ABSTRACT

ROBBBINS, REBECCA CLAIRE. Antibiotics in Pork Production. (Under the direction of Maria Correa and Glen Almond.)

The breadth of considerations that affect antibiotic use in swine rearing is covered in this thesis. Chapter 1 introduces the theme and its relevance to readers from veterinary, producer, industry and regulatory fields. Chapter 2 reviews diseases and disorders of swine where antibiotics have been used effectively. Minimizing the potential for development of bacterial resistance must be considered when using antibiotics in pork production.

The application of antibiotic stewardship to pork production is conducted with experimental and epidemiologic study designs. Chapter 3 presents an experiment to evaluate the feeding of a non-antibiotic plant-derived alkaloid to nursery age swine challenged with antimicrobial resistant Salmonella enterica serovar Typhimurium DT104. The plant alkaloid improved growth, intestinal health, and pathogen elimination among nursery pigs similar to a conventional antibiotic growth promoter. Chapter 4 shows the effect of antibiotic selection on a nursery group performance. Nurseries ≤4 weeks post-weaning, with moderate and severe disease, that received antibiotics for disease control, or displayed non-respiratory signs all had an increased odds for receiving an injectable antibiotic. Livability of groups that received chlortetracycline in the drinking water was 1.5% better than that of groups that received chlortetracycline with neomycin through the drinking water. Chapter 5 presents a position on residue limits and withdrawal times for the most commonly used antibiotics in swine health management. Over half of countries had an antibiotic residue limit set lower than Codex. High income countries had an increased odds of setting residue limits below Codex. A lower MRL increased the likelihood of a longer withdrawal time for only one of twelve antibiotics commonly used in swine.
Chapter 6 summarizes the implications of study findings for the veterinary, producer, industry and regulatory fields. Effective antibiotic stewardship being the result of evidence based medicine is supported by the study findings.
BIOGRAPHY

Rebecca C. Robbins was born on December 11, 1981 in Greensboro, NC. She resided with her parents Jim and Terri Robbins in Durham, NC. In 1998, she and her mother moved to Southern Pines, NC. She graduated from Pinecrest High School in 2000. Rebecca attended North Carolina State University where she obtained a Bachelors of Science degree in Poultry Science and a Bachelors of Science degree in Biology from North Carolina State University in 2004. Rebecca began a combined DVM/PhD program in the College of Veterinary Medicine in August 2004. She received her Doctorate of Veterinary Medicine in May 2009. She worked as an associate veterinarian for Banfield, the Pet Hospital August, 2009 through July, 2010 and staff veterinarian for Murphy-Brown, LLC from June, 2010 through May, 2013. Rebecca is currently employed by Seaboard Foods, LLC as the senior production veterinarian since June, 2013. Her PhD graduate work in population medicine was under the direction of Drs. Maria Correa and Glen Almond.
ACKNOWLEDGMENTS

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CHAPTER I.

INTRODUCTION TO ANTIBIOTIC STEWARDSHIP
The theme for the thesis was judicious antibiotic use for swine and pork. The antibiotic era began with the discovery of Penicillin\(^1\) which remains a widely used therapeutic in swine\(^2\). In 1994, the Animal Medicinal Drug Use Clarification Act authorized veterinarians within a veterinary-client-patient relationship to use drugs including antibiotics extra-label “when the health of an animal is threatened, or suffering or death may result from failure to treat\(^3\).” In addition, a veterinarian must establish and assure a sufficient withdrawal time is observed so that no residues result.

Additional regulations have continued to change how antibiotics are used in food animals. The use of antibiotics in food animals for the purpose of growth promotion was banned in the European Union and, effective January 1, 2017 the use of medically important antibiotics for this purpose is banned in the United States\(^4\). In addition, the over-the-counter label for antibiotics licensed for food animal species is also eliminated. Increasing regulations are the result of concerns that antibiotics administered to food animals are responsible for increasing antibiotic resistance\(^4,5\).

Veterinarians are sworn to “the protection of animal health and welfare, the prevention and relief of animal suffering, the conservation of animal resources, the promotion of public health, and the advancement of medical knowledge\(^6\).” Evidenced-based medicine supports judicious use of antibiotics but that is not sufficient. A method for continuous improvement is necessary (Figure 1). Antibiotic stewardship is a program that achieves judicious antibiotic use and improves patient health and well-being while minimizing the development and spread of antibiotic resistance microorganisms\(^6\).
The goal for the thesis was to conduct epidemiologic and applied experimental research that demonstrated antibiotic stewardship. With the intent that swine veterinarians could use the study results to develop evidence based antibiotic stewardship programs for their clients to ensure a healthy pig and a wholesome pork product. The goals were formulated from a swine veterinarian’s point of view.
Figure 1. Diagram of antibiotic stewardship program. When the need for an antibiotic has been established, a non-antibiotic alternative should be considered. If one isn’t found that is safe and effective, an antibiotic should be made through evidence-based medicine then the antibiotic should be used safely and effectively. There is purposely no endpoint because the decisions should constantly be re-evaluated.
REFERENCES


CHAPTER II.

SWINE DISEASES AND DISORDERS

Swine Diseases and Disorders

RC Robbins, Seaboard Foods, Guymon, OK, USA
G Almond, North Carolina State University, College of Veterinary Medicine, Raleigh, NC, USA
E Byers, Murphy-Brown, LLC, Warsaw, NC, USA

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Glossary

Autogenous vaccines  Vaccine made from microorganisms isolated from the animal it will be used on; in the United States these are killed and, by permit, are extended for use in a farm, production system, or region.
Farrow Process of parturition; location where a pig is born and stays till weaning, usually 3–4 weeks of age.
Grow-finish Phase of production that follows the nursery period where pigs reach slaughter weight; used to describe pigs from 10 to 30 weeks of age.
Histopathology ~ hist. The science and microscopic examination of formalin-fixed and paraffin-embedded sections of diseased tissues.
Lesion Visible (microscopic or macroscopic) deviation from normal.

In an approach to investigating any suspected disease or disorder in swine production, a history should be gathered first. Important history to understand from caretakers includes: age of pigs affected, duration of clinical signs, morbidity rate, mortality rate, treatments administered, response to treatments, and any other important information regarding previous diagnoses or disease in the affected group of animals. This is also the time to examine any production records that have been kept on the affected group of swine as well as previous groups for comparison. Records include but are not limited to: where the animals originated from; number in the herd; age; daily mortality; number treated; name of treatment, route of delivery and dose; feed and water usage; high–low temperatures; and vaccinations received or administered. After examining the production records and obtaining a history, proceed with a visual examination of the herd. Typically, it is a biosecurity custom to observe youngest groups first; however, in cases of suspected infectious diseases, it may be best to begin with the healthiest group advancing in order of increasing severity or prevalence.

Often, a definitive diagnosis is not achieved without an extensive clinical and pathological investigation. A post-mortem examination, or necropsy, of affected pigs should occur last. Any pigs recently deceased of natural causes should be examined to establish trends, with the understanding that submission of tissues from these animals may not yield valuable diagnostic results. Tissues for diagnostic evaluation should be collected from clinically affected pigs that are euthanized immediately before necropsy. Sampling of five or more pigs may be required to obtain a valuable diagnosis. When investigating signs referable to the central nervous system (CNS), it is important to preserve brain and spinal cord tissue for microscopic evaluation in cases of neurological disease; therefore, blunt force trauma and brain penetration by captive bolt are not preferred methods of euthanasia. At minimum, fresh and formalin-fixed tissue samples should include: brain, tonsil, heart, lung, lymph nodes, spleen, kidney, liver, and intestine. Additional samples that may be beneficial for diagnosis include: premortem whole blood and ethylenediaminetetraacetic acid-chelated blood (for serum chemistry and complete blood count), spinal cord, intact stifle and hock joints (remove the leg at the hip), intact eyeball with optic nerve attachment, urine, feed, and water. Consult a diagnostic lab regarding any additional samples that may be required in determining an etiologic diagnosis. The etiologic diagnosis should be based on consistent history, signs, and pathology derived from a list of differential diagnoses that are most common or most likely to occur in that herd or production system.

A treatment, control, or prevention program should be formulated simultaneously. Before using any chemical, pharmaceutical, or biologic in swine intended for food, know the domestic use guidelines, importer requirements or producer-packer agreements regarding withdrawal times, residue and tolerance limits, prescribing guidelines, and prohibited substances.

Central Nervous System Diseases and Disorders

This section will focus on a practical approach to investigating signs of neurological disease in swine summarized in Table 1. It is important to determine if clinical signs are consistent with CNS or peripheral nervous system lesions (PNS). Common CNS signs in pigs include behavioral abnormalities (most commonly stupor), ataxia, loss of righting, seizures or...
seizure-like activity (paddling), nystagmus, and blindness. Musculoskeletal disorders may clinically confuse or complicate perceived PNS signs and must be differentiated from each other.

*Streptococcus suis* is a gram-positive cocc i with 35 reported serotypes. Observational studies implicate sow as carriers and piglets are colonized as they pass through the birth canal (Amass et al., 1996). Disease occurs most frequently during the suckling and postweaning period. Commingle pigs from different herds, concurrent infection with porcine reproductive and respiratory syndrome (PRRS), and other stress factors may increase the risk of developing *S. suis* meningitis (Villani, 2002; Thavawongmuzech et al., 2000). Variable morbidity and mortality: mortality depends on early recognition and treatment. Clinical signs of *S. suis* meningitis include paddling, recumbency, nystagmus, and seizure. Isolation of *S. suis* from the lung, nasal secretions, or tonsils from normal pigs is clinically insignificant. In contrast, *S. suis* isolation from cerebrospinal fluid (CSF), meninges, joints, endocardium, or serosal surfaces with or without lesions is relevant (Pijpan, 1994). Few to no gross lesions may be observed during necropsy. Early recognition of clinical signs followed by injection with an antimicrobial that *S. suis* is susceptible is the most effective means of treatment. Administering an antimicrobial that *S. suis* is susceptible to in the drinking water has been proposed to control morbidity (Villani, 2002). Antimicrobial susceptibility patterns for *S. suis* isolates from regional diagnostic laboratories can be used to assist in selection of an appropriate antimicrobial while diagnostic tests are pending; ceftiofur is effective (Halbur et al., 2000). Commercial and autogenous vaccines are available but due to *S. suis* serologic diversity may not be effective (Halbur et al., 2000).

Haemophilus parasuis (HPS), also called Glässer's disease, causes bacterial meningitis, arthritis, and polyserositis similar to *S. suis*. Infections are not clinically or grossly distinguishable from *S. suis*. Definitive diagnosis is by bacterial isolation. However, HPS is a fastidious gram-negative rod and culture media must be supplemented with V factor for successful isolation. Owing to the difficulty in isolating HPS, Polymerase chain reaction (PCR) tests are a suitable alternative (Oliveira et al., 2001). Like *S. suis*, isolation from the airways has little significance unless lesions are present (Hoteffing, 1994). Antimicrobial susceptibility testing identifies ceftiofur or florfenicol that are typically effective first choice therapeutics (Oliveira, 2007b). Prevention may be achieved with medicated early weaning.

Edema disease results when a fimbrial (F18 or F4) and shiga-toxin (Stx-2e) positive strain of *Escherichia coli* successfully attaches to brush border receptors releasing toxin that damages blood vessels including those of the blood–brain barrier causing edema and encephalomalacia. Edema disease most commonly affects rapidly growing pigs, 2 weeks postweaning. Morbidity is moderate to high and mortality is high. Acute death of robust pigs, ataxia, eyelid swelling, and diarrhea are typical clinical signs (Rademacher, 2001). At necropsy, edema may be observed in the mesentery between the loops of the spiral colon and in the cardiac region of the gastric mucosa. Stomas are usually full of feed. Bacteriologic isolation of a β-hemolytic strain of *E. coli* from affected pigs with meningoencephalitis is not sufficient for a diagnosis. Genotyping is necessary to confirm that the *E. coli* isolated was F18 or F4 and Stx-2e positive and thus capable to induce such lesions. There is no effective treatment. Vaccination using an avirulent live culture of *E. coli* postweaning, thorough cleaning and disinfection between groups, and use of genetically resistance breeds that lack the fimbrial receptor are preventative (Fairbrother and Cyles, 2006).

Pseudorabies (PRV), also known as Azijnýka's disease, is caused by a herpesvirus. PRV was eradicated from the US commercial swine herd in 2004 (USDA APHIS, 2008). Feral swine are potential reservoirs. Cattle, sheep, dogs, and cats can also be infected with PRV. High morbidity is due to large quantities of virus shed in saliva and nasal secretions for several weeks following infection. Mortality is inversely related to age approaching 100% in neonates. Clinical signs are also age dependent. Neonates may die without signs. Suckling and recently weaned pigs are those that commonly exhibit ataxia, tremors, excess salivation, and seizures. At necropsy, the brain appears congested and hemorrhagic. Necrotic foci occur in the spleen, liver, lung, lymph node, and specifically tonsils. Histopathologic lesions are characterized by nonsuppurative meningitis and intranuclear inclusion bodies. PCR, virus isolation (VI), immunohistochemistry (IHC), or fluorescent antibody can be used to confirm the diagnosis. No specific treatment is available. Vaccination and eradication are effective for control (USDA APHIS, 2008). In areas free of PRV, suspicion of the disease should be reported to state and federal agencies as required.

Congenital tremors result when hypomyelination or demyelination of the brain and spinal cord. Clinical signs are clonic muscle contractions that cause a general tremor of the entire body. Pigs are affected at birth but severity subsides with
age (Dewey, 2006). Mortality is variable and is the sequela of malnutrition because piglets are unable to nurse. There is no known treatment or prevention.

Hypoglycemia occurs when piglets fail to nurse the sow. This condition is observed within 24 h after birth. There is a low morbidity but high mortality. Pigs may appear disoriented, ataxic, recumbent, or dead. On necropsy, affected piglets will have empty stomachs. Assigning an employee to attend farrowing to ensure piglets nurse will reduce incidence.

Water deprivation, also referred to as salt poisoning, is an idiopathic disease resulting from a period of inadequate water intake (Carson, 2006). High morbidity with variable mortality occurs. The disease is suspected when there is a history of power outage or poor management (Thacker, 2000). Fighting over water access is the first clinical sign and occurs within hours. Dog-sitting, opisthotonous, convulsions, and fighting over water follow and develop after 24 h without water. Removal of the brain from an affected animal reveals edema and eosinophilic meningoencephalitis with perivascular cuffing and this is pathognomonic (Gudmundson and Meagher, 1961). Serum or CSF with a sodium level above 160 mEq L⁻¹ may also be used for supporting evidence (Osweiler and Hard, 1974). Treatment of swine showing signs with an anti-inflamatory is variably effective. When water is restored, limit intake to short, 10–15 min intervals until all animals have had a chance to drink and fighting has ceased after which water can be provided ad libitum. Prevention is daily observation to ensure each animal can access water, adequate water delivery system, and equipping the facility with a generator or alternative method to deliver water during power outages.

### Gastrointestinal System Diseases and Disorders

Gastrointestinal diseases and disorders can occur in all ages of swine as summarized in Table 2. Most digestive diseases are referable to the gastrointestinal tract and result in diarrhea and occasional vomiting. Diarrhea is the result of an intestinal dysfunction caused by malabsorption, excessive secretion, or effusion. Unfortunately, this is not an exclusive characterization of diarrhea and overlap occurs (Mooser and Bielakager, 2007). Rather, differentials for diarrhea should be referable to age at onset and site of infection.

Gastric ulcers are noninfectious and result when glandular mucosa specifically the pars esophagica is traumatized by gastric acid. Gastric ulcers have a wide variety of causes but are most commonly associated with small feed particle size (Ayles et al., 1996) and interruption of feed intake whether caused by disease or poor management. It is common to see signs consistent with gastric ulceration increase following an acute PRRS or influenza outbreak. Morbidity and mortality vary with the scope of the underlying cause. Clinical signs include regurgitation, vomiting, pallor or jaundice, and acute death. An acutely dead pig with blood in its stomach is indicative of an active ulcer and is sufficient evidence for a diagnosis. In chronic cases, ulceration causes hyperplasia resulting in stricture of the pars esophagica and regurgitation. Feeding a coarse ground diet for 3 weeks significantly decreases severity (Ayles et al., 1996) but is impractical in modern production facilities.

Rotavirus is a nonenveloped RNA virus with a double-layered capsid allowing it to remain stable and infective in the environment for months and intrinsically resistant to some disinfectants. Four serogroups infect swine: A, B, C, and E (the latter only reported from the United Kingdom). In addition, infections with particular serogroups vary by age. Type C mostly in sucking piglets and Type A predominately in nursery pigs (Stephenson et al., 2013). Type A is the most prevalent serogroup. Severity of disease decreases with age and is self-limiting. The virus infects and destroys villous enterocytes resulting in villous atrophy. In response, crypt cells fill in the gaps but, because they are incapable of absorption, sucking piglets quickly lose body condition and have a gaunt or wasted appearance. Neither clinical signs nor gross lesions are pathognomonic although loops of small intestine appear thin-walled with moderate to large amounts of watery contents. A histopathologic report of blunted villi and crypt hyperplasia is suggestive of rotavirus infection. Infection with rotavirus can be confirmed by PCR or electron microscopy (EM). Enzyme-linked immunosorbent assay (ELISA) is also available but limited to detection of serogroup A. IHC and EM detect rotavirus and confirm its role in pathology, but both tests lack

### Table 2 Common gastrointestinal diseases and disorders of pigs

<table>
<thead>
<tr>
<th></th>
<th>Preweaning nursery</th>
<th>Postweaning nursery</th>
<th>Postweaning grow–finish</th>
<th>Mature</th>
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<tr>
<td>Gastric ulcer</td>
<td>++</td>
<td>+</td>
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<tr>
<td><em>Clostridium difficile</em></td>
<td>++</td>
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<tr>
<td><em>Clostridium perfringens</em> Type A (CpA)</td>
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<tr>
<td><em>Clostridium perfringens</em> Type C (CpC)</td>
<td>++</td>
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<tr>
<td>Salmonellosis</td>
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<td>Colibacillosis</td>
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<tr>
<td>Swine Dysentery</td>
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<tr>
<td>Porcine proliferative enteropathy (PPE) (ileitis)</td>
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</tr>
<tr>
<td>Rotarvirus</td>
<td>++</td>
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<tr>
<td>Transmissible gastroenteritis (TGE)</td>
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<tr>
<td>Porcine epidemic diarrhea (PED)</td>
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<tr>
<td>Whipworms</td>
<td>++</td>
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*Note: The first column provides the diseases. The remaining columns represent the respective phases of production. The frequency of the occurrence is + (occasional), ++ (common), and +++ (routine).*

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sensitivity. Treatment is supportive by administration of oral rehydration solutions. Acidifiers and antibiotics are sometimes administered to control secondary bacterial infections. Treatment success is variable and depends on the degree of malnourishment. Prevention among neonatal piglets is through ingestion of lactogenic virus neutralizing antibody from the sow, which is stimulated by administering feedback of Rotavirus positive piglet feces or intestines (Arruda et al., 2011) or a modified-live commercial Type A vaccine no less than 3 weeks before farrowing. A modified-live commercial Type A vaccine is also available for pigs. It does not induce cross-protection for other serogroups and may be cost-prohibitive.

TGE is caused by TGEV, a coronavirus that is heat labile at temperatures above 21°C, prone to dessication and photosensitization (Bay et al., 1952). The epidemic form causes acute disease in all age groups within as little as 18 h of infection. Morbidity and mortality is high, approaching 100%, in an epizootic outbreak. The severity of disease is age dependent but all ages will develop diarrhea (Mooser and Blikslager, 2007). Postweaning infections result in high morbidity but low mortality; the most significant economic losses at this time are caused by reduced average daily gain, market weights, and overall system efficiency. Necropsy reveals that the small intestine and colon are fluid filled, the small intestinal wall is thin almost translucent, and lactic acid is empty. Necrosis and atrophy are observed throughout the length of the villus. The colon and feces are spared. The endemic form occurs when susceptible animals are introduced to the herd or after maternal antibody wanes. Prior exposure to porcine respiratory coronavirus (PRCV) may cause false-positive antibody test results. A TGEV/PRCV differential ELISA is available. In an outbreak, sows are fed tissue of diarrheic pigs to stimulate herd immunity and new introductions of animals are stopped. After the exposure and a subsequent cool-down period of 4–6 months or after clinical signs cease, sentinel pigs can be introduced and monitored for seroconversion (Saif and Sestak, 2006). Absence of seroconversion indicates successful elimination of TGEV. Commercial vaccines are available but should be used with caution and only when elimination is not an option.

PED is caused by PEDV, a coronavirus that causes signs and histopathologic lesions indistinguishable from TGEV. Unlike TGEV, PEDV is more environment-resistant making elimination more difficult. The disease has been described in Europe, Asia, and, as of 2013, the United States. Prevalence of the enzootic form is approximately 50% (Chae et al., 2000). Morbidity approaches 100% and mortality is 80% or more in a naive sow herd resulting in 3–5 weeks of production losses. Clinical signs appear within 12 h; piglets develop a watery, fetid diarrhea leading to dehydration, metabolic acidosis, and death before caretakers are able to humanely euthanize them. Vomiting also occurs. The severity of disease is age dependent but all ages will develop diarrhea. Postweaning infections result in a high morbidity but low mortality; most significant economic losses at this time are caused by reduced average daily gain, market weights, and overall system efficiency. Viral shedding occurs up to 10 days postinfection. Reproductive failure and inefficiency is a sequel of an outbreak (Olanratmanee et al., 2010). TGEV/PEDV differential PCR is available to confirm a presumptive diagnosis of PED. Serum can be submitted for ELISA or immunofluorescent antibody but collected no sooner than 3 weeks after diarrhea was observed. Immunoprophylaxis using egg antibody or hyperimmune serum and supportive care including electrolyte administration have been used for treatment. In an outbreak, sows are fed tissue and feces from diarrheic pigs to stimulate herd immunity (Olanratmanee et al., 2010). Hygiene is the key to reducing environmental contamination. Preventing introduction of virus into a herd with biosecurity alone may not be sufficient because the virus has been found in aerosol up to 10 miles from a positive farm (Goede et al., 2013). Porcine coccidiosis is most often caused by Isospora suis. Farm hygiene, specifically farrowing rooms, and sow infestation influence the persistence of disease; however, age at infection rather than infectious dose has the greatest impact on severity (Worliczek et al., 2009). The prepatent period is approximately 5 days. Morbidity is variable and mortality is low. Pasty diarrhea, unthrift to potbellied appearance of 7–21 day old pigs, and below average wean weight is suspicious for coccidiosis. On necropsy, the small intestine often is thickened and the mucosal surface is necrotic and has an adherent pseudomembrane. Histopathologic examination of the affected portion of the intestine reveals larvae in the lamina propria. Sensitivity of fecal flotation is moderate. There is no effective treatment. Prevention is by oral administration of an anticoccidial (Maes et al., 2007). Heat treatment (flaming) of flooring may reduce environmental contamination. Concrete, rubber coated and plastic flooring in the farrowing crates are difficult to clean and disinfect so removal may be the only option.

Swine dysentery (SD) is a spirochete of the genus Brachyspira that is an oxygen-tolerant anaerobe giving it the ability to survive for long periods of time in manure, pits, and lagoons (Schwartz et al., 2012). Rodents, particularly mice, are known vectors and can serve as reservoirs. Brachyspira hyodysenteriae is the species known for causing SD. Other species of Brachyspira have been recently described in dysentery-like disease (Burrough, 2012). Incubation is 10–14 days but disease occurs in a 3–4 week cycle. Administration of tiamulin or lincomycin in the feed or water may alter the time to onset of signs after exposure. Morbidity is high and mortality is low to moderate characteristically causing disease in only the finisher and mature groups. Economic significance is mostly lost performance due to reduced daily gain and feed conversion. The specific mechanism of pathogenesis is not well understood but the spirochete does not invade the lamina propria. Clinical signs are the presence of mucohemorrhagic diarrhea containing flakes of frank blood or appearing as a generalized brick red to rust color. Lesions are mostly observed in the spiral colon where epithelial sloughing and mucosal invasion cause necrosis resulting in the formation of a pseudomembrane. The colonic walls may be thickened due to vascular congestion and mucosal hyperplasia. Bacterial culture produces strong β-hemolysis. PCR test for confirmation and speciation is recommended for any isolate with characteristic growth. Introduction of infected pigs and contaminated equipment or facilities are the source of infection. Pleuromutilins, like tiamulin, and the lincosamides, lincomycin, are effective for treatment. However, if the environment remains contaminated, clinical signs will recur. Depopulation has resulted in
successful eradication (Harms, 2011). Medicated elimination that combines thorough pulse medication with tiamulin, cleaning and disinfection, and employment of an aggressive rodent control program is also effective (Burrough and Sexton, 2013).

Escherichia coli is a gram-negative rod that infects all ages of swine but must express virulence factors to cause diarrhea. tschermeka coli colonize the small intestine by fimbrina that binds receptors on the villous surface of enterocytes. Enterotoxigenic E. coli (ETEC) then produce toxin(s) that increase osmolality leading to diarrhea (Mooser and Blikslager, 2007). ETEC is subdivided by fimbrina, toxin, and age of pig affected. Neonatal diarrhea (ND) is most common in pigs 0–7 days of age. The onset of postweaning diarrhea (PWD) caused by F18 is delayed, occurring 5–14 days postweaning, compared to that caused by F4 and its severity is indirectly related to wean age. Clinical signs are profuse diarrhea, rapid dehydration leading to emaciation, or death due to metabolic acidosis. Fluid-filled and hyperemic sections of jejunum and ileum may be present at necropsy but few consistent gross lesions occur. Intestinal contents have a distinctly alkaline pH. Isolation of large numbers of E. coli and with dense layers of rod-shaped bacteria covering villi seen on histopathology in samples from pigs with diarrhea is sufficient for diagnosis of E. coli but not ETEC. Genotyping is necessary to determine fimbrina and toxin types, which are essential to confirm diagnosis of ETEC.

Treatment of affected pigs/litters/groups includes administration of antibiotics and oral rehydration solution or electrolytes to correct hyperkalemia (Kiets et al., 2006). Control and prevention of ND is by passively derived lactogenic immunoglobulins from vaccinated females (Kohler, 1974). Prevention of PWD include selection of genetically resistant breeds lacking K88 and F18 receptors, administration of an oral avirulent live culture to stimulate active immunity or competitively exclude field strains (Genovese et al., 2000), feeding 2500 ppm zinc oxide postweaning and probiotics. Immunity and exclusion is unique to each farm; vaccines should include the prevalent genotype(s) causing the diarrhea.

Clostridium perfringens Type A (CpA) is a gram-positive bacillus and inefficient sporulator. Sows are regarded as the source of neonatal infection. Frequency of CpA diarrhea is on the rise in the USA. In uncomplicated cases, mortality is low whereas morbidity is high and below average weaning weights result. Cases of CpA diarrhea are associated with the expression of α and β2 toxin. CpA is cultured from the stomach and upper third of small intestine but does not bind intestinal epithelium causing few to no histologic lesions. Because of its ubiquitous nature and prevalence among healthy pigs, CpA may be an opportunist and its role as a primary cause of neonatal enteritis is not definitive. Large numbers (3 or 4 +) of gram-positive bacilli cultured from feces or intestinal contents of diarrheic pigs is suggestive of CpA. Genotyping by PCR to confirm presence of cpb2 gene in CpA isolates and rule out other causes of ND are supportive to the diagnosis (Bueschel et al., 2003). Treatment has variable success rates and is limited to administration of empirically selected antibiotics and oral rehydration solutions to affected pigs.

Control of CpA enteritis is best accomplished by preventing other causes of ND. Following a thorough cleaning, sporidical disinfectant should be applied to farrowing crates and equipment between litters and be allowed to dry before reloading. Feeding of bacitracin to sows has resulted in significant increases in weaning weights (Schults, 2007). A commercial CpA toxoid vaccine is available (Hammer et al., 2008). Autogenous whole cell vaccines are also in use. If vaccine is unavailable, feedback might be considered but should be pursued with caution (Robbins and Byers, 2013a).

CpC is a gram-positive bacillus and inefficient sporulator. Sows are regarded as the source of infection. Pathogenesis of type C is due to expression of β toxin leading to necrosis of intestinal epithelium resulting in hemorrhagic diarrhea or acute death of piglets less than 3 days of age. Gross necropsy reveals hemorrhagic and blood-filled loops of small intestine. A pseudomembrane may form on the luminal surface, and intestinal mucosa is edematous. Gross and histopathologic lesions in the presence of large numbers (3 + or 4 +) of gram-positive bacilli cultured from feces or intestinal contents warrant a presumptive diagnosis. Genotyping by PCR to confirm presence of the cpb gene is confirmatory (Senger and Uzal, 2005). Treatment of affected pigs is unrewarding due to the rapid and debilitating course of this disease. Prevention is accomplished by vaccination of gestating females with a commercial toxoid and ensuring piglets consume sufficient colostral antibodies to result in protection.

Clostridium difficile is a gram-positive bacillus that easily sporulates making it especially persistent as a pathogen. Infections of Clostridium difficile associated diarrhea leads to a 10–15% reduction in wean weights (Senger and Uzal, 2005). Although more than a third of piglet diarrhea involves C. difficile, it is the better known to cause healthcare-associated infections among humans. The pathogenesis of C. difficile infections is in response to the expression of toxins A and B. A watery diarrhea occurs in 1–7 days old piglets. Mesocolonic edema may be observed at necropsy. Clostridium difficile is difficult to culture and can be isolated from healthy piglets. Therefore, volcano lesions on histologic exam and confirmation of toxins in fecal contents by antigen ELISA are diagnostic. Treatment is ill-defined but is likely similar to that for CpA enteritis, because it is likely to be initiated based on clinical signs, which are similar. Autogenous vaccines are used to aid in prevention but efficacy is unclear.

PPE, commonly referred to as ileitis, is the general categorization of infections caused by Lautsonia intracellularis, an obligate intracellular bacterium. Because the bacteria cause lesions in the ileum, PPE is also referred to as ileitis. Sero-prevalence in grow-finish herds can reach 100%. PPE can further be divided into four clinical forms (Kroll et al., 2005). Porcine intestinal adenomatosis (PIA) is most common in 6–20 week pigs and causes little mortality. Porcine hemorrhagic enteritis (PHE) affecting pigs 28 weeks of age and older including breeding swine and can be associated with increased mortality and dark, bloody stools. Necrotic enteritis (NE) and subclinical ileitis, the most common form, occur among postweaning pigs. In all forms, transmission is by the fecal-oral route. Crypt enterocytes infected with L. intracellularis become hyperplastic. The altered ratio of villous and crypt enterocytes leads to malabsorption and subsequent increases in feed conversion and time to reach market weights. PIA results in variable degrees of thickened ileum that can be found at necropsy. The ileal lumen may contain a blood clot in PHE.
or pseudomembrane in NE. When diarrhea ranging in color from normal (PIA, NE, and subclinical) to dark-red or black (PHE) is observed, PPE should be considered as a possible cause. Subclinical ileitis usually causes no clinical signs (Gebhart, 2007). Histopathologic lesions containing intracellular S-shaped organisms are suggestive of Lawsonia infection but IHC should be used to confirm diagnosis. PCR is helpful to detect infection and is highly specific but moderately sensitive. Cross-sectional or longitudinal serologic profiling using a widely available ELISA is the best tool for determining timing of exposure. Treatment is with effective antibiotics, such as tylosin, administered by injection or in the feed or water. Control is by administration of a commercially available modified-live oral vaccine before infection or feeding antibiotics when infection is known to occur. Vaccination should take place at least 8 weeks before seroconversion (Walter et al., 2004).

Salmonellosis causing gastrointestinal disease in swine is most commonly associated with the species Typhimurium. Salmonella Typhimurium is commonly isolated from swine. Isolation of multidrug resistance strains of S. Typhimurium from swine at slaughter have garnered attention from public health and food safety professionals and it is this that make this infection significant to the pork industry (Foley et al., 2008). Some European Union member states have implemented meat-juice serologic monitoring at slaughter to assess on-farm Salmonella control programs. Pathogenesis of S. Typhimurium is similar to Salmonella choleraruis by invading enterocytes and subsequently macrophages leading to an infectious carrier state. Initial infection causes inflammation and cytokine release that result in watery, yellow diarrhea containing feed particles. Button ulcers may be visible on the mucosal surfaces of the colon and cecum on gross necropsy examination and, on histopathology, can be found to extend into the lamina propria. Bacterial isolation without using enrichment media and the presence of histopathologic lesions is consistent with a diagnosis of Salmonella enteritis. Treatment is with antibiotics administered symptomatically to diarrheic pigs. Antibiotic susceptibility of the isolate should be considered before initiating treatment. Rearing pigs on slatted floors, decreasing stocking density, and acidification of digesta are effective in reducing the prevalence of Salmonella infections in swine (Funk and Gebrayes, 2004; Boyen et al., 2008). Cross-protection with S. choleraesuis vaccine has been reported and reduces carcass colonization (Hasa et al., 2009).

Whipworm infestations of swine are the result of Trichuris suis infection. Pigs kept on pasture, in outdoor lots, or facilities with a history of T. suis diagnosis are at greatest risk for disease (Pittman et al., 2010a). The prepatent period is 6–7 weeks. The egg is not immediately infective, which requires 3–4 weeks in the environment. The infectious larva hatches from the egg and invades enterocytes in the small intestine and cecum. The entire life cycle of T. suis is completed in the intestine. Ulcerations in the mucosa and damage to capillary blood supply of intestinal epithelium lead to hemorrhage, anemia, and hypoalbuminemia. Clinical signs are depressed weight gain, increased feed conversion, bloody diarrhea, ill thrift, and death. Adult worms imbedded in the ileum, cecum, or proximal colon are sufficient for diagnosis of whipworms. Eggs are intermittently shed and thus not a reliable method of diagnosis (Pittman et al., 2010a). Treatment and control are synonymous and require administration of an effective anthelmintic like fenbendazole. Prevention is by steam sanitation and drying; however, eggs are resistant to common disinfectants and remain infective for years.

### Integument System Diseases and Disorders

The porcine integument or skin, like that of other domestic species, serves as a protective barrier between fragile internal tissues and harsh external hazards. Skin is comprised of layers (from external to internal): epidermis, dermis (superficial and deep), and subcutis. Blood vessels, hair follicles, sebaceous glands, and muscles are found in the dermis. Notably, the pig's skin does not contain sweat glands; therefore, modern swine facilities are outfitted with evaporative cooling systems for thermal regulation in hot climates. Skin diseases and disorders can be the result of viral or bacterial infections, parasite infestations, immunologic reactions, and idiopathic or iatrogenic causes that are summarized in Table 3 by their various macroscopic and microscopic lesions.

Greasy pig is a skin disease of swine caused by a toxin produced by Staphylococcus hyicus. A break in the skin is the typical sequela. Gilt litters reportedly have a higher incidence of this disease, presumably due to deficient maternal immunity. All ages of pigs may be affected but suckling and nursery pigs are most likely to develop disease. Affected pigs develop focal crusts on the face, neck, and axillary region, and the crusts may coalesce as the disease progresses. Affected areas are greasy to touch and may appear black due to dirt adhering to it. If pigs are untreated or fail to respond to treatment, the trunk and extremities may become involved. Pyrexia and lethargy can be observed in severe cases and are followed by growth reduction. Gross appearance of affected skin is rarely confused with other skin conditions of swine. Submission of formalin-fixed skin sections that include the junction of affected and unaffected layers of epidermis and dermis for histopathologic examination is needed for a diagnosis. The pathognomonic histologic lesion is exudative epidermitis.

| Table 3 Common integument diseases and disorders of pigs |
|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|
|                | Crust | Papule | Plaque | Pustule | Necrosis | Erythema | Scar | Scale | Vesicle |
| Greasy pig     | X     | X      | X      |         |          |          | X   |       |        |
| Erysipelas     | X     |        |        | X      |          |          | X   |       |        |
| Porcine dermatopathy and nephropathy syndrome (PDNS) | X | X | | X | | | |
| Sarcoptic mange | X | X | | | | | |

Note. The first column provides the diseases. The remaining columns represent the type of lesion that occurs.
The *S. hyicus* can be cultured from the surface of clinically normal skin sections. Treatment includes topical application of antimicrobials or disinfectants. Unaffected pigs with direct contact with affected pigs should also be treated to control spread. In cases where pigs exhibit systemic signs, administration of an injectable antimicrobial and anti-inflammatory is warranted. In the United States, no antimicrobials are labeled for the treatment, control or prevention of *S. hyicus* so all antimicrobial therapy is extra-label. Prevention should focus on facility hygiene and include a soap degreaser and disinfectant regime to reduce contamination. In addition, scarification of the skin of breeding age females with the farm-specific *S. hyicus* strain can reduce disease incidence in suckling pigs (Murray and Rademacher, 2008).

Erysipelas or diamond skin disease is caused by a soil-borne gram-positive bacterium, *Erysipelothrix rhusiopathiae*. This zoonotic pathogen is transmitted by migratory fowl, turkeys, and pigs. Humans may become sickened when direct contact with blood from affected animals contaminates an open wound (Brooke and Riley, 1999). The finding of lesions at slaughter results in partial or complete carcass condemnation (Bender et al., 2011). The disease is most common in growing, finishing, and breeding age swine. Bacterial emboli lodge in blood vessels causing vasculitis, thrombosis, and ischemia leading to lameness, abortions in gestating females, and raised, red to purple rhomboid skin lesions for which ersetipelas is best known. Skin biopsies from the affected area should include epidermis and dermis, but histologic lesions are only supportive. Bacteriologic isolation or PCR identifying *E. rhusiopathiae* confirms the diagnosis. Treatment with β-lactam antibiotics including penicillin is effective. Commercial bacteria and avirulent live cultures are available for prevention (Wood, 1984) or in the face of outbreaks to prevent the chronic form.

PDNS is associated with Porcine circovirus type 2 (PCV2) infection, but any disease process resulting in ischemia could cause result in PDNS. The condition is characterized by red to purple discoloration of skin that begins on the caudal surface of the hind limbs and the ventral surface of the abdomen resulting from ischemia. On necropsy, gross examination of the kidney cortex may be speckled with pinpoint, white foci caused by infarcted blood vessels. Pig of any age can be affected with PDNS, but it is more commonly observed during growing and finishing stages. Submission of fresh and formalin-fixed skin sections that include the junction of affected and unaffected layers of epidermis and dermis is required. There is no specific treatment or prevention; rather, diagnose the underlying cause to determine appropriate therapy (Figure 1).

Sarcoptic mange is the result of an allergic reaction to the saliva of ectoparasites, *Sarcoptes scabiei*. Mange may also be caused by *Demodex phylloides*. Mortality is low and morbidity is moderate. Economic losses are the result of reproductive inefficiency, growth reduction, and carcass condemnation. Infestation and subsequent clinical signs in the breeding herd, most notably a incessant scratching, develop following the purchase of infested genetic replacements. In addition, growing pigs placed in facilities that previously housed infected swine or facilities that reuse straw bedding or have solid wood partitions may also become infested. The mite is rare in modern, high-health swine operations. The burrowing mite causes red pustules and flaking skin. Individual pigs may develop signs in as few as 3 weeks but a herd may not show signs for several months. In the chronic stage, thick crusts develop at the corners of and inside the ears. Examination of a scraping from the crusts will reveal the mite (Averbeck and Stromberg, 1995). An ELISA test is used to determine prior exposure and determine the success of eradication programs. Treatment can be applied topically using an antiparasitic, such as amitraz, to temporarily alleviate clinical signs. Control and eradication programs utilize feeding or injection of ivermectins (Mohr, 2001).

**Musculoskeletal System Diseases and Disorders**

The musculoskeletal system is comprised of tendons, ligaments, muscles, and bones. Disorders and disease of this system are typically characterized by lameness. Lameness is any deviation in normal locomotion including favoring a limb or failure to bear weight on the limb. Neurologic conditions, which also cause changes in locomotion, may be ruled out by postmortem examination of articular surfaces and diagnostic testing. Investigation of musculoskeletal diseases and disorders should always start with the claws that are easily traumatized causing pain resulting in lameness. Flooring and genetics also influence the incidence of lameness. Common musculoskeletal diseases and disorders of swine can be divided into osteopathies and myopathies and summarized in Table 4.

*Mycoplasma hyorhinovae* colonizes upper airways and tonsils resulting in a carrier state. Transmission is vertical from sow to pigs and lateral between pigs (Ross and Spear, 1973). *M. hyorhinovae* is most often diagnosed during the grow–finish phase. Morbidity is variable but mortality is low. Clinical signs are a stiff gait and difficulty in standing, most often the stifle or elbow and less frequently the hock, hip, and shoulder. Signs often occur 2–3 weeks after a stressful event; lesions begin to resolve 7 weeks postinfection. The affected joint contains yellow or blood-tinged effusion with moderate
villous proliferation but is not always observed despite lameness and does not necessarily correlate with presence of histopathologic lesions. Aseptic collection of synovial fluid by needle aspiration or sterile swab or submission of the affected joint intact is recommended for diagnosis. PCR is the most sensitive test; culture requires special media and lacks sensitivity (Gomes Neto et al., 2012). Histopathologic examination of formalin-fixed synovium reveals nonsuppurative fibrous polynarthritides and lymphoplasmacytic perivascular synovitis. ELISA is also available. Lincomycinic has historically been an effective therapeutic choice (Burch and Godwin, 1984). Treatment should be initiated when lameness is first observed; however, spontaneous resolution is common. No commercial vaccines are available.

Mycoplasma hyorhinis is a ubiquitous bacterium that is an early colonizer of upper airways. Transmission is vertical from sow to pigs and then between pigs postweaning (Rovira, 2009). Infection can progress to polyarthritides, polyeserosis, and otitis in the pre- or postweaning phases; arthritis develops postweaning. Clinical signs include lameness, arthritis, and fever that develop 3–10 days after septicaemia occurs and persists for 10–14 days (Gomes Neto et al., 2012). Disease may become chronic resulting in ill thrift, reduced growth, and death. Articular surfaces may be eroded. In cases of lameness, synovial fluid and formalin-fixed synovium can be submitted. Alternatively, the entire affected leg can be submitted; disarticulate above the infected joint keeping the affected joint intact. Submission of fibrin or fibrin covered tissue(s) should be included for PCR testing to differentiate M. hyorhinis from other bacteria that form fibrin on serosal surfaces like HPS and S. suis (Rovira, 2009). Histopathology reveals nonsuppurative inflammation in affected tissues. Treatment is empirical.

Erysipelis is the result of a chronic E. rhusiopathiae infection causing arthritis and endocarditis that follows the initial septicaemia. Lameness and joint swelling is mostly noticeable in hock and carpal joints. Lameness may also occur in stifle and elbow but swelling cannot be appreciated. Synovial fluid appears serosanguinous and can be submitted for testing by bacterial culture or PCR. Alternatively, the entire affected leg can be removed to prevent contamination; disarticulate the leg above the infected joint. Histopathologic examination of formalin-fixed synovium reveals a proliferative synovitis. Other lesions that occur are nonsuppurative fibrous polyarthritides and erosion of cartilage that can progress to pannus and ankylosis. Treatment with β-lactamase antibiotics including penicillin is effective. An anti-inflammatory is added to a treatment program for pain management. Commercial bacterins or avirulent live cultures are available for control and prevention.

OCP is the result of a delay in ossification of articular cartilage, and represents the most common lesion among culled sows. Morbidity is most often reported in adult and breeding age pigs (Devey et al., 1993). Mortality is variable and is the result of humane euthanasia because the animal becomes nonambulatory. OC causes lameness, pain, and joint swelling. A noninfectious lameness most often affects the distal part of the humerus or femur. Lesions are typically bilateral and symmetrical. Diagnosis is made by ruling out other causes of lameness.

Rickets occurs as a result of phosphorus deficiency, vitamin D deficiency, or secondary to iron toxicity but is not caused by dietary calcium deficiency. The condition should be suspected when there is an increase in nonambulatory pigs and broken bones during the finishing stage particularly at and immediately before marketing. Occasionally, joint swelling in the nursery stage is observed. Rachitic rosy (enlargement of costochondral junctions) and soft bones are observed on necropsy. If rickets are present, a bone ash analysis of the second rib will be below normal. Feed analysis can identify low levels of vitamin D or phosphorus. Low levels of vitamin D or phosphorus serum chemistry also will occur (Madson et al., 2012). Supplementation of vitamin D is the only reported treatment and response that may be considered diagnostic. Prevention includes proper diet formulation for the stage of production.

MHD is a noninfectious disease of muscle caused by deficiency of vitamin E or selenium. It can occur if pigs are fed grain grown in selenium deficient soils (Devey, 2006). Clinical signs are limited to acute death of large, robust pigs. On necropsy, the heart muscle has a mottled appearance. Feed analysis, response to vitamin E supplementation, and ruling out other causes support diagnosis of vitamin E/selenium deficiencies like MHD (Hooser, 1996). Supplementation with selenium is impractical in the United States because of environmental regulations, and overzealous supplementation may cause toxicosis.

Splayleg is a noninfectious, congenital condition resulting from delayed myofibril development with no known cause. Splayleg has low morbidity and mortality as long as it is identified and corrected before it leads to starvation or being crushed by the sow. Treatment includes the use of nonslip
flooring in farrowing crates and application of harness or tape that holds the rear legs under the pig until it is strong enough to walk on its own.

Reproductive System Diseases and Disorders

Reproductive failure occurs when insemination fails to result in pregnancy or pregnancy fails to produce viable pigs due to infectious and noninfectious causes summarized in Table 5. Reproductive failure should be considered when a low conception or farrowing rate, irregular returns to estrus, abortions, stillbirths, or mummies persist at an abnormal rate. Infertility occurs when fewer than four embryos are present at the time of maternal recognition of pregnancy resulting in a regular return to estrus and reduced conception rate for that breeding group. Irregular returns to estrus result from embryonic death or early term abortion after implantation but before calcification of the fetuses. Embryonic death of some or all of the embryos will result in low total born or irregular return to estrus, respectively. Early-term abortion also will reduce farrowing rates. Mummies and stillborns can occur any time after calcification of the fetuses. The normal rates for mummies and stillborns are <0.5 and <1 pig per litter, respectively. Late-term abortions are classified as those occurring after 70 days of gestation. Total abortion rate should remain <2% of a breeding group. These are general guidelines; thus, familiarity with the herd’s normal reproductive performance is the most sensitive means to identify a reproductive problem.

PRRS is, at this time, known only to occur among swine. The estimated cost of PRRS to the US pork industry is US$664 million annually (Holtkamp et al., 2013). PRRS usually results when susceptible swine are infected with either the Leylstad or North American strains of PRRS virus (PRRSv), a member of the Arteriviridae family. Viremia lasts up to 42 days, but shedding of infectious virus can last much longer (Murtaugh and Genzow, 2011). PRRSV is most commonly transmitted by introduction of infected swine or contaminated fomites, use of contaminated semen, and aerosol. The pathogenesis of the reproductive form is believed to be arthritis of fetal umbilical cords during gestation (Lager and Halbur, 1996). Swine may show no signs when reinfected with a homologous strain. Conversely, infection with a heterologous strain will reproduce lesions and disease but is usually less severe than that of naïve swine (Murtaugh and Genzow, 2011). Clinical signs of PRRS in a breeding herd start with an epidemic of abortions followed by an increase in low viable piglets, stillbirths, and mummies. Abortions result due to fetal death or pyrexia of the gestating female. Sows and gilts may be anorectic, pyretic, or lethargic. Periparturient females may become agalactic. In severe outbreaks of PRRS, sow mortality also increases. In utero infection of feti can result in persistently infected piglets (Rossow, 1995). Perinatal mortality commonly increases and may remain above the herd average for weeks. Diagnosis can be made by submitting lung, spleen, and lymph node from fetuses or low viable piglets. Whole fetuses can also be submitted but should be refrigerated to prevent autolysis. Lesions are not pathognomonic so confirmatory testing such as PCR, IHC, or VI should be conducted. Tissues and thoracic fluid from stillbirths, aborted, or mumified feti can be submitted but may result in false negatives. Serum collected from aborted sows or low viable piglets and tested for PRRSV by PCR is another option for diagnosis. PRRSV ELISA indicates previous exposure but is not useful in a previously exposed herd. Treatment of PRRS is supportive. Anti-inflammatories to reduce fever and antibiotics for control and treatment of secondary bacterial pneumonia may be necessary. The most common methods for control include depopulation-repopulation and herd closure and rollover, also called load-close-homogenise, using commercial vaccine or herd-specific live virus exposure (Corzo et al., 2010). Periods of closure vary based on facility capacity but a minimum of 180 days is recommended. Commercial modified-live and killed vaccines are available but do not prevent infection and should be used in accordance with label and domestic guidelines.

PPV is sometimes described by the acronym SMED (stillborns, mummies, embryonic death, infantility). PPV is an enzootic infection of swine breeding herds in the United States. The virus is ubiquitous and is transmitted through ingestion of infected feces, afterbirth, or fetal tissue. The disease most commonly affects gilts and younger parity sows (Christianson, 1992). The pathogenesis is through damage to

| Table 5 | Common reproductive diseases and disorders of pigs |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
|                | Embryonic death | Abortion        | Mummies         | Stillbirths     | Infertility     | Weak born pigs  | Low total born  |
| PRRS            | ++              | ++              | +++             | +++             | ++              | +++             | ++             |
| Porcine parovirus (PPV) | ++              | ++              | +++             | +++             | ++              | +++             | ++             |
| Porcine circovirus type 2 (PCV2) | ++              | ++              | +++             | +++             | ++              | +++             | ++             |
| Leptospiruria   | ++              | ++              | +++             | +++             | ++              | +++             | ++             |
|                  | (Pomona)        | (Bralislava)    |                 |                 |                 |                 |                |
| PRV             | ++              | ++              | +++             | +++             | +++             | +++             | ++             |
| Brucellosis     | ++              | ++              | +++             | +++             | +++             | +++             | ++             |
| Carbon monoxide (CO) poisoning | ++              | ++              | +++             | +++             | +++             | +++             | ++             |
| Zearalenone     | ++              | ++              | +++             | +++             | +++             | +++             | ++             |
| Swine erysipelas| ++              | ++              | +++             | +++             | +++             | +++             | ++             |
| Autumn abortion syndrome (AAS) and seasonal infertility | ++              | ++              | +++             | +++             | +++             | +++             | ++             |

Note: The first column provides the diseases. The remaining columns represent the clinical signs. The frequency of the occurrence is: + (occasional), ++ (common), and +++ (routine).
the placental epithelium resulting in fetal death. Clinical signs of PPV range from low total born, mummies of various sizes, irregular returns to estrus, and females diagnosed pregnant but fail to farrow. Diagnosis is based on vaccination history, clinical signs, and PCR testing of mummified fetuses. PPV ELISA may provide diagnostic value if acute and convalescent serum samples are used. There is no effective treatment for PPV; however, commercial killed vaccines are available and very effective. Exposure of unbred females to tissue or cull sows from a seropositive herd has been used for immunization when vaccine is unavailable.

Leptospirosis is caused by infection by spirochete bacteria. *Leptospira* species may be zoonotic (*Leptospira canicola, L. icterohaemorrhagiae*), swine-adapted (*L. pomona* and *L. bratislava*), or incidentally infect swine (*L. grippotyphosa* and *L. hardyi*). Infection has been associated with exposure of swine to contaminated soil or untreated surface water, and exposure to urine from infected vectors, such as rodents. Infected swine can become carriers resulting in chronic disease. The pathogenesis is due to bacteremia resulting in transplacental infection followed by fetal death. Clinical signs include pyrexia, low conception rate, abortion, stillbirths, and low viability pigs resulting in increased prewean mortality. Diagnosis is made using dark field microscopy or IHC performed on tissues, particularly kidney, of aborted fets or stillbirths. Paired or matched serology for hemagglutination inhibition (HI) testing may be useful if suspected. Treatment with antibiotics, such as chlorotetracycline, may be pursued (*Henry et al., 1993*). Commercial killed bacterins are available to aid in prevention and should be given at least semiannually to breeding stock (*Christianson, 1992*) but may not be available in all countries. For example, federal regulations prohibit the use of these bacterins in France and The Netherlands (*Figure 2*).

PCV2 is a ubiquitous virus in swine facilities. Pigs become infected with PCV2 through ingestion (oral nasal contact). In addition, breeding females can become infected via insemination with contaminated semen (*Madson et al., 2009a*). Gilts and low parity sows are affected most often, whereas boars show no clinical signs. PCV2-associated reproductive failure may occur in conjunction with PPV. Infection results in variable lengths of viremia. PCV2-reproductive failure is due to transplacental infection of fetuses. Clinical signs depend on the stage of gestation when the infection occurs. Embryonic death, early term abortions, stillbirths, mummies, low total born, or low viable pigs can result from infection. Mummies may vary in size, like PPV, and measuring crown to rump length is useful to determine the time when that fetus was infected. PCV2-reproductive failure is diagnosed by the presence of viral antigen confirmed by IHC or deoxynucleic acid confirmed by PCR along with the presence of lesions in fetal tissue notably myocardial mineralization. PCR testing of fetal thoracic fluid is sufficient to diagnose in utero infection of piglets (*Madson and Opiensz, 2011*). Commercial killed baculovirus vectored vaccines are available and effective for prevention of disease but not infection or viremia (*Madson et al., 2009b*).

PRV or Aujeszky’s disease virus was eradicated from the US commercial swine herd in 2004; a comprehensive review is available (*USDA APHIS, 2008*). PRV is a member of the Herpesviridae family and, like other herpesviruses, infection can result in a carrier state or latency within nervous tissue with the potential for recrudescence. The pathogenesis of PRV results from viremia, and then replication and necrosis of epithelial tissue including the placenta (*Christianson, 1992*). The period of viremia gives PRV time to cross the placenta and cause fetal death. Clinical signs following acute infection include embryonic death, abortion, mummies, and stillborns. Necrotic foci can be found in fetal spleen, liver, lung, and lymph node. Histopathology is not definitive; IHC is required to confirm presence of antigen. Diagnosis may also be made through serology; a commercial ELISA test is available and can differentiate between exposure to the gene-deleted vaccine and wild-type virus used extensively in the US eradication. Commercial PRV vaccines are available but only should be used in accordance with federal guidelines.

Brucellosis is a zoonotic infection caused by the bacteria, *Brucella suis* biovars 1 and 3. *Brucella suis* is transmitted through direct contact with susceptible swine, ingestion of infected tissue, or fluids including milk and contaminated semen. Pathogenesis of *B. suis* is initiated when the mucosal epithelium is penetrated, thereby resulting in bacteremia that commonly persists for 5 weeks and results in placentalis among other lesions. Clinical signs of infection in gilts and sows include abortion with or without vaginal discharge, whereas boars have reduced libido and fertility. Bacteriologic isolation of *B. suis* from vaginal discharge or tissue confirms diagnosis. Serology reflects prior exposure (or vaccination) to *B. suis* but not for diagnosis of acute disease. The US commercial swine herd is Brucellosis free.

Swine erysipelas (SE) is a zoonotic, gram-positive bacterium, which is ubiquitous among swine. *Erysipelothrix rhusiopathiae* is the sole causative species. Carrier swine shed the bacteria in saliva, nasal discharge, and feces. Infection may result from direct contact with carriers, exposure to infected facilities or soil (*Wood, 1984*). A bacteremia lasting several days precedes lesions. Reproductive failure is most often due to abortion but infertility and low total born following high fevers or endometritis at the time of breeding is also possible.

![Figure 2](https://example.com/figure2.jpg)

**Figure 2** Stillbirths, fetus, pig. Fully developed piglet born dead. On necropsy, lungs are deflated and fail to float in water. Presumably caused by Leptospirosis sp. Infection; breeding herd was unvaccinated. Courtesy Dr. Rebecca Robbins.
Clinical signs including rhomboid skin lesions, high fevers, lethargy, inappetence, withdrawal, and response to treatment with penicillin of affected sows and gilts are suggestive of acute and subacute SE. Serology is available; availability is by veterinary diagnostic laboratory (VDL) and value is limited when vaccine is in use. Bacterial culture and histopathologic examination of fetal tissue is unrewarding for diagnosis of SE but is helpful to rule out other causes of abortion. In chronic SE, culture of *E. rhusiopathiae* from vulvar discharges was successful (Gertenbach and Bilkei, 2002). Treatment involves injections of antibiotics and anti-inflammatories. Commercial vaccines are available and effective.

CO poisoning induces hypoxia resulting in an increased number of stillborns (Hooser, 1996). Concentrations of >250 ppm are toxic. Malfunctioning heating units or poorly ventilated farrowing rooms are the cause. Diagnosis is done by ruling out infectious causes of stillbirths. Fetal blood or thoracic fluid can also be measured for CO concentrations.

Zearalenone is a lutetropic mycotoxin produced by *Fusarium roseum*. It binds estrogen receptors resulting in irregular returns to estrus, signs of estrus in prepubertal gilts, and reduced litter size (Hooser, 1996). Diagnosis is by detection of elevated levels in feed samples. However, definitive diagnosis is rarely possible because the contaminated feed has long been consumed by the time reproductive failure occurs.

AAS and seasonal infertility is a noninfectious cause of reproductive failure. The declining photoperiod and temperature fluctuations during the fall months result in declining progesterone levels. High-ambient temperature experienced during lactation and the postweaning period are suspected but not confirmed as a cause. Diagnosis is done by ruling out infectious causes and careful assessment of management, facilities, and reproduction records (Ruefl, 2006). Modern facilities that utilize gestation crates and evaporative cooling systems may improve but not prevent infertility during the fall months (Leman, 1992).

**Respiratory System Diseases and Disorders**

The respiratory system can be simply divided into upper and lower portions. The upper portion includes the nasal cavity and sinuses, throat, trachea, and bronchi for air conduction. The lower portion is the lung comprised of bronchioles and alveoli responsible for air exchange. The respiratory system is commonly involved in numerous infectious diseases of swine summarized in Table 6. The most notable infectious agents are the viral pathogens, PRRS and PCV2, which cause primary pathologic lesions to both the respiratory and the immune system. This damage to the immune system often leads to respiratory or systemic disease incited by secondary infectious agents. Such mixed respiratory infections can occur at any age, and when they occur in growing and finishing pigs, are termed porcine respiratory disease complex (PRDC). Multifactorial respiratory disease can obscure histopathologic lesions complicating the diagnostic process.

APP is a host-adapted, fastidious, and gram-negative encapsulated rod that is transmitted vertically from sow to piglet. Morbidity and mortality are strain-specific; virulence varies with expression of *Apx* and *Aap* toxins. Inhalation of strains of APP expressing *Apx* toxins results in lung lesions within 24–36 h. The disease is economically significant because mortality occurs during the latter part of the finishing phase, usually just before slaughter. Clinical signs of fever, lethargy, dyspnea, and acute death are common. Pigs found dead may have a frothy, blood-tinged discharge from the nose and mouth. Focal hemorrhage occurs in the diaphragmatic lung lobe, which is firm, and its appearance is likened to that of a bull’s eye. Fibrinous, necrotizing bronchopneumonia containing streaming leukocytes is a key histopathologic feature. Bacterial culture is difficult and requires nicotinamide adenine dinucleotide (NAD)-supplemented media so diagnosis is traditionally made on finding characteristic postmortem and histopathologic lesions. A PCR test is also available. In an outbreak, the entire population should receive antimicrobial therapy parenteral. Unlike many other gram-negative bacteria, APP is sensitive to a variety of antimicrobials: at Iowa State University VDL > 90% of isolates were sensitive to ceftiofur, enrofloxacin, florfenicol, tiamulin, tilimicin, and tulathromycin. Prevention is aimed at eliminating carrier swine through depopulation or by pulse medication (Marsteller and Fenwick, 1999).

*Actinobacillus suis* causes a hemorrhagic, necrotizing pneumonia during nursing and grow-finish phases. *Actinobacillus suis* infection has similar clinical signs and pathologic appearance to APP. Affected pigs are frequently observed in a dog-sitting position with elbows abducted. Unlike APP, lung lesions are random in their distribution and petechial

**Table 6** Common respiratory diseases and disorders of pigs

<table>
<thead>
<tr>
<th>Disease</th>
<th>Preweaning</th>
<th>Postweaning nursery</th>
<th>Postweaning grow–finish</th>
<th>Adult</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Actinobacillus pleuropneumoniae</em> (APP)</td>
<td>+</td>
<td>+++</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>Actinobacillus suis</em></td>
<td>+</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Atrophic rhinitis (AR)</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Ascaris suum</em></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>HPS</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Mycoplasma hyopneumoniae (MH)</td>
<td>+</td>
<td></td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>Porcine circovirus associated disease (PCVAD)</td>
<td>+</td>
<td>+++ (PMWS)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PRRS (respiratory form)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>Salmonella cholerasuis</em></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Swine influenza (IV)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

*Note:* The first column provides the diseases. The remaining columns represent the respective phases of production. The frequency of the occurrence is + (occasional), ++ (common), and ++++ (routine).
hemorrhages may be seen in other organs due to the septicemia that follows A. suis infection (Yao, 1993). A PCR test is available to help differentiate disease from APP and HPS (Oliveira, 2007a). Like APP, outbreaks should be treated by parental delivery of an antimicrobial; however, treatment of only those individual pigs with clinical signs is usually sufficient. Autogenous vaccines can be used for control but response is variable because most pigs are already seropositive at the time of vaccination.

*Ascaris suum*, the swine roundworm, is the most common parasitic infection of swine. The reduced growth performance and liver condemnations are responsible for economic losses (Stewart and Hoyt, 2006). The prepatent period is 40–53 days. Adult roundworms are present in the manure but it is the migration of larvae through the lungs, occurring 8–10 days after ingestion of an infective egg that causes respiratory signs. A persistent cough and dyspnea result due to verminous pneumonia. The liver develops whitish spots, called ‘milky spots’ that are the cause of condemnations but resolve within 25 days (Stewart and Hoyt, 2006). The presence of eosinophils is suggestive of a parasitic infection. Treatment and control is accomplished using anthelmintics: dichlorvos, fenbendazole, levamisole eliminate adults and larvae; pyrantel kills only adults. Proper cleaning and disinfection particularly removing fecal material between groups reduces potential for exposure but it is virtually impossible to get rid of *A. suum* once a premise is infested (Pittman et al., 2010b). It is necessary to prevent access to contaminated soil.

AR is described in two forms: progressive (PAR) and non progressive (NPAR). PAR is caused by toxigenic strains of *Pasteurella multocida*, whereas NPAR is the result of toxigenic strains of * Bordetella bronchiseptica*. In both forms, the bacteria attach to cilia in the nasal passages and the cytotoxic production causes hypoplasia of nasal turbinates. Clinical signs include sneezing, deviated snouts, and, in cases of PAR, bloody nasal discharge occurring in a large number of grow-finish pigs. Mortality is low but the reduced growth that results due to AR makes it economically important. Because the cytotoxins are responsible for AR, isolation of either bacterium from nasal passages is not sufficient for diagnosis. In addition, *B. bronchiseptica* and *P. multocida* colonize the lung leading to bronchopneumonia causing cough and dyspnea in pigs post-weaning, often part of PRDC (Hansen et al., 2010). Therefore, examination of nasal turbinates at slaughter is the recommended method for diagnosis of AR (Gatlin et al., 1996). Transmission is vertical; therefore, prefarrow vaccination of sows can protect piglets up to 16 weeks of age. If vaccination does not prevent AR, depopulation of the herd may be necessary.

HPS is also called Clässen’s disease. There are 15 serovars identified and prefer to colonize the nose (MacInnes et al., 2007). HPS may not actually result in pneumonia but does cause signs of respiratory disease including nasal discharge and dyspnea. In addition, fever, lethargy, and acute death are observed. On necropsy, one or all of the pleural, pericardial, epicardial, and peritoneal serosal surfaces become covered in fibrin. Effusion commonly occurs. Histopathologic lesions are described as fibrinopurulent. Definitive diagnosis is by bacteriologic culture on isolation media supplemented with V factor. Owing to the difficulty in isolating HPS, PCR testing is now available (Oliveira et al., 2001). Isolation from the airways in the absence of lesions has little significance (Hoeling, 1994). Cefotiofur, enrofloxacin, or tulathromycin delivered parenterally to affected animals are effective therapeutic drugs. Use of water-soluble antimicrobials is for control. Maternal immunity, medicated early weaning, and controlling infections with PRRS, PCV2, and influenza postpone or prevent disease onset (Rapp-Gabrielson et al., 2000). Commercial and autogenous vaccines are available but may experience limited efficacy due to serologic diversity; controlled exposure to low dose, live virulent culture is another option (Oliveira et al., 2004) (Figure 3).

MH is known to infect pigs in production systems worldwide causing reduced growth performance and mortality. The disease is classified as enzootic pneumonia or a component of PRDC. Both manifestations of MH cause paralysis of the mucusciliary escalator resulting in a severe cough and dyspnea known as thumping. Vertical and lateral transmission can occur, but, owing to its slow rate of transmission between pigs, the disease primarily occurs in grow-finish pigs (Meyns et al., 2004). In addition, time of colonization with MH and disease severity are directly related (Fano et al., 2007). On necropsy, well-demarcated (red to purple lobular consolidation occurs in the apical) diaphragmatic, and accessory lung lobes is visible. Histopathologic lesions characteristic of MH is bronchopneumonia with lymphocytic perivascular, peribronchial, and peribronchiolar cuffing. Because MH is difficult to isolate, PCR is the most sensitive method of detection. ELISA is available and is helpful in establishing herd status but must be interpreted in the context of vaccination as tests do not distinguish between antibodies produced subsequent to vaccination or field infection. Treatment of affected pigs with parental antimicrobials like enrofloxacin, tulathromycin, or lincomycin or administration of water-soluble lincomycin, tiamulin, or tetracyclines to affected groups is effective in outbreaks. Control can be achieved through pulse-medication in feed of chlortetracycline (Thacker et al., 2006) beginning

![Figure 3](https://example.com/figure3.jpg) Epicarditis, heart, nursery pig. Fibrin gives surface a granular appearance, caused by HPS infection. Note the enlarged (draining) mediastinal lymph nodes located cranial to the base of the heart and the excess thoracic fluid (reddish-brown) indicative of septicaemia. Courtesy Dr. Glen Almond.
1 week before the historical onset of disease (Maes et al., 2008). Commercial vaccines are whole cell bacterins marketed to reduce lesions but do not prevent disease or slow transmission rate. Simultaneous infection with PRRSV reduces efficacy of MH vaccination (Thacker et al., 2000; Thacker, 2000). Eradication from the herd is preventative but practically difficult to accomplish.

PCVAD is any disease process where PCV2 infection results in lesions and includes PMWS (Ellis et al., 1998) and PDNS. Infection with PCV2 is widespread. Morbidity and mortality is variable, often dependent on the occurrence of secondary infections and their virulence. Survivors of PCVAD remain stunted, owing to the economic significance of this collection of diseases. Clinical signs include wasting, dyspnea, depression, ill thrift, and diarrhea. Lungs are wet, heavy, and fail to collapse; pulmonary edema and lymphadenopathy also can be found at necropsy. Histopathologic results include presence of interstitial pneumonia, lymphoid depletion, enteritis, nephritis, and dermatitis. For a diagnosis of PCVAD the following must occur: PCV2 antigen within characteristic lesions and lymph nodes are depleted (Sorden, 2008). IHC is used to confirm presence of PCV2 antigen within the histopathologic lesion. PCR has little value in diagnosing PCVAD unless the herd is considered free. Commercial vaccines are very effective and available with flex labels for administration to sows and pigs and as 1 or 2 doses (Chae, 2012). Nonvaccinated, subclinically infected pigs have poor weight gain compared to their vaccinated counterparts (Kristensen et al., 2011); therefore, it is part of most vaccination protocols by US pork producers (Figure 4).

PRRS is the result of infection with the Leykastad or North American strain of PRRSV. The estimated cost of PRRS to the US pork industry is US$664 million annually (Holtkamp et al., 2013). PRRSV is the most commonly diagnosed viral respiratory pathogen at VDLs (Gauger, 2009). Infection is observed to increase susceptibility to other infections, particularly opportunistic bacteria. This apparent increased susceptibility to secondary and opportunistic infections is the result of the pathologic process in which PRRSV recruits and replicates in pulmonary alveolar macrophages, and then disseminates systemically (Rossow, 1998). Clinical signs are nonspecific including fever, lethargy, and dyspnea but not cough. Signs also depend on the type of secondary infection(s) present. Lungs fail to collapse and appear heavy, wet, and gray on post-mortem examinations. Lymphadenopathy is caused by hyperplasia of germinal centers. Interstitial pneumonia, alveoli are lined with hyperplastic type II pneumocytes and contain necrotic debris, whereas the lining of bronchi and bronchioles is normal (Rossow, 1998). Vasculitis also occurs. PCR is the most sensitive method for confirming infection. Owing to the genetic diversity of PRRSV, sequencing of the ORF5 region is a common adjunct to PCR testing. Sequences are then used to create dendrograms for use by production systems pursuing PRRS control and epidemiologic investigations (Murtaugh, 2012). PRRSV ELISA is helpful for establishing herd status: National Animal Health Monitoring Service reports that a large percentage of US herds are seropositive. Treatment is limited to maintaining pig comfort, minimizing stress, and controlling secondary infections. Commercial modified-live vaccines (MLV) are available and administration during the nursery phase significantly reduces mortality and improves growth performance during the grow-finish phase of production (Robbins et al., 2013b). MLV vaccines do replicate and should not be used in negative populations.

Salmonella cholerasuis is the swine-adapted Salmonella from the C1 serogroup and, unlike S. Typhimurium, is not a foodborne pathogen. Ingestion or inhalation of the bacteria causes a septicaemia resulting in low to moderate morbidity with high mortality within 1–2 days of infection that occurs postweaning, predominately during the grow–finish phase (Baskerville and Dow, 1973). Signs include high fever (>40 °C), lethargy, dyspnea, acute death, and cyanotic extremities and abdomen. The latter makes it impossible to differentiate clinically from classical swine fever virus (CSFV). Pleuropneumonia, interlobular edema, mediastinal, and tracheobronchial lymphoedema, and occasionally white foci in the liver are apparent postmortem (Turk et al., 1993). Acute histopathologic lesions that form in the lung are purulent bronchitis, lobular necrosis, and abscessation, whereas paratypohid nodules are observed in the liver. Isolation is best achieved from the draining lymph nodes, lung, or liver using selective culture media. Serogrouping and typing is necessary for speciation and diagnostic confirmation. Owing to the rapid onset of disease, parental treatment is recommended. Salmonella cholerasis isolates are commonly susceptible to cefotiofur. Increased hygiene particularly eliminating access to waste and vaccination is preventive (Vivsa et al., 2005).

SV4 is more accurately described as influenza, to encompass the infections occurring in swine, avian, and human species. Influenza virus is classified by its hemagglutinin and neuraminidase proteins; the three predominant strains in pigs are H1N1, H1N2, and H3N2. Rapid transmission and onset are characteristic in the experimental inoculation of one nonvaccinated nursery age pig resulted in 10.66 more becoming infected (Romagosa et al., 2011). Virus is shed for 3–5 days and uncomplicated lesions resolve 28 days postinfection (Gramer, 2007). Nasal discharge, fever, and lethargy occur but resolve quickly. Cough and dyspnea can last up to 2
weeks postinfection. PCR and VI detect virus for diagnosis of clinical cases. ELISA and HI detect antibodies; ELISA is helpful in establishing herd status, whereas HI is best for vaccination timing and measuring postvaccination titers (Allerson et al., 2008). Necrotizing bronchiolitis, bronchiolitis, and alveolitis as lesion resolves affected areas appear vacuolated. Pigs recover quickly so treatment should focus on maintaining pig comfort, minimizing stress, and controlling secondary infections. All licensed vaccines are killed; commercial and autogenous products are in use in the United States. Vaccination reduces lung lesions and rate of transmission, but does not prevent infection and is complicated by antigenic shift and drift. In the United States, it is typical to vaccinate the sows rather than pigs to control disease and infection (Allerson et al., 2013).

Diseases That Affect International Trade

Trade diseases are those listed by the OIE. When one of these diseases is suspected or confirmed, it results in closure of international market access, which would be economically devastating to import–export businesses. The primary method for managing diseases that affect trade is to prevent their introduction.

Foot-and-mouth disease (FMD) is caused by a picornavirus, FMDV, which causes mucosal lesions exclusively in cloven hoofed species. Clinical signs are excessive salivation, anorexia, and lameness causing high morbidity but low mortality. Gross lesions are vesicles at cutaneous junctions, on the snout, or in the oral cavity. Similar lesions can be caused by Seneca Valley virus, vesicular stomatitis, swine vesicular disease, and vesicular exanthema of swine; therefore, any blister in swine warrants diagnostic investigation. FMDV is highly transmissible within and between species.

African swine fever (ASF) is caused by ASFv; currently classified as an iridovirus. Soft ticks can act as reservoirs or vectors. Current outbreaks are reported throughout Eastern Europe and Russia that have been associated with improper garbage feeding. The virus damages blood vessels resulting in clinical signs and gross lesions consistent with septicaemia; including red to purple skin discoloration and enlarged spleen, liver, and lymph nodes. Excess blood and fluid in body cavities may occur.

Classical swine fever, historically referred to as hog cholera, is caused by CSFv, a pestivirus, eradicated from the United States in 1972. Transmission is associated with infected feeding, uncooked or undercooked garbage containing pork or pork by-products to swine. The virus remains infectious for months when refrigerated and years when frozen. Clinical signs are nonspecific and are easily confused with S. choleraesuis. The virus replicates rapidly in tansils, which makes it the ideal tissue to collect for diagnosis of CSFv.

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USDA APHIS.

CHAPTER III.

EFFECTS OF QUATERNARY BENZO(C)PHENANTHRIDINE ALKALOIDS ON GROWTH PERFORMANCE, SHEDDING OF ORGANISMS, AND GASTROINTESTINAL TRACT INTEGRITY IN PIGS INOCULATED WITH MULTIDRUG-RESISTANT SALMONELLA SPP

Effects of quaternary benzo(c)phenanthridine alkaloids on growth performance, shedding of organisms, and gastrointestinal tract integrity in pigs inoculated with multidrug-resistant *Salmonella* spp

Rebecca C. Robbins, DVM; Valeria C. Artuso-Ponte, DVM; Adam J. Moeser, DVM, PhD; W. E. Morgan Morrow, BVSc, PhD; Jerry W. Spears, PhD; Wondwossen A. Gebreyes, DVM, PhD

**Objective**—To evaluate effects of quaternary benzo(c)phenanthridine alkaloids (QBAs) against *Salmonella* spp and determine effects on growth performance, organism shedding, and gastrointestinal tract integrity in pigs inoculated with *Salmonella enterica* serovar Typhimurium.

**Sample**—36 *Salmonella* isolates and twenty 5-week-old pigs.

**Procedures**—Minimum inhibitory concentration of QBAs against the *Salmonella* isolates was determined. Pigs were allocated to 4 groups and inoculated with *Salmonella* organisms. Pigs received diets supplemented with 15 g of QBAs/1,000 kg of feed, 0.75 g of QBAs/1,000 kg of feed, or 0.4 g of chlorotetracycline/1,000 kg of feed or a nonsupplemented (control) diet. Pigs were weighed on day 0 and then weekly for 40 days. Fecal samples were collected to quantify *Salmonella* organisms. Gastrointestinal tract integrity was evaluated by measuring transepithelial resistance.

**Results**—In vitro, 9 of 36 (25%) *Salmonella* isolates were inhibited at 90 μg of QBAs/mL; all 36 were inhibited at 179 μg of QBAs/mL. Diets containing QBAs significantly decreased *Salmonella* spp shedding; shedding was lower 40 days after inoculation for pigs fed diets containing QBAs or chlorotetracycline than for pigs fed the control diet. Growth performance was similar for pigs fed diets containing QBA or chlorotetracycline. Gastrointestinal tract integrity was improved in pigs fed the diet containing 1.5 g of QBAs/1,000 kg of feed.

**Conclusions and Clinical Relevance**—QBAs and chlorotetracycline decreased *Salmonella* spp shedding but did not differ with regard to growth performance. Gastrointestinal tract integrity was better, albeit not significantly, in pigs fed diets containing QBAs. Further investigation into the role of QBAs and their mechanism as an immunomodulator is necessary. (Am J Vet Res 2013;74:1530–1535)

*Salmonella enterica* is a ubiquitous enteric pathogen estimated to cause > 1.4 million cases of illness in humans in the United States annually, of which 95% are estimated to result from foodborne transmission. In addition, the increased incidence of multidrug-resistant infections caused by phage types such as *S enterica* serovar Typhimurium DT104 and the isolation of this strain from swine may pose serious health risks to pork consumers.

The addition of antimicrobials to swine diets is a common practice in most parts of the world, including the United States. Chlorotetracycline is estimated to be the antimicrobial most widely used in feed for the nursery and growth and finishing phases of pork production. Feeding of antimicrobials, including chlorotetracycline, for growth promotion can decrease fecal shedding of enteric pathogens and could serve to improve preharvest food safety by minimizing carcass contamination during processing.

**Abbreviations**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tr>
<td>ADG</td>
<td>Average daily gain</td>
</tr>
<tr>
<td>DT</td>
<td>Determinant type</td>
</tr>
<tr>
<td>MPN</td>
<td>Most probable number</td>
</tr>
<tr>
<td>QBA</td>
<td>Quaternary benzo(c)phenanthridine alkaloid</td>
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<tr>
<td>TER</td>
<td>Transepithelial resistance</td>
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From the Department of Population Health and Pathobiology, College of Veterinary Medicine (Robbins, Moeser), and the Department of Animal Science, College of Agriculture and Life Sciences (Morrow, Spears), North Carolina State University, Raleigh, NC 27605; and the Department of Veterinary Preventive Medicine, College of Veterinary Medicine, The Ohio State University, Columbus, OH 43210 (Artuso-Ponte, Gebreyes).
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Address correspondence to Dr. Gebreyes (gebreyes.1@ncsu.edu).
Because of increasing concerns surrounding use of antimicrobials at subtherapeutic concentrations in food animals, the pork industry has funded investigations on alternatives to traditional antimicrobials used as growth or production promoters; those alternatives include herbs, immune modulators, and probiotics. Limited investigations have been conducted on the use of herbal extracts, particularly those containing isouquinolone alkaloids, for their antimicrobial properties. An isouquinolone alkaloid, QBA, reportedly has anti-inflammatory and antimicrobial properties and can decrease amino acid degradation, increase feed intake, and promote growth in swine. In 1 study, QBAs significantly decreased damage to the colonic mucosa and mitigated colonic inflammation when included in the diet of rats, which suggests a protective effect of QBAs on the colonic mucosa. Quaternary benzylphenanthridine alkaloid consists of sanguinarine and chelerythrine extracts. Neither QBAs nor any proprietary formulations have been evaluated for their effects on the antiobidiogram of foodborne pathogens, the ability to decrease bacterial fecal shedding, or improvement in gastrointestinal barrier function. Thus, the purpose of the study reported here was to evaluate the in vitro effects of QBAs on Salmonella isolates and compare the effects of QBAs with those of chlorotetracycline on growth performance, feed efficiency, and fecal shedding of Salmonella organisms in nursery-age pigs. Our hypothesis was that Salmonella spp shedding is influenced by feed-grade antimicrobials and that a nonantimicrobial alternative (QBA) could serve as a potential replacement to traditional antimicrobials.

**Materials and Methods**

**Animals**—Twenty 5-week-old crossbred gilts (mean ± SD body weight, 8.96 ± 0.26 kg) with negative results for culture of Salmonella spp in feces were used in the study. Only pigs that had received ceftiofur at the labeled dose and had 2 consecutive negative results for culture of Salmonella spp in fecal samples obtained on the day of ceftiofur treatment and 5 days after ceftiofur treatment were enrolled.

Pigs were housed individually at 26 ± 2°C in pens (3.05 x 0.76 x 1.37 m); there was a solid barrier between pens to prevent direct contact. Pigs were fed a 2-phase mash diet. The phase 1 diet (45% corn, 29% soybean meal, and 19.98% dried whey) was fed for 14 days. The phase 2 diet (62.5% corn and 30% soybean meal) then was fed for the remainder of the 40-day study. Diets were formulated on the basis of nutritional guidelines to meet or exceed dietary requirements for growing pigs. Feed and water were provided ad libitum throughout the study. Animal care was provided in accordance with a protocol approved by the North Carolina State University Animal Care and Use Committee.

**In vitro assay**—Inhibitory effects of QBAs on various strains of Salmonella spp were evaluated by use of the agar dilution method in accordance with standard reference methods recommended by the Clinical and Laboratory Standards Institute. Briefly, QBAs obtained from Macleaya cordata extract were diluted in sterilized deionized water to a concentration of 33 g/L and mixed with Müller-Hinton agar at concentrations ranging from 0 to 896 μg/mL. Thirty-six strains previously isolated from swine, including S. enterica serovars Typhimurium, Heidelberg, and Derby, that represented pan-susceptible and multidrug-resistant strains were tested. Spots of a suspension of fresh inoculum were placed on the agar with a replicator system. A control plate with no QBAs was also cultured. Plates were incubated for 24 hours at 37°C and visually evaluated for growth.

**Experimental design**—Pigs were allocated by use of a randomization procedure (ie, random numbers generated with software) to a room, a pen, and 1 of 4 experimental diets (5 pigs/treatment). Pigs were fed diets supplemented with 1.5 g of QBAs/kg of feed, 0.75 g of QBAs/2,000 kg of feed, or 59.4 g of chlorotetracycline/1,000 kg of feed or a nonsupplemented control diet. Dietary concentrations of QBAs and chlorotetracycline used in the study were fed as per label directions to improve rate of weight gain and feed efficiency. Pigs were challenged with Salmonella organisms on day 0 and euthanized (xylazine and pentobarbital; doses determined on the basis of body weight) on day 40. However, 1 pig fed the control diet and 1 pig fed the diet containing 0.75 g of QBAs/kg of feed were euthanized on days 6 and 13, respectively, because of conditions unrelated to the study.

**Challenge inoculation with Salmonella organisms**—Salmonella Typhimurium DT104 with a penta-resistant (ampicillin, chloramphenicol, streptomycin, sulfamethoxazole, and tetracycline) profile was used as the challenge strain. This isolate had been previously isolated from swine feces during the in vitro assay. The inoculum was grown to mid-logarithmic phase in Luria-Bertani broth with agitation at 37°C. On day 0, each pig received via oral drench 5 mL of inoculum containing 1.0 x 10⁸ CFUs (as determined by enumeration on Mueller-Hinton agar). This dose was consistent with doses used in another study.

**Salmonella isolation, antimicrobial susceptibility testing, and quantification**—Fecal samples were collected from each pig on days 2, 6, 12, 19, 26, 33, and 40. Salmonella organisms were isolated with tetraanti-biotic broth enrichment as described elsewhere. The antimicrobial resistance patterns for up to 5 Salmonella isolates were determined with commercially available antimicrobial susceptibility plates to confirm whether the challenge strain was the same as the strain cultured from the fecal samples. All isolates were evaluated for antimicrobial susceptibility with the Kirby-Bauer disk diffusion test. Quantification was conducted in accordance with the 3 dilution x 3 tube MPN method and calculated with an MPN calculator. Salmonella organism counts that were estimated as <1 CFU/g of feces by the MPN method were grouped into a single category for statistical analysis.

**TER—Transepithelial resistance** is a sensitive measure of intestinal barrier function and reflects the ability of epithelium to impart a resistance barrier (governed by tightness of the intercellular tight junctions). The TER was conducted...
in an Ussing chamber as described elsewhere. Briefly, fresh intestinal samples were collected from each pig immediately after the pigs were euthanized on day 40. Intestinal samples (sections of ileal tissue) were collected and mounted on an Ussing chamber. Mucosal barrier function was determined by measuring the electrical resistance per area of the intestine. Measurements were recorded as a continuous variable ranging between 0 and 80 Ω/cm², and TER values were compared between uninfected control pigs (2 pigs that originated from the same source farm and had the same genetic background as the other pigs and were tested and found to be negative for Salmonella spp) and Salmonella-challenged pigs receiving chlortetracycline or 1.5 g of QBAs/1,000 kg of feed. A TER of 40 Ω/cm² is considered typical for a clinically normal pig.

Neutrophil counts were determined as a measure of overall intestinal health and to confirm findings from the TER assay. Ileal tissues were fixed in formalin and stained with H&E. The extent of neutrophil infiltration was measured quantitatively with microscopic evaluation and quantified as the number of neutrophils per square millimeter.

Assessment of ADG and feed conversion ratio—Consumption of feed was calculated as weight of feed offered minus weight of unconsumed feed and reported as mean daily feed intake on a dry-matter basis. Pigs were weighed on days 0, 7, 14, 21, 28, 35, and 40 for calculation of weight gain, ADG, and the feed conversion ratio (ie, ratio of feed consumption to weight gain). Data for the 2 pigs euthanized prior to day 40 were not included in the statistical analysis for these variables.

Statistical analysis—The use of 1-tailed or non-directional tests increases statistical power to detect a difference in the expected direction. Investigators in other studies have found that inclusion of antimicrobials in diets of nursery pigs improves growth performance. Therefore, the expected outcome that chlortetracycline would improve growth and the feed conversion ratio of nursery pigs challenge-inoculated with Salmonella Typhimurium DT104 was assessed with a 1-tailed analysis. Pairwise comparisons of ADG, feed conversion ratio, and fecal shedding of Salmonella organisms were performed for pigs receiving diets supplemented with QBAs or chlortetracycline versus those for pigs receiving the control diet, pigs receiving diets supplemented with QBAs versus pigs receiving diets supplemented with chlortetracycline, and pigs receiving the diet supplemented with 1.5 g of QBAs/1,000 kg of feed versus pigs receiving the diet supplemented with 0.75 g of QBAs/1,000 kg of feed. Comparisons were conducted by means of a Wilcoxon rank sums test with standard statistical software. Spearman correlation coefficients for fecal shedding of Salmonella organisms within a treatment group were calculated with standard statistical software. Correlations between fecal shedding of Salmonella organisms and time after inoculation for each treatment group were plotted with commercial graphing software and examined. Significance was determined on the basis of 1-sided tests; values were considered significant at P < 0.05.

Results

In vitro assay—The in vitro inhibitory effect of QBAs on Salmonella isolates was determined (Table 1). Of 36 Salmonella isolates, 9 (25%) were inhibited at 90 μg of QBAs/mL; the remaining 27 isolates were able to grow at that concentration of QBAs. However, all 36 Salmonella isolates were inhibited at a concentration of 179 μg of QBAs/mL.

ADG and feed conversion ratio—Weight gain and feed efficiency were measured as ADG and the feed conversion ratio (Table 2). Pigs receiving QBAs supplemented with QBAs or chlortetracycline did not have a significantly better ADG or feed conversion ra-

<table>
<thead>
<tr>
<th>Serovar</th>
<th>Phage type of isolates</th>
<th>Total No.</th>
<th>90 μg of QBAs/mL</th>
<th>179 μg of QBAs/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Typhimurium</td>
<td>DT104</td>
<td>7</td>
<td>3</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>DT103</td>
<td>11</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>DT206</td>
<td>4</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>DT21</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>NA</td>
<td>NA</td>
<td>8</td>
<td>2</td>
</tr>
<tr>
<td>Derby</td>
<td>NA</td>
<td>3</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Heidelberg</td>
<td>NA</td>
<td>2</td>
<td>1</td>
<td>2</td>
</tr>
</tbody>
</table>

NA = Not applicable.

Table 1—Minimum inhibitory concentration of QBAs for 36 Salmonella enterica isolates from swine.

<table>
<thead>
<tr>
<th>Diet</th>
<th>Body weight (kg)</th>
<th>Mean daily feed intake (kg)</th>
<th>ADG (kg)</th>
<th>Feed conversion ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 0</td>
<td>Day 40</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.5 g of QBAs/1,000 kg of feed (n = 5)</td>
<td>8.58 ± 0.58</td>
<td>29.40 ± 3.38</td>
<td>1.90 ± 0.04</td>
<td>0.52 ± 0.08</td>
</tr>
<tr>
<td>0.75 g of QBAs/1,000 kg of feed (n = 4)</td>
<td>9.19 ± 0.57</td>
<td>31.25 ± 2.06</td>
<td>0.80 ± 0.43</td>
<td>0.55 ± 0.06</td>
</tr>
<tr>
<td>56.4 g of chlortetracycline/1,000 kg of feed (n = 5)</td>
<td>9.75 ± 0.76</td>
<td>31.70 ± 2.00</td>
<td>0.98 ± 0.07</td>
<td>0.55 ± 0.03</td>
</tr>
<tr>
<td>Unsupplemented control (n = 4)</td>
<td>8.98 ± 1.00</td>
<td>28.50 ± 3.50</td>
<td>0.83 ± 0.46</td>
<td>0.49 ± 0.09</td>
</tr>
</tbody>
</table>

Day of inoculation was designated as day 0. Feed was weighed daily, and pigs were weighed weekly. No significant (P > 0.05) differences in growth performance variables were detected among treatment groups.
Effect of diet on fecal shedding of *Salmonella* organisms was determined by measuring the number of CFUs per gram of feces obtained from each pig during the 40-day study. We assumed that the *Salmonella* colonies were indicative of fecal shedding of *Salmonella Typhimurium* DT104, given that pigs had negative results prior to challenge inoculation; no isolates were serotyped or phage typed after isolation. The distribution of pigs receiving a supplemented diet, whether with chlorotetracycline or either concentration of QBA, was significantly different from the distribution of those fed the control diet at days 26 and 40. Pigs receiving supplemented diets shed *Salmonella* organisms at a median of 9.3 CFU/g of feces on day 26 and in the range of 2.3 to 2.8 CFU/g of feces on day 40, compared with a median of 110 CFU/g of feces on day 26 and a range of 24 to 46 CFU/g of feces on day 40 for those receiving the control diet. In addition, the median number of *Salmonella* organisms shed on day 40 by pigs receiving QBAs in the diet was < 1 CFU/g of feces, which was significantly lower than that shed by pigs receiving chlorotetracycline (median, 9.3 CFU/g of feces). There was no significant difference in fecal shedding of *Salmonella* organisms between pigs receiving 1.5 g of QBAs/1,000 kg of feed or 0.75 g of QBAs/1,000 kg of feed at any time after inoculation.

Strong negative correlations were found between fecal shedding of *Salmonella* organisms and time after inoculation for each of the treatment groups (Figure 1). In each group, the time after inoculation accounted for > 25% of the variation in MPN of *Salmonella* organisms shed in feces (data not shown). Although the correlation for all treatment groups was $r \leq -0.50$, the strongest linear relationship ($r = -0.81$) was for the pigs fed 0.75 g of QBAs/1,000 kg of feed. Diets containing 1.5 g of QBAs/1,000 kg of feed, 0.75 g of QBAs/1,000 kg of feed, or 59.4 g of chlorotetracycline/1,000 kg of feed significantly decreased the number and expected duration of shedding of *Salmonella* organisms as determined on the basis of the inverse relationship. Although there was a decrease in the shedding of *Salmonella* organisms for the QBAs- and chlorotetracycline-treated pigs, compared with that in pigs fed the control diet, the overall differences were small.

Fecal samples from pigs fed the diet supplemented with 1.5 g of QBAs/1,000 kg of feed had a higher mean TER (62 Ω/cm²), which indicated enhanced health of the mucosal barrier, compared with the mean TER for pigs fed the diet supplemented with chlorotetracycline.

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**Figure 1**—Fecal shedding of *Salmonella* organisms (determined as the MPN) for growing pigs fed diets supplemented with 1.5 g of QBAs/1,000 kg of feed (A [n = 5]), 0.75 g of QBAs/1,000 kg of feed (B [4]), or 59.4 g of chlorotetracycline/1,000 kg of feed (C [5]) or fed an unsupplemented control diet (D [5]) and inoculated via oral drench with *Salmonella enterica* serovar Typhimurium DT104. Day of inoculation was designated as day 0. For each panel, the solid line represents the correlation for the data.
(31 Ω/cm²) or uninfected control pigs (2 pigs that originated from the same source farm and had the same genetic background as the other pigs and were tested and found to be negative for Salmonella spp [50 Ω/cm²]; Figure 2). This measurement was conducted on samples obtained at a single time point (day 40) and therefore did not provide conclusive results as to whether dietary supplementation with QBAs was protective or resulted in enhanced repair of barrier function following challenge inoculation with Salmonella organisms. There were no differences in results of histologic evaluation of the ileal samples among treatments, as determined on the basis of qualitative and quantitative neutrophil counts (results not shown).

Discussion

One of the objectives of the present study was to evaluate the in vitro effect of QBAs on Salmonella spp growth in cultures. The QBAs at a concentration of 90 μg/mL inhibited the growth of 5 serotypes and 4 phage types of Salmonella spp. These results agree with those of other studies2,23,25 in which QBAs were found to have antimicrobial effects, but to our knowledge, this is the first report of the minimum inhibitory concentrations of QBAs for Salmonella Typhimurium, Heidelberg, and Derby. Nevertheless, concentrations of QBAs used in the in vitro experiments were higher than concentrations typically included in feed, and more studies that involve the use of feed-grade antimicrobials at recommended concentrations are needed to determine the antimicrobial effects of QBAs in field settings.

The absence of a significant increase in ADG or decrease in feed conversion ratio for pigs receiving diets supplemented with QBAs or chlortetracycline in the study reported here may have been associated with the optimum housing and management conditions of pigs in a university research facility. Investigators in other studies21,22 reported that the true effects of growth promoters, especially those with antimicrobial properties, are often not realized in a research setting because of the cleanliness and strict biosecurity maintained in these facilities. Specifically, production performance for growing pigs raised on commercial farms has been reported to be up to 2 times as great as for growing pigs raised in research facilities when antimicrobials are included in diets at subtherapeutic concentrations.19 Pigs were not commingled or challenge-inoculated with multiple pathogens, so the effect of diet on growth performance may have been obscured. The authors also recognize that the effects reported in this study, or lack thereof, could have been attributable to random errors that may have been caused by the low sample size.

Growth promotants have cumulative benefits on pig performance and health.21,22 It has been estimated that 80% of diets formulated for growing pigs and 50% of finishing diets formulated for pigs contain at least 1 antimicrobial at a subtherapeutic concentration that is meant to increase growth and feed efficiency.21 In light of the historical benefits of antimicrobials as growth promotants as well as the current interest in identifying nonantimicrobial alternatives, we believed it was pertinent to compare the growth performance benefits attributable to a traditional feed-grade growth promotant (chlortetracycline) with benefits for a novel nonantimicrobial alternative (QBAs). This study revealed that benefits to pig growth and feed efficiency were similar regardless of the type of growth promotant administered. Further evaluation of pigs fed diets containing QBAs throughout the postweaning period may also reveal positive benefits, compared with benefits for diets containing chlortetracycline, on growth performance that could not be measured or were not significantly different in the present study.

The plant-derived alkaloids evaluated in this study were more effective than chlortetracycline for decreasing fecal shedding of Salmonella organisms at 40 days after inoculation. In addition, fecal shedding in pigs fed diets containing 1.5 g of QBAs/1,000 kg of feed and 0.75 g of QBAs/1,000 kg of feed was strongly correlated with time after inoculation (Figure 1), which suggested that the use of QBAs had an inverse relationship with fecal shedding of Salmonella spp after inoculation. The accuracy of the procedure for enumeration of Salmonella organisms (MPN calculation) was likely to have affected the analysis. Although this method is widely regarded as a standard microbiological procedure for quantification of bacteria cultured from food products, it does not appear to be appropriate for enumeration of large numbers of bacteria, considering that the highest value represented was 110 CFUs/g and that no higher numeric estimate could be obtained. Nonetheless, on the basis of these observations, QBAs appear to have an application as an in-feed intervention strategy to reduce the chance of antimetabolism, permamutagenesis, and postmortem contamination in pig rearing and slaughter facilities that results from pigs shedding high numbers of Salmonella organisms in the feces.

Certain pathological events, which include intestinal injury, enteric disease (e.g., salmonellosis), and stress, initiate the breakdown of intestinal barrier function, which is highlighted by increased gastrointestinal tract permeability as measured by the TER. Leaky in-
testines, indicated by a low TER, allow luminal agents such as bacteria, toxins, or antigens to freely traverse the intestinal epithelium and gain access to subepithelial tissues, which results in inflammation. Once bacteria, toxins, or antigens breach the subepithelium, they potentially gain access to the systemic circulation, which may result in septicemia, a sequela to multiple organ disease. In the present study, the TER for all groups was at or above the expected TER for the intestinal barrier in clinically normal pigs, despite challenge inoculation with Salmonella enterica serovars. In addition, the mean TER for Salmonella-challenged pigs fed 1.3 g of QBA/s/kg of feed was 12 and 21 Ω/cm² greater than that of un inoculated control pigs (2 pigs that originated from the same source farm and had the same genetic background as the other pigs and were tested and found to be negative for Salmonella spp) and Salmonella-challenged pigs fed chlorotetracycline, respectively. The QBA appears to have beneficial effects on intestinal barrier health, as indicated by an increase in TER. The potential mechanisms of action of QBA are not currently known, but hypothetically mechanisms include modulation of gastrointestinal tract flora, enhanced intestinal protection, and repair of the intestinal epithelium. The specific mechanism of action by which QBA improve intestinal health needs to be determined.

Although there are potential benefits of QBA to food safety and public health through reduction in the shedding of Salmonella strains from pigs, their effects on the bacterial ecology and molecular epidemiology of bacteria commonly found within swine production units are unknown. The use of antimicrobials in swine production units has been catalyzed as a possible selective pressure responsible for the expansion of antimicrobial resistance within bacterial populations; therefore, the potential influence of plant alkaloids (eg, QBA) with antimicrobial qualities on the prevalence of antimicrobial resistance patterns of Salmonella spp recovered from naturally infected pigs should always be considered. Therefore, evaluation of pigs fed QBA in a more representative production environment for the entire postweaning period is necessary before they can be confirmed as comparable or suitable alternatives to chlorotetracycline for reducing fecal shedding of Salmonella strains in commercial production units.

References

CHAPTER IV.

OBSERVATIONS ABOUT THE USE OF ANTIBIOTICS IN NURSERIES OF AN INTEGRATED SWINE PRODUCER
Introduction

Concerns over misuse of antibiotics in food animals leading to increasing antimicrobial resistant bacterial infections in humans necessitates that food animal veterinarians practice antibiotic stewardship. The American Association of Swine Veterinarians issued guidelines to aid swine veterinarians in judicious selection of antibiotics. These guidelines advise that scientific reports be considered to ensure the use of antibiotics is safe and effective.

Only approved antibiotics, those with a Food and Drug Administration (FDA)-issued New Animal Drug Application or New Drug Application registration number, can be administered to food animals. The FDA approves label claims which must be substantiated. A measurable difference must exist in the animal species that receives the antibiotic by the proposed route, dose, duration, purpose and indication compared to the one that did not receive the antibiotic before the antibiotic label is approved. Therefore, the efficacy of a FDA-approved antibiotic for its stated use is not being evaluated. Control trials conducted in research facilities frequently underestimate the benefits of antibiotic use.

Pigs receive proportionately more antibiotics during the nursery period, weaning to 44-60 pounds, compared to other production periods. The stress of weaning, waning maternal antibody and an inability to mount an effective innate immune response put the pig at higher risk for disease development during the nursery period. In addition, piglets are colonized as they pass through the birth canal with gram-positive Streptococcus suis. This early infection has led to the frequent diagnosis of S. suis during the nursery period.
Ceftiofur injections for treatment of clinical signs referable to S. suis has consistently improved nursery livability when compared to other injectable antibiotics (e.g. procaine penicillin and ampicillin), administration of antibiotics in drinking water (e.g. tiamulin, chlortetracycline plus sulfamethazine and amoxicillin), autogenous vaccination, and sham treatment\textsuperscript{8-11}. The purpose of the study was to describe the use of antibiotics in the nursery using an observational study design.

**Materials and Methods**

**Sample population**

From May 2009 to January 2010 the use of antibiotics delivered by water or injection to nurseries contracted or owned by a single integrated swine producer were observed. All groups of nursery pigs were housed in barns with similar ventilation, feeding, and waste-handling systems. All barns were all-in, all-out. All antibiotic therapy was applied to the group; the barn was the unit of interest. Antibiotics in the feed did not vary at the barn level so were not considered further. The livability, daily gain (GPD) and feed conversion (FG) for 661 barns was obtained; 211 groups received antibiotics by injection or through the water, 209 were graded for disease severity, and 107 were assessed for morbidity.

**Veterinary-Client-Patient Relationship**

All use of antibiotics was within a veterinary-client-patient relationship. All antibiotic administration, dose and duration of therapy were under the oversight and discretion of the herd veterinarian employed by the integrator.
Study design

A quasi-experimental design was used (Figure 1). Nurseries were not randomly allocated to different antibiotic regimens, rather antibiotic use of an integrated pork producer was observed. This type of study design is preferred when evaluating benefits of specific interventions on infectious disease\textsuperscript{12}. The study questions were formulated to quantify a subjective disease severity grade, identify risk factors for antibiotic use, and measure effect of antibiotic use on nursery livability in a natural setting. All data was collected and analyzed on group exposures and outcomes.

Data collection

The veterinarian and 3 production personnel employed by the integrator were responsible for selecting antibiotic regimens from a list provided by the aforementioned veterinarian. A standard data collection form was provided to each person. A single antibiotic use event was recorded on each form. The form requested that only one response from each category be selected. The only free text data was for the antibiotic regimen administered.

Exposure variables were categorical and outcome variables were continuous. There were 5 exposure variables of interest: antibiotic regimen (Table 1); route of administration (injection or drinking water); clinical signs referable to the body system affected (respiratory, central nervous system, enteric or systemic); disease severity (mild, moderate, or severe); and weeks post-weaning (\(\leq 4\) weeks or >4 weeks). Route of administration was a proxy for antibiotic regimen. There were 4 outcome variables: morbidity (percent of group displaying
clinical signs of disease); livability (percent of group that survived); gain per day (GPD, lbs); and feed conversion (FG).

Barn morbidity was determined by two assessors, not employed by the integrator, for the barn by tallying clinical signs known to be associated with diseases of certain body systems. Initially 100% of pens were tallied. However, it was determined that a random sample of 25% of pens was not statistically different (p>0.10) from the morbidity calculated when 100% of pens were examined (data not shown). A random number generator\(^a\) was used to determine the 25% of pens for assessment. For antibiotics administered by injection, the barn was assessed no more than 24 hours prior to the planned injection. For antimicrobials administered via drinking water, the barn was visited within 24 hours prior to or following the start of administration.

**Statistical analysis**

Statistical analysis was conducted in a standard statistical software package\(^b\). Non-parametric tests were used because sample data were not normally distributed. Mean, median and standard deviation (SD) were calculated for all continuous variables. When testing continuous outcomes, the Wilcoxon rank sum test was used for 2 samples and the Kruskal-Wallis analysis of variance for 3 or more samples. Pairwise comparisons calculated using Bonferroni adjustment for multiple comparisons. Spearman rank correlation coefficients were calculated for morbidity, livability, GPD, and FC among grades of disease severity and grades of disease severity for each clinical sign. Logistic regression was used to compute univariate odds ratios and their 95% confidence interval (CI) to determine the
likelihood of using an antibiotic delivered by injection. The p-value for association was calculated using the Wald test. Due to sparse data, clinical signs were dichotomized for computational purposes into a new category, non-respiratory. The chi-square test was used to determine strength of association between the frequency of sites in the study sample and the NAHMS sample using antibiotics delivered by injection or drinking water. Statistical significance for all testing was set at a p-value ≤ 0.05.

Results

Comparison of medicated and non-medicated nursery group performance

During the study period, less than a third (31.6%) of nurseries groups received an antibiotic by injection or water. The median livability, GPD, and FG was compared between non-medicated and medicated nursery populations using the Wilcoxon rank sum test (Table 2). Livability and GPD was greater for non-medicated nursery groups compared to medicated groups (p<0.001). FG was not different between non-medicated and medicated nursery groups (p=0.396).

Assessing circumstances for antibiotic use

Most (99%) of reported antibiotic use events also were graded on disease severity; 36.8% mild, 50.2% moderate, 12.9% severe. Livability was inversely correlated with increasing grades for disease severity (Spearman rank coefficient -0.41, p<0.001). GPD was inversely correlated with increasing grades of disease severity (Spearman rank coefficient -0.17, p=0.019). FG was not correlated with disease severity (Spearman rank coefficient -0.05, p=0.497).
The mean, median and SD were for livability, GPD and FG were calculated among grades of disease severity (Table 2). Variances in livability, GPD and FG among grades of disease severity were calculated using a Kruskal-Wallis analysis of variance test. Only livability and GPD varied by disease severity. Pairwise comparisons for grades of disease severity were made using a Bonferroni adjusted p-value calculated using the Wilcoxon rank sum test. Livability declined for each increasing grade of disease severity (p<0.02). GPD differed by disease severity but only for mild and moderate grades (p<0.02).

Morbidity was determined for 107 nursery groups that received a disease severity grade; 45 mild, 51 moderate, and 11 severe. Morbidity was correlated with increasing grade of disease severity for respiratory (Spearman rank coefficient -0.38, p=0.002) and systemic (Spearman rank coefficient -0.64, p=0.012) clinical signs. Morbidity for mild and severe grades differed from one another but not from the morbidity for moderate disease.

The morbidity for each disease severity grade was determined for enteric, systemic and respiratory disease (Table 4). Respiratory signs were the most common reason for antibiotic administration (69.08%) followed by systemic (24.15%) and enteric (6.76%). Morbidity was correlated with grades of disease severity for respiratory (Spearman rank coefficient -0.38, p=0.002) and systemic (Spearman rank coefficient -0.64, p=0.012) clinical signs, but were not correlated with morbidity for enteric signs.

The odds ratio for use of injectable antibiotics was determined for 4 exposure variables using logistic regression (Table 5). The odds of receiving an antibiotic by injection among nursery groups ≤4 weeks post-weaning was 4.34 times (95% CI 2.00, 10.00) the odds
of receiving an antibiotic by injection among nursery groups >4 weeks post-weaning. The odds of receiving an antibiotic by injection for moderate or severe grades of disease severity was 1.61 times (95% CI 1.06, 2.45) and 2.31 time (95% CI 0.95, 5.65) the odds of receiving an antibiotic when clinical signs were graded as mild. The odds of receiving an antibiotic by injection for the control of clinical signs was 3.44 times (95% CI 1.96, 6.25) the odds of receiving an antibiotic by injection for treatment of clinical signs. The odds of receiving an antibiotic by injection for non-respiratory signs was 3.20 times (95% CI 1.75, 5.86) the odds of receiving an antibiotic by injection for respiratory signs.

**Choose antibiotics with a measurable benefit**

Over-three quarters (78.13%) of nursery sites received antibiotics for respiratory disease. There was no difference in the observed proportion of sites that administered antibiotics for respiratory disease compared to those reported by NAHMS\textsuperscript{12} (p=0.40). Eight antibiotic regimens were administered at varying frequencies for treatment and control of respiratory signs (Table 6). NAHMS\textsuperscript{12} reported 3 times more antibiotic regimens in use for the purpose of treating respiratory signs than what was observed in this study.

The efficacy of an antibiotic regimen was determined by comparing median nursery livability. Because route of administration was a proxy for antibiotic regimen and disease severity and clinical signs were determined to be risk factors for administration of an antibiotic by injection, the efficacy of antibiotic regimens was determined for each clinical sign, purpose of administration and disease severity. Antibiotic regimens were compared within grades of disease severity using the Kruskal-Wallis test of analysis of variance.
Median livability did not differ between antibiotic regimens for the purpose of controlling mild (p=0.148) or moderate (p=0.257) respiratory signs. No antibiotics were administered for the purpose of controlling severe respiratory signs. The mean, median and SD was calculated for livability of nurseries that had differing grades of respiratory signs and received an antibiotic for the purpose of treatment (Table 7).

Pairwise comparisons of antibiotic regimens were made using a Bonferonni adjusted p-value calculated using Wilcoxon rank sum test. Antibiotic regimens with <2 observations were not considered for pairwise comparisons of median livability. Median livability was higher for nurseries that received chlortetracycline in the drinking water compared to those that received chlortetracycline and neomycin in the drinking water but did not differ from the livability of groups that received chlortetracycline in the drinking water for treatment of mild respiratory signs (p=0.033). There was no difference among antibiotic regimens for treatment of moderate or severe respiratory signs (p>0.05).

**Discussion**

The average nursery livability (96.67%) was 0.27% higher than the National Animal Health Monitoring System (NAHMS) overall average\(^\text{13}\) and 0.57% higher than that for nursery sites >5000 head (p<0.001). For 8 months (May-December) 32 nursery sites were observed for antibiotic use. Antibiotics were administered on 81.25% during the study period. Three-quarters of sites received an oral antibiotic during the study period; this was not statistically different than the proportion of sites use oral antibiotics reported by NAHMS\(^\text{13}\) (p=0.196). Less than half of the nursery sites (40.63%) received an injectable
antibiotic; this was 42.77% lower than the proportion reported by NAHMS\textsuperscript{13} (p<0.001). This significantly lower proportion of sites observed to use injectable antibiotics as compared to the rest of the industry was most likely due to a risk management plan the integrator had adopted to reduce the chance for broken needles in their pork products.

Antibiotic administration to a group may be delayed until a morbidity threshold is reached\textsuperscript{14}. We measured morbidity for grades of disease severity for enteric, systemic and respiratory signs in 31.62% of nurseries. Since threshold for grades of disease severity is likely to be modified by referable signs, morbidity was reported by clinical signs. The threshold for all grades of disease severity was numerically lower for systemic disease compared to respiratory and enteric signs. Morbidity for systemic and respiratory signs was correlated with increasing grade of severity. The frequency of enteric signs that were medicated with antibiotics was 62 and 18% lower than the frequency of respiratory and systemic signs medicated during the same period. The infrequent use of antibiotics for enteric signs may have been because a prior diagnosis for enteric signs had been obtained that would not indicate the use of antibiotics or the signs went un-noticed. Morbidity was quantified for grades of disease severity to estimate the prevalence of signs that may need to be present before caretakers would administer an antibiotic. Since disease severity and morbidity were not determined for non-medicated barns, accuracy of the observations could not be determined.

When a population is ill or is at risk of becoming ill, antibiotics may be administered to the entire population. It is easier to medicate a population through oral rather than
parenteral routes\textsuperscript{15}. This is likely the reason 55.2% of antibiotic administrations occurred via
the drinking water and 44.8% occurred by injection. We found that the odds ratio for
receiving an antibiotic by injection increased with disease severity. Although severe disease
was not statistically associated with injection, this is due to the lack of non-respiratory signs
classified as severe. Among barns that were administered antibiotics, 68.4% received
antibiotics for respiratory signs and 31.6% for systemic or enteric signs. The frequency of
respiratory disease rather than the ease of administration is likely responsible for the
preference for delivery of antibiotics via water than injection.

Nursery barns had an increased odds of receiving antibiotics by injection when they
were $\leq$ 4 weeks post-weaning and when the purpose of administration was for the control of
disease. Since pigs immediately post-weaning are likely at the highest risk for developing
disease, the increased likelihood of injection with an antibiotic for disease control may be
modified by age. Further analysis using a multivariate logistic regression model is warranted
to determine causality.

Oral drug delivery runs the risk of non-uniform dosing, waste, and failure to reach
therapeutic concentrations\textsuperscript{16}. Antibiotic treatment of nursery pigs with injectable ceftiofur
for enzootic bacterial infection was found to improve livability compared to administration of
chlortetracycline plus sulfamethazine and amoxicillin through the drinking water\textsuperscript{10}. Like
Dorr\textsuperscript{16}, the authors hypothesized that the antibiotic regimens delivered in the water may not
have performed as well because of failure to reach therapeutic concentrations. In the current
study, we failed to observe higher livability for injectable antibiotic regimens used to treat or
control mild, moderate or severe respiratory signs when compared to antibiotics administered in drinking water. In fact, chlortetracycline resulted in the highest livability when treating mild respiratory signs and chlortetracycline plus tiamulin resulted in the highest livability when treating moderate respiratory signs. All antibiotic regimens administered to control respiratory signs resulted in equivocal livability. There was not a negative control group in this study but presumably livability would be lower in barns that were identified with respiratory signs but didn’t receive antibiotics11.

Evidence based medicine has provided the medical field with a framework to make informed clinical decisions17. By using evidence based medicine inform their selection of antibiotics, the swine veterinarian has followed the judicious use guidelines. Judicious use is only one aspect of antibiotic stewardship. If United States consumers and regulators no longer regard food animal veterinarians as good stewards of antibiotics, this could lead to antibiotic bans like those in the European Union which have resulted in increased mortality in pigs post-weaning and accompanying welfare concerns18.
Figure 1. Quasi-experimental study design used to identify risk factors for and measure effect of antibiotic use in a nursery barn. Each nursery barn was not randomly allocated to an antibiotic regimen. Risk factors for each nursery barn were determined at the group-level.
Table 1. Antibiotic regimens used to medicate nursery barns between May 2009 and January 2010.

<table>
<thead>
<tr>
<th>Via drinking water</th>
<th>By injection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlortetracycline</td>
<td>Ceftiofur crystalline free acid</td>
</tr>
<tr>
<td>Chlortetracycline/tiamulin</td>
<td>Enrofloxacin</td>
</tr>
<tr>
<td>Chlortetracycline/lincomycin</td>
<td>Tulathromycin</td>
</tr>
<tr>
<td>Chlortetracycline/neomycin</td>
<td></td>
</tr>
<tr>
<td>Lincomycin</td>
<td></td>
</tr>
<tr>
<td>Neomycin</td>
<td></td>
</tr>
<tr>
<td>Potassium Penicillin</td>
<td></td>
</tr>
<tr>
<td>Spectinomycin</td>
<td></td>
</tr>
</tbody>
</table>
Table 2. Nursery livability, GPD, and FG of non-medicated and medicated nursery groups. Mean, median and standard deviation (SD) for key performance indicators. Differing superscripts indicate statistical difference of medians, p ≤0.05.

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>Livability (%)</th>
<th>GPD (lbs)</th>
<th>FG</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>Median</td>
<td>SD</td>
<td>Mean</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>Median</td>
<td>SD</td>
<td>Mean</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>Median</td>
<td>SD</td>
<td>Mean</td>
</tr>
<tr>
<td>Non-medicated</td>
<td>455</td>
<td>97.10</td>
<td>97.66(^{a})</td>
<td>2.65</td>
</tr>
<tr>
<td>Medicated</td>
<td>206</td>
<td>95.72</td>
<td>96.63(^{b})</td>
<td>3.49</td>
</tr>
</tbody>
</table>
Table 3. Nursery livability, GPD, and FG for mild, moderate and severe grades of disease severity. Mean, median and standard deviation (SD) for key performance indicators. Differing superscripts indicate statistical difference of medians adjusted for multiple comparisons, p≤0.05.

<table>
<thead>
<tr>
<th></th>
<th>Livability</th>
<th>GPD</th>
<th>FG</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>Mean</td>
<td>Median</td>
</tr>
<tr>
<td>Mild</td>
<td>76</td>
<td>97.13</td>
<td>97.58&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Moderate</td>
<td>100</td>
<td>95.30</td>
<td>96.10&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Severe</td>
<td>27</td>
<td>93.10</td>
<td>93.99&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>
Table 4. Morbidity for enteric, systemic and respiratory signs of mild, moderate and severe grades of disease severity.

Mean, median, and standard deviation (SD) for morbidity. *value could not be calculated because <2 observations.

<table>
<thead>
<tr>
<th></th>
<th>Enteric</th>
<th></th>
<th></th>
<th></th>
<th>Systemic</th>
<th></th>
<th></th>
<th></th>
<th>Respiratory</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>Mean</td>
<td>Median</td>
<td>SD</td>
<td>N</td>
<td>Mean</td>
<td>Median</td>
<td>SD</td>
<td>N</td>
<td>Mean</td>
<td>Median</td>
<td>SD</td>
</tr>
<tr>
<td>Mild</td>
<td>1</td>
<td>8.59</td>
<td>8.59</td>
<td>*</td>
<td>6</td>
<td>1.64</td>
<td>0.67</td>
<td>2.14</td>
<td>38</td>
<td>5.34</td>
<td>4.16</td>
<td>4.16</td>
</tr>
<tr>
<td>Moderate</td>
<td>7</td>
<td>4.50</td>
<td>3.73</td>
<td>3.68</td>
<td>10</td>
<td>1.15</td>
<td>0.94</td>
<td>0.75</td>
<td>34</td>
<td>6.17</td>
<td>5.41</td>
<td>3.76</td>
</tr>
<tr>
<td>Severe</td>
<td>2</td>
<td>4.79</td>
<td>4.79</td>
<td>4.85</td>
<td>2</td>
<td>0.81</td>
<td>0.81</td>
<td>1.15</td>
<td>7</td>
<td>8.51</td>
<td>8.70</td>
<td>4.60</td>
</tr>
</tbody>
</table>
Table 5. Likelihood that a nursery receives an antibiotic by injection for different exposure variables. Odds ratio (OR), 95% confidence interval (CI) and p-value of Wald test calculated using logistic regression. * referent.

<table>
<thead>
<tr>
<th>Exposure</th>
<th>Levels of exposure</th>
<th>OR</th>
<th>95% CI</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weeks post-weaning</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;4 weeks</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>≤ 4 weeks</td>
<td>4.34</td>
<td>2.00, 10.00</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Severity of disease</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mild</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>Moderate</td>
<td>1.61</td>
<td>1.06, 2.45</td>
<td>0.026</td>
<td></td>
</tr>
<tr>
<td>Severe</td>
<td>2.31</td>
<td>0.95, 5.65</td>
<td>0.065</td>
<td></td>
</tr>
<tr>
<td>Purpose of administration</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatment</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>Control</td>
<td>3.44</td>
<td>1.96, 6.25</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Clinical signs</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Respiratory</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>Non-respiratory</td>
<td>3.20</td>
<td>1.75, 5.86</td>
<td>&lt;0.001</td>
<td></td>
</tr>
</tbody>
</table>
Table 6. Proportion of nursery barns that received antibiotics for respiratory signs by purpose of administration.

<table>
<thead>
<tr>
<th>Antibiotic active ingredient</th>
<th>Route of delivery</th>
<th>Overall use (%)</th>
<th>Purpose of administration (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlortetracycline</td>
<td>Water</td>
<td>8.5</td>
<td>1.5</td>
</tr>
<tr>
<td>Chlortetracycline/Tiamulin</td>
<td>Water</td>
<td>36.6</td>
<td>13.6</td>
</tr>
<tr>
<td>Chlortetracycline/Lincomycin</td>
<td>Water</td>
<td>14.1</td>
<td>24.2</td>
</tr>
<tr>
<td>Chlortetracycline/Neomycin</td>
<td>Water</td>
<td>2.8</td>
<td>3.0</td>
</tr>
<tr>
<td>Lincomycin</td>
<td>Water</td>
<td>1.4</td>
<td>-</td>
</tr>
<tr>
<td>Ceftiofur crystalline free acid</td>
<td>Injection</td>
<td>7.7</td>
<td>15.2</td>
</tr>
<tr>
<td>Enrofloxacin</td>
<td>Injection</td>
<td>28.2</td>
<td>40.9</td>
</tr>
<tr>
<td>Tulathromycin</td>
<td>Injection</td>
<td>0.7</td>
<td>1.5</td>
</tr>
</tbody>
</table>
Table 7. Livability for different antibiotic regimens used for the purpose of treating respiratory signs by grade of disease severity. Different superscripts indicate statistical difference of medians adjusted for multiple comparisons (p ≤ 0.05). *antibiotic not used; † value could not be calculated because <2 observations.

<table>
<thead>
<tr>
<th></th>
<th>Mild</th>
<th>Moderate</th>
<th>Severe</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>Mean</td>
<td>Median</td>
</tr>
<tr>
<td>Ceftiofur</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>Chlortetracycline</td>
<td>4</td>
<td>99.11</td>
<td>99.13a</td>
</tr>
<tr>
<td>Chlortetracycline/lincomycin</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>Chlortetracycline/neomycin</td>
<td>2</td>
<td>98.27</td>
<td>98.27b</td>
</tr>
<tr>
<td>Chlortetracycline/tiamulin</td>
<td>15</td>
<td>97.10</td>
<td>97.55ab</td>
</tr>
<tr>
<td>Enrofloxacin</td>
<td>1</td>
<td>96.35</td>
<td>96.35</td>
</tr>
<tr>
<td>Lincomycin</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
</tbody>
</table>
Footnotes

a. Excel, 2007, Microsoft

b. Statistix, version 9.0, Analytical Software, Tallahassee, FL
REFERENCES


CHAPTER V.

FACTORS ASSOCIATED WITH ANTIMICROBIAL RESIDUE LIMITS FOR PORK
Abstract

United States pork producers are engaged in international trade which has opened access to many value-added markets. These markets can require the product to meet residue limits that may be more stringent than domestic limits or Codex. This is position paper that examined the MRL for 12 commonly used antimicrobials in pork production by 24 World Trade Organization members and Codex. Seventeen countries had at least 1 antimicrobial MRL set lower than Codex. Countries with GNI greater than USD $12,745 were 10 times more likely to have a MRL lower than Codex. The association between MRL and withdrawal time for the 12 antimicrobials was evaluated. A total of 551 product labels containing 1 of the 12 antimicrobials for an active ingredient were reviewed for the route of administration and withdrawal time. For only 3 of the 12 antimicrobials was a longer withdrawal time associated with a lower MRL. Withdrawal times varied within and among countries for the same antimicrobial. In a global economy, pork producers and veterinarians will need to know which residue limits differ from their domestic limits. In some cases, a longer withdrawal time than that found on the antimicrobial label may have to be observed to avoid violating another country’s MRL.

Introduction

United States pork has been a global commodity since 1994. Free trade agreements have facilitated a global economy that promotes interdependence of trade partners, provides guaranteed access to their exports and has abolished tariff trade barriers. Such agreements are credited for increasing the value of US hogs by $63 US per head. Global exports of US
pork are forecast to increase 4% in 2015. The US exports one-quarter of all US pork produced. Therefore, it is incumbent on the pork producer, packer/processor wishing to sell product abroad to be knowledgeable of their foreign customer’s sanitary and phytosanitary standards which include residue limits for pesticides and veterinary drugs.

Maximum residue limits for pesticides and veterinary drugs are established based on a risk assessment that incorporates husbandry, drug approval, legislation, consumption patterns, cultural practices, methods of residue measurement, and calculations for estimating limits of a pesticide or drug. The formation of Codex Alimentarius established an international food code that includes maximum residue limits (MRL) for veterinary drugs. Although the Codex has established residue limits for most veterinary drugs, the adoption of these limits is voluntary under the World Trade Organization Sanitary and Phytosanitary Standards (SPS) agreement; each country may establish its own MRL as long as it is scientifically founded. The exporting producer, packer, or processor that wishes to export product into another market with a discrepant MRL is responsible for ensuring that levels of pesticide or drug used in or on the food product must not exceed the importer’s MRL. Violation of residue limits result in product rejection and up to an outright ban to market entry.

The lack of harmonization in residue limits has the potential to alter trade patterns. For example, when Russia lowered the tetracycline MRL in pork to 10 parts per billion, 20% lower than the Codex MRL and 200% lower than the US MRL, there was a 60% reduction in pork exports from the US to Russia. When no MRL is set for drug-product combination, the
sensitivity of the test used by the importing country’s food safety authority will dictate the residue limit making detection of the drug in the target tissue violative. Because violation of residue limits is grounds for an importer to deny access to their market, the adoption of residue limits below those of Codex must be scientifically supported otherwise it is known as a non-tariff barrier to trade. However, the cost, knowledge and facilities required to validate and implement more sensitive residue testing methods may not be possible for developing countries leading to non-tariff trade barriers.

In addition to regulatory impediments described, consumers concerned about food safety are influencing market trends. Consumers are increasingly expressing concerns about antimicrobial use in food animals leading to antibiotic resistant infections and chemical adulteration of meat. The risk of any negative health effect for a Dutch consumer resulting from consumption of pork containing a tetracycline residue was calculated to be 1 in 33 million to 1 in 133 million, making microbiological hazards of pork consumption greater than that caused by chemicals. Despite the low risk to consumers associated with residues in pork, a survey of Belgian consumers found they planned to decrease their pork consumption by 27% because of their perception that pork was not free of harmful substances.

It is the responsibility of the food producer to observe a waiting period after the last dose is observed, called a withdrawal time. Withdrawal times are calculated with the consideration of the residue limit. The drug withdrawal time printed on the label is that which should prevent violative residues in the country the antimicrobial is being sold.
Therefore, if producers/processors/packers wish to export a food product like pork, they must know what the importers’ MRL for the drug is and will have to observe a withdrawal time long enough not to exceed the import country’s MRL. Often, to comply with international standards, US pork producers must observe longer withdrawal times than those required for domestic marketing\textsuperscript{17}. For some drugs and pesticides, producers must forego their use entirely if a suitable withdrawal time to ensure the product is residue free cannot be determined.

To evaluate the implications of antimicrobial residue limits on international trade, we surveyed the publicly accessible databases for label information. Using the aggregated data, we conducted statistical analysis to investigate if a country’s antimicrobial MRL is associated with the following: 1.) the antimicrobial’s withdrawal time and 2.) the country’s income. In addition, we wanted to determine if harmonization with Codex affects trade in pork as it was found to in other animal products trade\textsuperscript{18,19}.

**Materials and Methods**

**Data collection**

The data were organized hierarchically (Figure 1). All information had to be available through publicly accessible, free-of-charge, on-line resources. The sample population was 159 World Trade Organization member countries. The antimicrobials were selected from the NAHMS, Swine 2006 Part II: Reference of Swine Health Management Practices in the United States\textsuperscript{20}. Any antimicrobial formulation for which a primary active ingredient could not be identified was censored resulting in a total of 12 antimicrobials.
Residue limits for selected antimicrobials were obtained from the USDA Foreign Agriculture Service MRL database\textsuperscript{a}. The database contained residue limits for the 12 antimicrobials for 24 of the 159 WTO member countries. The MRL is the lowest value found for pork or pork by-product and is reported in parts per million in Table 1.

The trade volume of pork for 16 of the United States’ major trade partners was queried from the USDA Production and Supply Database\textsuperscript{e} and reported in Table 2. Trade data are from calendar year 2013, which was the most recent full year available. Each country was categorized as either an importer or exporter. A pork exporter was defined as a country where the exit of pork was greater than entry while a pork importer is a country where entry exceeds the exit of pork in 1000 ton units.

An income category for each of the 24 WTO member countries was assigned. The GNI published by the World Bank\textsuperscript{f} for the 24 countries were used to categorize countries by income. Countries with a GNI less than $12,746 per capita are considered developing and are referred to as low income in this study. All countries with GNI above $12,745 per capita are classified as high income. The income description for each country is listed in Table 2.

The labels of approved antimicrobial products were queried for 5 of the 16 countries that had MRL, income and trade information. The regulating authority that approves veterinary drugs including antimicrobials is listed in Table 2. Each veterinary regulating authority had a database\textsuperscript{g, h, i, j, k, l, m} that could be searched for any drug containing 1 of the 12 selected antimicrobials as a single active ingredient and labeled for use in swine. Since only 2 of the 12 antimicrobials for use in swine have been approved by the European Medicines
Agency, labels approved in Spain served as a proxy for EU members. Each product containing 1 of the 12 antimicrobials that had a unique registration number received an individual entry. The product was only entered if the label stated it was for use in swine. Label information of interest was withdrawal time (in days), route of administration (feed, water or oral suspension, and parenteral injection), purpose (treatment, control or prevention, growth improvement or a combination of these), and drug concentration.

**Statistical analysis**

Statistical analysis was conducted with standard statistical software. Because the data sets are small and do not follow a normal distribution, distribution free analysis was used and medians, rather than means, were reported. The Wilcoxon rank sum test was used to compare medians. An OR and 95% CI were used to estimate the effect and the precision of the effect measure for a potential risk factor. Residue limits equal to and greater than Codex were grouped based on findings from previous studies where harmonizing a MRL with Codex as well as increasing the MRL both increased trade. All hypothesis tests were evaluated at a 5% significance level; p-values are Pearson’s chi-square providing there were 5 or more observations per cell, otherwise p-values calculated using Fisher’s exact test are reported.

**Results**

**How do selected country’s withdrawal times compare to Codex?**

The MRL of the 24 countries for the 12 antimicrobials and the relationship to Codex are summarized (Table 2). All markets listed a MRL for bacitracin, carbadox, penicillin,
tiamulin and tulathromycin that was either harmonized to (equal) or less stringent (greater) than the Codex standard. Chlortetracycline and oxytetracycline had the most MRLs (43.75%) that were more stringent (lower) than Codex.

The median proportion of MRLs that were harmonized with or were less stringent than Codex was 91.29% (45.5, 100 95% CI). Less than one-third (29.17%) of countries had MRLs harmonized to Codex (Table 2). The United States was the only country to have more than half (58.33%) of its MRLs less stringent than Codex.

There were 54.17% of countries with at least 1 MRL less than Codex and 45.83% with >1 MRL more stringent (less) than Codex. Russia, South Africa and Brazil had set 54.55% of the MRLs more stringent than Codex. Mexico had the greatest proportion of MRLs (66.67%) that were more stringent than Codex standards. The probability of a country having multiple MRLs more stringent than Codex was 84.62% if the country had at least 1 MRL more stringent than Codex.

*Are trade and income associated with stringency of MRLs?*

When the proportion of a country’s MRLs that were equal to or greater than Codex was less than the median proportion of MRLs equal to or greater than Codex, the country was considered to have generally more stringent MRLs. A high income country was 10 times more likely to have more stringent MRLs than Codex than a low income country (1.45, 69.18 95% CI; p=0.019). There was no association of pork exports with MRL stringency (p=0.398).
Is length of antimicrobial withdrawal time associated with its MRL?

For the 5 countries for which label information was queried, there were a total of 574 registered products. There were 29 registered products (24 in Canada and 1 in Australia) that did not have complete label information (6 were missing concentration and 23 were missing WDT). Since the EU licensed only 2 products total, Spain served as a proxy for the EU member states. Spain had registered approximately twice as many products (194) as Australia (107), Canada (107) and US (93) and registered almost three times as many products as Chile (71). Slightly more than one-quarter (27%) of all registered products listed oxytetracycline as the active ingredient. Amoxicillin, tylosin and tiamulin accounted for a combined one-third (33%) of registered products. The remaining 40% of registered products contained bacitracin, carbadox, ceftiofur, chlortetracycline, lincomycin, penicillin, tilimicosin or tulathromycin as the active ingredient.

Forty percent of registered products were labeled for parenteral injection which was the most common route for administration that a product was registered (Figure 3). Spain and Chile registered the most injectables for the antimicrobials queried (Figure 4). Ceftiofur and tulathromycin were the only antimicrobials registered only for injection. The median withdrawal time for injectables is 16 days which was longer than that for products labeled for administration through water or feed, which is 5 days (Z=2.394, p<0.001).

Approximately one-third (33.62%) of registered products were for administration in the feed (Figure 3). Growth promotion as a reason for administration was only found on labels for antimicrobials administered in the feed. There were 60 products with a registered
There were 6 antimicrobials (amoxicillin, ceftiofur, lincomycin, tiamulin, and tulathromycin) that did not have growth promotion as a reason for use on the product label even though 3 of the antimicrobials (amoxicillin, lincomycin and tiamulin) are labeled for administration in the feed. Only 3 (Australia, Canada and US) of the 5 countries had labels with growth promotion as a reason for use. The US was the only country to register a product for all 6 of the antimicrobial products that had a growth promotion label and was the only country to register carbadox and penicillin for growth promotion.

Antimicrobial WDT was found to be discordant within and among countries. Despite stratification by route of administration of an antimicrobial, each country had at least 1 WDT where the median differed from the range (Tables 4-6). Lincomycin for injection was the only antimicrobial where the median and range within country was the same.

An increased odds of a country having a longer withdrawal time for an antimicrobial when its MRL for the same antimicrobial was lower than other countries was found to exist only for oxytetracycline (Table 7). A statistical association between a lower MRL and a longer withdrawal time was found only for tiamulin and tulathromycin; however, an odds ratio could not be calculated due to empty cells in the contingency table.

Discussion

Similarities and differences between MRLs for the US, EU and Codex have been previously published\textsuperscript{15}. However, this earlier comparison was of MRLs for beef liver and it is unclear how the authors decided which antimicrobial MRLs to compare. To our
knowledge, this is the first report that compares MRLs for the 12 most commonly used antimicrobials in post-weaning pigs in the United States and estimates odds ratios for exposure variables.

The positive association between increasing a MRL on the trade value e.g. exports for various commodities has been previously reported\textsuperscript{3,18,19}. When the residue limit for oxytetracycline was reduced by 1 unit, the trade in crustaceans was reduced by 170%\textsuperscript{18}. Farnsworth\textsuperscript{3} determined that greater public health expenditures, percent of agriculture imports and GDP per capita most consistently explained stricter MRLs. Here we report that there is an association between income and the stringency of the MRL. Like that observed for fruits and vegetables, we found that countries with higher incomes were more likely to have MRLs more stringent than Codex. Although the confidence interval for the odds ratio of income to unfavorable MRLs indicates some imprecision in the estimate, the lower limit does not cross 1 indicating that the effect observed is accurate.

Contrary to Farnsworth’s findings\textsuperscript{3}, we found that there was an increased odds of having more stringent MRLs if a country is an exporter. However, the association was not significant (p>0.05) and the lower limit of the confidence interval of the odds ratio crossed 1 suggesting that the odds ratio observed is not an accurate measure of the effect. There was not effect of trade flow on the stringency of MRLs for pork found in this analysis.

A pattern of stricter MRLs than those of Codex at the commodity and at the country-level was not previously found\textsuperscript{3}. In the current analysis, we found an increased probability of a country having multiple MRLs more stringent than Codex if at least 1 of the 12
antimicrobial MRLs was more stringent than Codex. It is likely that the United States has the greatest proportion of MRLs that are less stringent e.g. greater than Codex because the US actually uses a tolerance level rather than a MRL. The tolerance is calculated based on a marker residue (parent drug or key metabolite) rather than the total amount of residue (parent drug and its metabolites). Further discussion about the methods used to calculate tolerances and MRLs is beyond the scope of this discussion.

A potential pitfall of this analysis is our use of epidemiologic methods to compute effect estimates. The use of a gravity model would have allowed for determination of the effect of free trade agreements, distance between countries, colonial ties and other variables over time to be measured. However, a gravity model is an econometric model and beyond the scope of this study.

Because withdrawal times are calculated with consideration of MRLs, it seems reasonable that if a country’s MRL is lower than another’s then the withdrawal time for that antimicrobial in that same country is also lower than that in the other country. This tended (p<0.20) to be the case for chlortetracycline, lincomycin and oxytetracycline. In each of these cases, there was an increased odds of a longer withdrawal time when the MRL was lower than another country. The lower limit of the confidence interval for oxytetracycline was the only limit that did not cross 1 indicating the effect measure is accurate for the stated exposure-outcome relationship for this antimicrobial. For ceftiofur and tylosin a protective effect (or decreased) odds of a longer withdrawal time was observed. A possible explanation for the decreased odds for ceftiofur is most likely due to the formulation\textsuperscript{10}. An effect
measure could not be calculated for half of the antimicrobials (amoxicillin, bacitracin, carbadox, penicillin, tiamulin, and tulathromycin) because of too few observations.

The proportion of countries with all antimicrobial MRLs harmonized to Codex is less than one-third of those surveyed. All of these countries were not high income. Only one, Vietnam, was not an importer. Concerns that lower income and developing countries may be disproportionately affected when MRLs are more stringent than Codex was observed here for pork. This may not only occur because of the increased rigors of testing but the inability to find data to estimate acceptable residues. The databases from which these data were compiled are incomplete. There were 23 registered products without a withdrawal time—22 Canada and 1 Australia. There were 24 products with no purpose of use—19 Canada, 4 Chile, 1 Spain. There were 6 products with no concentration—3 United States, 2 Chile, 1 Spain. Such information is necessary to determine if a domestic withdrawal time can be extrapolated to achieve an international MRL.\(^{10}\)

Despite consumer fears about the harmful residues in meat, the incidence of residues in meat products is less than 1%\(^{21}\). The prevalence of antimicrobial residues in pork is even lower. Denmark estimates residues are present in 0.01% of its slaughter pigs\(^{22}\). From July to September 2014, the USDA found \(<0.1\%\) of the 5152 domestic slaughter pigs tested to have violative residues\(^{23}\). Therefore, despite the lack of harmonization in antimicrobial residue limits and the absence of a uniform relationship between residue limits and withdrawal times, pork is a safe source of protein.
Figure 1. Diagram of hierarchical data collection and points of analysis.
Figure 2. Percentage of 574 products labeled for use in swine by selected countries registration authority by active ingredient and route of administration.
Figure 3. Percentage of total approved products containing 1 of the 12 selected antimicrobials as its single active ingredient by route of administration for selected countries.
Table 1. MRL of selected antimicrobials for Codex and selected countries. No shading = greater (less stringent) than Codex; Light shading = equal to Codex; Dark shading = less (more stringent) than Codex; Black = No value reported

<table>
<thead>
<tr>
<th></th>
<th>Amoxicillin</th>
<th>Bacitracin</th>
<th>Carbarsox</th>
<th>Cefotiofur</th>
<th>Chlor-tetracycline</th>
<th>Lincomycin</th>
<th>Oxy-tetracycline</th>
<th>Penicillin</th>
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<th>Tilmicosin</th>
<th>Tular-thromycin</th>
<th>Tylosin</th>
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</table>
Table 2. Regulating authority for veterinary drugs in selected countries.

<table>
<thead>
<tr>
<th>Income group</th>
<th>Total pork imports (1000 tons)</th>
<th>Total pork exports (1000 tons)</th>
<th>Setting veterinary drug standard</th>
</tr>
</thead>
<tbody>
<tr>
<td>Argentina</td>
<td>Upper middle</td>
<td>18</td>
<td>1</td>
</tr>
<tr>
<td>Australia</td>
<td>High</td>
<td>183</td>
<td>36</td>
</tr>
<tr>
<td>Brazil</td>
<td>Upper middle</td>
<td>1</td>
<td>585</td>
</tr>
<tr>
<td>Canada</td>
<td>High</td>
<td>221</td>
<td>1246</td>
</tr>
<tr>
<td>Chile</td>
<td>High</td>
<td>51</td>
<td>164</td>
</tr>
<tr>
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<td>Spain</td>
<td>*</td>
<td>*</td>
<td>*</td>
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</tr>
<tr>
<td>Honduras</td>
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</tr>
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<tr>
<td>Japan</td>
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</tr>
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<td>Korea, Rep.</td>
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<td>388</td>
<td>2</td>
</tr>
<tr>
<td>Mexico</td>
<td>Upper middle</td>
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<tr>
<td>New Zealand</td>
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<td>Panama</td>
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<tr>
<td>Philippines</td>
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<td>Vietnam</td>
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</table>

* not listed; ♩ could not be determined.
**Table 3. Antimicrobials with a label for growth promotion in selected countries.** *All products are labeled for administration in feed; † No registered products in that drug class.*

<table>
<thead>
<tr>
<th>Antimicrobial</th>
<th>Australia</th>
<th>Canada</th>
<th>United States</th>
<th>Total</th>
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<td>Bacitracin</td>
<td>†</td>
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<td>4</td>
<td>7</td>
</tr>
<tr>
<td>Carbadox</td>
<td>†</td>
<td>†</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Chlortetracycline</td>
<td>†</td>
<td>5</td>
<td>5</td>
<td>10</td>
</tr>
<tr>
<td>Oxytetracycline</td>
<td>†</td>
<td>8</td>
<td>3</td>
<td>11</td>
</tr>
<tr>
<td>Penicillin</td>
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<td>†</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Tylosin</td>
<td>13</td>
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<td>8</td>
<td>29</td>
</tr>
<tr>
<td><strong>Total</strong></td>
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<td><strong>24</strong></td>
<td><strong>23</strong></td>
<td><strong>60</strong></td>
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</table>
Table 4. Median and range (in days) of WDT for selected antimicrobials labeled for administration by injection in selected countries*.

*There are no injectable preparations of bacitracin, carbadox, chlortetracycline, or tilimicosin labeled for swine by selected countries regulating authority; †No registered products in that drug class; ‡Median and range for withdrawal time is the same; §Only 1 product registered; ‖No withdrawal time found on label; ¶Spain used as a proxy for all EU member states.

<table>
<thead>
<tr>
<th>Country</th>
<th>Amoxicillin</th>
<th>Ceftiofur</th>
<th>Lincomycin</th>
<th>Oxytetracycline</th>
<th>Penicillin</th>
<th>Tiamulin</th>
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<td>2‡</td>
<td>18 (10, 42)</td>
<td>5 (5, 7)</td>
<td>14§</td>
<td>14§</td>
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<td>‖</td>
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<td>2‡</td>
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<td>8 (5, 10)</td>
<td>9§</td>
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<td>†</td>
<td>28 (12, 35)</td>
<td>8.5 (7, 10)</td>
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<td>14 (14, 21)</td>
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<td>6‡</td>
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<td>10.5 (6, 15)</td>
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<td>2‡</td>
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<td>5‡</td>
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Table 5. Median and range (in days) of WDT for selected antimicrobials labeled for administration by water in selected countries*. *There are no water soluble preparations of carbadox, ceftiofur, or tulathromycin labeled for swine by selected countries regulating authority; †No registered products in that drug class; ‡Median and range for withdrawal time is the same; §Only 1 product registered; ||Spain used as a proxy for all EU member states.

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<tr>
<th>Country</th>
<th>Amoxicillin</th>
<th>Bacitracin</th>
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<th>Lincomycin</th>
<th>Oxy-tetracycline</th>
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<th>Tiamulin</th>
<th>Tilmicosin</th>
<th>Tylosin</th>
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<td>†</td>
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<td>2‡</td>
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<td>†</td>
<td>6§</td>
<td>8.5 (7, 10)</td>
<td>†</td>
<td>7‡</td>
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<tr>
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Table 6. Median and range (in days) of WDT for selected antimicrobials labeled for administration in feed by selected countries*. *There are no feed-grade preparations of ceftiofur or tulathromycin labeled for swine by selected countries regulating authority;
†No registered products in that drug class; ‡Median and range for withdrawal time is the same; §No withdrawal time found on label; ||Only 1 product registered; ¶Spain used as a proxy for all EU member states.

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<th>Country</th>
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<th>Chlor-tetracycline</th>
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Table 7. Likelihood of a longer withdrawal time when antimicrobial has a lower MRL.

Odds ratio (OR), 95% confidence interval (CI) and p-value (chi-square or Fisher’s exact).

*Value could not be calculated due to 1 or more cells equal 0.

<table>
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<tr>
<th>Antimicrobial</th>
<th>OR (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
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<tr>
<td>Ceftiofur</td>
<td>0.22 (0.1, 3.97)</td>
<td>0.524</td>
</tr>
<tr>
<td>Chlortetracycline</td>
<td>13.50 (0.88, 207.24)</td>
<td>0.077</td>
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<tr>
<td>Lincomyin</td>
<td>9.50 (0.68, 131.87)</td>
<td>0.123</td>
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<tr>
<td>Oxytetracycline</td>
<td>4.76 (0.95, 23.86)</td>
<td>0.050</td>
</tr>
<tr>
<td>Tiamulin</td>
<td>*</td>
<td>0.030</td>
</tr>
<tr>
<td>Tilmicosin</td>
<td>2.67 (0.35, 20.50)</td>
<td>0.329</td>
</tr>
<tr>
<td>Tulathromycin</td>
<td>*</td>
<td>0.001</td>
</tr>
<tr>
<td>Tylosin</td>
<td>0.81 (0.16, 4.19)</td>
<td>0.804</td>
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Footnotes


m. http://www.accessdata.fda.gov/scripts/animaldrugsatfda/

n. Statistix, version 9.0, Analytical Software, Tallahassee, FL
REFERENCES


CHAPTER VI.
DISCUSSION
Antibiotic stewardship is achieved by judicious antibiotic use that minimizes the development and spread of antibiotic resistance microorganisms. To do so, results from experimental and epidemiologic studies as well as clinical knowledge must be applied to formulate a program ensures best outcomes for patients. The thesis demonstrated how following an antibiotic stewardship program can ensure a healthy pig and a wholesome pork product.

We conducted a clinical trial to evaluate quaternary benzo(c)phenanthridine alkaloids (QBAs) as a potential non-antibiotic for growth promotion. We also examined gastrointestinal health because that is another proposed benefit of using antibiotics at growth promotant levels. We determined that feeding of QBAs improved growth, intestinal health, and pathogen elimination among nursery pigs similar to feeding of chlortetracycline at the growth promoting level. This was a significant finding because the use of medically-important antibiotics like chlortetracycline for growth promotion will be banned as of January 1, 2017 in the United States.

Food animal veterinarians apply evidence to improve outcomes in animal populations. This makes epidemiologic methods particularly well suited to measure benefits of antibiotics to swine health. Using a quasi-experiment, we showed that age, disease severity, reason for administration and clinical signs were risk factors for injectable antibiotic use. In addition, we found livability of groups displaying and being treated for respiratory signs varied within disease severity by antibiotic regimen. Antibiotics delivered by injection did not increase livability compared with those delivered in the drinking water for any disease severity grade. We showed that observational data can be successfully used to evaluate antibiotic efficacy in nursery pig populations.
Safe concentrations of antibiotic residues have been established for pork and pork products worldwide. A survey of the United States’ major trade partners was conducted. Maximum residue limit (MRL) for the same antibiotic varies between countries and Codex. Income was a risk factor for a MRL being set lower than the Codex standard. A lower MRL was not associated with a longer withdrawal time for all antibiotics. Antibiotics that shared the same route of delivery had variable withdrawal times not only between, but also within country. The survey was limited by information availability.

Evidence must continually be updated to ensure that antibiotic stewardship programs are being fulfilled. Information on the effect of duration of therapy on livability is needed. Total volume and average daily dose of antibiotics in different phases of swine production should be considered. Risk communication and transparency will have to improve between pork importers and exporters to protect consumers and market access.
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