition, there appear to be "neutral" pigs that have a genetic and behavioral profile that contributes to them being resistant to performing or receiving pig-directed abnormal behaviors (Brunberg et al. 2013).

Recent studies have also indicated physiological differences associated with the tail biting behavioral phenotype. Both tail biter pigs and bitten pigs were found to have lower peripheral serotonin levels compared with neutral pigs (Ursinus et al. 2014). Differences between neurotransmitters in the brain regions of pigs that tail bite and those that are bitten have also been identified. Those pigs that tail bite show higher serotonin metabolism in the prefrontal cortex, while bitten pigs show changes in both dopamine and serotonin metabolism in their limbic cortex and striatum (Valros et al. 2015).

Regardless of the cause of the behavior, the removal of both the tail biter and tail bitten pigs is an important management strategy to both reduce the probability of social facilitation of tail biting and stem any increased harm to the pigs being injured.

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 most piglets perform some belly nosing, not all do, and there is a wide variation in the amount of belly nosing individual piglets perform.

Although belly nosing is most often associated with weaning at an early age (Fraser 1978; Worobec et al. 1999), the motivation behind the behavior is yet undetermined. It has been suggested that this behavior is the result of discomfort and stress in newly weaned piglets (Dybkjær 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. 2008a). Other factors associated with higher frequencies of belly nosing include the presence of certain forms of enrichment (Funbar) (Bulens et al. 2015), rearing entire male pigs (Tallet et al. 2013), and rearing pigs in artificial rearing systems (Rzezniczek et al. 2015).

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). Provision of environmental enrichment (EE) (Oostindjer et al. 2011; Rodarte et al. 2004; Waran and Broom 1993), suckling devices (Rau 2002; Widowski et al. 2005), alternative drinkers (Torrey and Widowski 2004), and rearing pigs in loose housing systems during lactation (Oostindjer et al. 2011) have been successful in reducing, but not eliminating, the behavior.

Lameness

Swine lameness on farm can result in negative affective states (i.e. pain) to individual animals (Jensen et al. 2012). Veterinarians and caretakers can use on-farm scoring methods to determine lameness level 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 analogue 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 analogue 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 endpoints of normal and "could not be more lame" for an individual's level of lameness (Quinn et al. 2007).

There has been interest in testing other lameness tools. Tools include the embedded microcomputer-based force plate system (Sun et al. 2011), the GAITFour pressure mat gait analysis walkway system (Karriker et al. 2013; Mohling et al. 2014a; Pairis-Garcia et al. 2015a), nociceptive threshold tests (Mohling et al. 2014b; Tapper et al. 2013), classification lameness tress (Abell et al. 2014), and behavior (Pairis-Garcia et al. 2015b; Parsons et al. 2015, 2016).

When considering behavior in more detail, Stienezen (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 the sows immediately before, during, and after their morning feed. Phenotypically normal (control) sows spent more time feeding and more time 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.

Pairis-Garcia et al. (2015a) noted that caretakers and veterinarians can use husbandry and management tools to provide supportive care for pigs experiencing lameness. Supportive care may include providing additional bedding or a rubber mat to create a more comfortable area for lying and resting (Elmore et al. 2010; Pluym et al. 2013). Campler et al. (2016) provided a practical case study for the use of mats in the farrowing/lactation house. This case study covered the cost, implementation, and longevity of mats. The authors concluded that (1) perforated rubber mats may provide an easy and inexpensive way to improve sow comfort in the farrowing stall; (2) mat size, cleanliness, cost, durability, and management are important factors to consider; and (3) rubber mats need to be placed properly under the sow and fastened properly to ensure maximum sow benefit.

Another approach for on-farm pain management is pharmacological techniques such as analgesics. NSAIDs are common analgesic medications used in livestock as they are easy to administer, long lasting, and cost effective. The pharmacokinetic profile of meloxicam (Pairis-Garcia et al. 2014) and flunixin (Pairis-Garcia et al. 2013) in mature sows has been determined. Paris-Garcia et al. suggested that meloxicam and flunixin meglumine are effective pharmaceutical interventions for alleviating pain associated with a chemically induced synovitis lameness model. Although analgesic drugs may be a key

tool to manage negative pain affective states associated with lameness at the time of writing, meloxicam and flunixin meglumine are not approved pain management treatments in swine in the United States.

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). This is likely because humans play a number of important roles for the pig. Humans act indirectly through their responsibility for the design of the environment and development of husbandry and management regimens. Caretakers also act directly by providing the day-to-day care of the animals. The human caretaker is the critical factor in the success or failure of a housing system and can impact pig welfare (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. Fear is defined as the general susceptibility of an individual to react to potentially threatening situations (Boissy et al. 2007), and fearfulness 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 to have reduced reproductive output (Hemsworth et al. 1987, 1989, 1993).

Human exposure is one of the most frightening events that farm animals are likely to experience (Boissy 1995). In swine production, humans may have little interaction with pigs other than situations that might be perceived as negative by the pig. These situations can include medically treating (Weimer 2012), castration, tail docking, restraining, and sorting (Waiblinger et al. 2006). With little opportunity to habituate, it is suggested that even domesticated animals may often perceive humans as predators (Suarez and Gallup 1982). However, previous positive experiences with humans such as gentle tactile interaction, talking, and food provision may decrease pigs' fear of humans (Brajon et al. 2015; Muns et al. 2015; Tallet et al. 2014). Additionally, genetic selection (Colpoys et al. 2014), pig sex (Colpoys et al. 2015; Reimert et al. 2014), and housing system (Reimert et al. 2014) have been shown to alter pig–human interactions.

Fatigued pigs

Transport losses due to injury, fatigue, or death represent significant animal welfare, regulatory, and economic concerns and are estimated to cost the US swine industry $46M annually (Ritter et al. 2009a). These dead and nonambulatory pigs are most commonly observed during unloading at the packing plant, but these losses can occur at any stage of the marketing process from loading at the farm to stunning at the plant. Transport losses at US packing plants include:

- Dead on arrival (DOA): A pig that died during transportation.
- Dead in yard (DIY) or dead in pen (DIP): A pig that died after unloading at the plant.
- Nonambulatory pig: A pig unable to move or keep up with the rest of the group at the plant.

There are two types of nonambulatory pigs observed under US commercial conditions: Fatigued pigs are pigs without obvious injury, trauma, or disease that refuse to walk at any stage of the marketing process from loading at the farm to stunning at the plant. Meanwhile, injured pigs have a compromised ability to move due to structural unsoundness or due to an injury sustained during the marketing process (Ritter et al. 2009a).

Incidence of dead and nonambulatory pigs at packing plants

According to national statistics reported by the Food Safety and Inspection Service (FSIS), the percentage of dead market swine at USDA-inspected packing plants has averaged 0.20% over the last 25 years (Ritter et al. 2017). Although national statistics are not available for nonambulatory pigs at the plant, a recent summary of 23 US commercial field trials involving 6.6 million pigs reported the following rates for transport losses at the plant: 0.25% for dead pigs and 0.44% for nonambulatory pigs. It is important to note that the vast majority of nonambulatory pigs in these studies were classified as fatigued (Ritter et al. 2009a).

The fatigued pig syndrome

Ivers et al. (2002) evaluated acute stress signs and metabolic parameters in 35 normal and 35 fatigued pigs during unloading at the packing plant. Fatigued pigs showed more clinical signs of acute stress including open-mouth breathing (44 vs. 0%, respectively), skin discoloration (77 vs. 0%, respectively), muscle tremors (83 vs. 3%,

respectively), and abnormal vocalizations (30 vs. 0%, respectively). Furthermore, fatigued pigs had higher stress hormone concentrations (cortisol, epinephrine, norepinephrine), higher creatine kinase values, and blood parameters consistent with metabolic acidosis. Controlled studies have demonstrated that the vast majority of fatigued pigs will metabolically recover if the stressors are removed and pigs are allowed to rest for 2–3 hours (Ritter et al. 2009a).

Porcine stress syndromes

It is interesting to note the striking similarities between the symptoms and metabolic characteristics of fatigued pigs to those of pigs with porcine stress syndrome (PSS) (see Chapter 3), which is caused by a C to T mutation at nucleotide 1843 of the RYR1 gene and is referred to as HAL-1843. Therefore, a commercial survey involving 2109 pigs was conducted at four Midwestern US packing plants to determine the impact of the HAL-1843 mutation on the incidence of dead and fatigued pigs at US packing plants. This study demonstrated that 98% of the normal pigs, 95% of the dead pigs, and 98% of the fatigued pigs evaluated were free of the HAL-1843 mutation (Ritter et al. 2008), suggesting that the HAL-1843 mutation has minimal effects on dead and fatigued pigs at the packing plant.

It is possible that other genes or mutations may be responsible for the fatigued pig syndrome. Recently, Nonneman et al. (2012) reported that a mutation in the dystrophin gene (DMD) was associated with death in pigs during routine handling and transportation. Additional research is necessary to understand if this new stress syndrome contributes to the fatigue pig syndrome.

Predisposing factors for transport losses

Transport losses are a multifactorial problem consisting of people (handling tools and handling intensity), pig (genetics, diet, ractopamine, gut fill, live weight, health status, and previous handling experiences), facility design (pen size, pre-sorting strategies, aisle width, distance moved, and loading ramp angle), transportation (trailer design, mixing of unfamiliar pigs, loading density, and length of journey), packing plant (waiting time at the plant, unloading procedures, distance moved, facility design, and lairage time), and environmental factors (season, temperature, relative humidity, and trailer settings for bedding, boarding, and misting), which have been reviewed by Ritter et al. (2012), Johnson et al. (2013), and Zurbrigg et al. (2017). A review by Ritter et al. (2012) concluded that transport losses are impacted by (1) the HAL-1843 mutation, (2) aggressive handling, (3) group size during handling, (4) facility design, (5) crowding pigs during transport, and (6) extreme hot and cold weather conditions.

Management strategies to reduce transport losses

Preslaughter stressors have additive effects on the stress responses (rectal temperature, blood lactate, and blood pH values) of market weight pigs (Ritter et al. 2009b). Therefore, removing or spacing out the stressors to allow the pig to return to homeostasis during the marketing process can improve the pig's well-being and can potentially reduce the risk of transport losses at the plant. Management strategies to reduce transport losses under US commercial conditions include better preparing pigs for transport, improving facility design, minimizing stress during handling, and optimizing transport conditions (reviewed by Ritter et al. 2012).

Aggression

The domestic pig is largely a social animal. In the wild or feral state, pigs are found either in a matriarchal group of one to five adult females and two or more ages of offspring from successive pregnancies (Barrett 1978). When males reach puberty, they leave the herd and travel either alone or in small groups (ex. 2 boars). Females and young males are clearly social, while adult males are often (but not always) solitary. As piglets from different mothers in a herd are born, they interact with other piglets with minimal aggression. They may play fight or have small skirmishes, but among prepubertal pigs, there are infrequent injuries from fighting. Boars do fight and can injure each other in the wild; however, injury is uncommon. And sows, generally, are dominant to boars most of the time. When sows are in estrus, they will allow boars into the group for mating.

When pigs moved from outdoor pastures (where litters were socialized from birth) to indoors, litters were often kept apart from birth through weaning. We have known for decades that aggression is common on commercial farms (Signoret 1962). This aggression is a function of the production system in that unfamiliar pigs are abruptly introduced without a preweaning socialization period.

For growing pigs, mixing-induced aggression 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 – and if they were previously housed individually or in small groups, they will fight. Post mixing aggression establishes a social hierarchy. This fighting is stressful but is reduced over time. McGlone (1986) showed that aggressive interactions

last for 19 hours after mixing pigs. He further reported that access to feed (or not) did not change the amount of fighting observed. Water access is more closely related to social stress. Aggressive behavior and the development of a dominance hierarchy will occur regardless of resources (feed, water, space, etc.). However, chronic or sporadic aggression may be due to limited resources. For example, even with established dominance hierarchies, growing pigs and sows that are limit fed (less than ad libitum) will show aggression. These challenges to the dominance order will occur, for example, when feed is given in limited amounts (Graves et al. 1978). Hunger makes pigs more aggressive, even if it is a few hours between meals (Kelley et al. 1980). Pigs may fight over limited resources such as feed, water, breeding mates, and/or nesting sites (Barnett et al. 1994; Csermely and Wood-Gush 1987; Edwards et al. 1994; Séguin et al. 2006). Interestingly, the social hierarchy is established without the need for all pigs to fight with each other. Mendl and Erhard (1997) mixed 4 pigs from 1 established group with 4 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 upon information gained from their own interactions and probably from interactions of other pairs. Preexposure that permits a mixture of visual (e.g. physical size), auditory (e.g. frequency or duration of vocalizations), and olfactory (McGlone 1985) cues could reduce fighting post mixing (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 and decreasing productivity (Mendl et al. 1992). During an aggressive act, a pig focuses its bites on its opponent's head and ears (Kelley 1980). When one pig submits to another pig in close quarters, it tries to protect its head and ears (McGlone and Curtis 1985). McGlone and Curtis (1985) also first reported the strong relationship between duration of aggression and the presence of wounds. Wound scores can be used in practice to determine the relative amount of aggression in groups of pigs.

A number of options have been explored to manage and reduce aggression in pigs. Pen shape has been reported to affect aggression in the short term. For example, pigs often use corners to "hide" (McGlone and Curtis 1985), and circular pens resulted in higher levels of aggression than square or rectangular pens. A solid barrier within the pen reduces the total number of aggressive interactions over a 12 hour post mixing period in sows (Edwards et al. 1993) and has longer-term benefits in sows. Barnett et al. (1993) compared adult pigs at mixing when placed into small rectangular pens (1.4 m^2/pig [15.07 ft^2]) for aggressive interactions and the consequent retaliations. The authors reported during the period of 15–90 minutes after grouping lower aggressive interactions, but the presence of stalls had no effects at this time. On the day following grouping, lying alone and standing were reduced, and concurrent lying and use of stalls (when present) were increased. In dynamic systems for sows, where subgroups are mixed into a larger resident group, dividing the pen into distinct lying bays, with one assigned to each subgroup on introduction, may have long-term advantages in reducing aggression by giving each subgroup its own "territory" (Bünger and Kallweit 1999).

Aggression can also be managed by adjusting group size. There are two hypotheses with regard to optimum group size: (1) that the number of fights will increase with the number of hierarchy positions to settle (Anderson et al. 2000; Schmolke et al. 2003) and (2) that pigs become less aggressive and may shift to a low aggressive social strategy in large social groups that may in turn provide potential benefits for the welfare of pigs under commercial production situations (Samarakone and Gonyou 2009). For a review on the impact of large groups on productivity, see Turner et al. (2003). However, to date, the optimum group size, parity balance within a group, body weight allocation, and space allowance remain relatively undecided in the United States. Probably very large groups of pigs (e.g. 1000 pigs in a large pen or building) never allow all pigs to establish a dominance order, so a small amount of fighting might be observed, or alternatively, pigs learn to not initiate aggressive interactions due to the large number of individuals in the group.

Chemical and nutritional interventions can be utilized to reduce aggression. 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 azaperone or saline. Injuries to the ears and total injuries were less severe in amperozide-treated pigs than in pigs on the other treatments. Amperozide-treated pigs spent less time eating on day 1 than 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) (Luescher 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.

Pigs have a well-developed sense of smell. Removal of the olfactory bulb significantly reduced pig aggression (Meese and Baldwin 1975), and pigs can tell one another apart by olfaction alone (Meese et al. 1975). McGlone (1985) and McGlone et al. (1987) found evidence that biological fluids change pig behavior including aggression. They proposed several pheromones that increase or decrease pig aggressive or submissive behaviors. In addition, they proposed that male odors might reduce aggression of prepubertal pigs.

McGlone and Morrow (1988) compared prepubertal crossbred pigs to determine the minimum dose of androstenone (5α-androst-16-en-3-one) that would reduce the level of agonistic behavior among dyads of newly regrouped pigs. The authors concluded that a single application of as little as 0.5 μg/pig androstenone reduced aggressive behavior among prepubertal pigs and, therefore, may be a way of reducing fighting among newly regrouped prepubertal pigs.

Boar presence can also impact aggression. Grandin and Bruning (1993) compared barrows and gilts at the packing plant with or without a mature boar in their lairage pens (via a pheromone effect similar to that reported by McGlone and Morrow [1988]). 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 post mixing 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.

Morrow-Tesch and McGlone (1990) identified skin secretions from sows that piglets could recognize. Pageat (1998) isolated maternal skin secretions (a mixture of fatty acids) that could be maternal–neonatal pheromones. McGlone and Anderson (2002) later showed that application of these putative maternal–neonatal pheromones reduced aggression and stimulated post weaning weight gain in pigs. Recently, Plush et al. (2016) showed that this maternal–neonatal pheromone slightly reduced aggression among group-housed adult sows; however, skin lesions were not reduced by this putative pheromone compared with a control group.

Early social experience may also play a role as a longer-term solution to reduce aggression at mixing (Pitts et al. 2000). Mixing 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 preexposure with and to other pigs. With repeated mixing, pigs that are remixed three or four times post weaning 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 preexposing 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 preexposure. Jensen and Yngvesson (1998) have also reported this preexposure 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). 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 approximately 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 enriching their environment with play objects could modify aggression frequency in intensively raised pigs.

In a review of literature, Johnson and McGlone (2011) showed that aggressive and submissive behaviors were lowly (but significantly) correlated. Aggression of sows toward piglets (a maladaptive behavior) is highly correlated (Knap and Merks 1987). 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. (2000) rediscovered the correlation between wounding and aggressive behavior (McGlone and Curtis 1985) and showed that part of this relationship is genetically determined. Post mixing 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. (2000) used a Bayesian approach to estimate the heritability of three traits associated with aggressiveness in pigs during the 24 hour post mixing: 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. In pigs (and other species), selection for increased ADG can cause an increase in aggressiveness (ADG and aggressiveness are correlated phenotypically; Vargas 1987). However, selecting groups of pigs for increased ADG and reduced aggression can improve ADG (Camerlink et al. 2013). One strategy to improve the welfare of pigs is to include behavioral measures such as aggression in selection programs (Rodenburg and Turner 2012). We know that feral pigs are more aggressive than most domestic pigs. Therefore, we do understand that behaviors can be selected that work better in a commercial setting. Consumers and retailers may prefer pork from pigs that do not show damaging behaviors toward each other. We should expect genetic improvement in animal welfare in the future, including selection for less stressful and damaging behaviors.

Influence of disease on behavior

As a result of behavioral changes, ill pigs are frequently subjects of investigation and bullying by their pen mates. Consequently, diseased and injured individuals comprise vulnerable populations within the swine operation, presenting unique behavioral and welfare needs (Millman 2007). Behaviors such as decreased feeding and drinking, decreased exploration, increased sleep, heat seeking, and lethargy are often the first clinical signs of disease that are observed by caretakers and veterinarians. These "sickness behaviors" are displayed by a wide range of vertebrate species in response to bacterial, viral, and protozoan pathogens and appear to be an evolved behavioral strategy that complements the innate immune system (Hart 1988). Sickness behaviors result when proinflammatory cytokines such as interleukin-1, interleukin-6, and tumor necrosis factor cross the blood–brain barrier or are produced by glial cells in the central nervous system (CNS) (Dantzer and Kelley 2007). These cytokines act as neurotransmitters, producing characteristic changes in physiology (e.g. fever) and behavior (e.g. anorexia).

Sickness behavior is organized as a motivational state and as such competes with other motivational states such as escape or vigilance for expression (Aubert 1999). Individual pig behavioral responses to particular pathogens will differ according to the pig's previous experiences and perception of its current environment. Understanding of the motivational factors that influence expression of sickness behavior can inform observation protocol and detection of ill individuals within a herd. Pigs infected with porcine reproductive and respiratory syndrome virus (PRRSV) spend more time lying relative to their uninfected pen mates (Escobar et al. 2007; Sutherland et al. 2007). Furthermore, PRRSV-infected pigs display lying postures that confer heat conservation (lying ventrally, lying in contact with other pigs) when housed in neutral thermal environments (24 °C), but not when housed at warmer (32 °C) temperatures (Sutherland et al. 2007). Although intake of water (Swinkels et al. 1994) and feed (Escobar et al. 2007) decrease during illness, affected pigs do not consistently reduce the amount of time spent at the feeder, perhaps due to social facilitation. In the absence of attentive observation, this behavior may provide the illusion of a healthy appetite and negatively impact detection of ill individuals in the pen.

Husbandry, handling, and housing of diseased pigs have important implications for animal welfare and pathogen transmission. Moving an ill or injured pig into a hospital can provide benefits in terms of minimizing the risk of bullying by healthy pen mates, reduced competition for resources, and ease of monitoring and medical intervention. However, social stress associated with mixing of unfamiliar pigs' results has been shown to increase shedding and transmission of *Salmonella typhimurium* (Calloway et al. 2006) and should be avoided when feasible. Furthermore, activation of the peripheral immune system has been shown to negatively impact spatial cognitive processing in piglets (Dilger and Johnson 2010), which could affect how compromised pigs respond to handling, and to navigate and find resources in novel environments. Hence, hospital pens need to be closely monitored to ensure ill and injured pigs are responding to treatment and for decision-making about humane endpoints when euthanasia is warranted (Millman 2015).

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 well-being 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, an 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, anxiety, and distress while rendering the animal rapidly insensible. The euthanasia process should result in rapid loss of consciousness, followed by cardiac or respiratory arrest and subsequent loss of brain function. Loss of consciousness should precede loss of muscle movement (AVMA 2013). Many 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 CNS, hypoxia, and physical disruption of brain activity (AVMA 2013). 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 cardiac and respiratory function. Physical disruption 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). If signs of sensibility are present subsequent to performing the euthanasia procedure, and it is safely possible, corrective action should be immediate. 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 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 the 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 the 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), though unconscious pigs may exhibit involuntary noises and movements. 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).

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 veterinary-administered anesthetic overdose, carbon dioxide, gunshot, penetrating and non-penetrating captive bolt, and electrocution (NPB 2016). Manually applied blunt force trauma for small pigs (<5.44 kg [12 lb]) is accepted (NPB 2016), but due to limitations of the method and aesthetics, active pursuit of alternatives is recommended (AVMA 2013, NPB 2016).

Carbon dioxide induces unconsciousness and anesthesia by altering the pH of cerebrospinal fluid and ultimately causes death by respiratory arrest (NPB 2016).

Specifically designed equipment is necessary to ensure pain and distress is minimized during the CO_2 euthanasia procedure, including an airtight container, with non-slip flooring, and gas regulator. Compressed gas in cylinders is the recommended source of CO_2. Other sources of CO_2, such as dry ice, fire extinguishers, or chemical reactions, are unacceptable (NPB 2016). Stocking density in the container should allow all pigs to lie down without being required to lie on top of another pig. Solitary pigs may display more distress behaviors than those euthanized in groups (Fiedler et al. 2016); however, practical considerations such as the size of the pigs relative to container and discrepancy in condition of the pigs may make euthanasia of a solitary pig preferable. Though important to consider stocking density as well as other factors such as ill health that impacts the respiratory system (e.g. swine respiratory disease), these factors have minimal impact on the efficacy of CO_2 euthanasia (Sadler et al. 2014a,b).

Although CO_2 is effective for causing death, this method is controversial (Mota-Rojas et al. 2012); loss of consciousness is not immediate (Chevillon et al. 2004), and vocalizations, signs of breathlessness, and active avoidance are sometimes observed during the inhalation phase (Raj and Gregory 1996; Rault et al. 2015). Although other gases such as nitrous oxide and argon have been successfully utilized in trials (Fiedler et al. 2016; Rault et al. 2015), there are practical considerations that make these alternatives prohibitive to implement, including attainability and difficulty in maintaining an efficacious gas concentration.

Gunshot, penetrating captive bolt, non-penetrating captive bolt, and blunt force trauma should inflict sufficient physical damage to both the cerebral cortex and the brain stem to cause immediate and irreversible brain damage leading to death. For all methods, restraint allowing proper application is necessary. A gunshot to the head can be effective for pigs (AVMA 2013; NPB 2016). 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). An alternative shot location is behind the ear, aiming toward the opposite eye (NPB 2016). It is imperative that the gun and load be sufficient to be effective with one shot. Neck and heart shots are not acceptable.

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 (Gregory 2007). Penetrating captive bolts can either stun or kill the pig. With the proper configuration, including gun design and charge, a penetrating captive bolt can be successful as a single step. Non-penetrating captive bolt guns do not cause sufficient brain damage to effectively stun or kill larger pigs and are only approved for pigs up to 31.57 kg (70 lb; NPB 2016). Similar to penetrating captive bolt, successful application of this procedure is depended on gun and charge configuration. For suckling piglets, physical methods of euthanasia may be the most practical for the caretaker. A non-penetrating 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. (2008b) 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 neonatal 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. 2013, 2014). 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.

Electrocution is another physical method of euthanasia. 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). Electric methods should only be used on pigs over 3 days of age.

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3

Genetics and Health

Caitlyn E. Bruns and Kenneth J. Stalder

Survival is a major economically relevant trait in the swine industry (Bergsma et al. 2008; Hermesch et al. 2014). Decreasing mortality at all production phases would increase profitability for the operation. Preweaning, nursery, and finisher livability has been trending downward over the last 6 years (Stalder 2016). Sow mortality has been increasing from 8 to 9% between 2010 and 2015 (Porth 2016). Given the mortality levels, there is opportunity for improvement. Genetic selection methods along with best management practices can be utilized to improve mortality throughout the swine industry.

Preweaning mortality

Preweaning mortality has increased by almost 1% per year for the last 6 years increasing to 17.4% in 2015 (Stalder 2016). The reason for this is not clear but may be attributed to the increase in litter size. Number weaned remained relatively flat not following the trend for increasing total born (Stalder 2016). Larger litters tend to have lower individual pig birth weights (Omtvedt et al. 1966; Roehe 1999). Lower pig birth weights observed in larger litters could result in higher preweaning mortality.

Genetic correlation estimates between total born and preweaning survival are varied. Nielsen et al. (2013) reported genetic correlations (SE) between total born and piglet mortality (including stillborns) to be 0.28 (0.06) and 0.22 (0.07) for Landrace and Yorkshire breeds, respectively. The genetic correlations reported by Putz et al. (2015) between total born and mortality to day 30 were not significantly different from 0. Additionally, Su et al. (2007) reported genetic correlations (SE) of –0.28 (0.12), –0.26 (0.12), and –0.43 (0.20) between total born and survival rate at birth, survival rate from birth to 5 days' post farrowing, and survival rate from 5 days' post farrowing to weaning for Landrace, respectively. The genetic correlation estimates for Yorkshire were –0.38 (0.16), –0.07 (0.23), and –0.527 (0.44) for total born and the survival traits (Su et al. 2007). The genetic correlations (SE) between the direct genetic effects of total born and proportion alive at birth and weaning were estimated to be 0.03 (0.01) and –0.26 (0.11) for Landrace, respectively, while the estimates for Yorkshire were not significantly different from 0 (Lund et al. 2002). The variation in genetic correlation estimates between studies suggests that the relationship between total born and preweaning survival may be highly dependent on the population. Differences in selection pressure over time may explain the varying relationship.

Selection for litter size and pig quality can be accomplished simultaneously. Using an alternative definition of litter size (e.g. number of pigs alive 5 days' post farrowing) can take advantage of desirable correlations with total born and preweaning mortality (Nielsen et al. 2013; Putz et al. 2015; Su et al. 2007). Genetic correlation estimates between total born and live pigs at 5 days ranged from 0.34 to 0.85, while the genetic correlation estimates between preweaning mortality and live pigs at 5 days ranged from –0.35 and –0.57 (Nielsen et al. 2013; Putz et al. 2015; Su et al. 2007).

Survival heritability estimates are low. Reported heritabilities for preweaning mortality ranged between 0.03 and 0.13 (Grandinson et al. 2002; Hellbrügge et al. 2008; Holl and Long 2006; Nielsen et al. 2013; Putz et al. 2015; Su et al. 2007). Selection for birth weight may be preferable when compared to preweaning survival. The heritability for the maternal genetic component of birth weight is greater than the heritability for survival. Reported heritability estimates for birth weight range from 0.04 to 0.10 for the direct genetic effect and from 0.15 to 0.26 for the maternal genetic effect (Grandinson et al. 2002; Holl and Long 2006; Hellbrügge et al. 2008; Putz et al. 2015). Since the birth weight maternal genetic effect has a higher heritability compared to preweaning survival, genetic progress can be made more rapidly in birth weight.

Diseases of Swine, Eleventh Edition. Edited by Jeffrey J. Zimmerman, Locke A. Karriker, Alejandro Ramirez, Kent J. Schwartz, Gregory W. Stevenson, and Jianqiang Zhang.

Selection for increased birth weight could indirectly improve preweaning survival. Putz et al. (2015) found the genetic correlation (SE) between pig birth weight and preweaning mortality to be –0.13 (0.11) and –0.14 (0.09) for Landrace and Yorkshire, respectively. These genetic correlations are not significantly different from 0, but the traits were not modeled with a maternal genetic effect. Hellbrügge et al. (2008) reported the genetic correlation (SE) between birth weight and survival rate to be 0.21 (0.16), but, again, the maternal genetic effect was not included in the model.

Based on the heritability estimates for both the direct and maternal genetic components, the maternal effect explains more variation in birth weight compared to the direct effect. It is important to understand the model used for birth weight when comparing genetic parameter estimates. Holl and Long (2006) estimated the genetic correlation (SE not reported) between the birth weight direct effect and the preweaning mortality direct effect to be –0.34, and the genetic correlation between the maternal effects was –0.16. The genetic correlation (SE) between the maternal genetic effects for birth weight and mortality was 0.18 (0.20) (Grandinson et al. 2002). These estimates are in opposite directions and suggest that genetic parameters are population specific and should be evaluated within a population before making decisions to include birth weight in a selection program. Knol et al. (2002) speculated that improving pig survival might not be possible by selecting on birth weight alone given the low genetic correlation estimates reported at that time. With greater estimates being reported in more recent years and the availability of more sophisticated tools like genomic selection, this may be worth reconsidering.

Birth weight is associated with improved performance throughout the pig's life. Pigs with greater birth weights were heavier at off-test and had greater growth rate (Fix et al. 2010a; Holl and Long 2006; Rehfeldt et al. 2008); however, heavier pigs at birth had lower loin depth and intramuscular fat percentage (Holl and Long 2006; Rehfeldt et al. 2008). Fix et al. (2010a) reported a quadratic relationship between birth weight and loin muscle area. Holl and Long (2006) found that pigs with greater birth weights had lower backfat at off-test, but Rehfeldt et al. (2008) and Fix et al. (2010a) reported no significant differences in backfat level for pigs with different birth weights. Fix et al. (2010b) observed a positive relationship between pig birth weight and survival to weaning, through the nursery, and through the finisher. Pigs with greater birth weights were higher quality at weaning and had a greater probability of being full value pigs at marketing when compared with pigs with lower birth weights (Fix et al. 2010b). In a study by Magnabosco et al. (2015), pigs in the lowest birth weight class (410–990 g) had the greatest preweaning, nursery, and finisher mortality compared with pigs in larger birth weight classes. According to Magnabosco et al. (2016), sows that weighed less than 1000 g at birth produced 4.5 fewer total pigs born over parities compared to sows that were heavier at birth. Additionally, the sows with the lowest weight at birth had the shortest productive life compared with heavier pigs (Magnabosco et al. 2016).

Nursery–finish mortality

Combined nursery and finisher mortality or mortality throughout a wean-to-finish system has increased by over 0.4% per year from 2010 to 2015 (Stalder 2016). In 2015, the average nursery, conventional finisher, and wean-to-finish mortality rates were 3.5, 4.5, and 6.7%, respectively (Stalder 2016). The combined nursery and finisher death loss is less than half of the preweaning mortality, suggesting that there is a larger opportunity to improve survival in the farrowing house compared with growing pig survival; however, the economic impact of finishing mortality may be greater depending on when the pig dies. Improving morbidity may provide another means to increase commercial swine producer profitability. Henryon et al. (2001) reported that time to treatment for growing pigs was heritable 0.10 for any disease, which was lower than heritability estimates (0.12–0.19) for time to treatment in individual disease categories. Heritability estimates for performance traits may be improved by accounting for challenge events that occurred during the finishing phase. Zumbach et al. (2008) reported a heritability estimate (SE) for carcass weight when not accounting for heat stress of 0.17 (0.01) and reported heritability (SE) estimates of 0.28 (0.01) and 0.14 (0.01) when grouping the pigs under heat stress and non-heat stress, respectively.

Disease resistance

Pigs that do not respond well to health challenges perform more poorly throughout the grow-finish phase and reduce producer economic efficiency. Additionally, improving disease resistance can improve consumer perception of the pig industry in general (Kanis et al. 2005). There is a genetic component to disease resistance; but oftentimes, this component is underestimated when field data has been used to conduct research. It is hard to guarantee that disease exposure probability was both consistent and sufficient for all pigs in a study; furthermore, clinical diagnoses are not always completely correct (Bishop and Woolliams 2010). Given that heterosis can greatly impact pig survival (Fahmy and Bernard 1972), it is important to understand what population was used to estimate genetic parameters and effects on disease resistance. Most commercial market hogs are

crossbred animals, while nucleus herds are composed of purebred animals. Survival rates are not the same among purebred and crossbred pigs (Fahmy and Bernard 1972), making selection for improved survivability at the commercial level difficult.

When considering incorporating a disease resistance component into a genetic program, it is important to understand the genetic parameters for the specific system in which the pigs will be raised. Many disease resistance studies that have been conducted have only included one line or one environment, meaning the results may not be applicable to other lines or environmental conditions. It is important to consider whether the study was conducted in a research or commercial setting (Guy et al. 2012). Additionally, understanding the selection program's goal is important. Improving disease tolerance may be more desirable than improving disease resistance. Hosts carrying a pathogen load may not experience any negative effects. Selecting for improved health and maintaining productivity when in a challenged environment may be accomplished with an emphasis on disease resistance and disease tolerance (Guy et al. 2012).

Lundeheim (1979) examined the genetic parameters associated with respiratory diseases (pneumonia, pleuritis, and rhinitis) in growing pigs. The heritability estimates ranged from 0.12 to 0.16, while genetic correlation estimates between the predisposition to a respiratory disease and growth and carcass measurements were low and not significantly different from 0 (Lundeheim 1979). The same study reported a negative phenotypic relationship between growth rate and lung lesions, enteric disease, locomotion disorders, and "failure to thrive." This suggests that an animal's health status affects its growth performance. Kadowaki et al. (2012) estimated the heritability for mycoplasma pneumonia score to be 0.07, which is slightly lower than the estimate from Lundeheim (1979); however the genetic correlation estimates between mycoplasma pneumonia score and growth and backfat were low and not significantly different from 0, which is in agreement with previous findings (Lundeheim 1979). Additionally, Kadowaki et al. (2012) demonstrated that pigs could be selected for improved mycoplasma pneumonia score while still increasing growth rate and maintaining backfat.

Immune response

Evaluating and selecting for immune response may be a way to improve the overall pig robustness in different health challenges. Pigs with more desirable immune response levels may perform better in health challenged environments regardless of the pathogens present. Clapperton et al. (2009) examined heritability for different biomarkers (acute-phase proteins and peripheral mononuclear leukocyte subsets) and their relationship with production traits. Heritabilities were estimated separately for pigs in a specific-pathogen-free (SPF) environment and non-SPF environment. The magnitude of the heritabilities was moderate for most biomarkers; however the heritability estimates were different when analyzing SPF versus non-SPF pigs (Clapperton et al. 2009). Alpha-acid glycoprotein was the only biomarker evaluated that was negatively correlated with growth rate in both SPF and non-SPF pigs. Additional negative genetic correlations existed between growth rate and monocytes in SPF pigs and growth rate and CD11R1 in non-SPF pigs. Henryon et al. (2006) estimated the heritabilities for IgG and haptoglobin serum concentration to be moderate, 0.16 and 0.14, respectively. The same study reported extremely high heritabilities for swine leukocyte antigen expression ranging from 0.46 to 1.23. Other studies have similarly reported moderate heritabilities for immune response measurements (Edfors-Lilja et al. 1994; Mallard et al. 1992).

Magnusson et al. (1998) reported that nursery-age pigs selected for high immune response had greater clinical arthritis scores in a challenged environment when compared with pigs selected for low immune response; however, there was no difference in weight gain in a challenged environment between the pigs selected for high and low immune response, and pigs selected for high immune response had higher weight gain in a non-challenged environment. This does not suggest a positive impact on growth performance when selecting for more desirable immune response.

Genetic advancements

Traditional selection for disease resistance and survivability is difficult due to challenges with measuring the traits in a nucleus herd. Nucleus herds have a higher health status when compared with commercial herds; therefore, nucleus animals are not exposed to as many pathogens. The approach to selecting for improved survivability has been to develop a pedigreed commercial herd with different pedigree ties to the nucleus herd. However, there are still challenges with this as well since the disease load is not constant or consistent over time. Disease resistance phenotypes are difficult to measure long term in order to incorporate them into a routine genetic analysis.

A single nucleotide polymorphism (SNP) is a location on the genome where variation exists between animals. These locations of variation can be used to estimate the difference in genetic potential between pigs. A PorcineSNP60 BeadChip with 60,000 SNPs can be utilized to incorporate genomic selection to estimate breeding values in swine genetic evaluations (Ramos et al. 2009). Genomic selection involves combining the estimated allele effects for each SNP analyzed with phenotypes to

calculate the expected breeding value for each pig (Meuwissen et al. 2001). Genomic selection has the most impact on traits that are difficult to measure, sex limited, or measured later in life (Goddard and Hayes 2007). Guo et al. (2015) examined the improvement in reliability when including genomic information when estimating breeding values for mortality up to day 5 post farrowing and were unable to show a significant improvement compared to traditional best linear unbiased prediction (BLUP) selection.

An alternative may be to focus on marker-assisted selection (MAS) to estimate the effect for one quantitative trait loci (QTL) and select to reduce the undesirable allele frequency (Knap 2005). Several markers have been previously identified to impact economically relevant traits like number born alive (estrogen receptor gene [ESR]), growth rate and feed intake (melanocortin-4 receptor [MC4R]), and meat quality (protein kinase adenosine monophosphate-activated gamma(3)-subunit [PRKAG3]) (Kim et al. 2000; Rothschild et al. 2007; Stalder et al. 2005). Porcine stress syndrome has been drastically reduced by incorporating a genetic marker into swine breeding programs (Stalder et al. 1997). The completion of the pig genome sequencing (Archibald et al. 2010) aided in the analysis of potential candidate genes.

Knowing the genome sequence can aid in studies exploring the allelic effects for genes expected to impact animal health. Boddicker et al. (2012) isolated a region on chromosome 4 that could reduce the impact of PRRS in a swine population. The marker was reported to explain 15.7% of the genetic variation in viral load and 11.2% of the genetic variation in weight gain in the populations evaluated. Edfors-Lilja et al. (1998) reported multiple QTLs that were associated with improved leukocyte counts and IgG levels for pigs immunized with an *Escherichia coli* vaccine. Reiner et al. (2002) showed that QTLs can affect the temperature and neurological symptoms when pigs were challenged intranasally with pseudorabies virus. Additionally, genes in the swine leukocyte antigen complex have been shown to influence antigen presentation and immune response (Lunney et al. 2009; Mallard et al. 1989). Utilizing markers found in these studies could aid in improving the pigs' response to disease challenges.

One thing that needs to be considered when determining the effectiveness for any QTL is the population that was studied as well as the pathogen strain used. The QTL effects can differ between swine populations and pathogen strains and may not be beneficial in all selection programs. A more recent approach to disease resistance is gene editing. Gene editing involves replacing a section of the genome with a synthesized sequence. Whitworth et al. (2015) validated the ability to edit the pig genome *in vitro* using clustered regularly interspaced short palindromic repeat (CRISPR) technology. Using the CRISPR technology, Whitworth et al. (2016) demonstrated that pigs edited with a mutation in exon 7 of CD163 displayed no response when infected with PRRS virus. Further investigations examining the long-term effects of the mutation need to be conducted. This may be the biggest discovery to improve PRRS disease resistance found to date. Given that PRRS has the greatest economic impact for any single disease in the swine industry, incorporating the edited gene into the population would likely increase swine industry profitability remarkably.

Sow productive lifetime

Most, if not all, economically important pork production traits including longevity are influenced by genetics and/or breeding system. Genetic effects can impact longevity through other important traits. Rydhmer et al. (1994) and Bidanel et al. (1996) have shown that genetics can impact age, weight, and backfat at puberty, which is 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 significant difference of 0.9 litters per sow or 12%. They also noted that 55.2% of culling in purebred sows occurred in the first three parities. During the first three parities, only 40.4% of the overall culling occurred in the crossbred sows. Jorgensen (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 greater culling for locomotion problems and reproductive failure. The difference in sow productive lifetime or parity at culling is particularly important for operations producing replacement gilts using an internal multiplication program. If the internal multiplication program involves purebreds or pure lines, producers must be aware of the expected differential lifetime or number of parities produced in order to maintain adequate replacement parent female and replacement pure line female populations.

Longevity may be influenced by the breed makeup of crossbred breeding females. Hall et al. (2002) noted that sows that were one-quarter Meishan had significantly greater mean productive lifetime days (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.

Heritability estimates for longevity or sow productive lifetime indicate that selection should be an effective method to improve this trait. However, those experienced at genetic improvement for any trait, but particularly those that are more lowly heritable like fitness and reproduction, recognize that improvement through selection alone will be slow. Sow longevity or sow productive lifetime falls into this type of category. Stayability is a measure that some researchers use to describe longevity. Simply, stayability is the ability of a sow to produce an additional litter after producing the previous litter. Reported longevity or productive lifetime heritability estimates range from 0.05 to 0.25 in swine depending on the trait evaluated (Engblom et al. 2009; Gou et al. 2001; Mészáros et al. 2010; Serenius and Stalder 2004; Serenius et al. 2006; Tholen et al. 1996).

In addition to the direct genetic influence on sow productive lifetime, the genetic relationship between economically important production traits like growth rate, backfat, and feet and leg soundness and sow productive lifetime can impact the phenotypic longevity results observed at the herd level. The relationships or correlations can differ depending on the population evaluated. Length of productive life and lifetime number of pigs born alive were positively associated in Finnish Large White and negatively associated in Finnish Landrace (Serenius and Stalder 2004; Serenius et al. 2008).

Improving sow productive lifetime involves choosing the correct genetic lines. 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 four litters, live pigs per sow life, and average sow life. Goodwin (2002) extended the analysis for the same maternal line study and found similar differences through the sixth parity. This seems to indicate that the producer's breeding stock source could impact their ability to retain sows in the breeding herd for longer periods of time or parities ultimately producing more piglets per sow lifetime.

Feet and leg soundness or lameness is usually the second largest identifiable reason for sows leaving the breeding herds, particularly the early parities 1, 2, and 3 (Douglas and MacKinnon 1993). Studies have identified a few factors that are significantly associated with improved sow productive lifetime, either positively or negatively, across several studies. 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 associated unfavorably with longevity (Jorgensen 1996). Numerous studies have shown that feet and leg conformation scores are moderately heritable traits (Bereskin 1979; de Koning 1996; Huang et al. 1995; Lundeheim 1987; Rothschild and Christian 1988a; Serenius et al. 2001) and some of these conformation traits have even been associated with sow longevity (Fernández de Sevilla et al. 2008, 2009; Serenius and Stalder 2004; Tarrés et al. 2006; Tiranti and Morrison 2006). Heritability estimates from these studies for various leg soundness scores range from 0.01 to 0.47, with many values above 0.15.

Rothschild and Christian (1988a) 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 feet and leg problems could be improved through proper selection. In a related paper, Rothschild and Christian (1988b) indicated that leg weakness problems are antagonistically correlated with backfat. This seems to indicate that some selection for feet and leg soundness is necessary to maintain adequate structure especially if there is a strong selection against backfat. Selection against backfat has been employed by most seedstock suppliers for several years and may help explain some of the feet and leg problems that many commercial producers see in the females in the breeding herd.

Lopez-Serrano et al. (2000) reported unfavorable genetic correlations between stayability from first to second litter and daily gain (–0.28 in Large White, –0.06 in Landrace) and stayability and backfat (0.22 in Large White, 0.24 in Landrace), while a favorable genetic correlation was found between stayability and leg score (0.08 in Large White, 0.19 in Landrace). Similarly, Brandt et al. (1999) and Jorgensen (2000) determined that leg quality had a significant influence on the productive life of sows. In further analyses, Jorgensen (2000) reported that "standing under position" also was negatively associated with sow longevity. Knauer et al. (2011) reported moderate genetic correlations (0.09–0.49) between stayability and sow structure visual scores and a negative genetic correlation (–0.14) between locomotion scores and stayability. Genomic regions with effects on a sow's body composition (e.g. body length, body depth, body width) and the structure (e.g. pasture posture) were identified by Fan et al. (2009). With further investigation into candidate genes, Fan et al. (2011) located specific markers with effects on sow body composition and structure.

Most research results that have been published to date where feet and leg soundness traits have been evaluated were conducted where sows were housed in gestation stalls. It is not clear if the same traits, additional traits, or a completely different set of feet and leg traits would influence productive lifetime if sows were housed in group loose sow housing facilities.

Recent advances in molecular biology may prove useful in improving sow productive lifetime. Mote et al.

(2009) reported that several genes including carnitine O-palmitoyltransferase I (CPT1A) and C–C chemokine receptor 7 (CCR7) were associated with improved survival or sow productive lifetime. That same study identified genes associated with reproductive performance including insulin-like growth factor binding protein (IGFBP1) and angiotensin I converting enzyme (ACE). Two genes were associated with both sow longevity and improved reproductive performance including CPT1A.

Onteru et al. (2011a) conducted a genome-wide association to determine genetic regions with influence on reproduction traits across multiple parities. Regions explaining a significant portion of genetic variation were found for total born, born alive, stillborns, mummies, and gestation length with different regions accounting for variation in litter size traits at different parities. With further investigation, Onteru et al. (2011b) found several gene regions with an effect on sow lifetime production (total number born, born alive, removal parity, and nonproductive days). These results suggest that genotyping information can be used to select for the components of sow longevity. In the future, swine breeders may be able to improve sow longevity using both traditional selection methods and advanced molecular tools to ultimately improve performance observed at the commercial level.

It is important to note that sow reproductive trait heritability estimates can vary when sows experience different health challenges. Additionally, the genetic correlation for the same trait when animals are exposed to different health conditions is not 1, suggesting that selection for increased performance in one condition is not directly correlated with improved performance in another condition. Lewis et al. (2009) reported different heritabilities for litter size and number of matings until conception when using data from a herd during a PRRS outbreak compared with using data from the same herd in a normal health status. Given that a sow's ability to stay in a commercial herd is dependent on her reproductive performance, this suggests that a sow's ability to tolerate a disease may impact her longevity in the breeding herd.

Because genetic line differences exist and the heritability for longevity traits is of sufficient magnitude that selection would be successful in improving sow productive lifetime, pork producers should have opportunities to choose lines that have improved sow longevity. At the same time, genetic suppliers can continue to improve this trait through selection. Many commercial producers have employed internal gilt multiplication systems. Genetic improvement of sow longevity must occur using purchased semen from their genetic supplier.

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4

Effect of Environment on Health

Michael C. Brumm

From the pig's point of view, the environment it lives in includes temperature, humidity, space, feed and water access, and air quality when housed in confinement facilities. Rather than document the impact of these variables on health, this chapter will discuss the known requirements that allow the healthy pig to grow and perform to its genetic potential.

Temperature

The pig is a homeothermic animal with a deep body temperature of 39 °C (Baxter 1984), meaning it has a constant core body temperature and will adapt behaviors to maintain this temperature. These behaviors are not only postural changes (huddling as an example when cold) but also changes in basal metabolism accomplished through a change in feed intake (more or less metabolic heat production) when forced to live in an environment warmer or colder than the thermal-neutral temperature zone.

At a constant body weight, heat production by growing pigs and lactating females has increased over the years as the rate of lean deposition and milk production has improved in response to improvements in nutrition, health, genetics, housing, etc. For growing pigs, this increase has amounted to about a 10–15% watt (W) increase at the same body weight increase every 10 years (Figure 4.1) (Brown-Brandl et al. 2004). Similar increases have been noted for early weaned pigs (Harmon et al. 1997). In lactation, the large increase in milk production to support larger litters has also resulted in a large increase in metabolic heat output (Pedersen 2002). This increase in metabolic heat production supports the conclusion that today's pigs are more sensitive to air temperature (especially warm or hot temperatures) than previous generations (Renaudeau et al. 2011).

At the same time, pigs have consistently demonstrated a preference for diurnal variation in temperatures (warmer during the days and cooler during nights). While this demonstrated variation is often 5–7 °C (Bench and Gonyou 2006; Morrison et al. 1987), fluctuations of ±4 °C during the first week after weaning have been demonstrated to increase post weaning scours (LeDividich 1981). Similarly, intermittent drafts of 0.99 m/s for 6 weeks after weaning resulted in increased respiratory distress (coughing, sneezing, and more pneumonic lesions; Scheepens et al. 1991). Brumm and Shelton (1988) and Johnston et al. (2013) have demonstrated no negative impact on pig health or pig performance when a reduced nocturnal temperature regimen is begun 1 week after weaning. This regimen often results in air temperatures in the early morning hours that are 4–5 °C lower than daytime (0700–1900 hours) temperatures.

Extended periods of heat stress have been demonstrated to impact intestinal integrity (Pearce et al. 2015), and heat stress *in utero* impacts postnatal body composition and growth potential (Johnson et al. 2015). Heat stress also impacts intestinal barrier function (Pearce et al. 2012), suggesting a negative impact on health.

Table 4.1 lists the recommended controller set points for facilities with mechanical ventilation. Generally this set point is considered to be at the lower end of the thermal-neutral zone. If temperatures in a production facility are lower than this, some type of supplemental heat is usually activated to maintain thermal-neutral conditions in the pig zone. As temperatures warm above this set point, devices such as fans, misters, or evaporative pads are activated to both remove heat and activate evaporative cooling mechanisms to assist the pig in dealing with the warmer conditions.

The low end of the thermo-neutral zone is normally considered the lower critical temperature (LCT), which is defined as the lowest air temperature that a given animal can maintain under homeothermic conditions without increasing metabolism to maintain body temperature (Hillman 2009). The suggested set points in

Diseases of Swine, Eleventh Edition. Edited by Jeffrey J. Zimmerman, Locke A. Karriker, Alejandro Ramirez, Kent J. Schwartz, Gregory W. Stevenson, and Jianqiang Zhang.

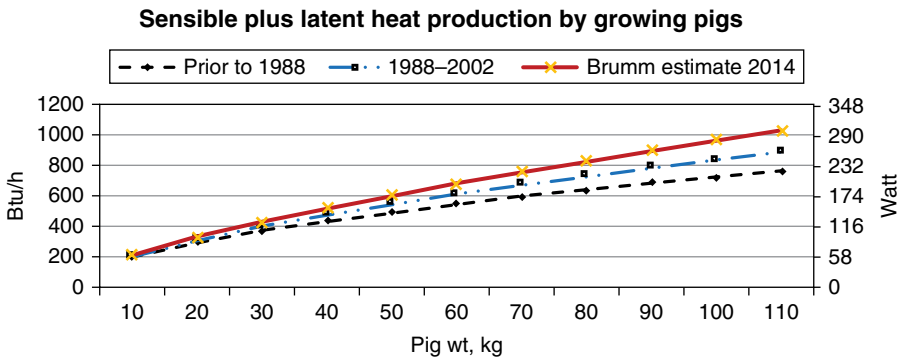

Figure 4.1 Total (sensible plus latent) heat production by growing pigs. *Source:* Adapted from Brown-Brandl et al. (2004).

Table 4.1 Recommended ventilation controller set points for fully slatted facilities.

Pig class or wt	Set point (°C)
Weaning (5–8 kg)	30
25 kg	22
50 kg	19
100 kg+	16
Individually housed gestating females	20
Group-housed gestating females	17
Lactating females week 3	20

Table 4.1 are generally within 1–2 °C of LCT for pigs housed in groups in fully slatted facilities without bedding, hovers, or other facility modifications that allow the pig to modify its LCT. When housed at temperatures in the pig zone at or slightly above the LCT, metabolic heat release to the environment is approximately 2/3 as sensible heat (air temperature) and 1/3 as latent heat (moisture). As temperatures rise, the pig must release a higher percentage of its metabolic heat as latent heat since the difference between skin surface temperature and air temperature lowers, which results in less heat dissipation via convection and conduction modes.

The exact LCT for any given pig or group of pigs will depend upon 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. As a consequence, when feed intake is reduced during an illness, the general recommendation is to increase air temperature in the pig zone to compensate. 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. In general, the goal of swine housing is to provide conditions that result in the least regulatory effort by the growing or adult animal (Hillman 2009).

Humidity

The desired humidity level in swine production facilities is 60–80%. Humidity fluctuates in a diurnal pattern in production facilities, most often being the lowest in morning hours and highest in the midafternoon due to pig activities increasing the amount of moisture that is evaporated from flooring surfaces. During hot weather, high humidity levels are associated with a high total enthalpy in the environment. Because of this high enthalpy, options for using evaporative cooling such as wetting of the pigs or evaporative pads in mechanically ventilated facilities are limited. As a consequence, pig performance is decreased as the pig modifies its behavior by reducing feed intake and activity to decrease total heat production.

The minimum ventilation rates in Table 4.2 are designed to maintain humidity levels at 60–80% in cold weather in full and partially slatted production facilities. Ventilation rates higher than the minimum are generally in response to the need to remove excess heat from a facility as a result of pig growth and/or warming outside temperatures.

Causes of death in ventilation failures

With a majority of the North American pig population housed in facilities with mechanical ventilation, ventilation failures due to equipment malfunction or electrical outages occasionally occur. In many instances these failures are accompanied by pig deaths.

Table 4.2 Recommended ventilation rates for swine production facilities.

Pig class	Weight (kg)	Cold weather minimum (m^3/h)	Mild weather rate (m^3/h)	Hot weather rate (m^3/h)
Weaned pigs	5–15	3.4	17.0	51.0
Growing pigs	15–34	5.1	25.5	76.5
Finishing pigs	34–68	11.9	40.8	130.0
Finishing pigs	68–135	17.0	59.5	200.0
Gestating females		20.4	68.0	425.0
Sow and litter		34.0	85.0	1100.0

Source: Adapted from MWPS (1990)

While it is common for producers and their advisors to talk about "suffocation" as a cause of death, the primary cause of death for all ages other than newly weaned piglets is hyperthermia (Robert et al. 2003; Zulovich and Bundy 1990), not lethal levels of carbon dioxide or other gases. Zulovich and Bundy (1990) demonstrated that at typical stocking densities of North American production facilities, death from hyperthermia can occur as rapidly as 30 minutes after a ventilation failure. In this simulation, only a minimal amount of air infiltration was necessary to keep predicted carbon dioxide and oxygen at reasonable levels. For all conditions simulated, relative humidity in the facility reach 100% within 2 hours of the simulated failure. In many situations where pig death occurs due to ventilation failure, smaller pigs (runts and sick pigs) often survive because their metabolic heat output is lower due to lower levels of feed intake. They do not reach critical core body temperatures before many of the fastest-growing (i.e. highest metabolic heat output) pigs have died and barn temperatures have begun to decline.

Christison and Heidenreich (1968) recorded rectal temperatures of 44.4 °C 5 minutes prior to death when a 25 kg pig was exposed to a 38 °C temperature for 5.25 hours. On-farm rectal temperatures of 41.1 °C have been recorded 4–5 hours after death associated with a ventilation failure event (MC Brumm, personal communication). Robert et al. (2003) conducted a series of experiments in a commercial nursery with 6.8 and 22.7 kg pigs in which the ventilation system was turned off and the rate of change in temperature and carbon dioxide concentration were recorded. For 6.8 kg pigs, they determined that air temperature would never reach a critical level to result in hyperthermia but carbon dioxide levels would become critical within 150 minutes. In the same facility with 22.7 kg pigs and no mechanical ventilation operating, the critical time before temperatures approached levels creating hyperthermia would be about 60 minutes, while the critical time for carbon dioxide levels was 95 minutes.

Pen space

The growing pig's spatial requirement in m^2/pig is well understood for both full and partially slatted facilities (Flohr et al. 2016; Gonyou et al. 2006). When space is defined by the allometric equation $m^2/pig = K * BW(kg)^{0.667}$ where K is a constant and BW is body weight, the generally recognized constant is 0.0336 (Gonyou et al. 2006a). Many welfare codes define the space requirement using a constant (K) of 0.035. In general, as space per pig is restricted, feed intake and daily gain decline with a less predictable response in feed conversion. Table 4.3 details the relationship between pig weight, pen space, and "K." The predicted impact on daily gain versus adequate space ($K = 0.0336$) is presented in Table 4.4.

There is limited data on the impact of space allocation on pig health. Turner et al. (2000) suggested that pigs in deep straw-bedded facilities given less space had a lower humoral response to an antigen challenge. In contrast, Oh et al. (2010) reported no impact of increasing pig numbers in a fixed dimension weaned pig pen on serum IgA and IgC. Hyun et al. (1998) have suggested that environmental stressors (temperature, space, and social regrouping) have an additive impact on performance.

Feeder space

A common limit to pig performance as sale weights increase is feeder access. Producers and equipment suppliers often size feeders based on the number of "holes" the feeder offers without regard to the issues surrounding the quality of the eating space or "hole."

The width of the feeding space is defined by the shoulder width of the pig. Petherick (1983) defined shoulder width in relation to pig body weight as width (mm) = $64.0 \times (\text{body weight, kg})^{0.33}$.

Table 4.3 Relationship between pig weight, pen space, and "K."

	"K"				
Pig wt (kg)	0.025	0.028	0.031	0.0336	0.035
	m^2/pig				
20	0.18	0.21	0.23	0.25	0.26
40	0.29	0.33	0.36	0.39	0.41
60	0.38	0.43	0.48	0.52	0.54
80	0.46	0.52	0.58	0.62	0.65
100	0.54	0.60	0.67	0.73	0.76
120	0.61	0.68	0.76	0.82	0.85
140	0.68	0.76	0.84	0.91	0.95

Source: Adapted from Petherick (1983).

Table 4.4 Estimated impact on daily gain when space per pig is restricted in fully slatted facilities.

Wt (kg)	Space/pig (m^2/pig) at $K = 0.0336$	K when space/pig is 0.66 m^2/pig	ADG versus $>K = 0.0335$ from 25 kg to stated wt
20	0.25	0.089	
40	0.39	0.056	No impact
60	0.52	0.043	No impact
80	0.62	0.035	No impact
100	0.73	0.0305	97.5%
120	0.82	0.0270	94.6%
140	0.91	0.0245	92.6%

Source: Adapted from Gonyou et al. (2006). Reproduced with permission of Oxford University Press.

To allow for pig movement at the feeder, feeder spaces should be sized based on 1.1 times the shoulder width. Table 4.5 lists the estimated shoulder width in relation to pig weight and the estimated width of feeder spaces based on these shoulder width dimensions. This table supports the recommendation that feeders for today's production facilities should have a minimum feeder "hole" width of 35.0 cm as slaughter weights in the United States continue to increase (Figure 4.2; NASS, USDA, 2018 and Figure 4.3).

Gonyou and Lou (1998) using pigs with weights up to 95 kg concluded that the best compromise for feeder depth (distance from the front lip of the feeder pan to the feed delivery point) for grow-finish pigs was 20–30 cm. They concluded that the ideal feeder depth for 95 kg pigs was 32 cm. This suggests that a majority of the feeders installed in production facilities in the United States and Canada today, which have depths of 20–25 cm, are limiting to pig performance as sale weights increase.

The problem with increasing feeder depth is that of smaller pigs at time of placement stepping into the feeder to access feed and then tracking feed out of the feed pan. However this has not been an issue with "wean-finish" feeders where pigs are grown from 5.5 kg to slaughter weight on the same feeder. In this author's experience, as sale weights increase, feeders must have at least 25.4 cm of depth from the front lip to the feed delivery device or agitator plate with 28–30.5 cm preferred for dry feeders. If the distance is less than this, pigs end up pushing against the feeder with the crown of their forehead and/or have difficulty accessing the feed agitator plate.

The table also highlights a common feeder problem with swine nursery units. Many nurseries now house pigs until 25 kg or heavier. The vast majority of feeders sold in North America for placement in swine nurseries still have feeder "holes" that are 15.25 cm × 15.25 cm. Based on shoulder dimensions, these feeders most likely limit feed intake or at the minimum limit pig access to feed by limiting the number of usable spaces that pigs can eat from unless the spaces/holes are 20.3 cm wide.

Similar sizing issues arise when drinking water is furnished in trough or cup/bowl drinkers. Pigs grow in three dimensions (length, width, and height), so the need to account for increases in head dimensions for these devices as slaughter weights increase is also a reality.

Table 4.5 Shoulder width of growing pigs in relation to pig weight and estimated feeder "hole" widths necessary to accommodate this dimension.

Pig wt (kg)	Shoulder width (cm)	1.1 × shoulder width (cm)
18.2	16.8	18.3
27.3	19.1	21.1
90.9	28.4	31.2
100.0	29.2	32.3
109.1	30.2	33.0
118.2	31.0	34.0
127.3	31.8	34.8
136.4	32.5	35.6
145.5	33.0	36.3

Source: Adapted from Petherick (1983).

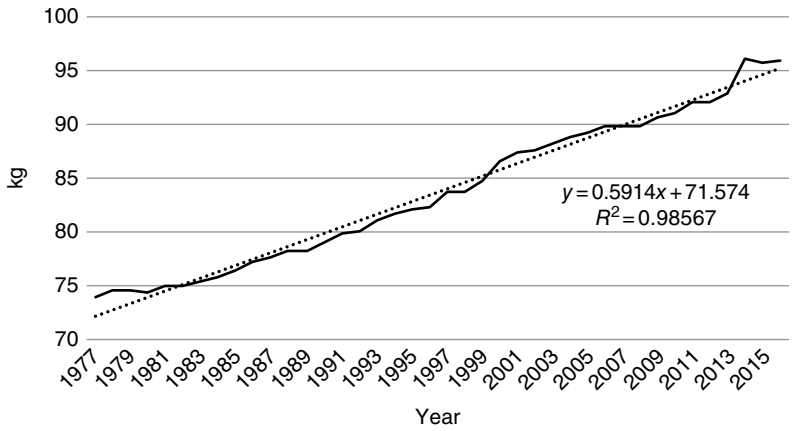

Figure 4.2 USDA-inspected barrow and gilt carcass weights by year. Data *source:* Livestock slaughter, NASS, USDA.

Figure 4.3 Twelve "hole" 2.13 m long double-sided feeder with marked pig (estimated to weigh almost 136 kg) destined for removal and sale immediately after the picture was taken from a pen of 125 finishing pigs.

Water

At birth, water accounts for 82% of the pig's empty body weight. By the time the pig weighs 240 pounds, water comprises only 51% of the empty body weight (Shields et al. 1989). In addition to body tissue and metabolic functions, water is used for (1) the adjustment of body temperature, (2) the maintenance of mineral homeostasis, (3) the excretion of the end products of metabolism (particularly urea), (4) the achievement of satiety (gut fill), and (5) satisfaction of behavioral needs (Brooks et al. 1989). Major sources of water for physiological needs, including growth, reproduction, and lactation, are

water from feedstuffs, water from metabolic processes, and drinking water. As a practical matter, drinking water is the major water source (Thacker 2001) Table 4.6.

Symptoms of water deprivation in swine include reduced feed intake, crowding around drinker devices, dehydration, increased heart rates, increased body temperatures, increased respiration, and death (Thacker 2001).

Water consumption for growing pigs has a distinct periodicity with a peak at the beginning and at the end of the feeding period when nose-operated drinkers are used. Water consumption between feeding periods peaked two hours after the morning feeding and one hour after the afternoon feeding (Olsson and Andersson 1985). Weaned pigs housed under conditions of constant light showed a diurnal pattern for water intake with higher consumption recorded from 0830 to 1700 hours as compared with the 1700 to 0830 hours time period (Brooks et al. 1984). Grow-finish pigs using nipple drinkers showed a large peak from 1500 to 2100 hours and a smaller peak between 500 and 1100 hours (Korthals 1998). The number of pigs in a group (pen) apparently influences water usage. In one study water usage was higher when pigs were housed in groups of 60 versus 20. Total drinking time per pig decreased when group size increased, even though the number of pigs per drinker was the same for both group sizes (Turner et al. 1999).

While there is very good evidence that a majority of water consumption is associated with eating activities in research settings, there is limited data on patterns of water usage in commercial facilities. Brumm (2006) documented drinking water disappearance patterns in production facilities in Minnesota and Nebraska. These facilities varied in the number of pigs per pen, the type of feeder and drinker, the type of ventilation, relative pig health, etc. The similarities between the winter and summer patterns at the sites suggests that two patterns of water usage exist, depending on the temperature in the facility (i.e. time of the year). In thermal-neutral conditions (generally air temperatures in the pig zone <26 °C), grow-finish pigs begin drinking water around 6 am, with a peak in drinking water disappearance in early afternoon and a gradual decline for the remainder of the day. This pattern is in agreement with published literature.

However, when pigs are growing in warm to hot conditions (air temperatures in the pen exceeding 26 °C for 1 or more hours per day), they alter their pattern of drinking water usage. Pigs begin drinking earlier in the day, with a morning peak from 0800 to 0900 hours. There is a decline in drinking water use midday with a second peak in drinking water use from 1700 to 2300 hours. followed by the decline into the night hours.

It is interesting to note that pigs shift to this pattern of drinking water use on the first day of air temperatures in the pig zone (>26 °C or so) and maintain the pattern for 3–5 days, even if these subsequent days have temperatures considered to be thermal neutral. This adaptation is often maintained for several days in anticipation that the heat stress event will be longer than a single day. This suggests that a shift in eating and drinking behavior is one of the first adaptations of the growing pig to heat stress. In the future, it may be possible to use this shift in drinking water usage as a predictor of a performance reduction due to heat stress in grow-finish pigs.

Figure 4.4 shows the typical daily water disappearance curve for pigs in a wean-finish facility equipped with bowl drinkers and offered corn–soybean meal-based diets ad libitum. Based on producer and veterinarian

Table 4.6 Recommended water flow rates from drinking devices.

Pig class	Flow (mL/min)
Nursery pigs	250–500
Grow-finish pigs	750–1000
Breeding animals	1000

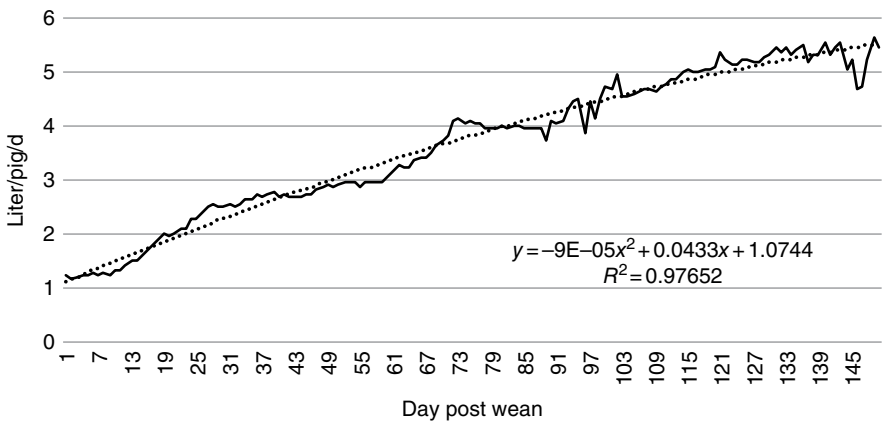

Figure 4.4 Typical drinking water usage for growing pigs. Data based on eight wean-finish groups of pigs from weaning (piglets 17–21 days of age) to day 150 post weaning.

observations, when daily water usage drops for 3 continuous days or drops more than 30% from day to day, this may indicate that a potential health challenge is occurring in the production facility (Brumm 2006).

Water–feed ratios (kg/kg) for liquid feeding systems typically range from 2.5 : 1 to 3.5 : 1 (English et al. 1988). Water–feed ratios ranging from 1.78 : 1 to 2.79 : 1 for pigs weighing from 18 to 114 kg and fed dry feed ad libitum have been reported (Brumm et al. 2000). The lowest reported water–feed ratios were with wet/dry feeders and bowl drinkers, whereas gate-mounted nipple drinkers had the highest ratios. With similar performance, this suggests that the major cause of differences in water–feed ratios between the various drinking devices is due to differences in water wastage, not differences in the amount consumed.

Water–feed ratios decrease as pigs grow. Recent on-farm data (MC Brumm, unpublished data) supports the conclusion that water–feed ratios decline as pigs grow, with a ratio as low as 1.5 : 1 common in facilities that use wet/dry feeders or stainless steel bowl drinkers in late finishing and offered corn–soybean meal-based mash diets ad libitum. Assuming similar water–feed ratios for both barrows and gilts, it follows that barrows drink more water than gilts since barrows eat more feed per day than gilts in mid to late finishing (NRC 2012). Pigs fed meal diets drink more water than pigs fed pelleted diets (Laitat et al. 1999), reflecting similar water–feed ratios and differences in feed conversion efficiency.

General recommendations exist for the number of pigs per drinking device (Midwest Plan Service 1983), but research to support these recommendations is limited. Researchers using 3- to 4-week-old weaned pigs reported a slight reduction in average daily gain and an increase in weight variation within pens of 16 pigs given access to 1 versus 2 nipple drinkers for 5 weeks' post weaning (Brumm and Shelton 1986). Generally, for groups larger than 10 pigs in a nursery and 15–20 pigs in a grow-finish facility, a minimum of 2 delivery devices is recommended (Brumm and Reese 1992).

Grow-finish pigs spent from 3 to 16 minutes per day at nipple drinkers when flow ranged from 1100 down to 100 mL/min (Nienaber and Hahn 1984). This suggests that pigs will exert some extra effort in order to obtain water. But it is not clear at what point having to wait for drinker access or exert extra effort impairs performance.

Lactating sows need considerable amounts of water, both for milk production and to remove the metabolic end products associated with this production (Thacker 2001). Water consumption (measured as disappearance) averaged 18 L/day with a range of 12–40 L/day (Lightfoot 1978). It is expected that as milk output by lactating sows increases due to advances in genetics, nutrition, and housing, this average value will increase, even as improvements in wastage are noted due to newer types of drinking devices.

Noise

The general noise level measured in mechanically ventilated pig buildings was 73 dB with naturally ventilated buildings averaging 10 dB lower levels (Talling et al. 1998). This noise level tends to be monotonous and continuous (Schaffer et al. 2001). Levels averaged 98 dB during transport (Talling et al. 1998). Loud fan noise (85 dB) has been shown to interfere with sow and nursing piglet communication, leading to disrupted nursings (Algers and Jensen 1985).

Kanitz et al. (2005) demonstrated that repeated exposure to 2 hours of 90 dB noise caused considerable alterations to the hypothalamic–pituitary–adrenal axis. They suggested that this alteration would have a substantial impact on the general vulnerability of the pig with respect to health, welfare, and productivity.

Stray voltage

Stray voltage is defined as the voltage between any two animal contact points. Most often these exist as neutral-to-earth voltages (Gustafson and Morgan 2004). These frequently occur in swine housing situations where there are large amounts of metal gating to provide a path for the electric current such as gestation stalls or farrowing crates or where there is metal flooring such as farrowing crates or weaned pig pens. Field reports indicate that when stray voltage is present, there is often a reluctance of pigs to drink from drinkers, a reduction in appetite, restlessness, increases in aggressive encounters, impaired growth, and a variety of health disorders (Robert et al. 1994a).

Current flow through gestating females was always higher on wet versus dry floors (Robert et al. 1994b). However, under controlled conditions, it has been difficult to demonstrate negative impacts on lactating female or growing pig performance or health (Robert et al. 1992, 1996).

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5

Differential Diagnosis of Diseases

Alejandro Ramirez

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 geographic 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. The World Animal Health Information Database (http://www.oie.int/wahis_2/public/wahid.php/Wahidhome/Home) 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 2 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 tests are not available. Chapter 6 reviews many of the different diagnostic tests available including important information on test performance and considerations in interpreting results. Collecting evidence and establishing causality is a critical step for proper diagnosis (see Chapter 8).

Digestive system

Chapter 15 covers valuable information regarding the digestive system including useful tables summarizing the mechanisms of diarrhea (Table 15.1), diagnosis of 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 5.1 and 5.2 respectively. The approximate age is given solely as guidance to help emphasize certain causes based on age of pigs and does not imply the cause to be restricted only to that age group. Table 5.3 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 5.4 and 5.5 summarize differential diagnosis lists for major respiratory clinical presentations.

Integumentary system

The integumentary system is reviewed in Chapter 17. Table 5.6 helps summarize the approximate age when specific skin diseases are more common. Tables 5.7 and 17.1 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 Tables 5.8 and 14.7.

Diseases of Swine, Eleventh Edition. Edited by Jeffrey J. Zimmerman, Locke A. Karriker, Alejandro Ramirez, Kent J. Schwartz, Gregory W. Stevenson, and Jianqiang Zhang.

Table 5.1 Approximate age at which certain causes of diarrhea in pigs are more common (also see Chapter 15).

1–2 days	3–4 days	5–6 days	1 week	2 weeks	3 weeks	1 month	2 months	3 months	4 months	5 months	6 months	Adults	Chapter
Clostridium difficile													51
Clostridium perfringens type A													51
Clostridium perfringens type *C*													51
Bovine viral diarrhea													39
Enterococcus spp.													47
Escherichia coli													52
Porcine reproductive and respiratory syndrome virus													41
Rotavirus													43
Teschovirus													40
African swine fever virus													25
Campylobacter spp.													64
Classical swine fever virus													39
High fever (any condition causing a high fever)													14
Porcine deltacoronavirus													31
Porcine epidemic diarrhea virus													31
Toxoplasma gondii													66
Transmissible gastroenteritis virus													31
	Hypoglycemia (agalactia)												18
	Cryptosporidium spp.												66
		Strongyloides spp.											67
		Eimeria spp.											66
		Cystoisospora suis											66
		Porcine sapovirus											29
			Iron toxicity										68
			Porcine astrovirus										27
				Adenovirus									24
					SBM								15
						Antibiotic-induced colitis							10
						Oesophagostomum dentatum							67
						Salmonella spp.							59
							Brachyspira pilosicoli						62
							Yersinia spp.						64
								Aflatoxin					69
								Arsenic toxicity					70
								Ascaris suum					67
								Bacillus anthracis					64
								Biotin deficiency					68
								Brachyspira hyodysenteriae					62
								Carbamate toxicity					70
								Chromium toxicity					68
								Dimetridazole toxicity					70
								Dipyridal herbicides					70
								Entamoeba spp.					66
								Fluorine toxicity					70

Table 5.1 (Continued)

1–2 days	3–4 days	5–6 days	1 week	2 weeks	3 weeks	1 month	2 months	3 months	4 months	5 months	6 months	Adults	Chapter
						Gastric ulcer							15
						Lawsonia intracellularis							58
						Monensin toxicity							10, 70
						Niacin deficiency							19, 68
						Organophosphate toxicity							70
						Porcine circovirus type 2							30
						Salt toxicity							70
						Selenium deficiency							68
						Sulfur toxicity							68
						T-2 toxin							69
						Trichuris suis							67
						Tryptophan toxicity							68
						Vitamin D toxicosis							22
						Vitamin E deficiency							68
						Vomitoxin							69
						Water quality							68
							Ovine herpesvirus 2						35

SBM, soybean meal hypersensitivity.

Table 5.2 Approximate age at which certain causes of vomiting in pigs are more common (also see Chapter 15).

1–2 days	3–4 days	5–6 days	1 week	2 weeks	3 weeks	1 month	2 months	3 months	4 months	5 months	6 months	Adults	Chapter
African swine fever virus													25
Classical swine fever virus													39
Porcine deltacoronavirus													31
Porcine epidemic diarrhea virus													31
Pseudorabies virus													35
Transmissible gastroenteritis virus													31
		HEV											31
		EEEV											46
			Yersinia enterocolitica										64
				Adenovirus									24
					Ascaris suum								67
					Diacetoxyscirpenol								69
					Stachybotrys atra toxin								19
					T-2 toxin								69
					Vomitoxin								69
					Strongyloides spp.								67
						Arsenic toxicity							70
						Atresia ani							15
						Bacillus anthracis							64
						Carbamate toxicity							70
						Cocklebur poisoning							70
						Fluorine toxicity							70
						Niacin deficiency							68

(*Continued*)

Table 5.2 (Continued)

1–2 days	3–4 days	5–6 days	1 week	2 weeks	3 weeks	1 month	2 months	3 months	4 months	5 months	6 months	Adults	Chapter
						Organophosphate toxicity							70
						Riboflavin deficiency							68
						Salt poisoning							70
						Selenium toxicity							68
						Thiamin deficiency							68
						Vitamin B_6 toxicity							68
						Vitamin D toxicity							68
							Rectal strictures						15
							Gastric ulcers						15
								Hairballs					15
								Foreign body					15

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

Table 5.3 Causes of rectal prolapses in pigs (also see Chapter 15).

Cause	Comments
Diarrhea	Abnormally acid stool in the rectum causes irritation, tenesmus, and prolapse. Refer to the section on diarrhea for differentiation between causes of diarrhea
Cough	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
Piling	Environmental temperatures too low. Abdominal pressure on the pig at the bottom of the pile produces prolapse
Zearalenone	Estrogens cause swelling of the perineal area, tenesmus, and prolapse
Floor design	Excessively sloped floors for crated sows cause increased pressure on pelvic structures as pregnancy progresses
Antibiotics	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
Inherited predisposition	Sporadic reports in the literature of herd outbreaks that occurred in the offspring of certain boars
Postpartum	Complex etiology surrounding farrowing
Prepartum	Constipation and pressure of heavily gravid uterus
Any condition that is associated with tenesmus	Urethritis, vaginitis, rectal or urethral injury post breeding, urethral calculi. Excess salt in the diet

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Table 5.4 Approximate age at which certain causes of pneumonia, respiratory distress, or coughing in pigs are more common (also see Chapter 21).

<1 week	1–4 weeks	1 month	2 months	3 months	4 months	5 months	6 months	Adults	Chapter
PCMV									35
Carbon monoxide toxicity									70
Dermatosis vegetans									17
Porcine reproductive and respiratory syndrome virus									41
Bordetella bronchiseptica									49
Porcine respiratory coronavirus									31
Clostridium tetani									51
Arcanobacterium pyogenes									64
Chlamydia suis									64
Nitrite toxicity									70
Coal tar toxicity									70
Methane toxicity									70
Pseudorabies virus									35
Toxoplasma gondii									66
Strongyloides ransomi									67
Classical swine fever virus									39
African swine fever virus									25
Nipah virus									37
	HEV								31
	Adenovirus								24
	Iron deficiency anemia (or blood loss anemia)								14
	Pasteurella multocida								57
	Haemophilus parasuis								54
	Actinobacillus pleuropneumoniae								48
	Actinobacillus suis								48
	Streptococcus spp.								61
	Influenza A virus								36
		Porcine stress syndrome							19
		Blue eye paramyxovirus							37
		Porcine lymphotropic herpesvirus							35
		Salmonella choleraesuis							59
		Clostridium botulinum							51
		Ascaris suum							67
		Metastrongylus spp.							67
		Paragonimus kellicotti							67
		Vitamin A deficiency							68
		Vitamin D toxicity							68
		Organophosphate toxicity							70
		Carbamate toxicity							70
		Chlorinated hydrocarbon toxicity							70
		Pentachlorophenol toxicity							70
		Dipyridal herbicide toxicity							70
		Fumonisin							69

(*Continued*)

Table 5.4 (Continued)

<1 week	1–4 weeks	1 month	2 months	3 months	4 months	5 months	6 months	Adults	Chapter
			Porcine circovirus type 2						30
			Erysipelothrix rhusiopathiae						53
			Mycobacterium spp.						63
			Mycoplasma suis						56
			Mycoplasma hyopneumoniae						56
			Hydrogen sulfide toxicity						70
					Gossypol toxicity				70
								CM	52
								Puffer	21

PCMV, porcine cytomegalovirus; HEV, hemagglutinating encephalomyelitis virus; CM, coliform mastitis; Puffer, puffer sow syndrome.

Table 5.5 Certain causes of sneezing in pigs (also see Chapter 21, especially Table 21.5).

Atrophic rhinitis	Chapter 49
Blue eye paramyxovirus	Chapter 37
Environmental contaminants:	
Ammonia	Chapters 4, 57
Dust, pollen, irritants	Chapter 4
Hemagglutinating encephalomyelitis virus	Chapter 31
Influenza A virus	Chapter 36
Mycoplasma hyorhinis	Chapter 56
Porcine cytomegalovirus	Chapter 35
Porcine reproductive and respiratory syndrome virus	Chapter 41
Pseudorabies virus	Chapter 35

Nervous and locomotor system

Chapter 19 reviews both the nervous and locomotor systems. It is important to note that many times diseases affecting either of these systems have similar general clinical presentations. Table 5.9 lists some causes of neurologic signs. It is important to use the right descriptive terms when describing clinical signs (see Table 19.5) to ensure proper differential diagnosis. Table 19.8 tries to further differentiate clinical presentations. Lameness conditions are summarized in Table 5.10.

Reproductive system

The reproductive system is summarized in Chapter 20. Possible causes of reproductive losses in pigs are summarized in Tables 5.11 and 20.7. Although not directly related to reproductive performance, but rather related to pregnancy, common congenital anomalies are listed in Table 5.12.

Zoonotic

An overview of preharvest food safety and zoonotic diseases is included in Chapter 12. Pig diseases with zoonotic potential are summarized in Table 5.13.

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 5.6 Approximate age at which certain skin diseases in pigs are more frequently seen (also see Chapter 17).

Weeks of age											
1	2	3	4	8	10	14	18	32	50	100	156
Infection of injury caused by trauma, ischemia, or surgical procedures											
Mange and lice											
Ringworm											
Insect bites from fleas, flies, and mosquitoes											
Sunburn or photosensitization											
Abscesses											
Necrobacillosis											
Epitheliogenesis imperfecta											
Teat and knee erosion											
Pustular dermatitis											
Thrombocytopenic purpura											
Dermatosis vegetans											
Staphylococcal acne											
Swinepox											
Acute generalized exudative epidermitis, local exudative epidermitis											
Pityriasis rosea											
Ear necrosis											
Parakeratosis											
Callus of the knee, fetlock, elbow, hock, or tuber ischii											
Porcine dermatitis and nephropathy syndrome											
Bursitis											
Erysipelas											
Dermatosis erythematosa											
Mastitis											
Shoulder ulcer callus											

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Table 5.7 Diseases affecting the skin of pigs (also see Chapter 17, especially Tables 17.1).

Location	Normal tissue	Proliferative or nonproliferative	Demarcation of lesions	Possible cause
Face	Elevated		Discrete	Staphylococcal acne
	Flat	Nonproliferative	Discrete	Necrotic stomatitis
Face and feet	Elevated		Discrete	Vesicular diseases[a]
Shoulder	Elevated		Discrete	Hematoma; callus
	Flat	Nonproliferative	Discrete	Ulcer
Knees, elbows, and hocks	Flat	Nonproliferative	Discrete	Knee erosions
	Elevated		Discrete	Callus
	Elevated		Diffuse	Bursitis
Ear	Elevated		Discrete	Hematoma
	Flat	Nonproliferative	Diffuse	Greasy spot behind ear
	Flat	Proliferative	Discrete	Ear necrosis
	Flat	Proliferative	Diffuse	Mange
Ear, eye, and udder	Flat	Nonproliferative	Diffuse	Photosensitization
Extremities	Flat	Nonproliferative	Diffuse	Cyanosis or reddening secondary to disease[b]
Dorsal	Elevated		Discrete	Fleas, flies, mosquitoes
	Elevated	Proliferative	Diffuse	Lumpy skin disease
	Elevated	Proliferative	Diffuse	Hyperkeratinization
	Flat	Nonproliferative	Diffuse	Sunburn
	Flat	Nonproliferative	Discrete	Epitheliogenesis imperfecta
Ventral abdomen	Elevated		Discrete	Pityriasis rosea, eosinophilic dermatitis
	Elevated		Diffuse	Urticarial mange
	Flat	Nonproliferative	Discrete	Transit erythema; teat necrosis
	Flat	Nonproliferative	Diffuse	Mastitis, benign peripartal cyanosis
Ventral cervical area	Elevated		Discrete	Jowl abscess, tuberculosis
	Elevated		Diffuse	Pharyngeal anthrax
Generalized	Elevated		Discrete	Pustular dermatitis, swinepox, infected injuries, neoplasia, abscess
	Elevated		Diffuse	Dermatosis vegetans
	Flat	Proliferative	Diffuse	Parakeratosis, demodectic mange, lice, sarcoptic mange, exudative epidermitis
	Flat	Nonproliferative	Discrete	Ringworm, dermatosis erythematosus, thrombocytopenic purpura, erysipelas
	Flat	Nonproliferative	Diffuse	Carbon monoxide toxicity, porcine stress syndrome, hypotrichosis, cyanosis, or reddening secondary to any bacteremia or viremia
	Flat	Nonproliferative	Discrete	Immune complex disorder possibly associated with circovirus

Source: Straw et al. (2006). Reproduced with permission of John Wiley and Sons.
[a] Foot-and-mouth disease, vesicular exanthema, vesicular stomatitis, swine vesicular disease, Senecavirus A, San Miguel sea lion virus, porcine parvovirus, and drug eruption.
[b] Salmonellosis, *Haemophilus parasuis*, *Actinobacillus pleuropneumoniae*, porcine reproductive and respiratory syndrome, colibacillosis, organophosphate toxicity, and hemagglutinating encephalomyelitis.

Table 5.8 Cause of anemia in pigs (also see Chapter 14, especially Table 14.7).

General	
Blood loss (acute or chronic)	Chapter 14
Chronic disease	Chapter 14
Gastric ulcer	Chapter 15
Hemorrhagic bowel syndrome	Chapter 15
Bacterial	
Lawsonia intracellularis	Chapter 58
Mycoplasma suis	Chapter 56
Salmonella spp.	Chapter 59
Deficiencies or toxicities	
Aflatoxin	Chapter 69
Anticoagulant toxicity (warfarin, brodifacoum, etc.)	Chapter 70
Coal tar toxicity (clay pigeons)	Chapter 70
Cobalt toxicity	Chapter 68
Copper deficiency and toxicity	Chapter 68
Folic acid deficiency	Chapter 68
Iron deficiency	Chapter 68
Niacin deficiency	Chapter 68
Trichothecenes	Chapter 69
Vitamin B_{12} deficiency	Chapter 68
Vitamin B_6 deficiency	Chapter 68
Vitamin E deficiency	Chapter 68
Vitamin K deficiency	Chapter 68
Zearalenone	Chapter 69
Parasites	
Fasciola hepatica	Chapter 67
Flea infestation	Chapter 65
Haematopinus suis	Chapter 65
Trichuris suis	Chapter 67
Macracanthorhynchus hirudinaceus	Chapter 67
Strongyloides ransomi	Chapter 67
Viral	
Bovine viral diarrhea virus	Chapter 39
Porcine reproductive and respiratory syndrome virus	Chapter 41

Table 5.9 Cause of neurologic signs in pigs (also see Chapter 19, especially Table 19.8).

General or congenital	
Brain or spinal cord injury	Chapter 19
Congenital malformations	Table 5.12
Congenital tremors	Table 19.9
Hypoglycemia	Chapter 19
Hypoxia/anoxia	Chapter 21
Middle ear infection	Chapter 19
Bacterial or protozoal	
Actinobacillus suis	Chapter 48
Clostridium botulinum	Chapter 51
Clostridium tetani	Chapter 51
Escherichia coli (usually 1–2 weeks post weaning)	Chapter 52
Haemophilus parasuis	Chapter 54
Listeria monocytogenes	Chapter 64
Streptococcus suis	Chapter 61
Toxoplasma gondii	Chapter 66
Other bacterial meningitis	Chapter 19
Deficiencies or toxicities	
Ammonia salt toxicity	Chapter 70
Arsanilic acid toxicity	Chapter 70
Arsenic toxicity	Chapter 70
Calcium deficiency	Chapter 68
Carbamate toxicity	Chapter 70
Carbon dioxide toxicity	Chapter 70
Carbon monoxide toxicity	Chapter 70
Chlorinated hydrocarbon toxicity	Chapter 70
Cocklebur toxicity	Chapter 70
Copper deficiency	Chapter 68
Dichlorvos toxicity	Chapter 70
Hydrogen sulfide toxicity	Chapter 70
Hygromycin toxicity	Chapter 19
Iron toxicity	Chapters 15, 68
Lead toxicity	Chapter 70
Magnesium deficiency or toxicity	Chapter 68
Mercury toxicity	Chapter 70
Niacin deficiency	Chapter 68
Nightshade toxicity	Chapter 70
Nitrate/nitrite toxicity	Chapter 70
Nitrofuran toxicity	Chapter 70

Table 5.9 (Continued)

	Organophosphate toxicity	Chapter 70
	Pantothenic acid deficiency	Chapter 68
	Pentachlorophenol toxicity	Chapter 70
	Phenoxy herbicide toxicity	Chapter 70
	Phosphorus deficiency	Chapter 68
	Pigweed toxicity	Chapter 70
	Riboflavin deficiency	Chapter 68
	Sodium chloride deficiency	Chapter 68
	Sodium fluoroacetate toxicity	Chapter 70
	Strychnine toxicity	Chapter 70
	Streptomycin toxicity	Chapter 19
	Vitamin A deficiency	Chapter 68
	Vitamin B_6 deficiency	Chapter 68
	Vitamin D deficiency	Chapter 68
	Water deprivation (salt poisoning)	Chapter 68
Viral		
	African swine fever	Chapter 25
	Atypical porcine pestivirus	Chapter 39
	Blue eye paramyxovirus	Chapter 37
	Classical swine fever	Chapter 39
	Hemagglutinating encephalomyelitis virus	Chapter 31
	Japanese encephalitis virus	Chapter 33
	Nipah virus	Chapter 37
	Porcine adenovirus	Chapter 24
	Porcine astrovirus type 3	Chapter 27
	Porcine cytomegalovirus	Chapter 35
	Porcine enterovirus	Chapter 40
	Porcine reproductive and respiratory syndrome	Chapter 41
	Porcine sapelovirus	Chapter 40
	Porcine teschovirus	Chapter 40
	Pseudorabies virus	Chapter 35
	Rabies virus	Chapter 45

Table 5.10 Approximate ages at which diseases causing lameness are more common (also see Chapter 19).

Age in months										
1	1.5	2	3	4	5	6	18	30	42	54
Trauma: muscle bruising, sprains, strains, dislocations, fractures										
Clostridium tetani or septicum infection										
Vesicular diseases: foot-and-mouth disease, vesicular exanthema, swine vesicular disease, vesicular stomatitis, San Miguel sea lion virus										
Streptococcus suis infection		Chronic suppurative arthritis due to *S. suis*, *S. equisimilis*, *M. hyorhinis*, *M. hyosynoviae*, *H. parasuis*, *Corynebacterium*, *Staphylococcus*								
Streptococcus equisimilis infection										
	Acute *Mycoplasma hyorhinis* infection									
	Haemophilus parasuis infection									
		Bursitis								
			Rickets							
			Acute erysipelas				Chronic erysipelas			
			Asymmetrical hindquarter syndrome							
			Foot rot							
			Back muscle necrosis							
				Osteochondrosis						
					Osteoarthrosis, degenerative joint disease					
					Epiphysiolysis					
						Brucellosis				
						Laminitis				
						Apophysiolysis				
							Osteomalacia			
								Tarsitis		
								Arthrosis deformans		
								Leg weakness syndrome		

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Table 5.11 Causes of reproductive losses in pigs (also see Chapter 20).

	Abortion	Weak births	Stillbirths	Mummification	Small litters	Chapter
General						
Genetics			X		X	3
High environmental temperatures	X	X	X		X	20
Management					X	20
Nutrition					X	20
Bacterial						
Actinobacillus spp.	X					48
Brucella suis	X	X	X			50
Burkholderia pseudomallei	X					64
Chlamydia spp.	X	X	X	X	X	64
Erysipelas rhusiopathiae	X			X	X	53
Lawsonia intracellularis	X					58
Leptospira spp.	X	X	X	X		55
Listeria monocytogenes	X	X	X			64
Mycoplasma suis	X				X	56
Salmonella spp.	X					59
Staphylococcus spp.	X					60
Streptococcus spp.	X					61
Toxicities and deficiencies						
Carbon monoxide toxicity	X	X	X			70
Fumonisin	X					69
Stachybotrys atra	X					19
Vitamin A deficiency	X	X	X			19, 68
Zearalenone					X	69
Parasites						
Toxoplasma gondii	X	X	X			66
Viral						
African swine fever virus	X	X	X		X	25
Blue eye paramyxovirus	X	X	X	X		37
Border disease virus	X	X	X		X	39
Bovine viral diarrhea virus	X	X	X		X	39
Classical swine fever virus	X	X	X	X	X	39
Encephalomyocarditis virus	X	X	X	X		40
Foot-and-mouth disease	X					40
Influenza A virus	X	X	X		X	36
Japanese encephalitis virus	X	X	X	X		33
Menangle virus			X	X	X	37
Nipah virus	X					37
Parvovirus	X[a]			X	X	38
Porcine adenovirus	X					24
Porcine circovirus type 2	X	X	X	X		30
Porcine cytomegalovirus		X	X	X	X	35
Porcine reproductive and respiratory syndrome virus	X	X	X	X	X	41
Pseudorabies virus	X	X	X	X	X	35
Senecavirus A		X				40
Teschovirus		X	X	X	X	40

[a] Parvovirus can cause abortions under rare and unique situations.

Table 5.12 Common congenital anomalies in pigs.

Defect	Prevalence (%)	Etiology	Diagnosis
Microencephaly	0.07	Heat stress midpregnancy	History of heat stress
		Unknown (most cases)	An agent affecting development in early or midpregnancy
Microphthalmia		Vitamin A deficiency	Multiple defects in affected litters; heavy neonatal mortality; history; diet analysis; serum and liver vitamin A analysis
		Hog cholera (HC) infection	HC infection in herd; virus isolation; fluorescent antibody test; serology; congenital tremor AI present in herd
		Heritable	Mode of inheritance uncertain; dominant gene (?)
		Unknown	An agent affecting embryos at 12–16 days of development
Neural tube defects (anencephaly, encephalocele, hydrocephalus, spina bifida)	0.04	Unknown	An agent affecting embryos at 12–16 days of development
		Vitamin A deficiency (hydrocephalus)	Multiple defects in affected litters; heavy neonatal mortality; history; diet analysis; serum and liver vitamin A analysis
Congenital tremor	0.20	HC virus (type AI)	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
		Type All (unidentified virus)	Hypomyelinogenesis of spinal cord; analysis of myelin lipids of spinal cord; small cross-sectional area of the spinal cord
		Type AIII	Monogenic sex-linked gene mutation in Landrace affecting only males and associated with defect in myelin sheath
		Type IV	Autosomal recessive gene in Saddleback affecting both sexes
		Pseudorabies (PR) virus	PR infection in herd; virus isolation; serology
		Neguvon (metrifonate, trichlorfon)	History of dosing sows in midpregnancy; hypoplasia of cerebrum and cerebellum; Purkinje cell loss; changes in neurotransmitters
	0.10	Tobacco stalks, jimsonweed, poison hemlock, wild black cherry	History of exposure to plants in early to midpregnancy
		Vitamin A deficiency	Multiple defects in affected litters; heavy neonatal mortality; history; diet analysis; serum and liver vitamin A analysis
Arthrogryposis		HC attenuated vaccine virus	History of vaccination during early pregnancy
		HC infection	HC infection in herd; virus isolation; fluorescent antibody test; serology; congenital tremor type AI in herd
		Paramyxovirus infection	Menangle virus infection during pregnancy
		Heritable	Recessive gene (?); autosomal recessive in Yorkshire pigs
		Unknown (most cases)	An agent affecting development in early or midpregnancy

Table 5.12 (Continued)

Defect	Prevalence (%)	Etiology	Diagnosis
Micromelia	0.10	Unknown	Possibly caused by limb vascular defects in early pregnancy
Cleft palate/harelip	0.07	Heritable	Possibly a recessive gene; cleft palate in Poland China pigs probably genetic
		Unknown (most cases)	An agent affecting development in early or midpregnancy
Deformed tail	0.08	Possibly heritable	Mode of inheritance uncertain; occasionally urogenital defect associated
		Unknown	Often associated with motor defects in hind limbs; vertebral defects
Myofibrillar hypoplasia	1.05	Heritable	Most common in Landrace, less in Large White; probably polygenic mode of inheritance; incidence modified by maternal stress, slippery floor, birth weight, or maternal nutrition
		Fusarium toxin	Higher mortalities than other forms; feed analysis
Inguinal hernia	0.40	Heritable	Mode of inheritance uncertain; incidence modified by environment
Umbilical hernia	1.00	Unknown	Possibly polygenic mode of inheritance
Anal atresia	0.40	Heritable	Possibly polygenic inheritance or an autosomal recessive or autosomal dominant form of transmission
Hypotrichosis		Heritable in some breeds	Mode of inheritance uncertain
		Iodine deficiency	Stillbirths and high neonatal mortality; enlarged thyroids; skin edematous; feed analysis
Epitheliogenesis imperfecta	0.05	Heritable	Possibly autosomal recessive gene; hydronephrosis associated
Dermatosis vegetans		Heritable	Autosomal recessive; associated with fatal giant cell pneumonia
Pityriasis rosea		Probably heritable	Mode of inheritance uncertain; affects young pigs, especially Landrace; benign and self-limiting
Von Willebrand's disease		Heritable	Recessive gene in Poland China pigs; excess bleeding from minor wounds; decrease in factor VIII and platelet retention time
Navel bleeding	0.14–1.2	Unknown	Cord is edematous; familial linkage
Cardiac defects	0.03	Unknown	Most cases recognized at 4–8 weeks; mostly males
Cryptorchidism	0.39	Probably heritable	Polygenic transmission; left testicle most commonly involved
Female genital hypoplasias, duplications	0.68	Probably a heritable component	Mode of inheritance uncertain; genital tract incomplete or duplicated
	0.06		
Male pseudohermaphroditism	0.2–0.6	Heritable	Mode of transmission uncertain; testicles in abdomen together with female tubular tract
True hermaphroditism		Heritable	Mode of inheritance uncertain; testicular and ovarian tissues usually with female tubular tract

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Table 5.13 Pig diseases with zoonotic potential (also see Chapter 12).

Bacterial	
Bacillus anthracis	Chapter 64
Brucella suis	Chapter 50
Campylobacter jejuni	Chapter 64
Campylobacter coli	Chapter 64
Escherichia coli	Chapter 52
Erysipelothrix rhusiopathiae	Chapter 53
Leptospira interrogans	Chapter 55
Listeria monocytogenes	Chapter 64
Burkholderia pseudomallei	Chapter 64
Salmonella spp.	Chapter 59
Staphylococcus aureus	Chapter 60
Streptococcus suis	Chapter 61
Yersinia pseudotuberculosis	Chapter 64
Yersinia enterocolitica	Chapter 64
Fungal	
Microsporum nanum	Chapter 17
Parasites	
Clonorchis sinensis	
Diphyllobothrium spp.	
Echinococcus granulosus	Chapter 67
Fasciolopsis buski	Chapter 67
Gastrodiscoides hominis	Chapter 67
Gnathostoma doloresi	Chapter 67
Gnathostoma hispidum	Chapter 67
Gongylonema pulchrum	Chapter 67
Macracanthorhynchus spp.	Chapter 67
Opisthorchis felineus	
Paragonimus spp.	Chapter 67
Sarcoptes scabiei	Chapter 65
Schistosoma japonicum	
Strongyloides stercoralis	Chapter 67
Taenia asiatica	Chapter 67
Taenia solium	Chapter 67
Trichinella spiralis	Chapter 67
Trichuris suis	Chapter 67
Protozoal	
Balantidium coli	Chapter 66
Sarcocystis suihominis	Chapter 66
Sarcocystis hominis	Chapter 66
Toxoplasma gondii	Chapter 66
Viral	
Encephalomyocarditis virus	Chapter 40
Ross River virus	Chapter 46
Influenza A virus	Chapter 36
Japanese encephalitis virus	Chapter 33
Kyasanur Forest disease virus	
Nipah virus	Chapter 37
Rabies virus	Chapter 45
Swine vesicular disease virus	Chapter 40
Vesicular stomatitis virus	Chapter 45

Source: Adapted from Glenda Dvorak.

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6

Diagnostic Tests, Test Performance, and Considerations for Interpretation

Jane Christopher-Hennings, Gene A. Erickson, Richard A. Hesse, Eric A. Nelson, Stephanie Rossow, Joy Scaria, and Durda Slavic

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 that 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 6.1).

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 6.1).

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 influenza A virus (IAV) serological testing and to serotype *Haemophilus parasuis* field isolates (Del Río et al. 2003). Although AGID continues to be used in some laboratories for *H. 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.

Bacterial culture and antimicrobial susceptibility testing (AST)

For detection of the majority of bacterial diseases, bacterial culture is the most common, but not necessarily the most rapid diagnostic method. It is routinely used by veterinary diagnostic laboratories to grow bacteria from clinical samples, providing evidence of their viability, in contrast to molecular diagnostic methods where detection

Diseases of Swine, Eleventh Edition. Edited by Jeffrey J. Zimmerman, Locke A. Karriker, Alejandro Ramirez, Kent J. Schwartz, Gregory W. Stevenson, and Jianqiang Zhang.

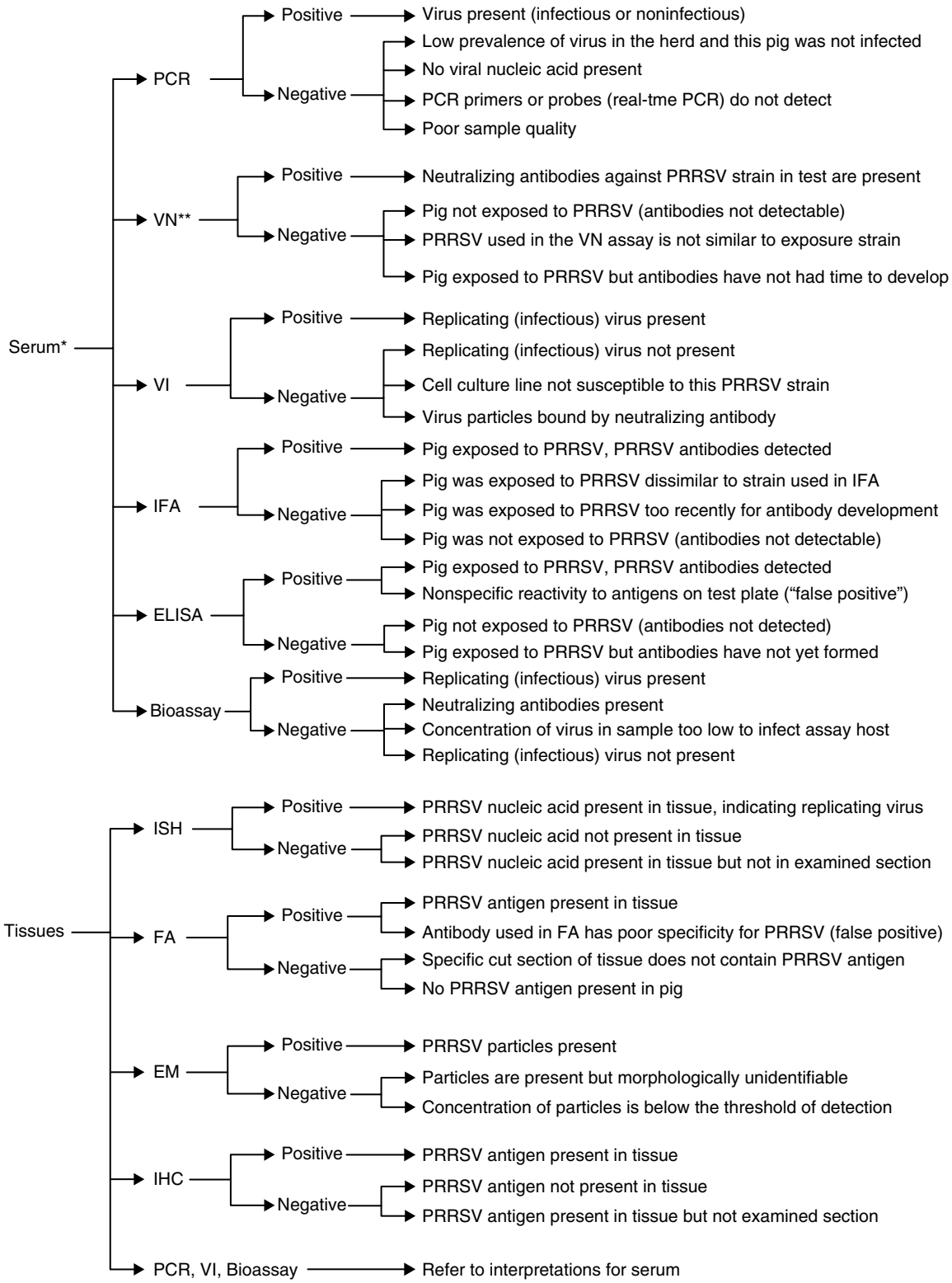

Figure 6.1 Possible interpretations of diagnostic test results using serum and tissues for PRRSV diagnosis. *Blood swabs and oral fluids can be tested by PCR. **VN, serum virus neutralization assay.

Table 6.1 Diagnostic tests for analyte types: infectious agent, antibody, antigen, or nucleic acid detection.

Antigen (or nucleic acid)-specific tests	Antibody-specific tests
	Agar-gel immunodiffusion (AGID)
Antigen ELISA	Antibody ELISA
Bacterial culture	
Bioassay	
	Buffered *Brucella* antigen test (BBAT)[a]
Clinical pathology[b]	
Complement fixation (CF)	Complement fixation (CF)
DNA sequencing	
Electron microscopy (EM)	
Fluorescent antibody (direct FA or indirect FA)	
Fluorescent microsphere immunoassay (FMIA)	Fluorescent microsphere immunoassay (FMIA)
	Fluorescent polarization assay (FPA)
	Hemagglutination inhibition (HI)
Immunohistochemistry (IHC)	
	Immunoperoxidase monolayer assay (IPMA)
	Indirect fluorescent antibody assay (IFA)
In situ hybridization (ISH)	
	Microscopic agglutination test (MAT)[c]
Parasite identification	
Polymerase chain reaction (PCR)	
Virus isolation (VI)	
	Virus neutralization (VN) or serum VN (SVN)

[a] For detection of *Brucella* sp. antibodies only.
[b] Indirect method of determining whether an antigen is present.
[c] For detection of *Leptospira* sp. antibodies only.

of nucleic acid does not discern between viable or nonviable organisms. Subsequently, bacterial growth is used for bacterial identification and subtyping (if applicable) to establish its significance. Not all bacterial isolates are associated with specific disease conditions, since some bacteria may be present in samples either as contaminants or commensals. Once the significance of bacterial isolates is established, their antimicrobial susceptibility pattern is determined to guide antimicrobial treatment decisions. Furthermore, isolates can also be saved for any future research or for vaccine production, making them a valuable source for retrospective studies and for disease prevention.

Before submitting samples for culture, it is important for clinicians to know how diagnostic laboratories process samples. There are a variety of artificial media, temperatures, and growth conditions that can be used to obtain bacterial growth from clinical samples (Markey et al. 2013). The conditions used primarily depend on sample type, animal age, and clinical history. Therefore, it is very important that the referring veterinarian provides this information at the time of sample submission to help guide sample setup and interpretation of results (Table 6.2). It is also beneficial to describe on the submission form any lesions observed and to specify if a particular bacterial disease is suspected. The next step is to select the appropriate bacterial test(s) if this option is available. Some laboratories offer a variety of bacterial cultures (e.g. aerobic, anaerobic, *Clostridium difficile*, *Mycoplasma*, *Brachyspira*, etc.) to help veterinarians make the selection at the time of sample submission. In general, a request for aerobic bacterial culture will ensure isolation of most porcine bacterial pathogens. They usually grow well aerobically on medium containing blood, and their growth is

Table 6.2 Guidelines for interpretation and troubleshooting of positive and negative bacterial culture results.

Pathogen	Analyte	Test	Outcome	Interpretation	Additional testing
Bacteria	Tissue Body fluids[a]	Culture in liquid or solid media	Positive	Bacterial agent is isolated from the sample	Identify agent to the species/subspecies/ pathotype level
	Blood Urine Feces		Negative	Bacterial agent is not isolated from the sample: • Antimicrobial treatment prior to sample collection • Inappropriate sample collection, submission, and processing • Commensal flora overgrowth • Other fast-growing pathogen overgrowth • Nonbacterial etiology	Submit samples for PCR or request IHC/PCR on histological sections/scrolls

[a] Cerebrospinal, thoracic, peritoneal, synovial.

improved in the presence of 5–10% CO_2. More fastidious aerobic pathogens (e.g. *H. parasuis* and *Actinobacillus pleuropneumoniae* biotype I) require additional nutritional supplements including nicotinamide adenine dinucleotide (NAD), whereas anaerobic bacteria (e.g. *Actinobaculum suis* and *Clostridium* spp.) must be grown in oxygen-free conditions, and therefore anaerobic culture must be requested. In some cases, enrichment methods are recommended for isolation of *Salmonella* and *C. difficile*. There are also a few swine bacterial pathogens that are very difficult to almost impossible to grow routinely. For example, *Mycoplasma hyopneumoniae* requires a specific medium, is extremely fastidious to grow, and can take up to 4 weeks to reach measurable levels (Thacker 2004). Similarly, *Lawsonia intracellularis* can only be grown in tissue cell culture that is not routinely available in veterinary diagnostic laboratories (Vannucci et al. 2012). Alternative methods such as polymerase chain reaction (PCR) or ELISA are available for detection of slow-growing or fastidious pathogens. More information about growth requirements and methods for culturing various porcine bacterial pathogens can be found in their specific chapters in this book.

When individual bacterial colonies (i.e. pure bacterial growth) are obtained, then bacterial identification and AST can be performed. Previously, the most common method of bacterial identification was based on specific growth characteristics, colony morphology, Gram staining, and a variety of biochemical tests using automated or manual identification systems. This relatively lengthy procedure took approximately 24–48 hours before bacterial identification was achieved. Although this approach is still in use in most diagnostic laboratories, it is being rapidly replaced by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS). In contrast to biochemical procedures, this is a very efficient and sensitive method where a small amount of bacterial growth is directly transferred to a stainless steel target plate and mixed with matrix solution to co-crystallize the bacterial proteins. The plate can accommodate between 24 and 384 samples, and it is then transferred into the MALDI-TOF instrument where individual samples are exposed to short laser pulses, resulting in protein ionization. Ionized proteins travel through a linear flight tube and are separated based on their mass-to-charge ratio plotted against signal intensity, which is then used to create a specific bacterial protein fingerprint. This fingerprint, in most cases, is unique for a given bacterial species. It has been well documented that for species-level identification, an accurate protein fingerprint is typically in the range of 2–20 kDa. The predominant proteins in this range are ribosomal proteins, which ionize well, are abundant in cells, and vary minimally under different growth conditions. Once the bacterial protein spectra are collected, they are compared with reference spectra in the MALDI-TOF MS database to generate the bacterial identification in real time. In addition to generating bacterial identification, the software also generates a numerical score to indicate how well-generated spectra match the spectra in the database. Typically, a high score indicates that a specific genus and species identification is reached (e.g. *Actinobacillus suis*), whereas a lower score may indicate only a genus level of identification (e.g. *Actinobacillus* sp.). However, the score levels can be influenced by many factors such as the thickness of bacterial smear applied on the target plate and the method used for smear processing. Smears that are too thick will yield lower scores or occasionally result in no spectra generated. In contrast, use of an "extraction method" where bacterial proteins are purified before they are applied on the target plate will result in the highest scores. Because the "extraction method" is time consuming, it is not used routinely for MALDI-TOF MS identifications. Instead, laboratories opt to use either a "direct transfer method" or an "extended direct transfer

method." With the "extended direct transfer method," 70% formic acid (FA) is applied on smears deposited on the target to partially extract proteins before the matrix is added. In some instances, this can improve score levels over the "direct transfer method" where only matrix is used, but it also adds to time and cost of running MALDI-TOF MS. If no results are obtained, in most cases the species reference spectra are not present in the database. Since 2011, the numbers of entries into the database almost doubled, and with each manufacturer's annual database update, the number of reference spectra keeps growing. Although reference spectra of the majority of swine pathogens are in the database, there are still some gaps that need to be filled. For example, no spectra for *Actinobacillus porcitonsillarum*, a bacterial species very closely related to *A. pleuropneumoniae* biotype I, are present in the manufacturer's database. Because MALDI-TOF MS is an open platform system, this issue can easily be rectified by creation of a customized database entry within the laboratory. All customized entries, however, have to pass rigorous multiple quality checks, which start with proper bacterial identification. Isolates used for spectra creation must be well characterized, and their identity, at minimum, confirmed by either 16S rRNA or other housekeeping gene sequencing. Next, an "extraction method" must be used for protein purification. Once reference spectra are created, they should also undergo extensive manufacturer's recommended internal quality control checks before being added to the customized database. In addition, it is highly recommended to add reference spectra from a minimum of five isolates of the same bacterial species to account for their natural variability. Aside from biochemical and MALDI-TOF MS, molecular methods can also be used for bacterial identification of individual colonies. The most frequent method used is 16S rRNA gene sequencing (Janda and Abbott 2007). 16S rRNA genes are present in all bacteria and are well conserved, making them a reliable marker for bacterial identification. However, when dealing with closely related bacterial species and subspecies, occasionally other genes (i.e. *rpoB*, *sodA*, etc.) may need to be sequenced because the 16S rRNA gene sequence may not provide enough information for speciation (Angeletti et al. 2015; Shin et al. 2015). For the most accurate bacterial identification, whole genome sequencing can be used, but its cost makes it still prohibitive for routine use.

To date, numerous studies have shown that MALDI-TOF MS bacterial identification is almost as accurate (if reference spectra are in the database) as molecular identification (Bizzini et al. 2010; Carpaij et al. 2011; Randall et al. 2015). When compared with biochemical identification, MALDI-TOF MS is superior, particularly for gram-positive bacteria for which routine biochemical identification was always challenging (Cherkaoui et al. 2011; Dupont et al. 2010). The MALDI-TOF MS bacterial identification is currently limited in its ability to differentiate between very closely related species and subspecies (Randall et al. 2015). This issue is gradually diminishing with each database update, as more reference spectra are added for each problematic species and subspecies. In addition, the software is being upgraded to distinguish slight differences between species and subspecies. In the meantime, MALDI-TOF MS is replacing biochemical methods for bacterial identification. In 2016, almost half of American Association of Veterinary Laboratory Diagnosticians (AAVLD)-accredited laboratories were using MALDI-TOF MS. It is expected that this number will increase in the future since the MALDI-TOF MS provides highly reliable bacterial identification within the same day, requires minimum handling time and technical expertise, can be automated (FA and matrix deposition), and is cost effective. However, successful bacterial identification using MALDI-TOF MS still depends on growing individual bacterial colonies from clinical specimens, and AST needs to be done separately. In the future, diagnostic uses of MALDI-TOF MS may include direct detection of bacteria in clinical samples and AST (Ferreira et al. 2011; Mailhac et al. 2017; Oviano et al. 2017).

The mere isolation of bacteria from clinical samples does not automatically imply their significance. It is important to critically evaluate the isolation of specific bacterial species in association with clinical signs and lesions to assess their relevance in morbidity and mortality. This is particularly true when additional characterization is required to differentiate between virulent and non-virulent bacterial pathotypes. For example, isolation of *Escherichia coli* from the gut does not indicate its association with enteritis. Further serotyping (i.e. for presence of F4 [K88]) and/or genotyping (e.g. detection of genes for fimbriae and toxins) must be done to establish this association (Osek 2001). Furthermore, the significance of isolation can sometimes be confirmed only by the detection of toxins as in cases of *C. difficile* (Moono et al. 2016).

Despite typical clinical signs and proper sample collection and submission, bacterial pathogens may not necessarily be isolated, which may happen more frequently than anticipated (Table 6.2). Antibiotic treatment and lack of refrigeration following sample collection are two of the most frequent causes of negative culture (Oliveira 2007). Lack of bacterial isolation may also result from overgrowth by commensal flora or contaminants, particularly in respiratory and intestinal samples (Fittipaldi et al. 2003). Additionally, if field veterinarians do not specify clinical signs, lesions, and a tentative diagnosis during submission, no isolation of bacterial species with specific growth requirements will likely be achieved. If in any doubt, it is highly recommended to call the laboratory

for help with any questions related to sample collection, storage, and submission.

In summary, bacterial culture is still a widely used diagnostic method for bacterial isolation, identification, and susceptibility testing. In contrast to bacterial isolation, which has not changed in decades, bacterial identification underwent significant improvements in the last few years with the introduction of the MALDI-TOF MS. The introduction of this technology allows diagnostic laboratories to achieve bacterial identification the same day when bacterial growth is detected. This can help veterinarians with empirical treatment decisions if pathogens are isolated or help reduce antibiotic use when no bacterial pathogens are present.

After the clinical significance of bacterial species is established, then AST is recommended to help guide treatment decisions. Some clinically significant bacterial species have predictable susceptibility patterns, which do not warrant AST. For example, currently, *Trueperella pyogenes* is predictably sensitive to penicillin, and this drug can be reliably used for its treatment without AST. In general, AST should be routinely performed for bacterial species with unpredicted susceptibility patterns (e.g. *E. coli, Salmonella* spp.) and for bacteria where resistance is reported or expected (e.g. *Pseudomonas aeruginosa*). In North America, AST is performed in veterinary diagnostic laboratories following guidelines published by the Clinical and Laboratory Standards Institute (CLSI). CLSI guidelines provide recommendations for testing, including preparation of bacterial suspensions, media use, incubation conditions, and a list of antimicrobial agents per animal species that may be considered for testing to standardize test performance. They also include result interpretation guidelines, which are specific for bacterial species–antimicrobial agent combinations (CLSI 2015). Therefore, it is important to know the identity of bacterial species when interpreting AST results. However, guidelines are not available for all clinically relevant bacteria that do not have predictable susceptibility patterns. For example, guidelines are not available for slow-growing bacterial species with fastidious growth requirements such as *H. parasuis*. There are two AST methods frequently used in veterinary medicine: agar disk diffusion and broth dilution (CLSI 2015). Agar disk diffusion, also known as the Kirby–Bauer method, is a qualitative method where a suspension of pure bacterial culture is streaked onto the surface of nutrient agar and then paper disks impregnated with antimicrobials are applied on top of it. The plates are incubated overnight, and a diameter of zone of inhibition (i.e. lack of bacterial growth around disks) is measured the next day. The agar disk diffusion method is highly flexible as any drug can be easily included and/or omitted from the testing. It is cost effective, but is not suitable for testing of all pathogens because some will grow poorly or will not grow at all on the media used. It is primarily used for testing of fast-growing aerobic bacteria (e.g. *E. coli*, *Salmonella*, *P. aeruginosa*, etc.) and some fastidious aerobic bacteria (e.g. *A. pleuropneumoniae*).

In contrast to agar disk diffusion, broth dilution is a quantitative test, and the results are expressed as the minimal inhibitory concentration (MIC). Broth dilution is performed in microbroth format where an equal amount of bacterial suspension is applied to each well of a 96-well MIC plate and incubated overnight. Veterinary specific formats of MIC plates are commercially available, and they contain antimicrobials approved for use in a particular animal species in a twofold dilution range. For example, the bovine/porcine MIC plate contains penicillin in range of 0.12–8 μg/mL and tiamulin in range of 1–3 μg/mL. Therefore each well on the MIC plate contains different antimicrobial concentrations. The presence of bacterial growth indicates that a bacterium is resistant to that particular drug concentration, whereas the absence of bacterial growth indicates susceptibility. The lowest concentration of drug that will inhibit bacterial growth is called the MIC, and it is expressed in μg/mL. It is necessary to achieve that concentration at the infection site during the course of treatment to inhibit bacterial growth. As concentration of drug can vary in different body systems and fluids, it is important to have pharmacokinetic studies done in order to determine if therapeutic concentrations can be achieved at target site. Unlike the agar disk diffusion method, the MIC method is more laborious, more expensive, and less flexible because it may take a few months before any antibiotic changes to the commercial plate format can be made.

Interpretation of AST results is performed following CLSI guidelines with values expressed as susceptible (S), intermediate (I), resistant (R), or nonsusceptible (NI). MIC values (μg/mL) can also be reported if the broth dilution method is used. S implies that a particular drug used in a dosage recommended by a manufacturer will likely successfully treat infection. R, on the other hand, means that infection likely will not be treated under the same conditions and usually indicates the presence of a resistance trait. I is defined as a "buffer zone," meaning that under certain conditions, the drug can be used for a favorable clinical outcome. For example, a drug can be used for treatment of infection in a site where it can be physiologically concentrated (i.e. urine) or when a high dosage of drug can be used. The NI designation is more complex, and it is reported for bacteria where values are defined for susceptible interpretative criteria only because of lack or rare occurrence of reported resistance. When AST results do not conform within defined values, the isolates are reported as NI to the specific drugs. That does not mean automatically that they have a resistance mechanism. It is possible that within the wild bacterial population, some isolates may have susceptibility values

above the ones defined as susceptible by the current CLSI guidelines (CLSI 2015).

There are numerous antimicrobial drugs approved for use in swine, but there is no need to test all of them individually. The CLSI recommends using one representative for a specific class of related drugs. For example, tetracycline is used as a representative of the tetracycline group (tetracycline, doxycycline, minocycline). If bacteria are found susceptible to tetracycline, they are considered susceptible to other drugs within this class. However, if they are resistant, then doxycycline and minocycline need to be tested individually as they have a broader antimicrobial coverage. For the macrolide class, clindamycin is used for susceptibility testing for both clindamycin and lincomycin. As the CLSI does not endorse any commercially available drugs, only nonproprietary names are used for reporting purposes.

Overall, AST results can be used to predict the effectiveness of treatment. However, AST is an *in vitro* test performed under optimal conditions for the bacterium–antimicrobial agent interactions. Therefore, it should be used only as a general guide for drug selection, and other aspects of appropriate treatment choice(s) should be evaluated, such as pharmacodynamics and pharmacokinetics of the drug, intracellular versus extracellular location of the bacteria, and site of infection. These and other aspects of antimicrobial drug selection are beyond the scope of this chapter, and for more information, the reader is referred to the "Drug Pharmacology, Therapy, and Prophylaxis" chapter of this book.

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 epidemic diarrhea virus [PEDV], porcine circovirus type 2 [PCV2], classical swine fever virus [CSFV], hepatitis E), and the conclusions are then compared to PCR results (Christopher-Hennings et al. 1995; Dee et al. 2014). 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

There are several *Brucella* sp. tests available including the buffered acidified plate antigen (BAPA), buffered Brucella antigen test (BBAT) or card test, rapid automated presumptive (RAP), rivanol plate agglutination test (RIV), standard plate test (SPT), standard tube test (STT), complement fixation test (CFT), and the fluorescence polarization assay (FPA). Most *Brucella* sp. serology assays use the cattle antigen, *Brucella abortus*, to test swine sera for *Brucella* sp. antibodies. A positive reaction is visually discerned by agglutination of the sera with the antigen. None of these tests are specific for *Brucella suis* since there is extensive cross-reaction between the *Brucella* species, which can cause false-positive reactions (Nielsen et al. 1999). The card test is the most commonly used screening test for pigs. Serum reacting with the card test can also be tested using other assays to corroborate or refute a positive result. The FPA is commonly used as the follow-up test to sera reacting with the card test. Although the FPA 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). State Board of Animal Health testing requirements vary from state to state as to what is an acceptable screening test and what follow-up test can be used for reacting sera. Some states will accept follow-up testing by state diagnostic labs, while some states insist that all follow-up testing be conducted by the National Veterinary Services Laboratory (NVSL). The NVSL result is accepted as the official final result. The best policy for *Brucella* serology testing is to know what screening test and follow-up test are required for a state or a foreign destination. State diagnostic labs will most commonly know the testing requirements for different states and countries.

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 *A. 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) can be used to visualize pathogens, particularly new and emerging viruses, when diagnostic reagents are not available (Chen et al. 2014). EM can also be useful in determining the cellular pathogenesis of an agent as an aid in developing intervention strategies. The strength of EM is its ability to identify a virus family in both antemortem and postmortem sample. The weaknesses of EM include its lack of sensitivity, inability to differentiate viruses within a family, dependence on a stable and detectable virus structure in samples, and the cost of maintaining the scope and hiring trained personnel. EM has a lower limit of detection with negative stain methods of approximately 10^6 virus particles per milliliter of sample and therefore is useful for detecting viruses in enteric cases since crude fecal suspensions routinely have greater than 10^6 particles of pathogenic viruses per gram of feces. Screening for known agents by EM has largely been replaced by PCR. For example, EM cannot adequately detect and differentiate group A, B, and C rotaviruses in comparison with PCR, but it can be used as a method to corroborate the identification of virus by other techniques. Next-generation sequencing (NGS) has the potential to further reduce the value of EM in pathogen discovery.

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 (the sample OD on the test well divided by the positive control OD). A "cutoff" level is designated for positive and negative results. 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 with 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. At the same time or 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 (lack of competition/blocking), whereas samples with specific antibodies show less color development (competition/blocking) with increasing antibody levels. This type of assay has been used for differentiation between pseudorabies virus G1- or gE-deleted vaccinated and infected pigs as well as with IAV, which uses a nucleoprotein antigen.

The greatest strengths of ELISA for antibody testing are high throughput volume, 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 may be 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 IAV, classical swine fever (CSF), group A rotaviruses, and PCV2. The primary strengths of antigen detection immunoassays are that they are generally rapid, are 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 coverslip 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 typically used to fix the assay plates when the cells are grown on plastic. Staining of virus isolation (VI) cultures is usually done at the first appearance of CPE or at a fixed time, usually 3–5 days post inoculation to detect non-cytopathic 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 obtaining accurate results, so these reagents should be fully characterized for their specificity and sensitivity. False-negative results can occur if the cut tissue section does not have any infected cells. A shortage of highly trained technicians, along with newer techniques like PCR and ELISA, has decreased this testing in some diagnostic laboratories. In addition, immunohistochemistry (IHC) and *in situ* straining techniques are used in place of FA to show a correlation with histopathology and specific cell types.

Fluorescent microsphere immunoassay

Simultaneous detection of multiple targets within one sample has been developed using the fluorescent microsphere immunoassay (FMIA). These assays use multiple beads, each having a distinct dye ratio to distinguish them from each other in a flow cytometric instrument (Luminex xMAP™, Luminex Corporation, Austin, TX). Individual beads are then coated with different antibodies or recombinant proteins to capture antigen (e.g. bead is coated with a specific antibody to the target antigen) or antibodies (e.g. bead is coated with the specific antigen to the antibody) within a sample, and the instrument measures the fluorescence of a secondary fluorescently marked antibody if the target molecule binds to the bead. Beads may also be coated with nucleic acid probes, which can then bind to a complementary DNA (cDNA) target. This method may or 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; Okda et al. 2015). 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).

Hemagglutination inhibition (HI)

An inherent structural capability of some viruses to bind or agglutinate red blood cells (RBCs) is referred to as hemagglutination. This capability can be used to detect the presence of antibodies that bind to hemagglutination-associated structures or epitopes on the virus inhibiting the virus' ability to hemagglutinate RBCs (e.g. adhere to RBCs). HI activity tends to correlate well with protection – the higher the antibody titer, the greater the level of protection. HI testing is currently used most extensively for IAV serological testing, evaluation of IAV strains for autogenous vaccine formulations, and detection of antibodies to porcine parvovirus (PPV) and hemagglutinating encephalomyelitis virus. In the HI test, if specific antibodies in the test serum bind the hemagglutinating portion of the virion, hemagglutination of RBCs (which are added to the test) is blocked, resulting in a "button" of RBCs at the bottom of a microtest 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 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 influenza viruses of swine, the test is subtype specific, which means that the H1 or H3 type of the viral test antigen must be cross-reactive with the type of IAV present in swine herds. HI tests can also be developed with a farm-specific strain. Monitoring infection with a specific strain can be more informative. 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 US 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 non-vaccinated 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. A universal flu ELISA test detecting, but not differentiating, subtypes would be preferable for obtaining plus/minus results.

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. As with most diagnostic tests, developing a specific plan for whom to test and what to test will result in superior results.

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. post weaning 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 [IFA]) and immunoperoxidase monolayer assay (IPMA) 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. Three of the most common IFA assays routinely used in swine diagnostics are for PRRSV, PEDV, and PCV2 (Madson et al. 2014; 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 aqueous 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), *L. 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 these strains 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 6.3 demonstrates the effect of strain variation on PRRSV IFA titer 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).

Laboratories that are able to maintain live *Leptospira* cultures for clinically relevant serovars affecting swine easily perform the MAT. 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 cDNA 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 since DNA or RNA probes specific

Table 6.3 Effect of strain variation on PRRSV IFA results.

	Days postchallenge					
	Day 0	**Day 4**	**Day 7**	**Day 11**	**Day 14**	**Day 28**
Pig # 1						
SD-23983	<20[a]	<20	40	640	1280	1280
Ingelvac PRRS MLV	<20	<20	40	1280	2560	2560
Ingelvac PRRS ATP	<20	<20	<20	160	320	640
Lelystad Isolate	<20	<20	<20	<20	<20	<20
Pig # 2						
SD-23983	<20	<20	20	640	1280	2560
Ingelvac PRRS MLV	<20	<20	20	640	1280	2560
Ingelvac PRRS ATP	<20	<20	<20	160	640	640
Lelystad Isolate	<20	<20	<20	<20	40	40
Pig # 3						
SD-23983	<20	<20	80	640	1280	2560
Ingelvac PRRS MLV	<20	<20	40	640	1280	2560
Ingelvac PRRS ATP	<20	<20	<20	160	320	1280
Lelystad Isolate	<20	<20	<20	<20	<20	40

[a] Endpoint titers expressed as the reciprocal of the greatest serum dilution showing detectable PRRSV-specific fluorescence.

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 longer 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 (PCR)

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 proteins, lipids, carbohydrates, or other materials that might be found in the clinical specimen, and it allows the PCR 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 is several commercially available extraction protocols designed for specific specimens (e.g. serum, tissues, cells, whole blood) and for the specific nucleic acid that is being extracted (e.g. total RNA, viral RNA, messenger RNA, DNA, total nucleic acid) 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, 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 culture for detection of bacterial pathogens previously treated with antibiotics (i.e. nonviable). In addition, it improves turnaround time from many days to a day for detection of certain fastidious viral and bacterial pathogens.

Gel-based polymerase chain reaction

Gel-based PCR uses agarose gels for detection of amplicons produced during PCR. The PCR 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 precasted 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 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 (UV) 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, 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 set up 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).

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. Most diagnostic laboratories to identify swine pathogens in clinical samples utilize two main signaling systems: 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.

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 6.2).

This needs to be considered when PCR results are obtained and used to evaluate the "infectivity" of clinical samples such as environmental samples. 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 VI and PCR detected serial dilutions of PRRSV, there was approximately a 3 log higher concentration by PCR (copy/mL) than by VI (tissue culture infective dose 50 [$TCID_{50}$]/mL) (Figure 6.3).

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 $TCID_{50}$/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 ($TCID_{50}$).

Multiplex polymerase chain reaction

Multiplex PCR refers to the simultaneous detection of multiple targets by PCR within a single sample. Multiple primer sets (or primer/probe sets for real-time PCR) are used to detect the multiple targets. A significant amount of optimization is needed to obtain similar sensitivities and specificities as detecting each target individually, thus somewhat limiting the number of targets that can be detected simultaneously. Multiplex PCR assays have been used in swine diagnostics to determine *Clostridium 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 IAV subtypes) within a single sample.