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Swine pathogen transmission: recognition, monitoring, and prevention

Alejandro Ramirez

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Swine pathogen transmission: recognition, monitoring, and prevention

by

Alejandro Ramirez

A dissertation submitted to the graduate faculty
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

Major: Veterinary Microbiology (Preventive Medicine)

Program of Study Committee:

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Iowa State University

Ames, Iowa

2011

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CHAPTER 1. GENERAL INTRODUCTION

Introduction

The goal of the swine industry is to profitably produce a consistent, high quality, wholesome, safe product. According to data from the USDA Foreign Agricultural Service in 2008, pork is the world’s most widely eaten meat compromising 40% of the meat consumed worldwide. The U.S. is the second largest pork producing country in the world, after China. In 2009, approximately 10,446,000 metric tons of pork, 10% of the world’s production, was produced in the U.S. However, the U.S. swine industry has undergone dramatic changes in the last two decades. Some of these changes have included a declining number of producers, increasing herd sizes, increasing sow productivity and farm specialization. In addition, emerging or re-emerging diseases have been recognized.

With changes in the structure of the swine industry there have also been changes in the roles of swine veterinarians. Swine veterinarians today focus more on preventive medicine and improving overall herd health rather than responding after disease occurs, the latter common in traditional “fire engine” practices of 20 years ago. Swine veterinarians now have a proactive role in anticipating problems and preventing disease while a responsibility to provide care to each pig. This is a challenge as resources (money and labor) are becoming more and more limited. Consequently, swine veterinarians are highly motivated to be innovative by utilizing modern technology, epidemiologic principals, biostatistics and improved diagnostic methods to better guide them through the prioritization and allocation of resources to improve the health and welfare of pigs.
One of the challenges of swine veterinary practice is that technology and disease diagnostics evolve at a faster pace than the knowledge can be applied. Considerable effort is required to allow for full interpretation of the clinical meaning and implications of new diagnostic tools. Research advancements and detection methods provide veterinarians the diagnostic tools to seek and detect the presence of “new” agents (porcine circovirus type 2, Torque teno virus genogroups 1 & 2, influenza A virus, etc.). A dilemma emerges when “new” agent(s) are detected but the context and clinical significance are not known. The question that follows is: So what now?

Historically, veterinarians often would look for a single pathogen and then interpret all of the clinical findings or pathology based on the presence or absence of the pathogen of interest. This process can lead veterinarians to over-attribute significance of a particular pathogen, or perhaps under-attribute the significance of others. A good example is the identification of porcine circovirus type 2 (PCV2) in the U.S. swine population. In the late 1990s, a new technology for serologic testing for specific antibody for PCV2 was developed; this tool allowed veterinarians to test for PCV2 infection. Many veterinarians in the field seized the opportunity to test for yet another “new” agent. The test results consistently demonstrated that most, if not all, pigs tested positive for PCV2. What did that mean? Was this a real pathogen? We now know the rest of the story as the Canadian and U.S. swine industries suffered a major disease epidemic with severe economic and welfare consequences starting in 2005 with the introduction of a new subtype of PCV2. The swine industry was not prepared.

Ironically, veterinarians in the U.S. swine industry did not give credence to the
occurrence of a severe disease epidemic which occurred in Europe in early 2000s. The Europeans described a post-weaning multisystemic wasting syndrome (PMWS) and associated it with PCV2. Because a lack of experience with PMWS and over-confidence in the new PCV2 serology technology, veterinarians in the U.S. ascribed the European disease experience to their pig-rearing methods or other diseases rather than a vigilant acknowledgement of an emerging viral infection. The U.S. swine industry and veterinarians had a diagnostic tool to diagnose an infection but it was not well-understood and, consequently, ignored a real threat through over-confidence in a limited technology. Technology had outpaced our knowledge. United States veterinarians were still thinking within a framework of Koch’s postulates and failed to recognize variation in virulence. The concepts of variation and accumulated evidence were not given serious consideration.

Swine veterinarians are not only concerned about pig health and welfare, but also need to be vigilant for issues that impact human health. Concerns are not only from a food safety standpoint, but also regarding public health and worker safety. The “industrialization” of livestock production occurred simultaneously with fewer people engaged in agriculture. Uninformed, often urban, populations become disconnected from agriculture and modern production methods. The lack of understanding and misinformation of news reports creates the sense that the consolidation of animal agriculture is causing more public health concerns. One of these is the concern that pigs can serve as the mixing vessel for influenza A viruses (IAV). Before 2009, the fear was that the next pandemic flu was going emerge from pigs and that the Midwest was at high risk because of the large concentration of pigs. There were also fears that the raising of large number of pigs in a small area would also promote the
development and propagation of a new pandemic strain of IAV. To date, there is little evidence to substantiate these fears and beliefs.

All veterinarians take an oath which states: “Being admitted to the profession of veterinary medicine, I solemnly swear to use my scientific knowledge and skills for the benefit of society through 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.” Public health is a veterinarian’s responsibility bound by oath. In practice this means veterinarians need to understand public concerns, issues or implications and take a proactive role. This is not without challenges in implementation or interpretation. The example of the recent pandemic IAV illuminates concerns at multiple levels. If pandemic IAV is found in swine, is it a threat for workers? What is the impact of detection on ability to market swine? How will regulators, public health officials and the public react to bits of information that may not have context? Indeed, there are risks to detecting agents or infections without context to accurately interpret impact or consequences.

On the other hand, a veterinarian’s oath is “to use scientific knowledge and skills for the benefit of society through the protection of animal health and welfare”; that is, if there is something important to find then we (veterinarians and swine industry) need to find it. Swine veterinarians value scientific information. Swine veterinarians are proactive in research in academic settings and in the field which allows continuous improvement of understanding through proper design of trials and correct interpretation of the significance of the findings. Ultimately, this will lead to a better understanding of intervention steps which
can help mitigate or ameliorate identified areas of concern. The goal is not just to find something, but rather find something and figure out what to do about it.

The most economically severe of all swine diseases, porcine reproductive and respiratory syndrome virus (PRRSV), continues to plague swine world-wide. Swine veterinarians lost some of their credibility with swine producers in the mid-1990s because, once again, technology outsmarted us. Initially, veterinarians classified herds as either positive or negative and matched herd health status based on a simple “yes or no.” Serology was used to identify which animals were infected and/or shedding virus versus those animals which presumably had sterilizing immunity, were not infected, and posed no risk to the rest of the herd. The repeated failure of diagnostic testing to accurately predict outcomes in the field compromised perceptions of profession competence. Today, based on ongoing discovery and research, swine veterinarians change tests, test strategies and interpretation in order to adapt to new knowledge and new situations. Concepts long-engrained in swine producers must also be changed based on new research findings. Although swine producers are right to question what swine veterinarians do, the profession remains committed to seeking and providing solutions for the elusive PRRSV.

Dr. Calvin Schwabe, in 1982, wrote an editorial in *Preventive Veterinary Medicine* which he labeled the lack of "economically and scientifically viable approaches to the new practice of intensive livestock production" as a "crisis" for preventive veterinary medicine. Perhaps recognizing the complexity of the health dynamic involved, he envisioned a future in which veterinary practice would be "a form of on-going on-farm research based upon surveillance ...." Yet, he was not privy to innovations yet to come.
In human diagnostic medicine, oral fluid specimens collected using simple, non-invasive procedures are used for the detection of a wide variety of infectious agents, hormones, toxins, and drugs. It was a stroke of ingenuity and innovation which allowed a team of researchers at Iowa State University (Zimmerman lab) to explore the application of this “new” diagnostic specimen in swine populations. The ease of collection and diagnostic value of specimens collected in this manner has provided increasing momentum for adoption. Recent research has shown that oral fluid specimens can be used to monitor the circulation of PRRSV, PCV2 and IAV in swine populations using polymerase chain reaction (PCR) assays. Oral fluid is increasingly being used to measure specific antibodies as surrogate for serum. This is providing veterinarians with valuable and practical tools with which to economically capture a large amount of information that can help us better understand pathogen excretion patterns and thus better understand disease ecology and transmission dynamics.

As veterinarians build on the knowledge and experience of the past and the increasingly complex technologies and innovations, basic concepts and questions must remain at the forefront. We need to obtain a better understanding of pathogen transmission (especially within-herd transmission) so that proper intervention practices may be developed and implemented mitigating exposure or the consequences of exposure (biological risk management). Preventive veterinary medicine is the key! To achieve this, it does require the development of newer and better technology practices that can maximize information collection more efficiently and cost effectively. As these new technologies become available (oral fluid surveillance), we will all need better training in the methodology and statistical interpretation of interactions with multiple infectious agents and
risk factors over time. This all needs to be done while still ensuring our industry continues to produce wholesome and safe food, provides for the welfare of animals, and ensures the safety of workers (zoonosis).

**Dissertation Organization**

This dissertation is prepared in an alternate manuscript format. It is composed of six chapters including the introduction, three separate scientific manuscripts, a literature review, and a general conclusion. One of the manuscripts has been published, one has been submitted, and one will be submitted with the intent for all of these chapters to be published in refereed scientific journals. The last chapter that includes the literature review has been submitted for peer review and is intended to be published on-line but not through a scientific journal. The Ph.D. candidate, Alejandro Ramirez is the primary author of all the manuscripts described.

The first manuscript describes the detection of possible transmission IAV from pigs to swine confinement workers and associated risk factors for seroconversion. This manuscript was published in the *Emerging Infectious Diseases* journal. The second manuscript estimates the within-herd rate of transmission of PRRSV and associated risk factors. This paper will be submitted for publication in the *Journal of Swine Health and Production*. The third manuscript proposes a new model for using oral fluid samples to monitor pig populations over time, more frequently, and efficiently. This paper has been submitted to *Preventive Veterinary Medicine* for publication consideration. The final paper
summarizes the current knowledge and understanding regarding biosecurity principles in swine operations and introduces the concept of biological risk management focused on routes of transmission. This paper has been submitted to the National Pork Board for peer review and future publication and dissemination to the swine industry (veterinarians and producers).
CHAPTER 2. PREVENTING ZOONOTIC INFLUENZA VIRUS INFECTION

A paper published in

*Emerging Infectious Diseases* 12:997-1000, 2006

Alejandro Ramirez, Ana W. Capuano, Debbie A. Wellman, Kelly Lesher, Sharon F. Setterquist, and Gregory C. Gray

Abstract

We evaluated 49 swine workers and 79 non-exposed controls for antibodies to swine influenza viruses. Multivariate modeling revealed that workers who seldom used gloves (OR=30.3) or who smoked (OR=18.7) were at highest risk of having evidence of previous H1N1 swine virus. These findings have value in pandemic influenza planning.
Introduction

In the United States alone, influenza viruses are estimated to annually cause 36,000 human deaths and 200,000 hospitalizations [1-5]. The current outbreaks of avian influenza in Asia and Eastern Europe remind us of the potential for these viruses to be zoonotic. As swine express sialic acids that can serve as receptors for swine, human, and avian influenza strains they are important in cross-species influenza transmission and the genesis of novel influenza strains. Previously reported human-to-swine and swine-to-human influenza transmission illustrate this potential [6,7].

Individuals who work in enclosed livestock buildings (confinement workers) are among the human populations with the highest risk of swine influenza virus infection. Their work involves close contact with large numbers of swine, and the frequent handling of sick pigs. The purpose of this cross-sectional study was to learn if these workers had evidence of previous swine influenza virus infection, and if so, to determine risk factors which cause them to be at infection risk.

The Study

Iowa is the United States’ number one swine-producing state, marketing an estimated 25 million hogs a year. From November 2004 to March 2005, we recruited confinement workers. Site selection was based upon the authors’ community contacts and opportunities to invite confinement workers to participate. Local veterinary clinics helped advertise the study and permitted enrollment at their facilities. This study was approved by the University of
Iowa’s institutional review board.

Workers were eligible to participate if they had worked in a swine confinement within the past 12 months prior to enrollment. Participants completed a questionnaire and permitted sera collection upon enrollment. The questionnaire captured demographic, medical, and occupational data including influenza immunization history, swine occupational exposures, and use of protective equipment (gloves and masks).

Non-exposed controls were enrolled during a concurrent study from among University of Iowa faculty, staff, and students [8].

Serum samples were studied using a hemagglutination inhibition (HI) assay against two recently circulating swine and one human influenza virus strains: A/Swine/WI/238/97 (H1N1), A/Swine/WI/R33F/01 (H1N2) and A/New Caledonia/20/99 (H1N1). The swine H1N1 strain represents a lineage of virus that has been circulating among US swine populations for the past 70 years. The swine H1N2 strain first appeared in the US swine populations in 1999. HI titer results are reported as the reciprocal of the highest dilution of serum that inhibited virus-induced hemagglutination of a 0.65% solution of guinea pig red blood cells for human influenza and 0.5% solution of turkey red blood cells for swine influenza.

Specimen laboratory results were studied for their statistical association with demographic, immunization, occupational, and other behavioral risk factors. Confinement workers were queried regarding the type of confinement work as well as the use of protective equipment. Because higher titers had low or no incidence in most of the population groups,
H1N1 titers above 1:10 were grouped. The resulting categories were <1:10, 1:10, and >1:10. Wilcoxon rank sum, as well as Chi-square statistic or two-sided Fisher’s exact test were used to access bivariate risk factor associations. Depending upon the nature of the data and modeling assumptions, proportional odds modeling or logistic regression was used to adjust for multiple risk factors. Final multivariate models were designed using a saturated model and manual backwards elimination. Analyses were performed using SAS software version 9.1 (SAS Institute, Inc., Cary, NC).

Questionnaires were individually completed by participants and made available in both English and Spanish versions. Site selections were based on personal contacts in three completely different geographic areas.

Results

Forty-nine confinement workers and 79 non-exposed controls were enrolled in the study. The distribution of ages was similar for the two groups but the confinement workers were more likely to be male and Hispanic, and less likely to have received influenza vaccination (Table 1).

Swine confinement workers were categorized by type of confinement work, frequency of contact with swine, use of gloves and use of masks. Among these, the gloves use question “When working with sick or diseased swine how often do you wear gloves?” explained the most variation in swine H1N1 antibody titers and was included in the best fit model. Workers who never or sometimes use gloves had markedly higher odds of elevated titers against swine H1N1 virus (OR=30.3, 95% CI=3.8-243.5) compared to the non-exposed
controls (Table 2). This group was also at statistically significant increased odds when compared to the other confinement workers who use gloves most of the time or always (OR=12.7, 95% CI=1.1-151.1 – data not shown). Subjects who reported smoking similarly had the highest odds of elevated titers against swine H1N1 virus.

Multivariate analysis also identified subjects who received the 2003-2004 flu shot as having significantly higher odds of elevated titers (≥1:10) against swine H1N1 virus (Table 2) as well as swine H1N2 (data not shown). Although cross-reaction with one of the viruses in the 2003-2004 vaccine or a circulating flu virus is a possible explanation, higher titers for all vaccinated subjects would have been expected (including controls) but not observed (Figure 1). We postulate this result represents other behavior or health related confounders not identified in the questionnaire of this study.

**Conclusions**

In concert with other swine occupational studies [8-10], these data suggest that swine confinement workers are at increased risk for zoonotic influenza infection. However, these are some of the first data to evaluate swine confinement workers, and our sample size was small, not likely representative of all swine workers, and exposure data were self-reported. Confinement workers, in contrast to other swine occupations, have proven to be difficult to reach, due to language barriers, on-farm policies regarding visitors (biosecurity protocols), and lack of trust in the public health sector.

Several studies have previously document smoking as a risk factor for human influenza virus infection [11-13]. However, we believe our data are the first evidence that
smoking also increases the risk of swine influenza virus infections. We postulate that this increased risk may be due to the worker inoculating his or her oral mucosa with swine influenza virus after handling pigs.

More interesting, we posit that this study’s chief unique contribution is the vivid evidence that use of gloves during swine confinement work noticeably decreases the risk of swine influenza virus infection. Thus a rather simple personal protective measure might do much to reduce swine-to-man virus transmission. Future larger studies of swine confinement workers are needed to validate our findings and to better quantify risk factors for this particular population.

Individual behavior is known to strongly influence influenza virus transmission [5]. The national strategy for pandemic influenza highlights the importance of worker education and emphasizes the responsibilities of individuals in preventing the spread of pandemic virus [14]. Should virulent novel zoonotic influenza virus enter swine confinement facilities and spread among concentrated swine populations, the impact would be grave. It thus seems imperative that surveillance for zoonotic influenza virus be routinely conducted among agricultural workers and that they should be encouraged to use personal protective equipment like gloves, to often wash their hands, as well as to avoid smoking in or around swine facilities. Further it seem appropriate that such workers be included in state and federal pandemic plans as a high risk group for the receipt of annual influenza vaccines, pandemic use of antivirals, and receipt of pandemic vaccine.
Acknowledgements

This work was funded by The University of Iowa’s Center for Emerging Infectious Diseases. Serological assay work was made possible in part by a grant from the National Institutes of Allergy and Infectious Diseases (NIAID-R21 AI059214-01). We thank the veterinarians and staff from Valley Veterinary Center in Cherokee, Iowa, and the Winthrop Veterinary Clinic, Iowa, for use of their facilities for this project. We would also like to thank Father James Miller and Hector Hernandez from the Hispanic Ministries in the St. Patrick’s Catholic Church in Hampton, Iowa, for their assistance in recruiting study subjects. Special thanks are due to the phlebotomists who volunteered to help with this project especially Amy Kapanka of Hawkeye Community College and Carrie Ducommun from Larrabee, Iowa.

We thank Kendall Myers for her assistance with study data, Professor Christopher Olsen from the School of Veterinary Medicine at the University of Wisconsin – Madison, for providing the swine viruses as well as positive swine sera for controls, and Alexander Klimov of the Centers for Disease Control and Prevention in Atlanta, GA for providing human viruses. Finally, thanks to Professor James Roth and the staff at the Center for Food Security and Public Health at Iowa State University for their support.

References


## Tables

**Table 1. Study population characteristics.**

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<td>113</td>
<td>36 (78.3)</td>
<td>77 (97.5)</td>
</tr>
<tr>
<td><strong>Served in the military?</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>12</td>
<td>5 (10.4)</td>
<td>7 (8.9)</td>
</tr>
<tr>
<td>No</td>
<td>115</td>
<td>43 (89.6)</td>
<td>72 (91.1)</td>
</tr>
<tr>
<td><strong>Take medications that weaken the immune system?</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>3</td>
<td>2 (4.2)</td>
<td>1 (1.3)</td>
</tr>
<tr>
<td>No</td>
<td>122</td>
<td>44 (91.7)</td>
<td>78 (98.7)</td>
</tr>
<tr>
<td>Don't know</td>
<td>2</td>
<td>2 (4.2)</td>
<td>0 (0)</td>
</tr>
<tr>
<td><strong>Have heart or vascular disease?</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>3</td>
<td>2 (4.2)</td>
<td>1 (1.3)</td>
</tr>
<tr>
<td>No</td>
<td>124</td>
<td>46 (95.8)</td>
<td>78 (98.7)</td>
</tr>
<tr>
<td><strong>Received the 2003-2004 flu shot?</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>43</td>
<td>8 (16.7)</td>
<td>35 (44.3)</td>
</tr>
<tr>
<td>No</td>
<td>84</td>
<td>40 (83.3)</td>
<td>44 (55.7)</td>
</tr>
<tr>
<td><strong>Received the 1976-1977 flu shot?</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>2</td>
<td>1 (2.1)</td>
<td>1 (1.3)</td>
</tr>
<tr>
<td>No</td>
<td>119</td>
<td>41 (85.4)</td>
<td>78 (98.7)</td>
</tr>
<tr>
<td>Don't know</td>
<td>6</td>
<td>6 (12.5)</td>
<td>0 (0)</td>
</tr>
<tr>
<td><strong>Smoked 5 or more packs of tobacco product in past year?</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>14</td>
<td>9 (18.4)</td>
<td>5 (6.3)</td>
</tr>
<tr>
<td>No</td>
<td>114</td>
<td>40 (81.6)*</td>
<td>74 (93.7)</td>
</tr>
</tbody>
</table>

*Significantly different than controls at $\alpha=0.05$
Table 2. Odds ratios for increased serological response against swine H1N1 influenza virus by hemagglutination inhibition assay.

<table>
<thead>
<tr>
<th>Variables</th>
<th>N</th>
<th>Titer ≥1:10 n (%)</th>
<th>Titer ≥1:20 n (%)</th>
<th>Univariate OR (95% CI)</th>
<th>Multivariate OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age group (y)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;29</td>
<td>40</td>
<td>3 (7.5)</td>
<td>1 (2.5)</td>
<td>1.2 (0.2-6.1)</td>
<td>3.5 (0.4-30.6)</td>
</tr>
<tr>
<td>29-42</td>
<td>46</td>
<td>3 (6.5)</td>
<td>1 (2.2)</td>
<td>reference</td>
<td>reference</td>
</tr>
<tr>
<td>&gt;42</td>
<td>42</td>
<td>9 (22)</td>
<td>6 (14.6)</td>
<td><strong>4.2 (1.1-16.8)</strong>†</td>
<td>6.1 (0.9-41.3)</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>63</td>
<td>13 (21)</td>
<td>7 (11.3)</td>
<td><strong>8.4 (1.8-38.7)</strong> †</td>
<td>7 (0.9-52.1)</td>
</tr>
<tr>
<td>Female</td>
<td>65</td>
<td>2 (3.1)</td>
<td>1 (1.5)</td>
<td>reference</td>
<td>reference</td>
</tr>
<tr>
<td>Swine exposure</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Swine workers who use gloves sometimes or never</td>
<td>34</td>
<td>12 (35.3)</td>
<td>7 (20.6)</td>
<td><strong>21 (4.4-100.8)</strong> †</td>
<td><strong>30.3 (3.8-243.5)</strong> †</td>
</tr>
<tr>
<td>Swine workers who use gloves most of the time</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>or always</td>
<td>14</td>
<td>1 (7.1)</td>
<td>0 (0)</td>
<td>2.8 (0.2-34.2)</td>
<td>2.4 (0.1-40.9)</td>
</tr>
<tr>
<td>No swine exposed controls</td>
<td>79</td>
<td>2 (2.6)</td>
<td>1 (1.3)</td>
<td>reference</td>
<td>reference</td>
</tr>
<tr>
<td>Smoked in past year &gt;5 packs?</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>14</td>
<td>4 (28.6)</td>
<td>3 (21.4)</td>
<td><strong>4 (1.1-14.5)</strong> †</td>
<td><strong>18.7 (2.5-141.3)</strong> †</td>
</tr>
<tr>
<td>No</td>
<td>114</td>
<td>11 (9.7)</td>
<td>5 (4.4)</td>
<td>reference</td>
<td>reference</td>
</tr>
<tr>
<td>Received the 2002-2003 flu shot?</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>36</td>
<td>4 (11.4)</td>
<td>1 (2.9)</td>
<td>1 (0.3-3.4)</td>
<td>---</td>
</tr>
<tr>
<td>No</td>
<td>91</td>
<td>10 (11)</td>
<td>7 (7.7)</td>
<td>reference</td>
<td>---</td>
</tr>
<tr>
<td>Received the 2003-2004 flu shot?</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>43</td>
<td>6 (14.3)</td>
<td>3 (7.1)</td>
<td>1.6 (0.5-4.8)</td>
<td><strong>16.3 (2.5-107.4)</strong> †</td>
</tr>
<tr>
<td>No</td>
<td>84</td>
<td>8 (9.5)</td>
<td>5 (6)</td>
<td>reference</td>
<td>reference</td>
</tr>
<tr>
<td>Elevated titer human H1N1 (titers ≥ 1:40)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>39</td>
<td>2 (5.3)</td>
<td>1 (2.6)</td>
<td>0.3 (0.1-1.5)</td>
<td>---</td>
</tr>
<tr>
<td>Negative</td>
<td>89</td>
<td>13 (14.6)</td>
<td>7 (7.9)</td>
<td>reference</td>
<td>---</td>
</tr>
</tbody>
</table>

* Using proportional odds model, these titers were grouped: <1:10, 1:10; >1:10
† Significant odds for increased serological response, p-value <0.05
CHAPTER 3. ESTIMATING WITHIN-HERD TRANSMISSION OF PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS (PRRSV) AND ASSOCIATED RISK FACTORS FOR TIME-TO-SEROCONVERSION IN GROWING PIGS

A paper to be submitted to

*Journal of Swine Health and Production*

Alejandro Ramirez, Chong Wang, Scott Hurd, Kenneth J. Stalder, Jeffrey J. Zimmerman, Claudia Muñoz-Zanzi, James A. Roth, Locke A. Karriker

**Summary**

*Objective:* This study was conducted to evaluate the within-herd rate of transmission of porcine reproductive and respiratory syndrome virus (PRRSV) and risk factors affecting the rate of seroconversion in growing pigs post-weaning.

*Materials and methods:* Twenty six barns were selected to participate and 20 pigs per barn (for a total of 520 pigs) were housed together in a pen, tagged and tested for PRRSV
antibodies for approximately 24 weeks, sampling every two weeks from the time of enrollment in the nursery or wean-to-finish building until marketed. At the start of the project, these pigs were also tested for PRSSV circulation using PCR.

**Results:** A total of 101 (22%) pigs were identified to be viremic for PRRSV by PCR at enrollment. The cumulative mean incidence density rate for PRRSV seroconversion for all pigs was 2.57 per 100 pig days. This means that on average, it took pigs 39.0 days (95% CI, 35.3 to 43.2) from enrollment to seroconvert to PRRSV as detected by ELISA. On average it would take 3.6 bleeding periods (between 7 and 8 weeks) from when the first pig in a group was identified to have seroconverted until the last pig in the group seroconverted. Cox proportional hazard multivariate modeling identified enrollment PRRSV PCR positive result (HR=34.0, 95%CI 8.06 to 143.44) and number of PCR positive pigs in the cohort at enrollment (HR=1.66, 95%CI 1.23 to 2.23) as significant risk factors for decreased time-to-seroconversion.

**Implications:** PRRSV is not highly contagious. The smallest pigs in the group are not more likely to be PCR positive or seroconvert to PRRSV sooner than the largest pigs in the group. PRRSV ELISA S/P values at weaning are not a predictor of viremia or time-to-seroconversion. Serologic PRRSV homogeneity is dynamic and difficult to achieve even in a small group of pigs with constant exposure to each other.
Introduction

Porcine reproductive and respiratory syndrome virus (PRRSV) continues to be recognized as the most significant swine disease in North America with an annual impact of over $560 million a year.\textsuperscript{1} Much of the previous research has focused on better understanding the epidemiology, transmission, and risk factors associated with PRRSV and breeding herds. As the North American swine industry moves towards PRRSV eradication, currently there has been more interest in better understanding the dynamics of PRRSV transmission post weaning. Holck and Polson\textsuperscript{2} reported the average cost of PRRSV in growing pigs to range between $6.25-15.25 per pig. Neumann et al\textsuperscript{1} attributed 35.9\% and 52.2\% of the annual swine industry losses to the nursery and growing phases of production respectively with losses due to increased mortality, lower average daily gain and decreased feed efficiency. With over 22 years since PRRSV was first reported in the field,\textsuperscript{3,4,5} the epidemiology and dynamics of viral transmission are still not fully understood.

Once a herd is infected with PRRSV it tends to circulate within a herd perpetuated by a cycle of transmission from dam to her pigs, then by pig-to-pig contact at later stages of production. Under field conditions in which susceptible and infectious pigs are mixed, a large proportion of the contact population may become infected. Thus, Dee and Joo\textsuperscript{6} reported 80 to 100\% of pigs in 3 swine herds were infected by 8 to 9 weeks-of-age and Maes\textsuperscript{7} found 96\% of market hogs from 50 herds to be sero-positive. Regional evaluation of PRRSV antibodies conducted in swine at slaughter from 2005 – 2008 has consistently identified over 2/3 of pigs as seropositive for PRRSV.\textsuperscript{8,9} These data support practitioner’s experiences of dealing with high numbers of PRRSV positive finishing pigs in the Midwest implying extensive viral
transmission is occurring.

Field research has found marked differences in PRRSV transmission rates between groups, pens, and even rooms of animals in infected herds. Houben et al\textsuperscript{10} even found transmission to vary within litters, with some littermates seroconverting as early as 6 to 8 weeks and others as late as 10 to 12 weeks of age with some still free of PRRSV infection by the end of the 12 week study period. Le Potier et al\textsuperscript{11} found a lack of seroconversion in young sows on endemically-infected farms using in-herd gilt replacements, i.e., these animals had lived among PRRSV for 10 months or more without becoming infected. Results of a large study on PRRSV transmission in nursery pigs in seven commercial swine herds identified two distinct patterns of transmission.\textsuperscript{12} In farms with rapid spread 50% of the pigs were infected between 4 and 7 weeks of age and \( \geq 90\% \) of pigs were infected by 8.5 weeks of age. In slow spread farms, only 20 to 40\% of pigs were infected by the end of the nursery phase.\textsuperscript{12} Difference in infection patterns between close-contact groups have also been reported by others.\textsuperscript{13,14}

There are many studies demonstrating shedding of the PRRSV in oral fluids, semen, nasal secretions, urine, mammary secretions, and feces.\textsuperscript{15,16,11,17,18,19} There have also been attempts to quantify the PRRSV minimum infectious dose for intranasal and intramuscular administration\textsuperscript{20} as well as the ID\textsubscript{50} dose for oral, nasal, and aerosol routes of transmission.\textsuperscript{21,22} Persistently infected animals, based on testing tonsils, has been demonstrated to last for over 150 days\textsuperscript{23,14} with one study even finding viral RNA at 251 days post-infection although the bioassay was negative from both the blood and tonsil samples.\textsuperscript{24} The latter study also concluded there was a distinct drop in proportion of animals harboring
virus around 3 to 4 months post-inoculation. A study conducted in Great Britain in 2003-2004 identified PRRSV herd persistence was associated with larger herd (>250 sows), in pig dense areas, with repeated introductions of infected animals.

When performing field studies with weaned pigs, maternal antibodies complicate the interpretation of serological data. The role of antibodies, especially maternal antibodies, in providing protection against PRRSV is still not fully understood. There have been conflicting reports depending on the parameters measured as well as the outcome evaluated. Maternal antibodies do seem to be protective, especially when evaluating neutralizing antibodies, although not all pigs seem to be protected. Maternal antibodies for PRRSV are usually detectable until 4-10 weeks of age. A recent study calculated the half-life for maternal PRRSV serum neutralizing antibodies to be 3 weeks expecting negative pigs by 10.5 weeks of age. This also coincides with other studies identifying seroconversion to occur between 6 and 12 weeks of age.

As calls for regional eradication continue to gain momentum, a better understanding of PRRSV transmission in the wean-to-finish periods is necessary. The objective of this study was to evaluate the within-herd rate of transmission of porcine reproductive and respiratory syndrome virus (PRRSV) and risk factors affecting the rate of seroconversion for growing post-weaning pigs.

**Materials and Methods**

Experimental procedures used in this study were approved by the Iowa State University Institutional Animal Care and Use Committee (4-05-5869-S).
A multi-site, prospective study was performed involving repeated sampling (nursery through market) of a cohort of pigs at multiple barns. A total of 26 barns were initially enrolled and 20 pigs per barn were individually tagged for a total of 520 pigs. The barns were sought based on personal contacts and selected on: a) known history of PPRSV seroconversion, b) willingness to participate in the study, and c) proximity to resources to collect blood samples reliably. Pigs were selected from each barn based on convenience with the requirement that the study cohort pigs had to be penned together throughout the study.

Barn characteristics were collected at enrollment included number of sow farms commingled in nursery (by room, barn, and site), whether they practiced all-in-all-out between groups (by room, barn, and site), total number of pigs (in room, barn, and site), minimum and maximum age of pigs at enrollment (in room, barn, and site), and average number of pigs per pen.

Study pigs were individually weighed at enrollment by using a commercially available, battery operated, digital scale. Pigs were later categorized within a barn (cohort) based on their initial weight as follows: SMALLEST (lightest 7 pigs), MIDDLE (middle 6 pigs), and LARGEST (heaviest 7 pigs).

Serum samples were collected at enrollment and thereafter every 2 weeks until pigs were sent to market. Serum samples were submitted to the Iowa State University Veterinary Diagnostic Laboratory for testing. All samples were tested shortly after collecting in order to provide results back to the barn veterinarians in a timely manner. All samples were tested for antibodies against PRRSV virus using a commercial enzyme-linked immunosorbent assay.
(ELISA) kit and protocol (HerdCheck ® 2XR, IDEXX, Westbrook, Maine, USA). Results were recorded as serum-to-positive (S/P) values, positive or negative based on the manufacturer’s recommended cutoff (≥0.400 is positive), as well as categorically based on S/P value into: NEGATIVE (<0.400), LOW (0.400 to 0.999), MEDIUM (1.000 to 1.999), and HIGH (≥2.000) as previously described. All initial serum samples were also tested for PRRSV viremia via reverse transcriptase polymerase chain reaction (PCR) using a commercially available kit (Tetracore Inc., Rockville, MD, USA) as previously described.

A specific ELISA serial number (kit 1) had been reserved for testing all study samples. Unfortunately due to a logistic issue it was necessary to switch to a second serial number (kit 2) approximately half way through the study. The timing of the use of kit 2 was selected to coincide with the enrollment of the last 10 barns to minimize effects of the transition. A subset of 90 previously collected samples were selected for kit agreement comparisons based on convenience and ensuring variability in S/P values. This subset of samples was then tested at the same time using both kits. Test agreement was assessed as described by Bland and Altman. In brief, PRRSV EELISA S/P values were transformed using natural log and paired sample t-test was performed. A Bland-Altman plot was also created by plotting the average of both kit’s log transformed ELISA S/P value for each sample (x-axis) by the actual difference in the log transformed ELISA S/P values obtained for each sample (y-axis).

Seroconversion was defined as the first time when an increase from negative titers to a positive titer (S/P ≥0.4) was detected and maintained for at least 2 consecutive periods. For pigs that did not have a recorded negative sample (i.e. possible maternal antibodies)
seroconversion was defined as the first change in S/P values resulting in an upward trend that was maintained for at least 2 consecutive periods. ELISA S/P results for each pig were examined manually to identify the time of seroconversion using the above definitions.

For pigs to be considered in the final analysis the barn had to meet the following three inclusion criteria which was established before any barns were enrolled in the study:

1. All pigs in the study cohort had to have been kept together in a single pen throughout the entire study period (nursery and finishing).

2. Pigs could not have been vaccinated for PRRSV.

3. At least one pig in the cohort (barn) had to demonstrate exposure to PRRSV by seroconverting at some point during the study period.

Incidence density rates (IDR) were computed for each barn individually as well as a cumulative rate for all barns combined to allow for the comparison of event rates amongst barns including differences in pen characteristics. The IDR were calculated by taking the number of pigs in the group that seroconverted to PRRSV during the study period and dividing it by the sum of all individual pig-days until seroconversion was detected for each pig in the group. The 95% CI were calculated using OpenEpi® Version 2.3 (available at www.openepi.com).

Kaplan-Meier survival curves for time-to-seroconversion were calculated using JMP® Version 8.0 (SAS® Institute Inc., Cary, NC, USA) for variables of interest. The log-rank test was used to identify differences between groups since censored data was included. Survival analyses were performed to study the effects of explanatory factors on time-to-
seroconversion by using Cox regression models using SAS® Version 9.2 (SAS® Institute Inc., Cary, NC, USA). Explanatory variables evaluated are listed in Table 1. Both univariate and multivariate regression analyses were performed. An interaction variable with time was created for enrollment variables that could change over time by using the natural log transformation of the time-to-seroconversion. A stepwise forward procedure was applied for multivariate Cox regression model selection. A robust sandwich covariance matrix estimate was used to account for the dependence within site to maximize partial likelihood estimates under an independent working assumption to account for the intracluster dependence. For all statistical analysis, significance level was set at $\alpha = 0.05$.

Results

**Herds and serology**

A total of 26 barns (520 pigs) were enrolled in the study from farms that expected pigs to seroconvert to PRRSV. Barns were sampled between September 2005 and September 2006. A total of 3 (12%) barns were excluded from analysis due to unexpected events which resulted in them not meeting our pre-determined inclusion criteria described above. Two barns were excluded as unexpectedly all pigs (n=40) stayed seronegative though the entire study period. One barn was excluded as all pigs were inadvertently vaccinated for PPRSV using a modified live commercial vaccine (Table 2). Table 3 summarizes some general characteristics for the study population. Of the 5,282 samples collected from the 23 barns (460 pigs) used in the discussion and analysis, 3,922 (74%) were ELISA positive for PRRSV and 1,360 (26%) were negative (Table 4). A total of 101 (22%) pigs were identified to be viremic for PRRSV by PCR at enrollment. Figure 1 shows the mean PRRSV ELISA values
for study pigs over time. A total of 33 (7%) pigs died during the study with an additional 6 (1%) that were unaccounted. There were 34 (7%) pigs that appeared to have seroconverted to PRRSV before enrollment (left censored) and 16 (3%) pigs died before seroconversion could be identified (right censored). Table 5 summarizes censure data for the study. No sampling periods were missed on any of the study cohorts.

PRRSV ELISA kit comparison

Of the 23 barns in the study, PRRSV ELISA kit 1 was used exclusively in 2 barns (8.7%), kit 2 exclusively in 10 (43.5%), and both kits 1 & 2 in 11 (47.8%) barns. Manual review of the data revealed that there were only 3 (0.7%) pigs who’s timing of seroconversion was possibly affected by the kit change. In all other study pigs either a single kit (n=240, 52.2%) was used for their entire period, they had already seroconverted or been censored before the kit change occurred (n=191, 41.5%), or they had a well-defined serologic pattern that appeared not to be affected by the change (n=26, 5.7%). Figure 2 shows the Bland-Altman agreement plot for the natural log transformation of the ELISA S/P ratios on a subset of 90 samples ran simultaneously on both PRRSV ELISA kits 1 and 2. This agreement had a correlation of 0.945 and the mean of the difference was not statistically different from zero ($P<0.05$).

Incidence density rate and weeks to positive

A summary of the incidence density rate (IDR) per 100 pig days is provided in Table 6 sorted from lowest to highest IDR. The mean IDR for all pigs was 2.57 events per 100 pig days. This means that on average, it took pigs 39.0 days (95% CI, 35.3 to 43.2) from enrollment to seroconvert to PRRSV as detected by ELISA. The overall mean days to
seroconversion for all barns ranged from 13.8 (95%CI, 8.9 to 22.5) to 105.0 (95%CI, 67.8 to 171.8). Information regarding the number of PRRSV PCR positive pigs at enrollment as well as the number of pigs per pen is provided for each barn. Figures 3 and 4 summarize the barn distribution of PCR positive pigs and number of pigs per pen.

On average it took 3.6 bleeding periods (between 7 and 8 weeks) from when the first pig in a group was identified to have seroconverted until the last pig in the group was detected to have seroconverted. The range varied from 2 (4 weeks) to 7 (14 weeks) periods (Figure 5).

Survival analysis

Three interesting Kaplan-Meier (K-M) survival curves are worth reporting. The survival curves (time-to-seroconversion) for pigs that were PRRSV PCR positive at enrollment were significantly different ($P<0.001$) than those PCR negative (Figure 6).

Statistical analysis revealed no significant differences ($P<0.05$) in the survival curves between the categorical ranking, within a group, of the pigs based on their enrollment weight (Figure 7). Even when splitting the ranking into 10 different categories, there still was no difference in the seroconversion curves between the different groups of pig sizes within a barn (data not shown). Chi squared analysis did not show any statistical association ($P<0.05$) between size of pig and PRRSV PCR result at enrollment.

Kaplan-Meier survival curves (time-to-seroconversion) for the magnitude of initial ELISA S/P value did not show any statistical differences ($P<0.05$) between all these four groups (Figure 8). At enrollment, there was no statistical association ($P<0.05$) between any
of the PRRSV ELISA categories and PRRSV PCR result. Multivariate survival analysis results using Cox proportional hazards regression are summarized in Table 7.

**Discussion**

This study was designed to specifically target pigs from flows known to be seropositive for PRRSV. This characteristic increased the likelihood that study subjects were exposed to homologous PPRSV. The data from this study confirms that usually PRRSV is not a rapidly contagious virus. Contagiousness was defined as having a shorter time to seroconversion. A delay in seroconversion could be cause by lack of sufficient viral exposure or simply a delay in the immune response. Currently, ELISA PRRSV seroconversion has been reported to be first detected within 10 – 14 days with 100% seropositive by 14 days post inoculation even when evaluating different PRRSV pathogenic strains and inoculation doses. This would suggest the delay in seroconversion is more likely explained by lack of sufficient exposure rather than by a delay in immune response. Our data goes further and provides the range of days to seroconversion (Table 6) observed in the barns from our study. Overall our data show it took just over 5 weeks (39 days, 95% CI 35.3 to 43.2) for all pigs in a cohort of 20 to seroconvert. This is quite an impressive number, and short confidence interval, considering all barns housed the 20 pigs in the cohort together in a single pen so contact time between pigs was frequent. The average number of pigs per pen were 38 indicating that in over half of the study barns we were monitoring over 50% of the pigs in the pen. This prolonged period to reach seroconversion can have multiple implications in the field setting.
As expected, the most significant risk factor contributing to quicker time-to-seroconversion (hazard) was whether the study pig was identified to be viremic for PRRSV at the start of the study which was also evident in the univariate Kaplan-Meier (K-M) survival curve in Figure 6. It is also important to note that the Cox proportional hazard multivariate modeling did recognized that the effect of the initial PRRSV PCR result declined over time (HR<1). Besides the individual pig itself being viremic, for every PCR positive pig in the cohort at enrollment time the HR of 1.66 (95%CI, 1.23 to 2.23) would increase exponentially. So having three more pigs would provide a mean HR of $1.66^3$ or 4.57. The more PCR positive animals in the group, the more likely other pigs will get exposed to PRRSV sooner. The effect of this variable also decreased slightly over time.

Being the smallest pig is not a disease. There were no statistical differences in the K-M survival curves among the different pig sizes within a cohort. The multivariate Cox proportional hazards model also did not show the smallest pigs as having a higher risk for seroconversion. It was the middle sized pigs that did have a statistically significant HR=1.12 (95%CI, 1.04 to 1.20) compared to the smallest pigs and HR=1.23 (95%CI, 1.06 to 1.44) compared to the largest pigs in the cohort. Our results also showed that pigs of different sizes within a cohort were not more or less likely to be PCR positive for PRRS at enrollment than the other sized pigs. This is in agreement with Cano et al\textsuperscript{40} in that in the farrowing house, piglet weight was not a risk factor for detecting PCR positive pigs.

In univariate K-M survival analysis, there was no effect of the magnitude of the PRRSV ELISA S/P value when categorized as negative, low, medium, or high (Table 7) on the rate of seroconversion. The multivariate Cox proportional hazard model did identify high
S/P values (≥2.000) as protective (delaying seroconversion). In our study, at enrollment, pig ELISA S/P categories were not statistically associated with the pig’s PCR result. Similar results have been reported in older pigs.41

The HR results for waterer type and space per pig in the nursery are difficult to interpret. For every 1 m² provided per pig the HR=49.04 (95%CI, 4.78 to 503.41). The wide confidence interval suggests a higher sample size may be needed to refine the magnitude of the effect of this variable. It also does not make biological sense that providing more space would increase the hazard for seroconversion. The estimates for waterer type also seem to lack power to detect differences between nipple and cup waterers (data not shown) but suggest a possible protective effect from nipple waterers. This is an interesting area that should be investigated as reservoir type waters could be doing exactly that, holding virus and facilitating its spread. Further studies need to be done before any conclusions can be supported regarding both of these variables.

Limitations of this study include not being able to determine the exact timing of seroconversion as sampling was done in 2 week intervals. Not all pigs were enrolled at the same age, but the selection of survival analysis does allow for this consideration. Ideally it would have been better to freeze back all serum samples and run the PRRSV ELISA on all samples at the same time. Unfortunately it was necessary to report results back on a timely manner in order to provide value to the herd veterinarian and herd owner who volunteered their barns for participation in the study. The change in ELISA kit serials was not ideal, but our analysis and evaluation of the data suggest that its impact was minimal if any (<1% of pigs possibly affected). The study was also not designed to account for PRRSV strain
differences and their possible effect on time-to-seroconversion.

**Implications**

- PRRSV virus seems to move relatively slowly in a confined population.
- The smallest pigs in the group are not more likely to be PCR positive or seroconvert to PRRSV sooner than the largest pigs in the group.
- PRRSV ELISA S/P values (negative, low, medium, or high) at weaning are not a predictor of viremia or time-to-seroconversion.
- Serologic PRRSV homogeneity is dynamic and difficult to achieve even in a small group of pigs with constant exposure to each other.

**Acknowledgements**

Authors would like to thank Lori Layman for her great help in organizing and executing this project. We would also like to recognize the owners and veterinarians for all 26 barns for their willingness to participate in this study. This project was supported by Pork Checkoff funds distributed through the National Pork Board, P.O. Box 9114, Des Moines, IA.

**References**


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Tables and Figures

Table 1. Explanatory variables evaluated for possible effect on porcine reproductive and respiratory syndrome virus (PRRSV) time-to-seroconversion based on ELISA S/P values in a cohort of 20 pigs house together from enrollment in the nursery phase through marketing

<table>
<thead>
<tr>
<th>Variable description</th>
<th>Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pig’s start weight</td>
<td>Categorical (SMALLEST, MIDDLE, LARGEST)</td>
</tr>
<tr>
<td>Pig’s starting PRRSV ELISA S/P value</td>
<td>Categorical (NEGATIVE, LOW, MEDIUM, HIGH)</td>
</tr>
<tr>
<td>Pig’s starting PRRSV PCR result</td>
<td>Categorical (YES, NO)</td>
</tr>
<tr>
<td>Number of PRRSV PCR positive pigs in cohort</td>
<td>Numerical (integer)</td>
</tr>
<tr>
<td>Number of sow farms comingled in the nursery room</td>
<td>Numerical (integer)</td>
</tr>
<tr>
<td>Number of sow farms comingled on the site</td>
<td>Numerical (integer)</td>
</tr>
<tr>
<td>Age difference of pigs in nursery room</td>
<td>Numerical (integer)</td>
</tr>
<tr>
<td>Age difference of pigs on site</td>
<td>Numerical (integer)</td>
</tr>
<tr>
<td>Number of pigs per pen in nursery</td>
<td>Categorical (SMALL, LARGE)</td>
</tr>
<tr>
<td>Nursery space per pig (m²/pig)</td>
<td>Numerical (continuous)</td>
</tr>
<tr>
<td>Nursery waterer type</td>
<td>Categorical (CUP, NIPPLE, BOTH)</td>
</tr>
<tr>
<td>Wean-to-finish barn</td>
<td>Categorical (YES, NO)</td>
</tr>
<tr>
<td>All-in-all-out by site</td>
<td>Categorical (YES, NO)</td>
</tr>
</tbody>
</table>

PRRSV = porcine reproductive and respiratory syndrome virus; ELISA S/P = enzyme-linked immunosorbent assay serum-to-positive; PCR = polymerase chain reaction
Table 2. Summary of pertinent nursery phase barn characteristics for all 26 barns initially enrolled in porcine reproductive and respiratory syndrome virus (PRRSV) longitudinal seroconversion study and whether they were included or excluded in the final analysis

<table>
<thead>
<tr>
<th></th>
<th>Excluded* (%)</th>
<th>Included (%)</th>
<th>Total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of barns</td>
<td>3 (11.5)</td>
<td>23 (88.5)</td>
<td>26</td>
</tr>
<tr>
<td>Number of sow farms per barn</td>
<td>1.3</td>
<td>3.3</td>
<td>3.1</td>
</tr>
<tr>
<td>Number of sites practicing AIAO</td>
<td>0 (0)</td>
<td>10 (43.5)</td>
<td>10 (38.5)</td>
</tr>
<tr>
<td>Number of pigs per barn</td>
<td>1,517</td>
<td>2,291</td>
<td>2,201</td>
</tr>
<tr>
<td>Number of wean-to-finish sites</td>
<td>2 (66.7)</td>
<td>16 (69.6)</td>
<td>18 (69.2)</td>
</tr>
<tr>
<td>Average number of pigs per pen</td>
<td>256*</td>
<td>38</td>
<td>63</td>
</tr>
<tr>
<td>Number of large pen barns‡</td>
<td>2 (66.7)</td>
<td>11 (47.8)</td>
<td>13 (50.0)</td>
</tr>
<tr>
<td>Number of barns with cup waterers</td>
<td>3 (100)</td>
<td>19 (82.6)</td>
<td>22 (84.6)</td>
</tr>
<tr>
<td>Number of barns with nipple waterers</td>
<td>0 (0)</td>
<td>4 (17.4)</td>
<td>4 (15.4)</td>
</tr>
</tbody>
</table>

* Barns were excluded from analysis for the following reasons: 2 barns unexpectedly stayed negative for PRRSV for the entire grow-finish period and one barn inadvertently vaccinated pigs with a modified live PRRSV vaccine.

‡Number of pigs per pen for the three excluded barns were 612, 123, and 32.

AIAO = all-in-all-out; PRRSV = porcine reproductive and respiratory virus
Table 3. General enrollment characteristics for 460 pigs (23 barns with 20 pigs per barn) used in porcine reproductive and respiratory syndrome virus (PRRSV) longitudinal seroconversion study

<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th>Min</th>
<th>Max</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (days)</td>
<td>27.8</td>
<td>18</td>
<td>43</td>
<td>6.94</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>7.25</td>
<td>4.13</td>
<td>14.68</td>
<td>1.786</td>
</tr>
<tr>
<td>Initial PRRSV ELISA S/P value*</td>
<td>0.535</td>
<td>0.000</td>
<td>4.146</td>
<td>0.7109</td>
</tr>
<tr>
<td>Initial PCR positive pigs per barn†</td>
<td>4.4</td>
<td>0</td>
<td>13</td>
<td>5.13</td>
</tr>
</tbody>
</table>

*HerdCheck ® 2XR, IDEXX, Westbrook, Maine, USA.
†Tetracore Inc., Rockville, MD, USA.

Min = minimum; Max = maximum; SD = standard deviation; PRRSV = porcine reproductive and respiratory syndrome virus; ELISA S/P = enzyme-linked immunosorbent assay serum-to-positive; PCR = polymerase chain reaction
Table 4. Distribution of 460 study pigs (23 cohorts of 20 pigs each) categorized based on their starting porcine reproductive and respiratory syndrome virus (PPRSV) ELISA S/P values (negative, low, medium, or high) and viremia

<table>
<thead>
<tr>
<th>ELISA S/P*</th>
<th>n (%)</th>
<th>n (%)</th>
<th>n (%)</th>
<th>n (%)</th>
<th>Total n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>284 (61.7)</td>
<td>95 (20.7)</td>
<td>49 (10.7)</td>
<td>32 (7.0)</td>
<td>460 (100)</td>
</tr>
<tr>
<td>Low 0.400 – 0.999</td>
<td>56 (12.2)</td>
<td>23 (5.0)</td>
<td>14 (3.0)</td>
<td>8 (1.7)</td>
<td>101 (22.0)</td>
</tr>
<tr>
<td>Medium 1.000 – 1.999</td>
<td>49 (10.7)</td>
<td>49 (10.7)</td>
<td>49 (10.7)</td>
<td>49 (10.7)</td>
<td>49 (10.7)</td>
</tr>
<tr>
<td>High ≥ 2.000</td>
<td>32 (7.0)</td>
<td>32 (7.0)</td>
<td>32 (7.0)</td>
<td>32 (7.0)</td>
<td>32 (7.0)</td>
</tr>
</tbody>
</table>

*HerdCheck ® 2XR, IDEXX, Westbrook, Maine, USA.

†Tetracore Inc., Rockville, MD, USA - PCR positive results are summarized based on the pig’s ELISA S/P value.

PRRSV = porcine reproductive and respiratory syndrome virus; ELISA S/P = enzyme-linked immunosorbent assay serum-to-positive; PCR = polymerase chain reaction
Table 5. Summary comparing the number of pigs seroconverting to porcine reproductive and respiratory syndrome virus (PRRSV) or censored based on reason for removal from longitudinal study involving 23 barns each with 20 pigs housed together in the same pen from nursery through market

<table>
<thead>
<tr>
<th>Removal reason</th>
<th>Total n (%)</th>
<th>Left censored †</th>
<th>Seroconverted ‡</th>
<th>Right censored §</th>
</tr>
</thead>
<tbody>
<tr>
<td>Death</td>
<td>33 (7.2)</td>
<td>2 (5.9)</td>
<td>15 (3.7)</td>
<td>16 (100)</td>
</tr>
<tr>
<td>MIA</td>
<td>6 (1.3)</td>
<td>0 (0)</td>
<td>6 (1.5)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Lost tags</td>
<td>29 (6.3)</td>
<td>0 (0)</td>
<td>29 (7.1)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Marketed</td>
<td>372 (80.9)</td>
<td>26 (76.5)</td>
<td>346 (84.4)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>TOTALS</td>
<td>460 (100)</td>
<td>34 (7.4)</td>
<td>410 (89.1)</td>
<td>16 (3.5)</td>
</tr>
</tbody>
</table>

*Percentages are based on the total for the column with the exception of the last row with totals which represents the percentage based on total number of pigs in the study (n=460).

†Left censored was defined as seroconversion having occurred before enrollment.

‡Seroconversion to PRRSV was defined as an increase from negative titers to a positive titer (S/P ≥0.4) that was maintained for at least 2 consecutive periods. For pigs that did not have a recorded negative sample (i.e. possible maternal antibodies) seroconversion was defined as the first change in S/P values resulting in an upward trend that was maintained for at least 2 consecutive periods.

§Right censored was defined as pig did not seroconvert by the time it was removed from the study.

PRRSV = porcine reproductive and respiratory syndrome virus; MIA=missing in action (i.e. pigs became unaccounted for)
Table 6. Calculated porcine reproductive and respiratory syndrome virus (PRRSV) seroconversion incidence density rates (IDR) per 100 pig days for all 23 barns in longitudinal study of 20 pigs per barn housed together in a pen from nursery through market

<table>
<thead>
<tr>
<th>Barn ID</th>
<th>No. pigs/PCR positive</th>
<th>Total days to event*</th>
<th>No. pigs seroconverted</th>
<th>No. pigs left censored†</th>
<th>IDR (per 100)</th>
<th>Average days to seroconversion 95% CI</th>
<th>Average pigs per pen</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>0</td>
<td>1890</td>
<td>18</td>
<td>0</td>
<td>0.95</td>
<td>105.0, 67.8, 171.8</td>
<td>18</td>
</tr>
<tr>
<td>23</td>
<td>13</td>
<td>365</td>
<td>6</td>
<td>6</td>
<td>1.64</td>
<td>60.8, 29.2, 150.8</td>
<td>30</td>
</tr>
<tr>
<td>14</td>
<td>0</td>
<td>935</td>
<td>20</td>
<td>0</td>
<td>2.14</td>
<td>46.8, 30.8, 74.5</td>
<td>55</td>
</tr>
<tr>
<td>15</td>
<td>0</td>
<td>812</td>
<td>18</td>
<td>0</td>
<td>2.22</td>
<td>45.1, 29.1, 73.8</td>
<td>55</td>
</tr>
<tr>
<td>7</td>
<td>5</td>
<td>717</td>
<td>16</td>
<td>2</td>
<td>2.23</td>
<td>44.8, 28.2, 75.7</td>
<td>25</td>
</tr>
<tr>
<td>16</td>
<td>0</td>
<td>848</td>
<td>20</td>
<td>0</td>
<td>2.36</td>
<td>42.4, 27.9, 67.5</td>
<td>55</td>
</tr>
<tr>
<td>12</td>
<td>0</td>
<td>814</td>
<td>20</td>
<td>0</td>
<td>2.46</td>
<td>40.7, 26.8, 64.8</td>
<td>20</td>
</tr>
<tr>
<td>11</td>
<td>0</td>
<td>807</td>
<td>20</td>
<td>0</td>
<td>2.48</td>
<td>40.4, 26.6, 64.3</td>
<td>40</td>
</tr>
<tr>
<td>22</td>
<td>8</td>
<td>439</td>
<td>12</td>
<td>4</td>
<td>2.73</td>
<td>36.6, 21.5, 67.5</td>
<td>31</td>
</tr>
<tr>
<td>21</td>
<td>0</td>
<td>653</td>
<td>18</td>
<td>0</td>
<td>2.76</td>
<td>36.3, 23.4, 59.3</td>
<td>48</td>
</tr>
<tr>
<td>20</td>
<td>0</td>
<td>725</td>
<td>20</td>
<td>0</td>
<td>2.76</td>
<td>36.3, 23.9, 57.7</td>
<td>48</td>
</tr>
<tr>
<td>17</td>
<td>0</td>
<td>652</td>
<td>18</td>
<td>1</td>
<td>2.76</td>
<td>36.2, 23.4, 59.2</td>
<td>48</td>
</tr>
<tr>
<td>18</td>
<td>4</td>
<td>590</td>
<td>17</td>
<td>1</td>
<td>2.88</td>
<td>34.7, 22.1, 57.6</td>
<td>47</td>
</tr>
<tr>
<td>13</td>
<td>0</td>
<td>655</td>
<td>19</td>
<td>0</td>
<td>2.90</td>
<td>34.5, 22.5, 55.6</td>
<td>55</td>
</tr>
<tr>
<td>5</td>
<td>3</td>
<td>646</td>
<td>19</td>
<td>0</td>
<td>2.94</td>
<td>34.0, 22.2, 54.9</td>
<td>21</td>
</tr>
</tbody>
</table>

*Event was defined as seroconversion (see Table 5 footnote) or censure.
†Left censored was defined as seroconversion having occurred before enrollment.

PRRSV = porcine reproductive and respiratory syndrome virus; IDR = incidence density rate; PCR = polymerase chain reaction; CI = confidence interval
Table 6. Calculated porcine reproductive and respiratory syndrome virus (PRRSV) seroconversion incidence density rates (IDR) per 100 pig days for all 23 barns in longitudinal study of 20 pigs per barn housed together in a pen from nursery through market (continued)

<table>
<thead>
<tr>
<th>Barn ID</th>
<th>No. pigs PCR positive</th>
<th>Total days to event*</th>
<th>No. pigs sero-converted</th>
<th>No. pigs left censored†</th>
<th>IDR (per 100)</th>
<th>Average days to seroconversion</th>
<th>Average pigs per pen</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Mean 95% CI</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Lower</td>
<td>Upper</td>
</tr>
<tr>
<td>4</td>
<td>0</td>
<td>653</td>
<td>20</td>
<td>0</td>
<td>3.06</td>
<td>32.7 21.5 52.0</td>
<td>55</td>
</tr>
<tr>
<td>19</td>
<td>2</td>
<td>574</td>
<td>18</td>
<td>1</td>
<td>3.14</td>
<td>31.9 20.6 52.2</td>
<td>48</td>
</tr>
<tr>
<td>9</td>
<td>11</td>
<td>255</td>
<td>8</td>
<td>6</td>
<td>3.14</td>
<td>31.9 16.8 68.6</td>
<td>25</td>
</tr>
<tr>
<td>26</td>
<td>13</td>
<td>420</td>
<td>14</td>
<td>2</td>
<td>3.33</td>
<td>30.0 18.3 52.7</td>
<td>31</td>
</tr>
<tr>
<td>6</td>
<td>10</td>
<td>359</td>
<td>12</td>
<td>4</td>
<td>3.34</td>
<td>29.9 17.6 55.2</td>
<td>34</td>
</tr>
<tr>
<td>10</td>
<td>9</td>
<td>322</td>
<td>12</td>
<td>4</td>
<td>3.73</td>
<td>26.8 15.8 49.5</td>
<td>32</td>
</tr>
<tr>
<td>25</td>
<td>11</td>
<td>395</td>
<td>16</td>
<td>2</td>
<td>4.05</td>
<td>24.7 15.5 41.7</td>
<td>31</td>
</tr>
<tr>
<td>24</td>
<td>12</td>
<td>248</td>
<td>18</td>
<td>0</td>
<td>7.26</td>
<td>13.8 8.9 22.5</td>
<td>31</td>
</tr>
<tr>
<td>TOTAL</td>
<td>101</td>
<td>14774</td>
<td>379</td>
<td>33</td>
<td>2.57</td>
<td>39.0 35.3 43.2</td>
<td>38.4</td>
</tr>
</tbody>
</table>
Table 7. Parameter estimates and hazard ratios (HR) for all variables identified as significant by Cox proportional hazards multivariate regression analysis for time-to-seroconversion to porcine reproductive and respiratory syndrome virus (PRRSV) in 23 cohorts each with 20 pigs (n=460) housed together in a pen from nursery through market

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Estimate</th>
<th>SE</th>
<th>P</th>
<th>HR</th>
<th>LCI</th>
<th>UCI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pig PCR result*</td>
<td>3.52642</td>
<td>0.73442</td>
<td>&lt;0.0001</td>
<td>34.00</td>
<td>8.06</td>
<td>143.44</td>
</tr>
<tr>
<td>Pig PCR result (time)†</td>
<td>-1.04311</td>
<td>0.19265</td>
<td>&lt;0.0001</td>
<td>0.35</td>
<td>0.24</td>
<td>0.51</td>
</tr>
<tr>
<td>Barn PCR number‡</td>
<td>0.50401</td>
<td>0.15258</td>
<td>0.001</td>
<td>1.66</td>
<td>1.23</td>
<td>2.23</td>
</tr>
<tr>
<td>Barn PCR number (time)</td>
<td>-0.13273</td>
<td>0.04454</td>
<td>0.0029</td>
<td>0.88</td>
<td>0.80</td>
<td>0.96</td>
</tr>
<tr>
<td>Pig start weight category§</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>middle vs smallest</td>
<td>0.11169</td>
<td>0.03704</td>
<td>0.026</td>
<td>1.12</td>
<td>1.04</td>
<td>1.20</td>
</tr>
<tr>
<td>middle vs largest</td>
<td>0.20790</td>
<td>0.07830</td>
<td>0.0079</td>
<td>1.23</td>
<td>1.06</td>
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<td>0.0029</td>
<td>0.35</td>
<td>0.18</td>
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* Tetracore Inc., Rockville, MD, USA.
†(time) = interaction variable with time.
‡ Number of PCR positive pigs in the cohort at enrollment.
§ Pigs were categorized within a barn (cohort) based on their initial weight as follows: smallest (lightest 7 pigs), middle (middle 6 pigs), and largest (heaviest 7 pigs).
** Space calculated as m²/pig.
†† HerdCheck ® 2XR, IDEXX, Westbrook, Maine, USA) categorized based on serum-to-positive (S/P) values.
‡‡ Waterer type as either a cup (cup, bowl, wet/dry feeder), nipple, or both (cup and nipple).

PPRSV = porcine reproductive and respiratory syndrome virus (PRRSV); HR = hazard ratio; 95% CI = 95% confidence interval for hazard ratio; LCI = lower confidence interval estimate; UCI = upper confidence interval estimate; ELISA = enzyme-linked immunosorbent
Figure 1. Median porcine reproductive and respiratory syndrome virus (PRRSV) IDEXX HerdCheck® 2XR ELISA serum-to-positive (S/P) values for positive (S/P ≥ 0.400) and negative (S/P < 0.400) serum samples from 460 study pigs (23 barns with 20 pigs per barn housed together in a single pen) sampled every 2 weeks to detect the rate of seroconversion from nursery through market.
Figure 2. Bland-Altman agreement plot between the two different serial number kits used in determine the porcine reproductive and respiratory syndrome virus (PRRSV) ELISA S/P values for the samples described in Figure 1. The solid red line is the calculated mean difference between the natural log transformation of results obtained for the same sample’s ELISA S/P for each serial kit. The dotted red lines encompass the 95% CI for the mean difference. Paired sample t-test did not show statistically significant difference between both test kits (P>0.05)
Figure 3. Distribution of the grouping of the number of pigs within a cohort ($n=20$) testing positive for porcine reproductive and respiratory syndrome virus (PRRSV) at the time of enrollment via polymerase chain reaction (PCR) for each of the 23 study barns.
Figure 4. Distribution of the average number of pigs per pen for all 23 barns analyzed in longitudinal study to detect the rate of seroconversion to porcine reproductive and respiratory syndrome virus (PRRSV)
Figure 5. Distribution of the range of the number of 2-week periods needed for all pigs in a cohort to complete seroconversion to porcine reproductive and respiratory syndrome virus (PRRSV) for all 23 barns studied. See Table 5 for a description of study and definitions used to determine seroconversion. For this graph, period 1 was defined as the first time period when the first pig in the cohort was determined to have seroconverted to PRRSV.
Figure 6. Kaplan-Meier survival curve comparing for the number of days until seroconversion to porcine reproductive and respiratory syndrome virus (PRRSV) was detected between pigs that were polymerase chain reaction (PCR) positive for PRRSV and those testing negative at enrollment. The study involved 460 pigs in cohorts of 20 pigs housed together in the same pen in 23 barns from enrollment until marketed. See Table 5 for definitions on seroconversion. The log-rank test identified these two groups as having statistically different survival curves (P<0.001)
Figure 7. Kaplan-Meier survival curve comparing for the number of days-to-seroconversion to porcine reproductive and respiratory syndrome virus (PRRSV) was detected between pigs of different starting weights within a cohort. Pigs were categorized within a barn (cohort) based on their initial weight as follows: SMALLEST (lightest 7 pigs), MIDDLE (middle 6 pigs), and LARGEST (heaviest 7 pigs). See Figure 5 and Table 5 for study information and definitions on seroconversion. The log-rank test did not identify any statistical differences in the survival curves between all three weight groups (P>0.05)
Figure 8. Kaplan-Meier survival curve comparing the number of days-to-seroconversion to porcine reproductive and respiratory syndrome virus (PRRSV) detected between pigs with different levels of PRRSV ELISA serum-to-positive (S/P) values at enrollment. Pigs were categorized based on S/P value into: NEGATIVE (<0.400), LOW (0.400 to 0.999), MEDIUM (1.000 to 1.999), and HIGH (≥2.000). See Figure 5 and Table 5 for study information and definitions on seroconversion. The log-rank test did not identify any statistical differences in the survival curves between all four categorical grouping of PRRSV ELISA S/P values (P>0.05)
CHAPTER 4. SURVEILLANCE OF PIG POPULATIONS USING ORAL FLUIDS

A paper submitted for publication in

*Preventive Veterinary Medicine*

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**Abstract**

Currently there is a lack of a cost effective method to efficiently and routinely collect informative diagnostic specimens on multiple agents over multiple time periods from pig populations. The objective of this study was to evaluate if oral fluids collected by barn personnel could be used as a method of surveillance based on PCR testing. Approximately 12,150 pigs in 10 wean-to-finish barns on 10 different farms were monitored for the circulation of porcine circovirus type 2 (PCV2), porcine reproductive and respiratory syndrome virus (PRRSV), influenza A virus (IAV), and Torque teno virus genogroups 1
(TTV1) and 2 (TTV2) using oral fluid specimens. Oral fluid samples were collected from 6 pens at each site starting at the time of pig placement (~3 weeks of age) and continuing thereafter at 2-week intervals for a period of 18 weeks. Data were analyzed both on a pen basis as well as a barn basis. Overall, 508 (85%) samples were PCR positive for PCV2, 73 (12%) for PRRSV, 46 (8%) for IAV, 483 (81%) for TTV2, and 155 (26%) TTV1 during the study period. The estimated arithmetic means of the quantitative PCR-positive oral fluids for PCV2, PRRSV, and IAV were $1 \times 10^{4.62}$, $1 \times 10^{4.97}$, and $1 \times 10^{5.49}$ per ml. With a single exception, all barns were positive for PCV2 and TTV2 at every sampling point in the study. Pathogen circulation varied among barns, particularly for IAV and PRRSV. Chi squared analysis of the cumulative distribution of pen level agent combinations between all 10 barns indicated they were statistically different from each other. The most commonly observed patterns were PCV2+TTV2 (239, 40%), PCV2+TTV1+TTV2 (88, 15%), and PCV2 alone (66, 11%). Although weekly mortality was the only herd health parameter available for evaluation, the Cox proportional hazard survival analyses showed that the issue of herd health is complex and will require thoughtful experimental design and analysis. This “proof-of-concept” project showed that a variety of pathogens circulate both intermittently and continuously in pig populations and demonstrated that barn health is highly variable, even among barns in the same production system. Oral fluid sampling and testing may ultimately meet the objective of being a more convenient and cost effective means for pig barn surveillance.
1.0 Introduction

Over the latter half of the 20th century, commercial pig production in most parts of the world changed from small, extensive (outdoor), labor-dependent, enterprises to large, intensive (confined), production systems. Technification of pig production achieved higher production efficiencies (Hayenga, 2000), but also introduced a "new class of diseases" characterized by complex, multi-factorial etiologies (Bäckström and Curtis, 1981; Stärk, 2000). At about the time this change began to occur, Dr. Calvin Schwabe (1982) wrote an editorial in which he labeled the lack of "economically and scientifically viable approaches to the new practice of intensive livestock production" a "crisis" for preventive veterinary medicine. Perhaps recognizing the complexity of the health dynamic involved, he envisioned a future in which veterinary practice would be "a form of on-going on-farm research based upon surveillance ...." Thirty years after Schwabe's editorial, this vision remains largely unfulfilled, primarily for lack of a method to efficiently and routinely collect informative diagnostic specimens from pig populations. Infectious diseases monitoring and surveillance remain limited to narrowly focused research or government-funded programs and more cost effective approaches are always sought. Thus, heirloom methods of diagnostic sampling impede the full application of analytical methods (epidemiology, disease ecology, and economics) to the solution of infectious disease issues in contemporary pig populations (Dohoo, 1993; James, 2005).

In human diagnostic medicine, oral fluid specimens collected using simple, non-invasive procedures are used for the detection of a wide variety of infectious agents, hormones, toxins, and drugs (Streckfus and Bigler, 2002; Tabak, 2001, 2007; Prickett and
When used in infectious disease surveillance, e.g., HIV (Connolly et al., 2004) and measles virus (Carr et al., 2009), oral fluid testing has facilitated the safe, efficient, and low-cost of high volume diagnostic sample collection. For example, in the United Kingdom, oral fluid specimens were collected from 11,698 children at home by their parents and mailed to the laboratory for antibody testing (Bartington et al., 2009). Not so unlike humans, oral fluid samples are easily collected from individual pigs or pigs grouped in pens by allowing them to chew on cotton rope suspended in the pen for 20 to 30 minutes, manually extracting the fluid from the rope, and decanting the sample into a tube for submission to the laboratory (Pricket et al., 2008a; Kittawornrat et al., 2010). Research has shown that oral fluid specimens can be used to monitor porcine reproductive and respiratory syndrome virus (PRRSV) (Prickett et al., 2008a,b; Kittawornrat et al., 2010), porcine circovirus type 2 (PCV2) (Prickett et al., 2011), and influenza A virus (Irwin et al., 2010) circulation in swine populations using polymerase chain reaction (PCR) assays. The objective of the study described herein was to determine if Dr. Schwabe's vision of "on-going on-farm research based upon surveillance" could be achieved through the routine collection and testing of oral fluid samples from contemporary swine farms.

2.0 Materials and Methods

2.1 Experimental design

Approximately 12,150 pigs housed in 10 wean-to-finish barns on 10 different farms, but under a unified management system, were monitored for PCV2, PRRSV, influenza A virus (IAV), and Torque teno virus genogroups 1 (TTV1) and 2 (TTV2) circulation using oral fluid specimens. Oral fluid samples were collected from 6 pens at each site starting at
the time of pig placement (~3 weeks of age) and continuing thereafter at 2-week intervals for a period of 18 weeks. Oral fluid specimens were primarily collected by persons working on-site and were then mailed to Iowa State University Diagnostic Laboratory to be tested for the pathogens of interest by PCR-based assays. Serum samples were collected from 5 pigs in each of the 6 pens during the first week of placement and in the week following the last oral fluid collection. Serum samples were tested for antibodies against PCV2, PRRSV, and IAV. Daily mortalities were recorded by barn workers on a barn sheet and summarized for each week. At the study termination, diagnostic data for PCV2, PRRSV, IAV, TTV1, and TTV2 infections were analyzed in relation to mortality over time.

2.2 Barns and pigs

Animal housing, feeding, handling, and veterinary care were under the supervision of Murphy-Brown L.L.C. Health Assurance and Welfare personnel. Pigs monitored in the study were farrowed in breeding herds located in Colorado, Illinois, Oklahoma, and Utah USA, weaned at approximately 21 days of age, and transported to one of 10 barns in the upper Midwest USA. Pigs were vaccinated against PCV2 with a killed commercially available vaccine at 18 to 25 days of age, but were not vaccinated against IAV, PRRSV, or TTV 1 or 2. There were no special animal treatments needed for this study. Any and all treatments or interventions performed were part of the routine care administered to animals by their caretakers.

The 10 barns in the project were on 10 different farms. Each wean-to-finish barn was one of three barns on the farm site, with individual sites spread over approximately 645
kilometers. The barns housed approximately 12,150 barrows and gilts, with 9 barns of
~1,100 animals and one barn of 2,250 animals. Barns were divided into 18 (n = 1), 20 (n =
1), 36 (n = 1), 40 (n = 2), 50 (n = 4), or 60 (n = 1) pens. All barns had fully slatted floors,
deep-pitted manure handling systems, automated feeding systems, and nipple drinkers. Nine
of the barns were curtain-sided and one was tunnel ventilated.

2.3 Biological samples

2.3.1 Oral fluid samples

An oral fluid sample was collected from each of the same 6 pens in each barn at 2-
week intervals. Within each barn, the 6 selected pens were spaced approximately equidistant
to each other over the length of the barn, with three pens distributed on each side of the
center walkway. Oral fluid sampling was conducted on Mondays starting the week the pigs
were placed in the barns and continued each 2 weeks for 18 weeks thereafter (6 pens \times 10
barns \times 10 samplings = 600 oral fluid samples). To standardize and simplify oral fluid
collection on the farm, a metal bracket with a 2.5 cm hole in the center of the horizontal
surface was fixed in each pen. Bracket structure and placement depended on building
structure. Brackets in the tunnel-ventilated building in the study were bolted to a gate in each
pen. Brackets in the naturally ventilated buildings (n = 9) were hung from a rafter above
each pen. To collect the oral fluid sample, one end of the cotton rope (1.6 cm diameter) was
knotted and the opposite (unknotted) end threaded through the hole in the horizontal surface
of the bracket. The rope was then cut to length so that the loose end hung shoulder-high to
the animals. Each collection period lasted 30 minutes, after which the lower, wet portion
(portion with oral fluids) of the rope was inserted into a single-use, disposable plastic bag and
cut from the remainder of the rope. The rope was manually squeezed while inside the plastic bag to release the oral fluid, then the corner of the bag was cut and the sample decanted into a 50 ml centrifuge tube. Samples were chilled and shipped immediately following collection.

For shipment, samples were placed in an insulated shipment container with frozen ice packs to the diagnostic laboratory. Upon arrival at the laboratory, samples were centrifuged at 9000 x g for 10 min, decanted into 5 ml tubes and frozen at -20°C until assayed.

To facilitate uniform sampling, 10 "collection kits" were assembled and stocked at each of the 10 barns. Each collection kit contained all of the materials necessary for one collection: pre-cut lengths of three-strand cotton rope 1.6 cm in width (n = 6), single-use disposable plastic bags (n = 6), 50 ml centrifuge tubes (n = 6), disposable scissors, a permanent marker for labeling tubes and bags, and a pre-paid, pre-addressed, insulated shipping container and ice packs for shipping samples to the laboratory following collection. In addition, a pictorial step-by-step guide to the process (oral fluid collection, processing, packaging, and shipping) was detailed on a laminated poster (0.9 m x 1.2 m) displayed in each barn. Investigators demonstrated the process to caretakers, after which barn personnel assumed responsibility for collection. Effort was devoted to this phase of the project to ensure sample quality and timely collection.

2.3.2 Serum samples

Blood samples were collected from 5 different pigs conveniently selected pigs in each of the 60 monitored pens at the time of pig placement and during the week of the final oral fluid collection for a total of 600 serum samples. Samples were collected using a single-use
blood collection system (Becton Dickinson, Franklin Lakes, New Jersey, USA). Blood samples were centrifuged at 1000 x g for 10 min, after which the serum was harvested and stored at –20°C.

2.4 Laboratory assays

Biological samples were appropriately processed by laboratory personnel and stored frozen in the laboratory until the study was completed. Laboratory testing was performed after all samples were collected. To avoid test bias, tubes were randomized (www.radom.org), renumbered, and submitted as a single set of samples.

2.4.1 Polymerase chain reaction assays

The presence of PCV2, PRRSV, IAV, TTV1, and TTV2 in oral fluid samples was determined using PCR assays. Viral nucleic acids were extracted using commercial kits and the KingFisher® 96 magnetic particle processor (Thermo Scientific, Waltham, MA, USA). TTV nucleic acid was extracted using the MagAttract® Virus Mini M48 kit (Qiagen®, Valencia, CA USA), as directed by the manufacturer. Porcine circovirus type 2, PRRSV, and IAV nucleic acid extraction was performed using the MagMAX™ viral RNA isolation kit (Applied Biosystems, Foster City, CA USA) with modifications to the manufacturer’s protocol described by Chittick et al. (2011).

A quantitative real-time reverse transcription (RT)-PCR assay was used to detect PRRSV or IAV RNA. The PRRSV PCR was performed using Ambion® AgPath-ID™ PRRSV One-Step RT-PCR kit (Applied Biosystems) on the ABI 7500 instrument (Applied Biosystems) and analyzed according to manufacturer’s recommendations. A standard curve
was generated using the genomic copy standards provided in the kit (1 x 10^5 to 1 x 10^{10} genomic copies per ml) and the results were expressed as PRRSV genomic equivalents per ml (log_{10}). Polymerase chain reaction for IAV was performed using the QuantiTect® Probe RT-PCR Kit (Qiagen) in a 20 μl reaction volume using 4μl of extract on the ABI 7900HT Sequence Detection System (Applied Biosystems), as previously described (Sim et al., 2009). A set of IAV preparations, each with a known virus titer was used to generate a standard curve.

For the detection of PCV2 DNA, a quantitative PCR was performed as previously described (Opriessnig et al., 2003). In brief, the PCR reaction consisted of 50 μl of a mixture that contained 25 μl of TaqMan® Universal PCR Master Mix (Applied Biosystems), 5 μl of extract from each sample, and primers and a probe at a final concentration of 0.4μM each and 0.2μM respectively. Each plate was run in GeneAmp® 5700 Sequence Detection System (Applied Biosystems) under conditions specified by the manufacturer for the master mix used.

To detect TTV1 and TTV2 DNA in a differential manner, a gel-based nested PCR was performed following the procedures, conditions, and settings described by Martinez et al. (2006). The Taq PCR Core Kit #201225 (Qiagen®) was used for the initial PCR setup. A primary and nested round of PCR was run to amplify TTV genetic material in the samples (Applied Biosystems). The presence of TTV1, TTV2 or both in samples was visualized using agarose gel electrophoresis and compared to molecular weight standards 260 bp and 230 bp respectively.
2.4.2 Serum antibody assays

Serum samples were assayed for the presence of anti-PRRSV antibody using a commercial ELISA (HerdChek® PRRS Antibody 2XR Test Kit, IDEXX Laboratories, Inc., Westbrook ME USA) following the instructions provided by the manufacturer. Data management and calculations were performed using software provided by the manufacturer with S/P values ≥0.40 considered positive.

Serum samples were tested for the presence of anti-IAV antibody using a commercial ELISA (FlockChek® Avian Influenza MultiS-Screen Antibody Test Kit, IDEXX Laboratories, Inc.). This ELISA is an epitope-blocking assay in which labeled monoclonal antibody against influenza A nucleoprotein (NP) is blocked from binding to the NP-coated microtiter plate if the serum sample contains antibody against NP. Ciacci-Zanella et al. (2010) evaluated the AI-MS ELISA using serum samples from 304 negative control pigs and 149 serum samples from 84 animals pigs experimentally inoculated with a variety of IAV isolates (H1N1, H1N2, H2N3, H3N2) and reported the diagnostic sensitivity and specificity of the assay at 94.6% and 99.3%, respectively, at a sample-to-negative (S/N) cutoff of ≤0.60. In this study, pig serum samples were tested for anti-influenza antibody according to the instructions provided by the manufacturer. Data management and calculations were performed using software provided by the manufacturer, with S/N values ≤0.60 considered positive.

Serum samples were tested for anti-PCV2 antibody using an ELISA described by Nawagitgul et al. (2002). In brief, serum samples were diluted 1:100 in 5% non-fat dry milk
(NFDM) in phosphate buffered saline (PBS) and assayed in two wells coated with partially-purified recombinant open reading frame two (ORF2) protein that had been produced in baculovirus-infected cells. To measure background reactivity, serum samples were concurrently assayed in two wells coated with baculovirus-infected insect cells (SF-9 cells, Invitrogen 11496-015, Carlsbad, CA USA). Controls consisted of PCV2 antibody-positive serum diluted in PBS to the appropriate range of reactivity and antibody-negative serum from a cesarean-derived, colostrum-deprived pig determined to be free of PCV2. Antibody-antigen reactions were detected using horseradish peroxidase-labeled anti-swine IgG antibody (KPL, Gaithersburg, MD USA) diluted 1:2000 in PBS followed by the addition of tetramethylbenzidine (TMB, KPL 52-00-03). The reaction was read at 405 nm and measured as optical density (OD). The results were corrected for non-specific reactivity and reported as sample-to-positive (S/P) values. Serum S/P results $\geq 0.30$ were considered positive for anti-PCV2 antibody.

3.0 Data Analysis

Statistical analyses were performed using SAS® Version 9.2 (SAS® Institute Inc., Cary, NC, USA). Data was analyzed both on a pen basis as well as a barn basis. A barn was considered positive for an agent when any pen out of six was positive. Differences in the distribution of virus combination patterns among barns were tested using Chi-square tests. The association for the presence or absence between the five different viruses (PCV2, PRRSV, IAV, TTV1, TTV2), was assessed through pairwise comparisons using the Cochran–Mantel–Haenszel test stratified by barn and pen. If a significant association was identified, differences in odds ratios between barns and pens were evaluated using the Breslow-Day
Test for homogeneity. Data were subjected to survival analysis using the PHREG procedure in SAS used at a barn level to evaluate the association between the number of positive pens samples and the mortality for each agent. Mortality information was captured on a weekly basis. Oral fluid samples were collected every two weeks. To better associate the weekly mortality with an oral fluid sampling, for the weekly periods when samples were not taken, the results from the previous and the next sampling period were averaged. Both univariate and multivariate estimates for hazard ratio were calculated associating weekly oral fluid results (number of pens positive for each of the five viral agents) and the mortality for the following week. Barn was used as a fixed effect in the model.

4.0 Results

Samples were collected between June and November of 2007. Field personnel did not report any difficulty in collecting, processing, packaging, and submitting samples to the diagnostic laboratory. No samples or sampling periods were missed and samples arrived at the laboratory in good condition.

4.1 Serum antibody results vs. oral fluid PCR results

Analysis of the PCR data in the context of serum antibody results (Table 1) showed general agreement in herd infection status between the two assays with three inconsistencies:

1. Cumulatively, 127 (71%) of the 180 oral fluid samples collected from barns 1, 8, and 9 were PCR positive for PCV2, including 16 (89%) of the 18 samples collected at week 18. However, only 30% (barn 1), 37% (barn 8), and 13% (barn 9) of the animals tested serologically positive at week 18 (Table 1). On a barn level basis, conclusions on the PCV2
status of the barn were the same for oral fluids and serum testing.

2. Barn 8 had one PRRSV PCR-positive and 59 (98%) PCR-negative oral fluid samples over the course of the observation period. Serologically, 30 of 30 pigs in barn 8 were negative for PRRSV serum antibodies at week 18. On the basis of this evidence, the single PRRSV PCR positive oral fluid was considered a false positive and reclassified as negative. On a barn level basis, conclusions on the PRRSV status of the barn would be different for oral fluids (barn considered positive) and serum testing (barn considered negative).

3. A comparison of serum samples collected the week of placement vs. week 18 post-placement in barns 1, 7, and 10 showed that IAV seroprevalence had increased over the observation period (Table 1). However, no IAV PCR-positive oral fluid samples were obtained from these herds. These data suggest that the IAV PCR had produced an unknown number of false-negative oral fluid results over the course of the observation period. On a barn level basis, conclusions on the IAV status of the barn would be different for oral fluids (barn considered negative) and serum testing (barn considered positive).

4.2 Pen-level results

The PCR data on the 600 oral fluid samples are summarized in Table 2 and Figure 1. Overall, 508 (85%) samples were positive for PCV2, 73 (12%) for PRRSV, 46 (8%) for IAV, 483 (81%) for TTV2, and 155 (26%) TTV1. Fifteen (3%) of the samples were negative for all 5 agents, 117 (20%) were positive for one agent, 289 (48%) for 2 agents, 149 (25%) for 3 agents, 27 (5%) for 4 agents, and 3 (0.5%) for all 5. Twenty-four of 32 possible outcomes (a
binary outcome for each of five different PCRs or $2^5$) were observed, with combinations of agents more common early in the post-weaning period (Table 2). The most commonly observed patterns were PCV2+TTV2 (n = 239, 40%), PCV2+TTV1+TTV2 (n = 88, 15%), and PCV2 alone (n = 66, 11%). Cumulatively, one of these three patterns were detected in 393 (65%) of the 600 samples.

4.3 Barn-level results

Barns were considered positive if one or more pen samples were PCR positive for the agent of interest (Figure 2). On that basis, all barns were positive for PCV2 and TTV2 at every sampling point in the study except for one PCV2-negative sampling from barn 8 and one TTV2-negative sampling from barn 6. All barns were positive for TTV1 at least once, and 9 of 10 (90%) barns were positive for PRRSV at least once during the observation period. Seven of 10 (70%) barns were PCR-positive for IAV at least once, but as discussed in Section 4.1, IAV was determined to have circulated undetected in the remaining 3 barns on the basis of the serum antibody response of pigs in this barn. For TTV1, but even more so for PRRSV and IAV, the pattern of circulation (onset and duration) was highly variable among farms.

Overall, Chi squared testing identified there were differences in cumulative distribution of pen level agent combinations between all 10 barns (p < 0.001). Pairwise comparisons of the presence or absence of viral agents stratified by barn and pen only identified statistically significant positive associations between PRRSV and TTV1 ($p = 0.018$) and between PCV2 and IAV ($p = 0.002$). No other associations between agents were
identified as statistically significant at $p \leq 0.05$.

4.4 Quantification of viral shedding

The arithmetic means of quantitative PCR-positive oral fluid pen samples over time are shown in Figure 3. The arithmetic means for PCV2, PRRSV, and IAV were $1 \times 10^{4.62}$ (based on 510 PCV2-positive samples), $1 \times 10^{4.97}$ (based on 73 PRRSV-positive samples), and $1 \times 10^{5.49}$ (based on 46 IAV-positive samples) genomic equivalents per ml. No IAV-positive pens were detected in any barn at 10 and 12 weeks post-placement. Quantification of TTV1 or TTV2 was not possible as only a gel-based PCR (i.e., qualitative) was available.

4.5 Association of mortality with agent detection

The average weekly mortality rate for all barns over the study period was 2.04 pigs per 1000 (Figure 1). Univariate and multivariate survival analysis using Cox proportional model examined the association between PCR-positivity for infectious agents and mortality are summarized in Table 3. The hazard ratio is the ratio of the instantaneous conditional risk for mortality given a pig has survived up to that point. The univariate analysis identified statistically significant hazard ratios (HR) for TTV1 (HR = 1.089; $p = 0.022$) and IAV (HR = 1.160; $p < 0.001$) with PRRSV (HR = 1.075; $p = 0.052$) being close to statistically significant. The multivariate analysis did not show any statistically significant interactions among other pathogens. Multivariate hazard ratios for TTV2 (HR = 0.805; $p = 0.008$) and IAV (HR = 1.203; $p < 0.001$) were the only two identified as statistically significant.

5.0 Discussion

Swine producers and veterinarians routinely make herd health decisions with little
baseline information. These decisions are balanced between disease losses that occur when necessary treatment is withheld and costs that occur when the unneeded or mis-timed treatment is applied. Swine health researchers experience similar limitations in the breadth and depth of data collected for clinical trials and field studies and often are unable to account for potentially significant interactions among pathogens. The lack of complete and timely infectious disease information primarily stems from the inconvenience and cost of acquiring diagnostic samples, i.e., the labor and expense of routinely and periodically collecting and testing statistically meaningful numbers of blood, feces, or nasal swab samples from individual pigs. To address this shortcoming, we evaluated a method of surveillance based on PCR testing of biweekly collections of pen-based oral fluid samples by farm personnel.

Oral fluid sampling was easily implemented by producers and caretakers. With the exception of one set of samples from one barn that were collected a few days late, all samples arrived in the laboratory via the mails as scheduled and in good condition and no sampling periods were missed resulting in 100% compliance. For future animal health emergencies, it should be noted that oral fluid sampling by barn personnel completely eliminated the risk of moving pathogens between sites during the process of specimen collections.

Diagnostic assays for oral fluids are well-established in human diagnostic medicine (Prickett and Zimmerman, 2010), but are still in the process of development and validation for swine oral fluid specimens. In this study it was determined that the PCR assay used for the detection of IAV produced an unknown number of false-negative results. This shortcoming will be rectified as diagnostic assays for oral fluid are further refined (Chittick et al.,
In addition to PCR-based assays, adaptation of antibody assays to porcine oral fluid specimens is an important development (Prickett et al., 2011). Polymerase chain reaction assays are useful for documenting circulation of a pathogen in a population, but isotype-specific antibody assays could inform regarding the stage of herd immunity. In addition, antibody testing may allow for surveillance at a lower cost than is currently possible with PCR assays. As validated PCR and antibody assays become available for oral fluid-based surveillance of swine populations, the repertoire of oral fluid testing will hopefully include pathogens of international concern, e.g., foot-and-mouth disease virus (Eblé et al., 2004), classical swine fever virus (Weesendorp et al., 2009), African swine fever virus (Greig and Plowright, 1970), and others. This cost-effective approach could make disease prevention and control accessible to more parts of the world where pigs are an economically important food source.

One or more pathogens were detected by PCR-based assays in 98% of the pen samples (585 out of 600). In general, PCV2 and TTV2 were both found in > 70% and PRRSV, IAV, and TTV1 detected in < 33% of the samples at each time period. Re-breaks with PRRSV and IAV in barns were observed using oral fluid samples. It is unlikely that the agent-specific prevalences observed in this study are universally found across commercial farms in the USA. Rather, the observed differences in infection rates among agents underline the fact that sample size should take into account the agent’s expected prevalence. In this study we arbitrarily chose to 6 samples per barn per period.

Given that the 10 farms were under a centralized management system, a certain
degree of uniformity among barns was expected in the virus circulation patterns. Unexpectedly, pathogen circulation varied among barns, particularly for IAV and PRRSV. For example, PRRSV was detected in 7 of 10 biweekly samplings from Barn 3, but only 1 of 10 samplings in Barn 5. In the case of Barn 5, 5 of the 6 (83%) oral fluid samples were PCR-positive at the time of the one positive sampling and PRRSV infection was subsequently confirmed by serology. Interestingly, the field data did not coincide with the development of herd immunity and PRRSV excretion cessation reported in the literature (Wills et al., 1997; Molina et al., 2008). In the case of Barn 3, PRRSV excretion in oral fluids was much longer than would have been predicted from the research and in Barn 5, much shorter. Whether these observations are a function of virus isolate, “population”, or some factor waiting to be identified remains an open question. Finding the answer may require the re-examination of assumptions regarding infectious diseases in swine populations. As noted by Diez Roux and Aiella (2005), “Populations are usually viewed as collections of individuals, rather than as meaningful entities with inherent properties….”

Although mortality was the only herd health parameter available for evaluation, the analyses showed that the herd health is complex and will require thoughtful experimental design and analysis. For example, univariate and multivariate analyses arrived at different conclusions regarding the role and statistical significance of TTV1, TTV2, and PRRSV. It is interesting to postulate that the protective nature of TTV2 identified in the multivariate analysis could be attributed to an immune response that has been modified due to previous exposure to a different agent. Although we did not identify any interactions in our model, it could be due to some other factor that was not evaluated in our study. Arriving at a
definitive conclusion concerning which analysis was more correct was not among the objectives of this study. Of relevance is the fact that field studies not accounting for pathogen variety and the complex circulation will almost certainly produce mistakes in analysis and interpretation of their experimental data.

The addition of infectious disease data to the production data typically collected in contemporary swine herds will result in large, complex datasets. Although site personnel can collect oral samples, animal health specialists and veterinarians will need to integrate the infectious disease data with production data, perform the analyses, and integrate their discoveries with actions and interventions to improve herd health. While this development will mandate training in statistical and analytical methods, it does not eliminate the need for veterinarians to “walk the barns”. Clinical skills and observational powers will continue to remain important tools in the armamentarium.

6.0 Conclusion

This study was a “proof-of-concept” project to determine whether the collection and PCR testing of pen-based oral fluids was practical and useful. Repeated sampling over time showed that a variety of pathogens circulate both intermittently and continuously in pig populations and demonstrated that barn health is highly variable, even among barns in the same production system. In addition, the data suggested that concepts of pathogen circulation and herd immunity based on small populations of pigs in research settings may not accurately describe the situation in the field. Farver et al. (1985) expressed the hope that animal health specialists would use “disease prevalence surveys to monitor the health of the
food animal population in an attempt to anticipate a health problem long before it becomes a full-blown epidemic." Oral fluid sampling and testing may ultimately meet this objective.

Acknowledgments

This study was supported in part by Pork Checkoff funds distributed through the National Pork Board, P.O. Box 9114, Des Moines, IA USA, the PRRS CAP USDA NIFA Award 2008-55620-19132, and Pfizer Animal Health, Inc. We would like to thank A. Clark for technical assistance during this project.

Conflict of Interest Statement

No conflicts of interest are reported by any of the authors.

References


Table 1. Serum antibody positive results at weeks 0 and 18 post-placement from 30 pigs (5 pigs per pen) in 10 wean-to-finish barns in a study designed to evaluate circulation of porcine reproductive and respiratory syndrome virus (PRRSV), porcine circovirus type 2 (PCV2) influenza A virus (IAV), and Torque teno virus genotypes 1 and 2 (TTV1, TTV2)

<table>
<thead>
<tr>
<th>Barn</th>
<th>PCV2 ELISA&lt;sup&gt;a&lt;/sup&gt; Week 0&lt;sup&gt;d&lt;/sup&gt;</th>
<th>PCV2 ELISA&lt;sup&gt;a&lt;/sup&gt; Week 18</th>
<th>PRRSV ELISA&lt;sup&gt;b&lt;/sup&gt; Week 0</th>
<th>PRRSV ELISA&lt;sup&gt;b&lt;/sup&gt; Week 18</th>
<th>IAV ELISA&lt;sup&gt;c&lt;/sup&gt; Week 0</th>
<th>IAV ELISA&lt;sup&gt;c&lt;/sup&gt; Week 18</th>
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<tr>
<td>1</td>
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<td>9 30%</td>
<td>11 37%</td>
<td>27 90%</td>
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<td>18 60%</td>
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<td>29 97%</td>
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<td>28 93%</td>
<td>18 60%</td>
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<td>29 97%</td>
<td>9 30%</td>
<td>26 87%</td>
<td>27 90%</td>
<td>29 97%</td>
</tr>
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</table>

<sup>a</sup>PCV2 antibody assay performed as described by Nawagitgul et al. (2002).

<sup>b</sup>HerdChek<sup>®</sup> PRRS Antibody 2XR Test Kit, IDEXX Laboratories, Inc.

<sup>c</sup>FlockChek<sup>®</sup> Avian Influenza MultiS-Screen Antibody Test Kit, IDEXX Laboratories, Inc.

<sup>d</sup>Weeks post-placement
Table 2. Count of PCR-positive pen-based oral fluid samples (n = 600) from 10 wean-to-finish barns over time in a study designed to evaluate circulation of porcine reproductive and respiratory syndrome virus (PRRSV), porcine circovirus type 2 (PCV2) influenza A virus (IAV), and Torque teno virus genotypes 1 and 2 (TTV1, TTV2)

<table>
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<th>IAV</th>
<th>TTV1</th>
<th>TTV2</th>
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<td>23</td>
</tr>
</tbody>
</table>

^aDashes (—) indicate agent not present

^bDots (●) indicate agent present

^cTotals represent total number of pens over all time periods (out of 600) testing positive for the specific agent

^dTotals represent 6 pens in each of 10 barns for a total of 60 samples per testing period
Table 2. Count of PCR-positive pen-based oral fluid samples (n = 600) from 10 wean-to-finish barns over time in a study designed to evaluate circulation of porcine reproductive and respiratory syndrome virus (PRRSV), porcine circovirus type 2 (PCV2) influenza A virus (IAV), and Torque teno virus genotypes 1 and 2 (TTV1, TTV2) (continued)

<table>
<thead>
<tr>
<th>PCV2</th>
<th>PRRSV</th>
<th>IAV</th>
<th>TTV1</th>
<th>TTV2</th>
<th>Number of PCR-positive pens by weeks post-placement</th>
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<tr>
<td>508c</td>
<td>73</td>
<td>46</td>
<td>483</td>
<td>155</td>
<td>60d</td>
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Table 3. Univariate and multivariate hazard survival analyses for the association of number of pens testing PCR positive for each agent and barn mortality for the following week in a study designed to evaluate circulation of porcine reproductive and respiratory syndrome virus (PRRSV), porcine circovirus type 2 (PCV2) influenza A virus (IAV), and Torque teno virus genotypes 1 and 2 (TTV1, TTV2)

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<th>Multivariate analysis</th>
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<td>p-value$^b$</td>
<td>HR</td>
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<tr>
<td>IAV</td>
<td>1.160</td>
<td>&lt; 0.0001</td>
<td>1.203</td>
</tr>
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</table>

$^a$HR=hazard ratio

$^b$Chi squared
Figure 1. Virus circulation and mortality rate over time in 10 wean-to-finish barns (12,150 animals) in a study designed to evaluate circulation of porcine reproductive and respiratory syndrome virus (PRRSV), porcine circovirus type 2 (PCV2) influenza A virus (IAV), and Torque teno virus genotypes 1 and 2 (TTV1, TTV2).

Virus circulation was determined at 2-week intervals by PCR testing of pen-based oral fluid samples (6 pens per barn) in 10 barns for porcine circovirus type 2 (PCV2), Torque teno virus genogroups 1 and 2 (TTV1, TTV2), porcine reproductive and respiratory syndrome virus (PRRSV), and influenza A virus (IAV) starting at placement at approximately 3 weeks of age.
Figure 2. Presence of porcine circovirus type 2 (PCV2), Torque teno virus genogroups 1 and 2 (TTV1, TTV2), porcine reproductive and respiratory syndrome virus (PRRSV), and influenza A virus (IAV) in barns over time.

Barns were considered positive if one or more of the 6 pen-based oral fluid samples were PCR positive for the agent of interest. A colored filled dot indicates the presence of the select agent at the specific timeframe post placement at 3 weeks of age.
Figure 3. Arithmetic means of genomic equivalents per ml of oral fluid samples positive by quantitative PCR for porcine circovirus type 2 (PCV2), porcine reproductive and respiratory syndrome virus (PRRSV), and influenza A virus (IAV)

Study involved testing 6 pens each in 10 different barns every 2 weeks starting with placement at approximately 3 weeks of age.
CHAPTER 5. SWINE BIOLOGICAL RISK MANAGEMENT

A paper for peer review and publication by

National Pork Board

Alejandro Ramirez, Pam Zaabel
Introduction

The United States (U.S.) swine industry has undergone dramatic changes in the last two decades. Some of these changes have included a declining number of operations, increasing herd sizes, increasing sow productivity and farm specialization. Regionalization of the industry is also occurring with a large number of sows located in the southeastern U.S. including Oklahoma and North Carolina, while the Midwest, especially Iowa, has become a central location for feeding pigs to market.

The following graphs from United States Department of Agriculture’s (USDA) National Agricultural Statistic Service (NASS) data demonstrate the direction the industry has taken over the past several decades. The first graph includes years 1900-2010 (Figure 1) and demonstrates the changes in the December hog inventories in the U.S. which do vary somewhat from year to year. The second graph includes years 1965-2010 and highlights the decrease in the number of farms with hogs, followed by an increase in the number of hogs per farm. These trends within the U.S. swine industry have a direct impact on and highlight the importance of mitigating disease spread on a farm, as well as between farms, as failures to control disease spread could have more costly consequences.
Since 1900, the total number of all hogs (breeding and market) in the U.S. based on December 1 inventory has generally fluctuated between 50 and 70 million with 2010 inventories being at just over 64.3 million. The lowest inventories occurred in 1934 with just over 39 million hogs and the highest occurred nine years later in 1943 with 83.7 million.

The total number of farms with hogs in the U.S. has been steadily decreasing. The number of farms dropped from 1.06 million farms in 1965 to 60,460 by 2010 (Figure 2). That is just over a 94% decrease in farm numbers in 45 years. During this same time frame, the average number of hogs per farm has increased from 48 to 1,064 head.
The swine industry is recognized as being proactive and innovative in regards to biosecurity measures. A 2008 study by Moore et al\textsuperscript{1} identified the poultry industry as having the most extensive and consistent set of biosecurity recommendations available online followed by swine. The awareness of disease transmission is recognized by the industry, but unfortunately these practices are not always effectively implemented. With that in mind, it is best to address disease transmission from the perspective of biological risk management (BRM).
Importance of Swine Biological Risk Management

The objective of the swine industry is to profitably produce a consistent, high quality, wholesome, and safe product for consumers around the world. The U.S. is the second largest pork producing country in the world, after China. In 2009, approximately 10,446,000 metric tons of pork, 10% of the world’s production, was produced in the U.S. According to data from the USDA Foreign Agricultural Service in 2008, pork is the world’s most widely eaten meat compromising 40% of the meat consumed worldwide.

Export markets have contributed to the profitability of the U.S. pork producer for several years. According to the annual study conducted by University of Missouri economists Ron Plain and Glenn Grimes, exports contributed $40.56 for every pig sold in the U.S. during 2008. One only has to look at the following table containing data from the U.S. Meat Export Federation to see how exports have increased over the past ten years.
Table 1. Total U.S. Pork Exports from 1999 to 2009, including variety meat, according to the U.S. Meat Export Federation.

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<th>Year</th>
<th>Volume (Metric tons)</th>
<th>Value ($Billions)</th>
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The emergence of the H1N1 influenza strain caused exports in 2009 to fall below the record levels of 2008. However, 2009 exports were still above 2007 levels and continued to make a positive contribution to hog prices.

Zoonotic diseases are not the only diseases which can have a huge impact on the swine industry. In 1991, postweaning multisystemic wasting syndrome (PMWS) was first
identified in Canada. The disease was characterized by wasting pigs, anemia, fever, enlarged lymph nodes, high morbidity (4 – 60%), and variable mortality (4-20%). By 1997, PMWS was being reported as a sporadic condition in several locations throughout Canada and the U.S. At the same time, France and Spain were observing a similar syndrome which spread to other European countries. Soon it was reported that a different porcine circovirus strain (now called porcine circovirus type II or PCV2) was associated with this new disease. This new syndrome appeared to be sporadic in North America, yet common in Europe. Clinical signs in Europe were observed in the nursery phase of production, while in the U.S. clinical signs associated with PMWS were more frequently documented in the early to mid-finishing phase of production. In the U.S., the swine industry initially ignored the problem, determining it was a “European” issue. Most hogs tested positive for PCV2 during the initial testing in the U.S., yet no clinical signs were being noted. Therefore, the conclusion was that PMWS was not important. Some clinicians even started calling it the “circus virus.” While the European swine industry organized a significant research effort, the U.S. practically ignored the issue. The attitude by many practitioners was that the problem was not related to “our” industry. PCV2 submissions to the Iowa State Veterinary Diagnostic Laboratory increased from the late 1990 and peaked in 2002. This led many to believe the problem was over; especially considering it was never identified as a “major” issue of concern. Then in early 2005, Canada started to have a severe PMWS outbreak with high mortality. Initially, many in the U.S. were thinking this outbreak was just a late peak in cases for the Canadians; just like cases had peaked in the U.S. in 2002. By mid-summer, as health conditions in Canada deteriorated and large numbers of Canadian hogs continued entering the U.S. to be fed-out to
market, the U.S. swine industry was in high alert. This disease which had a significant impact elsewhere finally seemed like it was making its way to the U.S. Now, it was not a matter of “if” it will arrive in the U.S., but rather “when.” In November of 2005, many large operations in the U.S. noted a dramatic increase in early finishing mortality, starting in North Carolina where mortality was reaching 50 - 60% in some units. Within a few weeks, the PMWS epidemic was upon the U.S. industry. Many operations began averaging over a five-fold increase in their average mortality early in the finishing phase. At that time, no vaccines or treatment protocols could stop or mitigate the consequences of the syndrome renamed porcine circovirus associated disease (PCVAD). Although PCVAD is a multifactorial disease, it is believed that a small mutation in the PCV2 virus had a dramatic impact in the virulence of the pathogen. Why did we ignore this virus for so long? How did this virus spread so quickly across the U.S.? Many experts believe that biosecurity lapses in a highly interconnected industry are to blame.

**Risk Perception**

The category of risk perception examines what those involved with the industry think about the real and potential risks of infectious and zoonotic diseases. These perceptions may be influenced by on farm experiences, or by what owners, managers, and employees have read in magazines, on the internet, or in the paper. This is where one may encounter many of the obstacles and challenges to educating about risk management. Many individuals have negative perceptions associated with risk management, most of which are based around ideas of disbelief or economic concerns. Common negative beliefs include the following:
• I already know this information.

• We have always done it this way.

• I’ve already had most every disease on this farm.

• I don’t have enough time to mess with this.

• It’s too complicated.

• It won’t make a difference.

• It’s too expensive.

• I don’t have the space.

• Our animals were tested once and we found nothing. It was just a waste of money.

• Our farm is pretty safe.

Risk of disease transmission cannot be totally eliminated, but attention paid to biological risk management can reduce risks and their consequences. While it is difficult to prove and measure the benefit of things that don’t happen, counter-arguments tend to fall into three categories: there is a risk, it is economically worthwhile to prepare, and the overall impact must be considered.

• Infectious/zoonotic disease outbreaks can and do happen.
• Prevention is always less costly than treatment.

• Protecting your financial investment and your future assets from liability is worthwhile insurance.

• Protecting employees saves time and money.

• A biological risk management plan established and followed can reduce the risk of disease transmission to an acceptable level for minimal to no disruptions in continuity of operation during a foreign animal disease event.

• A focus on preventative medicine helps to maximize public and environmental health of your community.

• Prevention of disease through awareness and management of infectious disease risk is an important part of improving animal welfare.

Risk Assessment

To increase its effectiveness and completeness, a comprehensive risk assessment should be performed from a variety of perspectives. First and foremost, the general herd characteristics and farm policies should be examined through pre-assessment questions in order to gather enough information to better understand the specific characteristics of that particular farm.

In reviewing this material, it is imperative to understand that attention is focused on routes of transmission, not specific disease entities. Assessing risk based on routes of
transmission provides a more complete and holistic approach while avoiding emphasizing specific diseases. Any references made to specific diseases, syndromes or infectious agents in this material is for illustrative purposes only, and no specific recommendations are suggested as to vaccination, treatment or testing procedures. This focus will make the information applicable to a variety of audiences and remain relevant even as scientific advances improve our understanding of diseases.

**Risk Management**

This document illustrates the best available “standard operating procedures” for a wide range of management practices. Each veterinarian should perform a thorough assessment of each operation to identify opportunities for improvement. Management recommendations should focus on which ones are most practical, applicable, and economically feasible. Most recommendations can be implemented independent of others. This will result in tailoring the BRM program for each producer based upon his/her preferences, resources, risk perception and risk tolerance. Some suggestions may not be feasible for a given facility; but recognizing what is optimum helps establish long term goals.

**General Practices**

*Internal vs. External*

Traditionally BRM is divided into external and internal risks. The objectives are different. When looking to mitigate external risks, the objective is to prevent the introduction of a new disease or strain into an operation. On the other hand, internal BRM is designed to minimize the spread of diseases which are already present within an operation.
purpose of this paper, we will not make the distinction between internal and external BRM. Instead, our focus is the route of transmission. Veterinarians and producers can refer to internal vs. external risks when prioritizing the management recommendations identified pertinent to a particular operation based on their specific needs and goals.

**Location**

The farm/unit location is likely the single most important risk factor for new disease introduction. Although there is evidence that some bacteria and viruses may move by airborne routes, the actual range of spread by infectious particles or perhaps aerial vectors is unknown. The range of transmission is likely highly variable and dependent upon meteorological phenomena as well as local topography and will be discussed further under the aerosol section. We do know that in high pig density areas, disease agents often find their way to near-by locations even when stringent safety measures are in place (area spread). Epidemiological field studies often fail to confirm the source of new disease agents. We also know that indirect contact between the pigs and the outside world is greatly increased in these high density areas. In a recently published study, the number of direct livestock contacts (number of times animals were moved from one farm to another) per month for some pig farms in California was an average of 0.2/month, but the indirect contact rate (number of times people or vehicles that had visited more than one facility and therefore could act as fomites) was 807/month.\(^3\) In areas of high pig density many, if not most, of the indirect livestock contact rate would be associated with pigs from other farms. Although in an ideal world one would select a location as far away from other pigs as possible (especially for higher health segments of the industry – see next section on health pyramids), many times
this is not practical, feasible, or even an option. High traffic areas (especially those used by others to transport pigs) can be a risk for aerosol transmission of different diseases. With the concept of BRM, one must realize the current limitations of all operations and concentrate on other ways one can try and mitigate disease transmission.

Iowa has been the number one state in pig numbers for many years. Because of this, most genetic companies have elected to locate their genetic nucleus herds outside this state. Locating these high health herds as far away from other pigs as possible minimizes the opportunity for exposure to swine pathogens though the air or area spread.

**Health Pyramid**

The swine industry has been proactive in establishing production pyramids to be able to produce high quality, high health status breeding stock. These pyramids are referred to as genetic or health pyramids. Technically, genetic pyramids focus only on the structure of genetic breeding (parents, grandparents, F1s, etc.), while health pyramids focus primarily on the health status of different operations. In essence, the concept is the same, and therefore we will address them simply as health pyramids. The number of operations in each category of a pyramid increases as you move down the pyramid. For example, one genetic nucleus can supply nine nurseries which may feed into 18 different finishers, etc. (1→9→18→etc.). The exact numbers are not important, but rather the concept that more operations are being impacted as you move farther down the base of the pyramid. The following diagram helps visualize a typical health pyramid.
The goal of a pyramid is to designate specific farms for specific production needs. Not only does this allow maximizing genetic pools, but also an opportunity to focus on health issues at these sites. As operations look at maximizing their investment returns, health pyramids help prioritize resources. The hierarchal nature of the pyramid highlights a key biological risk management factor. The basis for the continued success of a “disease free” herd relies primarily on its ability to continue to have a “disease free” source of replacement animals. The advent of a better understanding of the porcine reproductive and respiratory syndrome (PRRS) disease emphasized the importance of this key point. Because of this, all of the major genetic suppliers in the U.S. recognize that they needed to provide PRRSV naïve animals. There is a distinction between a naïve animal and a negative animal. The
definition of a naïve animal is an animal that has never been exposed to a particular disease agent. A negative animal just implies that the animal tests serologically negative. However, this particular animal could have, at one time, been exposed to a particular agent and has become serologically negative over time. We have also realized within the past 15 years that not all positive animals are equal. This is especially true when looking at diseases such as PRRS in which there are multiple strains circulating. Just because an animal has been exposed to a particular strain does not imply in any way that it would be resistant or less susceptible to a different strain. Herds that are negative do not provide the same sense of safety as a naïve herd. With non-naïve herds, there is always the question of whether they are truly negative or just have a low incidence of disease which is not being detected through routine monitoring programs. Because of this, heavy emphasis is placed on obtaining naïve replacement animals.

Through the development of health pyramids, the industry has created a method to become more efficient at producing healthy replacement animals, but has also opened themselves up to a risk of disseminating new diseases to a larger number of operations. The use of a centralized location for producing replacement animals requires the dissemination of these animals to several other respective farms. So, in other words, a single genetic nucleus farm can provide replacement animals to several dozen commercial sow farms. A leak of an undetected infectious agent from the genetic nucleus to several dozen different sow farms can then spread the disease to many more nurseries, which can then spread the disease to a larger number of finishers. To minimize this cascading effect, operations must implement monitoring programs in order to quickly detect the emergence or re-emergence of any
disease of concern. The earlier a disease is detected, the quicker a new plan can be implemented to redirect pig flow, so as to minimize the consequences of a disease break downstream.

This health pyramid concept is also applicable for all swine operations when establishing work orders. Although small operations might not be relying on a different location for providing their replacement animals, they can utilize the same concepts in establishing animal and people movement within their operation. Chore order could be related to the health status of a particular stage of production within a farm. At the top of this “health pyramid”, we would have the breeding herd. Within the breeding herd, we prioritize the healthiest animals as those in the farrowing house, followed by the gestation animals, nursery animals, and finally the finishing animals. Within each of these groups of animals, we prioritize health from youngest to oldest. The older animals are the more likely to have been exposed to more disease agents; and therefore, we would consider them to be a lower health risk. The concept again is the same. We are trying to minimize the downstream effects of disease by controlling disease toward the top of the pyramid. So, our chore order in this case would be as follows:
Work order

Breeding Stock     First – Highest Health Concern
Farrowing (Youngest → Oldest)
Gestation
Nursery
Finisher     Last – Lowest Health Concern

This chore order would change if the health status of any one of the groups changes so as to visit any potentially infected animals last, thereby minimizing the opportunity for spreading disease to other groups.

**Routes of Transmission**

Pathogenic agents can be spread from animal to animal, animal to human, or human to animal through a variety of transmission routes. Animals or humans can acquire disease causing agents through aerosol, oral, direct contact, fomites, or vectors. Pathogen exposure to swine can occur in any of the aforementioned methods, and many times it occurs in more than one way. Many disease agents can survive for extended periods of time in dust or organic matter. This survival time is specific for each pathogen and dependent on many factors including temperature, light exposure, humidity, and environmental pH. While environmental contamination is not a route of transmission in itself, it must always be
considered when developing a BRM plan. Each route of transmission will be discussed separately, and some general management/control strategies will be included.

**Aerosol**

Survival of swine pathogens in air is dependent on the pathogen load, temperature, and humidity. Most swine pathogens will survive for at least a few minutes after a point exposure to air. Influenza A virus (IAV) has been reported to survive for 15 hours in air. The duration of air contamination is extended in buildings in which pigs are continuously shedding organisms. Foot-and-mouth disease virus was detected in air for 5 days, and porcine respiratory coronavirus was detected in air for 6 days in rooms housing infected pigs. The distance that pathogens can be transmitted by aerosol is widely based on anecdotal evidence and computer modeling.

Airborne transmission of pathogens is facilitated by prevailing wind velocity and direction, cloud cover, and humidity. Increasing the distance between infected and susceptible animals will decrease the chances that transmission will occur because pathogen concentration in air decreases exponentially with increasing distance. Increasing distance between hogs within an operation, between other operations, wildlife, and newly introduced animals will help minimize exposure by the aerosol route. The distance that pathogens can travel and be transmitted by aerosol is not completely understood, but experimentally, pathogens rarely travel more than 2 miles in air. Farms located within 1.25 miles of > 4 farms were almost 3 times more likely to experience two or more respiratory disease outbreaks/year than farms located within 1.25 miles of ≤4 other farms. Herds within 3.2 km
of an infected herd had the highest risk for aerosol transmission of *Mycoplasma hyopneumoniae*. Environmental and management practices are the most consistent means of control against respiratory disease, which is the main type of disease transmitted by aerosol.

Proper indoor air quality and ventilation practices are essential for swine health and well-being, in part, by diluting and removing harmful contaminants from buildings. Proper ventilation is important to reduce dust and feed particles in the air. These particles can carry bacteria and viruses and can increase aerosol transmission of disease. Dust reduction protocols can be implemented, such as adding 1% fat to feed or sprinkling oil on building surfaces, which also decreases bacteria levels. It is important to ensure adequate air flow from a fresh source, thus displacing air that has high concentrations of organisms. Utilizing proper ventilation to keep humidity low (40-60%) can reduce the water droplets available for pathogens to travel in. Aerosol droplets with infectious agents in a low humidity environment will not allow the organisms to live; however, in an environment with moderate humidity, the pathogens can remain viable and infective. At very high humidity, the droplets will pick up water, increase in size, and precipitate out of the air, thus making it easy for transmission of the organisms. Overall, air quality depends on a number of factors including density, the cubic capacity of the building, the lower critical and upper critical temperatures, concentrations of gasses and levels of dust. Air quality can be monitored based on the content of certain gasses, particulate matter, and airborne microbes in the air around or in swine facilities.
Air filtration systems are currently being used in higher health status herds (especially boar studs) to help minimize aerosol transmission of PRRSV. The use of HEPA (99.97% DOP at ≥ 0.3 microns) filters in Europe and MERV 16 filters (95% DOP at ≥ 0.3 microns) in the U.S. has been quite successful in several operations. High Efficiency Particulate Air (HEPA) and Minimum Efficiency Reporting Value (MERV) are standardized ways to rate the effectiveness of air filters with HEPA filtration having higher standards based on dispersed oil particulate (DOP) testing rate. The PRRSV is about 0.065 microns small which is much smaller than the HEPA or MERV filters can handle. But more important than particle size is how the virus is transported in aerosols. Bioaerosols, generally 0.4 – 0.7 microns in size, will be filtered out by these systems. The cost of implementing an air filtration system including initial construction costs is between $180 - $200 per sow or boar.\(^{16}\) The use of filtration systems in grow-finish buildings is rare today due to the high costs. As new and cheaper filter alternatives are developed, the use of this technology may dramatically increase. The implementation of these filtration systems also requires a complete revamping of an operation’s entire BRM program as aerosol transmission of disease is not the main or only way for disease transmission to occur.

**Oral**

Oral transmission can occur through the consumption of contaminated feed, water, or any item in the environment that pigs may contact via their mouth. Feed and water troughs/feeders, pen gating and other objects that pigs can gain access to and lick can serve as means for disease transmission via the oral route. As a general rule, viruses do not replicate outside animals as they require living host cells, but bacteria can replicate quickly.
The average time for many bacteria to double in numbers is approximately 20 minutes, and an organism like *Salmonella* can increase in numbers from one organism per mL in liquid feed to up to 200,000 organisms per mL in just 48 hours at 82°F. The goal of mitigating oral transmission of disease involves minimizing or eliminating the opportunity for oral exposure to occur, as well as minimizing the pathogen load (fewer exposures with fewer organisms each time).

**Colostrum**

Piglet survival is highly dependent on receiving an adequate amount of colostrum from the dam. Consuming colostrum which contains large amounts of the dam’s antibodies provides protection to the piglet during its first few weeks of life. Colostrum would not be as important if we were able to place young pigs into a pathogen free environment. But that is not possible in the production system. Therefore, ensuring adequate colostrum intake helps to protect the piglet by providing immunity against some of the pathogens in the environment. In an ideal world, every dam would produce enough colostrum for all her piglets, and every piglet would consume enough colostrum to provide adequate protection. However, in some cases, the dams do not milk well or piglets do not nurse. Care needs to be taken when cross fostering piglets. While this management technique has advantages of evening out litters, it also has a major health disadvantage of spreading disease. Many experts would advise against cross fostering after the piglet is 24 hours old. After 24 hours, the piglet may no longer be receiving colostrum from the dam to protect it against the pathogens that the nurse sow could be shedding.
Feedstuff

Feed can serve as a possible source of pathogens (viruses and bacteria) as well as mycotoxins (toxins produced by fungi). All operations should be very careful about acquiring products from clean sources as well as making sure that all diets are properly formulated to meet all macro and micro nutrient needs. Operations need to ask their feed providers which procedures/tests are implemented on their end to ensure source ingredients are not contaminated. Their testing protocols and frequency of testing are also important to know and verify (ask for reports). Operations also need to have their own protocols for collecting feed samples from every feed delivery as well as a mechanism for periodic testing for pathogen contamination including mycotoxins. Feed suppliers should have HACCP (Hazard Analysis and Critical Control Points) programs implemented to ensure product quality. Feed companies obtaining International Standard Organization certification (e.g. ISO 9000) are indications of verified high standards in production practices.

Distillers Dried Grains (DDGs)

From a disease standpoint, DDGs do not pose a risk of a disease introduction into a herd. However, when utilizing DDGs in a swine ration, producers do need to be concerned about nutrient balancing and the quantity of mycotoxins that may be present in the feedstuff. Mycotoxins, especially aflatoxins, are known to suppress the immune system of animals, making them more susceptible to diseases. Mycotoxins are concentrated some 3 times in DDGs. Other mycotoxins such as zearalenone mimic estrogen and therefore can present in a sow herd as a reproductive failure issue that is non-infectious.
Food Waste (Garbage) Feeding

Feeding human food waste or garbage to livestock has occurred over the years evolving from a means to utilize household waste to utilizing food waste from restaurants, schools, nursing homes, and grocery stores. According to the USDA, there were 2,783 licensed garbage feeders in the U.S. and nearly 1,964 in Puerto Rico (USDA-APHIS, VS, 2008). One concern related to feeding livestock food waste is the potential to transmit disease to the pigs, especially foreign animal diseases. Therefore, food waste to be fed to pigs must be heat treated as mandated by the 1980 Swine Health Protection Act. Heat treated garbage can only be fed from a facility in which the operator has a valid license for the treatment of garbage. However, regulations relating to food waste or garbage feeding can vary by state. In a state which prohibits the feeding of garbage to swine, a license under the Act will not be issued to any applicant. Therefore, in some states, feeding treated or untreated waste is against the law.

Diseases may be spread to other livestock or humans if swine consume contaminated meat in improperly treated food waste. Livestock diseases which may be transmitted to swine through food waste include classical swine fever, foot and mouth disease, African swine fever, and swine vesicular disease. Other pathogens of concern that could spread to humans are Salmonella, Campylobacter, Trichinella, and Toxoplasma. Food waste must be cooked as described below.

- Food waste shall be heated throughout at boiling (212°F or 100°C at sea level) for 30 (thirty) minutes.
• It shall be agitated during cooking, except in the steam cooking equipment, to ensure that the prescribed cooking temperature is maintained throughout the cooking container for the prescribed length of time.

It is the presence of meat in food waste that necessitates cooking; all table or plate scraps resulting from the handling, preparation, cooking, or consumption of food require cooking before feeding to swine (except for those produced and fed upon household premises). The act does not require the cooking of non-meat food waste or byproduct items (e.g. bakery waste, vegetable waste, etc.).

In 2009, an Interim Rule was adopted concerning the Swine Health Protection Act. This rule added another type of material to the list of exempted materials. “Processed products” may be exempt from the cooking requirements. According to the rule, if industrially processed products contain meat, they meet the definition of garbage and must be heated throughout at boiling or an equivalent temperature for 30 minutes to be eligible for feeding to swine. However, in some cases, the procedures used to process such materials are controlled and monitored in such a way that it is possible for the processors of the materials to demonstrate that the materials have been heated throughout to at least 167°F for at least 30 minutes, making the additional “margin of safety” of heating the material boiling unnecessary.21

For production units utilizing food waste as a feedstuff for their animals, care needs to be taken to ensure the correct nutrient balance is maintained. In many instances, the type of the food waste on a day by day basis may vary and, hence, so does the nutrients included
with that particular food waste. If pigs aren’t maintained on a correctly balanced diet, production can be poor and diseases may be more of a concern.

Spray-dried Animal Plasma (SDAP)

The use of spray-dried animal plasma has become a nutrient source that more producers are trying. Concern has been expressed over the possibility of this nutrient source spreading disease to pigs. In a study published in the Journal of Animals Science in 2005, inclusion of SDAP in the diet improved growth of pigs without seroconversion. Spray-drying conditions used in this study were effective in eliminating viable pseudorabies and PRRSV from bovine plasma. Additionally, other studies have demonstrated that the higher drying temperatures for extended times eliminate viable pathogens for swine vesicular disease and classical swine fever viruses in addition to Salmonella and E. coli bacteria. Producers should make sure they acquire SDAP from a reputable source that has quality control measures in place to make sure drying temperatures and times are high enough to ensure viable pathogens have been eliminated.

Water

When considering the risk of water contamination, it is important to identify the source of water for each operation. Rural water sources are safe for drinking as water must meet specifications for human consumption. Deep well water is usually safe as natural filtering occurs. However, some deep well water can contain high levels of bacteria, which generally cause a digestive upset to animals (and humans) when first exposed to the new water source. The use of surface water (ponds, lakes, etc.) is a major concern for disease
transmission from birds and other wildlife. Pathogens such as influenza viruses, leptospirosis, and avian tuberculosis are just a few that have been suspected to be transmitted to pigs though contaminated surface water.

Water chlorination is used as a way to help kill pathogens in the water. This is especially important when surface water is being used for drinking purposes on an operation. The process of water chlorination is simple, but it does require individual farm testing as the pH of water has a great effect on the ionization of the chlorine molecule. It is the free chlorine which is active against the bacteria and viruses. Routine testing of water chlorination on farm can be achieved through the use of swimming pool kits.

The use of individual nipple waters is more likely to minimize disease spread via water. Cup waters and especially troughs can serve as a source to spread pathogens between different animals. For example, PRRSV has been shown to have lasted for 11 days in a water system after pigs left the facility. The importance of disinfection between groups cannot be emphasized enough in order to prevent the spread of disease.

Bedding

In some production units such as hoop style structures, bedding is required. Bedding can serve as a source of pathogen introduction. The bedding, whether corn stalks, straw, wood chips or other material, needs to be from a pig-free source where it has been protected from birds and rodents. The bedding could serve as a vector for pathogens to enter a herd, as well as transmit a disease between groups. Care needs to be taken to remove all the bedding between groups, so as to prevent disease spread.
Although unusual, corn stalk bedding baled when containing high moisture may harbor high levels of mycotoxins. As discussed in the DDG section, high levels of mycotoxins can affect the immune status as well as reproductive performance of the animals housed with this bedding source.

**Manure Handling**

Decontamination of manure pits is not practical on a routine basis, but may be necessary to reduce the risk of pathogen transmission when manure pits are contaminated. Long-term storage of manure for at least six months at 4°C without addition of new manure should reduce virus titers by 1 to 2 log₁₀ units per month. The Federal Ministry of Agriculture in Germany provides two options. Forty to 60 liters of a 40% solution of lime hydrate per cubic meter of liquid manure can be used at temperatures between 0 and -10°C. Sixteen to 30 liters of a 50% solution of sodium hydroxide per cubic meter of liquid manure can be used at temperatures between 0 and 10°C. Manure should be stirred prior to, during, and for 6 hours after chemical disinfection. The duration of exposure of manure to chemicals should be at least 4 days and preferably 1 week.²⁴ The PRRS virus can survive in lagoons for up to 14 days in 4°C,²⁵,²⁶ while IAV can survive up to nine weeks at similar temperatures. Some of the other pathogens that can survive in manure or lagoons include *Ascaris suum*, *Brachyspira hyodysenteriae*, Pseudorabies virus, *Salmonella*, and Transmissible Gastroenteritis virus (TGE).

**Direct Contact**

Direct pig-to-pig contact is one of the most effective methods of disease spread.
Litters may be combined in the nursery, pens are mixed in the grower and finisher, and sows and gilts may be penned together during part of the gestation period. Any time animals come into contact with other animals, disease may be transmitted. Pig-to-pig contact can be limited or minimized through a variety of management approaches. Limiting pig-to-pig contact helps limit the exposure to any pathogens which are being shed at that time. Management approaches may include vaccination, changes to pig flow such as all-in all-out, herd closure, parity segregation, and isolation and acclimation procedures.

**Vaccination**

Vaccines are commonly used in swine production to mitigate the effects of disease and disease transmission. There are many different products available in many different formulations and all have different strengths and weaknesses. Vaccines can play a critical role in all BRM programs but it is important to remember that vaccines cannot prevent infection and therefore cannot be used as the sole prevention program. All pork producers need to work very closely with their herd veterinarian in determining which vaccines they should use as well as when they need to be using them as part of their BRM planning. It is also important to remember that pigs don’t all respond equally to vaccination. This can be due to many reasons including, improper administration of the vaccine, nutritional differences, differences in stress levels, and simply biological variation in immune response.

**Pig Flow**

**All-in-All-out**

All-in-all-out (AIAO) production is one of the greatest technologies that has been
implemented in the swine industry, having had a tremendous positive influence on the health status of animals. The objective of AIAO is to group animals either on the basis of a room, barn, or site by which all animals in the group are completely moved out before the next group of animals is allowed to move in. Being able to practice AIAO by site is better than by barn which is still better than by room. In essence, this coordinated movement creates a break in pig flow. This break in pig flow is critical for several reasons, the most important being that the new pigs will not be in contact with the previous group. Pig-to-pig transmission is probably the greatest way to spread disease. By removing the previous group of pigs before the next group arrives, a break in pig-to-pig disease transmission is created. Having a break also allows the area that these animals occupied (room, barn, or site) to be fully emptied so that cleaning and disinfection can be performed and the room allowed to dry. Cleaning and disinfection will also be discussed in more detail later in the paper, but it is evident that by cleaning the environment and allowing it to dry, the amount of organisms the new group of animals could be exposed to is reduced.

AIAO management has proven to be economically beneficial due to the ability to limit disease spread from one group of pigs to another. AIAO production can improve the feed efficiency, weight gain, days-to-280 lbs, and respiratory health of pigs compared to continuous flow production. In a study by Scheidt et al (1995),27 lung lesions at slaughter were 54% less prevalent and 80% less severe in pigs raised AIAO than the same source pigs raised under continuous flow conditions. Average daily gain and feed efficiency were also significantly better in the AIAO group. Other studies have shown that a $1-$5 savings per pig produced may be realized when using AIAO growing-finishing in remodeled facilities.27
Continuous Flow

The opposite of AIAO is continuous flow in which animals are constantly being added to a group. With continuous flow production, buildings or rooms are not completely emptied before new animals are brought in. Producers vary with how they manage continuous flow production. When pens are emptied, the pens may or may not be cleaned, disinfected and allowed to dry before filled with new animals. Surrounding pens may remain filled with animals. So, while the pen may be AIAO, the entire room or building is not. Therefore, animals which remain in the room or building may shed pathogens and infect the newly introduced animals. The reverse may also be true. The newly introduced animals may shed pathogens and infect the remaining animals. Typically, older animals have been exposed to more pathogens than younger animals. Therefore, when new younger animals are introduced into a continuous flow unit, these animals are exposed to the pathogens that the older animals are shedding. Diseases tend to cycle through these rooms. Disease can be difficult to control in a production unit when utilizing continuous flow methods. For this reason, AIAO should be practiced at all times.

Off-Site Production

With the advent of AIAO production, it is important to create some separation by age with pig groups. Because of this, many of today’s operations function with multiple sites each specialized in a different stage of production. From a BRM standpoint alone, ideally, one would be able to have two or three sites of production. Site 1 is considered the breeding and gestation herd. Pigs are kept on this site until weaning time; then they will move to site
2. If site 2 is a wean-to-finish site, pigs will remain there until market. If site 2 is a nursery-only site, pigs are moved to a finishing site (site 3) at a later date. Being able to completely go AIAO by site in both nurseries and finishing buildings can be very beneficial in helping break disease cycles. Many operations are not able to go completely AIAO by site and then have to rely on going AIAO by building. Finally, if AIAO by building is not possible, there are still advantages for trying to go AIAO by room.

**Early Weaning**

Early weaning may include many different approaches such as isowean, segregated early weaning, and medicated early weaning. The concept behind early weaning is that the pig consumes colostrum from the dam which helps protect it against disease. While the pig is protected with the antibodies from the colostrum, it is weaned and moved into a clean facility where it is less likely to be exposed to pathogens. If the pig remains with the dam, eventually the colostral antibodies will no longer be protective, and there is a greater risk of disease transmission vertically from the dam to the pig. With that in mind, producers have weaned pigs at varying ages trying to determine when the pigs can be taken from the dam and thrive on their own, while reducing the risk of becoming infected with pathogens from the dam. Several bacterial diseases have been eliminated in today’s production systems through the early weaning of pigs. At times, pigs will be weaned as young as 7-10 days of age instead of 18-21 days of age. At weaning, pigs are moved to a separate site that has been treated as AIAO. Under special circumstances, early weaned pigs will be treated with antibiotics to maximize the probability of eliminating certain bacterial pathogens, termed
medicated early weaning. Production units that utilize early weaning do so as a method to control disease, and strict AIAO needs to be adhered to. It is important to realize that although some pathogens can be controlled or even eliminated through early weaning, others cannot (including PRRSV, *Streptococcus suis*, *Haemophilus parasuis*). Diseases not eliminated by early weaning are usually associated with infected fetuses being born (in-utero transmission) or transmission at the time of birth, such as in the case of *S. suis*.28

**Herd Closure**

Of course, one way to eliminate disease introduction into a herd through replacement animals would be to close the herd. The concept of herd closure is just as it sounds. The herd would be closed to the introduction of new live animal genetic stock and to animals leaving and returning to the farm for any reason. As with any management approach, herd closure has its advantages and disadvantages. While closing the herd eliminates the introduction of disease through replacement animals and returning animals, the herd is unable to introduce new genetic stock. The herd would need to devise a system in which they produce all their own replacement animals. Semen can be utilized from outside sources, but in many cases, a herd would need to maintain two genetic lines. Semen from maternal lines may be used to produce replacement animals for the herd while semen from terminal lines would be used to produce market animals. Herd closure requires managing these two sets of animals differently in most cases. However, the benefit would be to eliminate disease entry from replacement animals.

From a disease standpoint, when a herd is closed to eliminate an existing disease, a
key objective for herd closure is to eliminate susceptible populations (i.e. young pigs) on the same site. In this sense, herd closure will occur for a specific time period and cannot go on indefinitely as new breeding stock (either purchased or home raised) will need to be introduced at some time point. If a herd is to be closed to eliminate an existing disease, it must ship all weaned animals off-site during this period of closure. Even if this is a farrow-to-finish site, weaned pigs must be removed from the site. This will create a break in pig age which is critical in creating a gap in new susceptible animals. This break in susceptible animals is vital in eliminating the opportunity for the disease to find new animals to infect and therefore the disease will “burn out” with time. Proper planning to discuss how replacement animals can be managed is critical to the success of the operation. Having off-site locations where replacement gilts can be developed and possibly even be bred can help minimize breeding herd down time. A key consideration will be evaluating the risk of having off-site animals exposed to different disease or strains. Once the herd is re-opened for animal introductions, these off-site animals need to be brought into the farm just like all other replacement animals, requiring a minimum of 30 days of isolation.

The duration of the herd closure for disease purposes will be dependent on the pathogens that are targeted for control/elimination. For example for PRRSV, many have identified that 200 days from the last known exposure is needed for better success rates. That means that for 200 days, no animals will be allowed to enter the herd. Herd closures for less than 200 days can be successful, but field reports indicate they are less likely to be successful.
**Parity Segregation**

Parity segregation involves separating gilts from sows. The pigs from gilts’ litters are also separated from sows’ offspring in the nursery. Production units that practice parity segregation do so out of concern that the gilts’ pigs may be infected with more pathogens than older sows’ pigs. If the older sow has been exposed to more pathogens and has built up better immunity, she will pass that protection on to her piglets in the colostrum. However, a gilt, which has not been exposed to as many pathogens, may not pass the colostral protection on to her pigs.

Providing sufficient facility space is a limiting factor for some production units when considering parity segregation. The success of parity segregation can be maximized by keeping the offspring from these gilts separate from older parity offspring throughout all of the growing phases.

**Replacement Animals and Animals Returning to the Farm**

When replacement animals need to be introduced into a herd, care should be taken to reduce the risk of disease introduction to the main herd. Anytime new animals are brought into a herd, a risk of disease introduction is possible. Veterinarians should always conduct a “Vet-to-Vet” consult before any new animals are purchased for additions to a herd. This is especially true regarding breeding stock animals. Care should also be taken when animals from a herd leave to attend an exhibition and then return to the original herd. During the exhibition, animals are exposed to a large number of other pigs as well as humans which can all pose as a source of infection to pigs at the show. A study of Australian pig shows in 2006
indicated that pigs were 40 times more likely to be fed swill (human food including meat) by
the public than by staff or exhibitors even considering the biosecurity concerns of foot and
mouth disease and classical swine fever in that country.\textsuperscript{29} Awareness of human influenza
transmission from humans to pigs was raised in 2009. Not only was there concern that pigs
carry influenza and could possibly transmit it to people, but also the pigs could be exposed to
influenza from the human visitors. Animals returning home or replacement animals
introduced into a herd can carry new pathogens or isolates onto the production site.
Protocols need to be in place and followed to minimize the risk of a herd becoming infected
with one of these pathogens. Incoming animals should never be allowed to enter into the
herd directly and be co-mingled with herd animals.

Of course, the risk of exposure varies with the potential pathogen. However, one has
no way of knowing which pathogens other animals may be shedding at an exhibition. When
purchasing replacement animals, sellers should provide information on the health of the
swine, such as routine vaccination and worming procedures, and diagnostic results, so that
their suitability for the herd can be assessed; and where necessary, appropriate treatments and
vaccinations administered. This information is usually obtained from a “Vet-to-Vet” consult.

When either a herd purchases replacement animals from more than one source or the
exhibition animals return home from multiple shows, the risk of pathogen exposure increases
for the herd. For example, studies have shown that herds purchasing stock from more than
one source per year were almost three times more likely to become infected with
\textit{Mycoplasma hyopneumoniae} than herds purchasing from a single source.\textsuperscript{30} A study by Maes
et al (2001)\textsuperscript{31} also identified frequency of gilt replacements as a risk factor for lung lesions at slaughter house in farrow-to-finish herds.

While incoming animals may not appear to be showing any signs of disease, they may be carrying the disease, otherwise known as incubation. The incubation period is the time between when an animal becomes infected and when that animal shows signs of disease. In some cases, many days may pass before an infected animal shows signs of disease. One model estimated that six to 30 days would pass before clinical signs of TGE were detected in a herd after the introduction of a single carrier pig.\textsuperscript{32} Isolating incoming animals prior to introduction into the herd minimizes the risk of disease entry.\textsuperscript{33} The duration of the isolation period can vary greatly between farms but should always be a minimum of 30 days. Factors which determine the length of the isolation period include the health of the farm from which the replacement animals come and the health status of the receiving farm. Animals returning from an exhibition could be exposed to a large number of pathogens, therefore, making their isolation period longer. During isolation, animals can be observed for clinical signs of disease, tested for pathogens they may be carrying, and acclimated to organisms already present in the breeding herd.\textsuperscript{33} Diligence needs to be taken when caring for animals in isolation. Because they may be carrying and shedding various pathogens, employees responsible for these animals need to take care of them at the end of the day and not return to the main herd until the following day. Separate coveralls and boots may be left at the facility. Equipment utilized in the isolation facility should remain there and not be utilized in the other buildings, so as not to risk carrying diseases on the equipment from isolation animals to those in the main herd.
Where should an isolation facility be located in relation to the main herd? This is a great question which has been debated with no scientific data to give a precise location. On some production sites, the isolation unit is located in a room of one of the buildings or may be its own separate building located off site. Keeping some physical separation is helpful in emphasizing these units need to be treated differently. Unfortunately, the layout of the site might restrict the separation distances as well as the actual location for this facility. No matter what the location is, many experts do agree that the isolation facility should be treated as being separate from the main herd. Therefore, this facility should have its own feeding system, manure disposal, and attention to the personnel caring for the animals.

While placing animals in isolation helps to protect your herd, those newly introduced animals can also become infected with pathogens present on your farm. Acclimatization introduces new breeding stock to viral and bacterial pathogens present on the receiving farm.\textsuperscript{33} Acclimatization may be done in the same facility as isolation, although with only one group of animals at a time.\textsuperscript{34} The Swine 2000\footnote{33} study found that 84.1\% of sites that isolated new breeding females vaccinated them as part of the acclimatization process. Other practices used commonly to acclimate gilts were: exposure to cull females (49.0\% of sites); feedback of feces from other swine (25.1\% of sites); feedback of mummies/placentas/stillborns (11.3\% of sites); and exposure to sick pigs (7.7\% of sites).

The vaccination schedule for replacement animals can be arranged with the herd veterinarian.\textsuperscript{34} The vaccines utilized will vary by herd depending on the diseases present in the herd, diseases affecting animals in the area, and vaccines available and their efficacy.
The duration of the acclimation period is dependent on the disease of greatest concern. Because of PRRSV, many facilities today utilize at least 60 days of acclimation to allow animals to be fully exposed to the virus as well as “cool down” (stop shedding virus) before they can be introduced to the rest of the herd.

Throughout the isolation and acclimation periods serologic monitoring can be utilized as a tool to determine the immune status or possible exposure of the animals to various diseases. Blood can be collected and tested for a variety of diseases. In many cases, collecting samples and testing for the immune response to a disease at different points in time help determine the exposure of an animal and how their body is responding to that disease. For example, when replacement animals arrive on site, samples may be collected to detect their immune status to diseases that the veterinarian or producer is concerned about. Some animals may have a low titer, high titer or no titer evident at that time. Therefore, in many situations, the animals are retested 2-4 weeks later, and their titers compared. The immune response of those same animals may look different at that time. Now, the replacement animals need to be exposed to the diseases that are currently present in the main herd. Again, serologic monitoring is a useful tool to determine when and if the exposed animals are mounting an adequate immune response to the diseases of concern as well as to make sure the animals are not shedding virus (viremic) so as to be able to enter the main herd.

In many instances, to minimize the time needed for isolation/acclimation, some operations will actually start the acclimation process shortly after the animals arrive on farm at the same time that the isolation period is going on. Strictly speaking, from a BRM
standpoint, focusing on external biosecurity is not the best practice. Animals are being exposed to the site’s own agents shortly after arrival. It will become difficult to differentiate clinical signs due pathogens external to the operation (bad) or those already present in the operation (good). An operation needs to evaluate the benefits vs. the risks of running concurrent isolation/acclimation periods.

Age at entry is dependent on the health status of incoming animals as compared to the health status of the recipient herd. Some veterinarians are recommending entry as early weaned pigs. This decreases the likelihood of introducing new diseases (as animals have less time to get exposed to different agents) and allows ample time for the pigs to become acclimated to the recipient herd’s diseases. When the pigs are acclimated or exposed to the recipient herd’s diseases, they will have plenty of time to get over the disease and stop shedding the disease agents. Farms with limited isolation facilities on-site have tended towards more traditional age at entry (60 days prior to breeding). A successful isolation/acclimitization program does not allow disease to get from the isolation unit to the main herd while fully exposing these new animals to all pathogens already present on farm.

The cleaning and disinfection of isolation facilities is a controversial topic, as these facilities are usually used for acclimation purposes as well. On one hand, the goal of acclimation is to expose animals to pathogens already present on the farm. By cleaning and disinfecting the isolation/acclimation facility, disease exposure is minimized. On the other hand, not cleaning the facility can create an environment that may be too contaminated, which in turn, could overwhelm the replacement gilt’s immune system and create clinical
disease. It may be appropriate to clean the isolation/acclimation facility possibly once or twice a year. This would allow continuation of disease exposure without overwhelming the system too much. The buildup of manure is also tough on equipment, so some cleaning will extend the life of the facility/equipment. If a facility is used strictly as an isolation facility, it is imperative that it be fully cleaned and disinfected between groups to prevent disease exposure from one group to the next. It is also important to note that if, at any time, the isolation/acclimation building has to be emptied because of unexpected disease exposure, the facility must be fully cleaned and disinfected before the next group of animals is allowed to enter.

**Semen**

Artificial insemination (AI) reduces the risk of disease transfer between the boar and breeding female. However, certain pathogens can be transmitted in semen. Parvovirus, PRRSV, Brucella, pseudorabies virus, and many other disease agents have been isolated from semen of infected boars.

Currently 90% + of all breeding on farms is done via artificial insemination. This has allowed for quicker genetic advancements and more efficient use of boars. This broad practice makes semen a significant risk for spreading diseases simultaneously to many different operations.

Porcine reproductive and respiratory syndrome virus can be transmitted via fresh and diluted semen, and infected boars can become long-term carriers of the virus. Currently, the U.S. boar industry is focused primarily on PRRSV transmission via semen. Boars can shed
PRRSV in semen as early as 3 days after infection, they do not necessarily show clinical signs and they can test positive via polymerase chain reaction testing (PCR) for up to 92 days. Boar studs need to be monitored routinely for PRRSV.

There are many other important swine pathogens that have been found in semen from infected boars. Although PRRSV is currently the biggest concern in the U.S., awareness of other pathogens is important. Very few boar studs currently test for other pathogens other than PRRSV. For example, leptospirosis has been reported in swine from all parts of the world. The disease mainly causes reproductive problems in breeding herds (abortion, stillborn piglets and infertility). Clinical symptoms and infertility may also occur in acutely infected boars. Venereal transmission is thought to play an important role in the spread of infections.

The PCV2 virus has also been linked to a number of other disease conditions, including reproductive failure (late term abortions and stillbirths). However, PCV2-associated reproductive disease under field conditions does not seem to be common.

To prevent possible spread of infectious diseases via AI, several precautionary measures should be undertaken in AI centers. First, individual hygiene and general sanitation procedures are important. Personnel collecting semen or coming into contact with any materials need to understand that they can be a source of contamination or act as a carrier in transferring contamination. To minimize the bacterial load originating from the boar, the ventral abdomen should be clean and dry. Preputial fluids, which can contain high numbers of microbes, should be evacuated prior to exteriorization of the penis for semen collection.
Detailed lists of measures that can be taken to minimize the risk of contamination are reported by Althouse et al.\textsuperscript{40} To reduce the unavoidable presence of bacteria in the ejaculate and to prolong in vitro longevity of sperm, preservative levels of antimicrobials are an essential constituent of any semen extender. Apart from a possible dilution effect of pathogens, semen processing and addition of antimicrobials, however, do not eliminate viruses. Monitoring for bacterial contamination of the extended semen samples may constitute an important part of a control program. Harmless organisms that have no negative influence on sperm quality and sow herds do not need to be monitored, unless they exceed a certain threshold or would have an indicator function.

The health status of the animals should be checked daily. However, as indicated before, clinical examination alone is insufficient, since clinically normal boars can shed pathogens (e.g. CSF virus, FMDV, PRRSV, PRV, and \textit{Brucella suis}) in their semen. Vaccination of the boars can be considered for some pathogens; for example, vaccination against parvovirus may help to reduce shedding of the virus following infection.

Disease surveillance in boar studs is a continuous process. Animals are constantly being monitor by testing the semen (especially for bacteria) as well as the blood (especially for PRRSV). Diligence is necessary in these facilities as they have potential to infect a large number of sows if a disease agent is shed in the semen. Serologic monitoring is utilized on a regular basis as part of the protocol of disease surveillance in these facilities. It is critical that all operations purchasing semen from outside their production unit utilize a source that is reputable and progressive in their disease monitoring. It is important to know the exact
monitoring program the stud implements to better evaluate the operation’s risk for disease introduction through semen. Relationships with the boar stud should also be strong enough that the operation and its veterinarian are kept informed of any changes in their monitoring program. Periodic communications with the stud can help make sure monitoring programs are being implemented as planned. As with all BRM programs, there is no single perfect program that will eliminate all risks. The goal of all BRM programs is to minimize and mitigate as much risk as possible. All monitoring program do add costs to the production of semen.

_Fomites_

_Facilities and Equipment_

Fomites include objects such as equipment for sorting and treating animals, feeders, boots, and clothing that can become contaminated with pathogens and infect another animal. Survival of an infectious dose of a pathogen on fomites and subsequent transmission to a susceptible host is dependent on many factors. Some pathogens do not survive outside of the pig. Others can survive for weeks or longer in manure.\textsuperscript{41}

Care must be taken to minimize the risk of disease introduction or the occurrence of disease spread via fomite contamination. One way to reduce this risk is through good sanitation. Therefore, diligence and attention to detail is essential. Equipment needs to be washed thoroughly to remove all manure and dirt that is present. A study by Kauffold et al (2005)\textsuperscript{42} showed that ultrasound equipment used in swine operations is commonly contaminated with both bacteria as well as PRRSV residues. Although this study did not
demonstrate whether this PRRSV was infective or not, it did show that equipment can become contaminated even when plastic or household cling-film is used. This study highlights the importance of minimizing the sharing of equipment, even valuable equipment, between farms.

Another important area that may be overlooked is equipment which comes into direct contact with animals. This is especially true regarding equipment used to process baby pigs (e.g. tail docking and castration). Work by Alvarez et al in 2002 demonstrated that dipping equipment momentarily in Nolvasan® disinfectant did not significantly reduce the median aerobic bacterial counts on the cutting blades of the equipment. Wiping the blades with a clean cloth was actually quite effective. This study highlights the contact time disinfectants need to be effective. To allow for sufficient contact time, different sets of equipment need to be used in a rotational basis. It probably will not be practical to allow for equipment to be fully disinfected between each pig, but longer contact time can be achieved by switching equipment between litters of pigs. Although the use of a clean towel was quite effective under research conditions, it would be hard in a regular field setting to have a clean cloth available at all times.

Some pathogens can survive in and be spread through dirt and manure contamination. After the dirt and manure are removed, an effective disinfectant should be utilized. Which disinfectant is most effective on this farm? That is a tough question. While pathogens on each farm vary, some disinfectants are more effective against certain pathogens. Review the labels on the disinfectants currently being used. Are the farm pathogens of concern listed on
the label? If not, review labels of other disinfectants to see if there is a disinfectant that will kill the pathogen of interest. After a disinfectant has been selected, follow the directions on how to best utilize the disinfectant to kill the pathogen. Using the product too diluted or not allowing for enough contact time can reduce the effectiveness of the product.

**The ideal disinfectant has the following characteristics:**

- Proven broad-spectrum activity and efficacy under farm conditions
- Fast acting to rapidly kill highly infectious agents
- Temperature stable and active at extremes of heat or cold
- Stable after dilution, especially for footbaths
- Suitable for porous and non-impervious surfaces
- Active in the presence of organic matter
- Versatile for use in footbaths, vehicles and surfaces
- Safe for environment, animals and operative staff
- Government approved for notifiable diseases
- Easy to store
- Cost effective
Footbaths are another effective way of preventing disease transmission if used properly. In some cases, footbaths serve their purpose of reducing the risk of spreading disease that may be present on boots into a facility. However, many times that is not the case. Employees and visitors are busy and may bypass the footbath by stepping over it or quickly stepping through it on their way into the facility. The disinfectants used in footbaths are not effective if there is contamination on the boots or if contact time of the disinfectant on the boot is inadequate. Therefore, for a footbath to be effective, contamination should be scrubbed off boots before the footbath is used. A boot brush should be available, so that before personnel step into the footbath, manure and contamination can be removed. Also, the brush will apply disinfectant on all surfaces of the boot while standing in the footbath. Scrubbing plays a major role in decontamination of boots as shown in Table 2 below.46,47 Selection of a proper disinfectant not inactivated by organic material as well as maintenance of the footbaths is another concern.48,47,49 At any given time on many farms, one could inspect footbaths and find that a majority are poorly maintained, containing contamination. Footbaths need to be cleaned with fresh disinfectant added on a daily basis to be effective.
Table 2. Bacterial counts from sole of boots after respective treatments

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean bacterial count</th>
</tr>
</thead>
<tbody>
<tr>
<td>No foot bath</td>
<td>$2.78 \times 10^8$</td>
</tr>
<tr>
<td>Step though disinfectant</td>
<td>$1.76 \times 10^8$</td>
</tr>
<tr>
<td>Stand in disinfectant for 2 min</td>
<td>$2.59 \times 10^7$</td>
</tr>
<tr>
<td>Scrub in disinfectant for 30 sec</td>
<td>20</td>
</tr>
<tr>
<td>Scrub in water for 30 sec</td>
<td>$1.04 \times 10^5$</td>
</tr>
<tr>
<td>Scrub in water for 30 sec then step through disinfectant</td>
<td>120</td>
</tr>
</tbody>
</table>

Aside from correctly cleaning and disinfecting all fomites, several other procedures can be utilized to reduce the risk of disease transmission. If possible, avoid sharing equipment among premises. When treating sick animals, utilize new needles and clean snares and sorting panels between animals if possible. Handle biologics aseptically. Disposable boots and coveralls are a great alternative when visiting different sites. Caution must be taken when removing these for disposal as accidental contamination of the hands can occur. For operations with different sites, having a set of boots and coveralls that stay at each site can also be very helpful in mitigating the transmission of disease between sites.

Some operations require anyone (except for on-site employees) getting out of their vehicles to have some type of disposable foot covering such as plastic boots. These plastic boots are placed over footwear while still inside the vehicle just before taking the first step on the ground. The use of this protective covering will minimize the opportunity of any contamination from having direct contact with the site traffic area.
Not all boots are created equal. When purchasing boots, it is very helpful to look at the tread on the bottom of the boots. Each brand has a different pattern. There are also great differences in how easy these boots can be cleaned. Boots with a very narrow tread are very difficult to clean even when using a brush. This is especially true when you have been walking on these boots for some time and the manure/dirt has been compacted heavily in these crevices. Boots with wide tread are much easier to clean thoroughly. This will speed up the cleaning time, and most likely improve your BRM compliance as well. It may also be helpful to use different color boots (and coveralls) for different sites or purposes. For example, blue boots and red coveralls are to be used when taking animals to the dead pile (rendering, compost or incinerator). This helps with compliance in different ways. First of all, it serves as a reminder for all employees that changes in clothing and boots are necessary. It also allows anyone from a distance to know whether proper BRM practices are being followed. Employees are also aware that others can identify when they do not follow protocol; and therefore, they themselves are more likely to follow the rules. The hassle with dealing with special orders and different suppliers does make it more difficult to implement these color codes, but the benefit of a better BRM program are definitely worth it.
Regarding people and fomites, the following summarizes from the safest practice to the most risky:

- Shower-in and shower-out with complete change in boots and coveralls **Safest**
- Change in boots and coveralls and washing hands
- Change in boots and coveralls
- Change in boots
- Simply “clean & disinfect” boots worn elsewhere **Most risky**

The concept of shower-in and shower-out will be discussed in greater detail under the employee section of the BRM. Hand washing is the single most important measure to reduce the risk of disease transmission to humans. It is also a great way to minimize the spread of disease from animal to animal. Hands should be washed between different animal contacts (different age, different barns, different sites) and after contact with secretions, excretions, or tools/equipment contaminated by them. Hands should also be washed before working in the office or eating. Proper installations (sink and water supply) and supplies (soap and towels) are critical in making sure workers have good access to hand washing equipment as well as ensure their use.

Besides boots and coveralls being a source of contamination, other equipment and supplies arrive almost daily. The risk of disease transmission from these is unknown.
Research by Dee et al (2002) has shown that cardboard boxes can become contaminated with PRRSV especially in the winter when the virus can survive longer in snow. This snow can melt once inside the building which can then serve as a source of contamination to anything placed on the floor. Because of the risk supplies and equipment can pose to an operation, several BRM techniques can be implemented. Probably the most common one is a double bagging system. With this system, operations will ask supplier to double bag everything they purchase. Once arriving to the farm, the outside bag will be opened and the inside bag will then be grabbed from someone in the clean side to carry in the supplies. This will prevent the box and outside bag from entering the facility as they are considered dirty. For larger pieces of equipment, this practice is not practical. A second method used by some operations is to build a small room where all products entering the facility can be fumigated with a proper disinfectant. This process does require a special room as well as means to make sure that the fumigation plume reaches all of the outside surfaces of all equipment. This is difficult to do in one step as all supplies need to be placed on some type of heavy duty screen so provide better access to the bottom of the equipment/supplies. To alleviate this, many operations will actually run the fumigation process twice with a rotation of all supplies/equipment occurring once between each of the fumigations. For equipment/supplies too large to fit in the fumigation room, a simple manual decontamination process needs to occur.

**Loading**

The loading area can prove to be an area of disease exposure for any farm. Design and location should be carefully considered to ensure that any vehicles loading or unloading
pigs are kept on the dirty side of the unit. In fact, some production units designate areas as a clean area and a dirty area with an actual painted line separating the two areas. This painted line serves as a continuous reminder that a separation is necessary to maximize BRM. Personnel and equipment in the clean area must stay on the clean side while the truck driver and any loading equipment from his truck must remain on the dirty side. Once an animal crosses over from the clean side to the dirty side, it must not be allowed to turn back. The same is true for employees. If a production employee crosses over the line, they must re-enter the facility by following the set protocol which may include showering, changing coveralls and boots, or hand washing as if they were entering for the first time that day.

Materials used for the loading bay must be easy to clean and disinfect. The truck driver should wear clean clothes and boots each time, especially with each individual farm. The loading facility should be washed and disinfected, ideally after each use, and should not drain into the building. If cleaning of the load-out facilities is done by farm personnel, cleaning should be done at the end of the day so that personnel do not need to re-enter the building that day.34

Ideally, every load-out should be constructed to prevent entry by truck drivers. Load-out areas should have a roof and fencing installed to the roof’s edge. The chute gate should have a guillotine mechanism which prevents animals from re-entering the load-out holding area. “Sacred zones” should be designated to truck drivers by management. These areas are strictly off limits to the drivers.52 It is extremely helpful to have a great working relationship with your truck driver so they, too, are very familiar with your BRM practices without
requiring a full explanation every time someone shows up to deliver or load-out hogs.

**Transport Vehicles**

Many trucks enter and exit a production site in one day. Feed trucks, delivery vehicles, farm employees, veterinarians, and trucks to load-out pigs can all put a production site at risk for disease exposure. The recovery of *Salmonella* from the truck swabs taken in one study suggest that feed trucks might serve as a potential source of contamination.\(^{17}\) A 1995 study by Bech-Nielsen et al. suggested that PRV virus was transported from swine markets to farms, by either the farmer or trucker.\(^{53}\) Proper sanitation methods between loads, including washing with a disinfectant and hot water, should be implemented to minimize the likelihood that the truck would contribute to contamination of subsequent loads.\(^{17}\) Vehicles can potentially transmit swine pathogens when manure containing disease agents are adhered to tires or the vehicle frame. There is evidence that *Actinobacillus pleuropneumoniae*, TGE, PRRSV, and *Streptococcus suis* can be spread by contaminated vehicles.\(^{35,54}\)

It is recommended that vehicles be specifically designated for different jobs. For example, a trailer that is used to move animals within a farm should be different than the one used to deliver pigs outside the farm. A trailer used to transport isowean pigs should be different than the one that is used to take market pigs or cull animals to slaughter. Some larger systems also separate their transport vehicles into PRRSV status as much as possible. Transport trailers are dedicated for animal movements to and from PRRSV negative systems. If a PRRSV negative trailer has to be used in a PRRSV positive system, the trailer will then have to undergo additional cleaning and disinfection steps and also be required to have down
time so it can completely dry before it is allowed back into the PRRSV negative system. The more specific and dedicated tasks that trailers can be assigned, the less likely it will be that they can serve as a fomite for disease transmission, while still maintaining some operational effectiveness.

Providing an enclosed, well lit, and heated building for shelter will greatly improve the quality of the washing. Federal regulations require that all the wastewater be captured in an approved holding facility. If bedding is used, it must be held until it can be disposed of properly or applied to agriculture land. Proper slope for washing out the trailer is necessary. A minimum of 2% to 3% slope to get the wash water to run out is recommended. A pressure washer with high pressure and hot water is needed. Recommendations include using a minimum of 2,000 psi with 4 gallon per minute of water. An accurate metering device is important in the application of the soap and disinfectant.

Trucks which arrive on the farm contaminated with manure should be excused from the property and invited to return when the trailer has been cleaned and disinfected. All employees on a farm, regardless of their position, should have the authority to reject any vehicle that is not clean.
**Procedures for Cleaning and Disinfecting Transport Vehicles:**

1. Bedding and large debris should be completely removed before entering the wash area.

2. The use of detergents is highly recommended to reduce washing time by loosening debris. Normally it is applied on low pressure and by soaking the entire trailer at once. This will provide some time to loosen debris. However, don’t allow the soap to dry or it will be harder to rinse.

3. Start rinsing and cleaning the trailer from the top down. The trailer cab must not be forgotten.

4. Rinse and clean each deck from front to back and ceiling down starting with the top deck. All trailer areas and equipment need to be fully cleaned including unloading ramps, sorting boards, paddles, and boots after every load.

5. After the trailer has been rinsed inside and out, apply the disinfectant at the appropriate dilution rate. Start on the inside of the trailer and finish on the outside. Disinfectant should be applied at low pressure because many of the metering devices will not dilute properly on high pressure.

6. Clean out the inside of the cab. Wash and disinfect the floor mats.

7. After disinfection, park the truck on a slope so all the remaining water can drain out. Allow enough time for the trailer to fully dry.
The disinfectant and cleaning protocol a system uses depends on many factors including the diseases present on the farm, the stage of production of the animals hauled, where the animals were hauled to, and what the trailer will be used for next. Dee et al (2006) tested a protocol using conditions found on commercial swine production units, for sanitation of 1:150 scale models of commercial transport vehicles contaminated with PRRSV. The group concluded that high-pressure washing of transport trailers, followed by 90 to 120 minutes exposure to either modified potassium monopersulfate or quaternary ammonium chloride disinfectants applied with a hydrofoamer is likely to eliminate residual infectious PRRSV.

Following sanitation, the vehicle must be allowed adequate drying time after disinfection. As with facilities, this is the most important step in the sanitation protocol to completely inactivate the virus. The use of high volume warm air can decrease the amount of time needed for drying. The Thermo-Assisted Drying and Decontamination (TADD) system developed by PIC is recommended to achieve a dry trailer in the shortest amount of time. Studies have indicated that 120 minutes of high volume warm air applied via the TADD method can effectively remove PRRSV from contaminated surfaces in transport trailers. One system heats and holds at a minimum of 142°F for 10 minutes before flushing with fresh air.

The use of truck-mounted tire sanitizers have also been evaluated for effectiveness in minimizing bacterial contamination found in tires. Although this type of system theoretically
should be effective, two different studies have shown that there is too much variability in bacterial contamination during different seasons and therefore their effectiveness is hard to prove. Cold weather conditions also make it challenging to consistently implement.\textsuperscript{59,60}

Aside from implementing biosecurity measures to keep trucks clean, controlling traffic is another way to control disease entry. Installing a perimeter fence can prevent uninvited visitors, whether human or animal, from entering your production site. Keeping the fence locked also controls entry of vehicles and allows for employees to monitor those vehicles entering the site. Entry can be limited to only select individuals. Also, all trucks can be inspected for cleanliness. Any truck which does not pass inspection can be refused entry. Keeping the gate locked and having an employee unlock the gate for those entering, does allow that employee to monitor whose driving onto the site. If no gating is available, then proper signage at the entrance of the site is critical in helping limit traffic. Although signs will not prevent unauthorized entry, they will help limit access to those who unintentionally would be wandering on-site.

Parking

Restricting entrance to the site as well as establishing an area for designated parking is very helpful in controlling traffic on site. Placing the parking area in an obvious location, preferably with signage, will prevent visitors from pulling their vehicles too close to livestock buildings. This is especially true during inclement weather when people’s tendencies are to minimize their discomfort without thinking about the risks posed to the livestock. Having vehicle traffic close to where animals are located can increase the risk for
disease transmission including aerosol transmission.

**Employees**

Employees provide another conduit for pathogen entry into a production site. Research has proven that diseases such as foot and mouth disease (FMD), *Mycoplasma hyopneumoniae*, and IAV have been transmitted from people to pigs. People wearing clothing or boots contaminated with manure from sick animals can be a source of pathogens. However, exposure doesn’t only occur through contaminated clothing and boots. People can carry pathogens on their skin or in nasal passages. Sellers et al. (1971) reported that FMDV could be transferred by human beings, from infected pigs, to susceptible cattle. Results from Seller's work appear to be the origin for the "48 hour rule" (down time or time to be away from pigs before making contact with the next group of pigs) used by many producers even though different viruses and bacteria may be harbored for longer or shorter periods by humans. Goodwin and others (1985) could not recover *Mycoplasma hyopneumoniae* from the breath or hair of exposed personnel, but could recover the organism from clothing over a 24–48 hour period post-infection. However, recent work by Amass and Batista appear to refute these results, indicating shorter recovery times of FMDV from people and the inability of personnel to spread *Mycoplasma hyopneumoniae* from infected to naïve herds, despite multiple attempts over extended periods of time. Recent reports by Otake and Alvarez demonstrated the inability of personnel to harbor or transmit PRRSV and TGEV, respectively, following completion of basic sanitation protocols. The former study demonstrated that if PRRSV was to be detected on personnel, it was only present on the palms of the hands. In 2009 the pandemic novel H1N1 virus was documented in Canada
to have been transmitted from human to pigs. This incident help remind everyone that
down times are important for zoonotic purposes; and therefore, company policies need to be
flexible to allow employees to stay home when they are sick without concerns of losing their
job.

Having a visitor log is important in helping keep track of people traffic on site as well
as making sure visitors and repair personnel sign off on how long it has been since they have
had contact with pigs. Having people sign a sheet helps emphasize the importance of your
operation’s BRM program.

Presumably, showering and hand washing both remove contamination, but
showering appears to do a better job. Hand washing lowers the dose of pathogen but might
not lower the dose sufficiently to prevent pathogen transmission. No set time is
recommended for hand washing or showering because the time needed will depend on the
extent of contamination. A good rule of thumb is to wash until you do not see any visible
contamination. The efficacy of medicated soap varies according bacterial type. Alcohols
are not effective on visibly contaminated hands. Wearing gloves can decrease the gross
contamination of hands but does not remove the need for hand washing.

Showers for employees and visitors can be laid out in a variety of ways. In most
cases, the shower is placed at the only entrance into the building so the shower facility cannot
be bypassed. The employee may pass through a door to an area where they will undress,
leaving their clothes and jewelry in a designated area. This street side is considered the dirty
side. The employee would then step into the shower. The employee must shower, washing
body and hair. After showering, the employee steps into the next room which has clean undergarments, coveralls, and boots. This barn side is called the clean side. If at any time the employee needs to return to the dirty side, they must take another shower before returning to the clean side. Some of the shower layouts have curtains or doors with or without locks. Employees’ privacy needs to be considered when designing what will work for each production unit. Unfortunately, due to the nature of human beings to take shortcuts, showers can be viewed as a nuisance, resulting in personnel skipping the showering process completely or partaking in a cursory rinsing.

Operations with shower facilities also need to have washers and dryers on site. Being able to do laundry on site ensures that clean apparel will be available, minimizes the tracking of contaminated clothing off-site, and prevents bringing items from off-site into the operation. Preventing the contamination of off-site premises is very important especially from a public health standpoint. It is best to leave all contaminated clothing at the site to minimize taking any pathogens home where others, including children, could be exposed to zoonotic pathogens.

However, a newly recognized weakness, particularly in cold climates, is the anteroom area, located just prior to the entry of the actual shower facility. The anteroom is the section of the farm encountered immediately upon entry through the main door. Here, boots and coats are removed and stored throughout the day. Work by Dee et al (2002) indicated that the anteroom floor could serve as a site of PRRSV survival and a contamination point for shipping parcels that frequently enter swine farms, including styrofoam semen coolers,
toolboxes, cardboard containers for pharmaceutical shipments, and lunch pails. While the frequency of parcel contamination was significantly higher during cold weather versus warm weather, these studies emphasized the need for biosecurity protocols to focus upon the anteroom area throughout the year.

One other method that can be used to minimize disease introduction/spread without using a shower (or in addition to a shower as an extra BRM practice) is utilizing the so called “Danish system or boot exchange.” In this system, a solid bench is built to create a physical separation between two sides of a room. As a person enters the room from the dirty side, they will then sit on the bench, remove their footwear and street clothing and then swing their legs over the bench to the opposite side (clean side) where they can now put on clean coveralls and boots provided by the farm. The person will then exit the building through a door on the opposite side from where he/she entered. This bench serves as a physical barrier that will prevent the accidental stepping over into the clean side.

Another area of concern identified by these studies was the infamous “pass-through window”, the sliding pane of glass that separates the office area (clean area) from the anteroom (contaminated area). This so-called “barrier” is frequently abused, being left open, allowing for shaking hands of visitors with farm personnel and for the introduction of containers that have set on the potentially contaminated anteroom floor.

Employees who remain on site during their workday need to be provided with a designated kitchen area. Disease can be brought onto a site in meat products if those products are carried into the production units. This risk is minimized if employees have an
area where they can eat lunch away from the buildings, so that food is not carried into one of 
the production units. For example, classical swine fever and FMD can be transmitted in 
improperly cooked meat products such as sausage. If contaminated product were carried into 
a production unit and the pigs consumed scraps, the pigs could become exposed to classical 
swine fever. Granted while this type of scenario is a little less likely than other disease 
introductions, it is possible and would have a devastating impact. Changes in trade policy 
that permit importation of animal products from designated regions of countries with FMD 
could increase the chance of inadvertent introduction of the virus, and the threats of 
agroterrorism that include the release of exotic animal diseases increase the chance of 
intentional introduction of FMD into the U.S. 70,71

Limiting farm access to essential personnel is one method of controlling human 
contact. Security measures such as perimeter fencing and monitored entrances can also be 
used to prevent unauthorized access of people to your farm. 41 It is also helpful to have all 
doors on site locked. This added security will prevent accidental entry by an unauthorized 
individual. It also serves as a deterrent for those who are looking for a quick way to get in 
and out of a place.

Many operations will also restrict employees from owning or working with other 
pigs. This practice helps ensure that accidental cross contamination from one group of pigs 
to the other is minimized. This is especially important because many times when animals 
first pick up a disease, they may be shedding the organisms for some time before they 
actually show clinical signs of illness. So simply agreeing to stay away from sick hogs will
not be sufficient.

Some operations also restrict employees from living with other individuals that may work with pigs from a different company. The concern is that by having different people from different swine operations share a common living space, cross contamination can occur possibly putting both operations at risk. This could be a risk, but currently there is no science to support this. As long as employees do not bring home any contaminated clothing or boots, the risk is truly non-existent. The risk of zoonotic transmission from pigs to humans then to another human and then back to pigs although theoretically possible is also highly unlikely and has not been ever reported to date. There are many other BRM practices (especially shower in and shower out practices) to be implemented on farm, which are closer to the pigs, to mitigate this particular risk more effectively.

One other important BRM tool related to employees has to do with human food. As discussed under the garbage feeding section, human food especially imported cured meats can pose a risk for swine disease transmission. In the case of imported products, there is concern regarding diseases such as African swine fever, CSF and FMD. Because of this, it is important to have strict rules that prohibit employees from eating in any area where pigs can accidentally get access to these foods. In some operations, all meat products are prohibited in the buildings and are allowed only in office areas that are located away from animal premises. Some human food for employees contained in cans has been fogged in the same way other products are as they are brought onto the site. Another method utilized is to double bag the lunch so the outside bag can be removed in the entry room while someone on
Visitors

Employees are not the only people entering a production site during the day. Many visitors frequently enter production sites. These visitors may prove to be someone of very low risk of carrying a pathogen; however, they may also be carrying a new strain of PRRSV for example. Entry should always be limited to essential personnel only.

According to the USDA’s National Animal Health Monitoring System (NAHMS) report, about 80 percent of sites in 2006 did not allow anyone except employees to come in contact with areas where swine were housed, compared to about 65 percent of sites in 2000. As mentioned previously, a California study did show large swine farms as having some 807 indirect contacts with pigs per month with 95% of these occurring through people.

The Ohio State University Extension published a Factsheet titled, “On-Farm Biosecurity: Traffic Control and Sanitation” in which visitors were divided into three different categories including low-risk, moderate-risk, and high risk visitors. The level of risk depends on the possibility of exposure to other animals, especially swine, the frequency in which they visit farms, and the type of contact they have with animals. Low-risk visitors are those who come from urban areas or others who don’t have any livestock contact. This group of people has minimal risk of introducing disease into the farm. Moderate-risk visitors would be those that have little or no contact with animals but routinely visit farms. Salesmen, repair people, feed and fuel delivery people are examples. This group does present a moderate risk of disease introduction. Finally, high-risk visitors would include
anyone who is in direct contact with animals and their bodily secretions/excretions. This group would include livestock haulers, livestock-owning neighbors, processing crews, and veterinarians. This last group does pose a higher risk for disease introduction.

In all cases, providing a shower facility along with requiring a complete change in boots and coveralls (preferably provided by your own site) will dramatically decrease the probability that an accidental introduction will occur. Visitors should be restricted from entering pens or having direct contact with animals unless necessary. Providing gloves as an added safety measure can also be beneficial. All equipment and tools brought in by visitors should be cleaned and disinfected as discussed in the fomite section previously. This includes tools brought in for maintenance by outside contractors/servicemen.

International Visitors or U.S. Citizens Traveling Abroad

On type of visitor that has not been addressed is the visitor from a foreign country or U.S. citizen who has recently traveled abroad. The risk that this type of visitor could pose would depend on a number of factors including the following: the diseases present in their country or the country they were visiting, if they visited any livestock production sites, if they came in direct contact with any animals, and if they entered the U.S. with any clothing they wore on those sites. For example, a visitor from China who lives in a large city and never has had contact with livestock would pose a low risk even though the country they come from has classical swine fever. In this case, they have never come in contact with pigs. However, a U.S. citizen who visits a hog production site in Brazil and then returns to the U.S. would pose a much greater risk. In most cases, the U.S. citizen would bring their own
clothes back home and potentially carry disease into the U.S.

Human diseases in foreign countries need to be considered also when determining the precautions necessary when allowing foreign visitors. The 2009 pandemic influenza situation reminded many production units to go to greater lengths to protect their pigs from zoonotic diseases. According to their website, the National Pork Board recommends that if entry of a foreign visitor is essential, consider requiring that these people use face masks, or preferably properly fitted, valveless N95 respirators, and gloves, upon entering and while inside a swine housing facility. The bottom line is to understand the background of the visitors entering your production unit and react accordingly.

The National Pork Board distributed a pamphlet titled “Are you hosting international visitors to your farm?” reminding producers of precautionary measures to take when hosting an international visitor. The following points were included in the pamphlet:

- Did you know that many swine viruses can survive on clothing, footwear or equipment for weeks and in some cases months?

- Supply a complete set of coveralls, hairnet and boots. If they are disposable, immediately collect them in a plastic bag and incinerate them. If they are not disposable, immediately wash them in a solution of chlorine bleach (30 ml of bleach to 1 gallon of water) or other disinfectant.

- Do not allow cameras, equipment, food items or other items that cannot be properly disinfected into areas where livestock are present.
• Visitors should have had no contact with livestock for at least 48 hours prior to visiting your pork production site. If visitors are from a Foot-and-Mouth Disease infected country they should have no contact with livestock for at least 5 days prior to visiting.

• Utilize shower in / shower out, if possible.

• Supply a dust mask for all visitors to wear. The mask should be tight fitting and have two straps to secure it over the mouth and nose.

• All visitors should completely wash their hands, including scrubbing fingernails, with a disinfectant soap prior to entering the farm and again before exiting.

• Control the traffic flow and allow visitors only in carefully selected areas. Do not allow them to unnecessarily handle the livestock.

As we continue to learn more about how disease transmission occurs, these procedures and the farm policy should continue to be updated to prevent the introduction of a disease by international visitors.

**Carcass Disposal**

Unfortunately, even the most productive sites need to dispose of dead pigs. However, the method to dispose of these animals and the biosecurity precautions taken may determine
if the production site exposes themselves to a pathogen from the outside or spreads disease internally. Farms can choose between on-farm disposal techniques and off-farm carcass disposal. Either choice has its benefits and risks; therefore, each production site needs to determine which technique can be managed better at their site. Again this is the focus of a BRM.

It has been estimated that a 1,000 sow farrow-to-finish operation with annual mortalities of 7% in the sow herd, 10% pre-weaning, 5% nursery, and 2% finishing will produce approximately 85 dead pigs a week. Disposing of all these animals in an appropriate, timely, biosecure, and environmentally correct way requires some planning.

On-farm disposal techniques may include burial, incinerations, and composting. The major advantage of an on-farm system is biosecurity. Outside mortality collection trucks are not required to visit the farm, nor do farm trucks have to risk contamination when delivering mortality carcasses to a rendering plant or central collection site.

Burial was used extensively when operations were smaller because it was an inexpensive and efficient method of mortality disposal. Due to today’s larger size operations as well as environmental concerns, many states limit the number of animals that can be buried on a per acre basis, making this practice impractical. The major disadvantage of burial is the possibility of contaminating groundwater, particularly in areas with sandy soils and a high water table. In colder areas it is difficult, if not impossible, to dig trenches when the ground is frozen. Also, predators can uncover carcasses if they are not buried deep enough, which is unsightly and increases the risk for the spread of diseases.
Incineration has become quite popular in recent years. In the past, incineration generated the most public complaints in the U.S. Limited size of the equipment for dealing with larger animals (late finishing as well as adults) and limited space to deal with sporadic high mortality problems are of major concerns with most operations. Incineration eliminates all pathogens but high operational costs and incineration's potential to contribute to air pollution (if not properly maintained and operated) decreases its usefulness for widespread use as a mortality carcass disposal option.

Composting uses organic by-products such as dead pigs, straw, or sawdust and converts them into an odorless, inoffensive, generally pathogen-free product that can be used as a soil amendment or organic fertilizer. Composting pigs has gained a lot of popularity in the past few years due to its lower operational costs and better environmental sustainability. There are a lot of great resources available for properly building and maintaining a successful composting pile. Composting does work even in cold areas, although it is slower, but it does require proper management to be successful. Under normal circumstances, if proper composting practices are used, rodent and scavenger activity will not be a problem. It is always good though to take additional steps to minimize any possible rodent or scavenger activity. See the next section on vectors. In areas with large amount of wildlife, fencing off the compost pile area might be necessary to create an additional separation between the operation and wildlife.

Rendering and landfill are the two main opportunities for off-farm carcass disposal. To use a rendering service, farm personnel should deliver dead pigs to an off-site point where
the renderer can pick them up. Be sure rodents and other animals do not have access to the
dead pigs. 

Landfill opportunities are rapidly decreasing as municipal authorities refuse to accept
carcasses. With landfill tipping fees of $10-50 per ton, costs are becoming prohibitive in
areas that still allow this practice. Landfills are most often used when death losses exceed
everyday disposal capacity or under disaster situations. For producers with access to a
protein recovery plant, rendering has been, and will continue to be, the best means for
converting swine carcasses into a nutritionally valuable and biologically safe protein by-
product meal. Unfortunately, the number of rendering facilities operating in the U.S. is
decreasing, especially among small local plants that accept mortality carcasses. Many
rendering plants have closed because of more stringent EPA regulatory action and/or because
of the depressed world prices for fat, protein, and hides. As a consequence, the remaining
plants are further apart making it cost-prohibitive to transport carcasses to these locations for
disposal, leaving on-farm composting as the primary new means to dispose of carcasses.

Biosecurity guidelines need to be respected with either on-farm or off-farm carcass
disposal. Because carcasses can serve as a reservoir for disease, they should be disposed of
daily. Employees need to be cautious in their work duties not to dispose of the carcass on-
site then track back into any groups of pigs. Care must also be taken when delivering
carcasses to an off-site point (preferably some type of dead box located off site) so as not to
track back into the herd. Options for the employee may be to dispose of carcasses on-site or
haul the carcasses to the off-site location at the end of the day and not re-enter the production
until the following day. If employees need to re-enter, they need to follow the set protocol which may include showering, changing coveralls and boots, or hand washing as if they were entering for the first time that day.

**Vectors**

Rodents, feral animals, pets, birds and even insects can be sources of pathogens for pigs. The disease that each of these vectors transmits varies as may the route in which transmission occurs.

**Rodents**

When rodent infestations are not diligently managed they quickly become severe, which, in turn, can pose significant economic problems to a swine producer. Rodents consume and contaminate feed, gnaw on structural, mechanical, electrical and various utility components, and weaken concrete slabs and walkways via their burrowing activities. Norway rats and large populations of mice are particularly destructive to building insulation. Rodents can also play a significant role in the maintenance and transmission of swine diseases such as leptospirosis, trichinosis, toxoplasmosis, erysipelas, swine dysentery, and others. Mice and rats can spread or accelerate the spread of established diseases from contaminated areas to uncontaminated areas via their droppings, feet, fur, urine, saliva, or blood. As an example, mice may travel through infected manure and then contaminate the food and water of healthy animals several hundred feet away, or introduce a disease to nearby uninfected barns. Consequently biosecurity cannot be assured if rodents are tolerated in or around swine facilities.79
Rodents have been implicated in the transmission of many disease agents including pseudorabies, *Bordetella* (atrophic rhinitis), encephalomyocarditis virus, leptospirosis, *Salmonella*, swine dysentery, *Toxoplasma*, and *Trichinella*. Facility sanitation plays a critical role in controlling rodent populations. It is obviously impractical to eliminate all food sources for rodents in and around swine facilities. Still, feed spills, or equipment malfunctions that provide rodents with unlimited amounts of food should be removed or repaired as soon as possible. Easily accessible harborage is also one of the key elements that allows for rodent explosions. Any exterior debris such as old equipment, junk piles, old boards, and the like should be eliminated. Controlling weeds is also important. Weeds provide rodents with food, water, nesting material, and cover from predators. By maintaining an uncluttered 3-foot weed-free graveled perimeter around buildings, rodents cannot use these areas. Gravel should be at least 1 in. in diameter and be laid in a band at least 3 ft. wide and 1/2 ft. deep. Rats and mice can be eliminated or severely reduced in numbers by using poison baits (rodenticides) and/or rodent traps. In the majority of cases involving established infestations, rodenticide baits strategically placed based on the results of the rodent inspections will provide the most cost effective control.

**The three keys to effective control using rodent baits are:**

1. Installing fresh baits in the rodent's high activity areas as determined from the inspections and/or rodent signs (droppings, gnaw marks, etc.);

2. Placing out enough bait points to ensure the rodents readily encounter the baits during their nightly travels to gather food;
3. Matching the right bait formulation (e.g., pellets, vs. blocks, vs. packets, etc.)
to the specific area needing to be baited. A casual approach of putting out
baits in corners of barns and buildings, or simply stuffing rodent bait packets
down rat burrows will have little long term effect on rodent population
reduction, regardless of the bait brand used.

To treat exterior rat burrows in a cost effective and safe manner, loose bait pellets can
be inserted directly into the burrow, or permanent bait stations containing blocks, packets or
loose pellets can be established nearby the burrows. Stuffing bait packets or blocks down
burrows and caving in the burrow is often inefficient and sometimes hazardous because rats
often kick out some, or all of the baits applied in this manner. Rejected baits on top of the
ground the next morning, may be found by dogs, cats, wildlife and even inquisitive
children.\textsuperscript{79}

For minor infestations of rats and mice, or to stem off an infestation from new
incoming rats or mice, the use of traps, placed strategically where rodents have been noticed
is very effective, and inexpensive. But traps are too labor intensive for anything beyond a
minor infestation.\textsuperscript{79}

\textbf{Feral Swine}

Feral swine, while abundant in the southeastern U.S., Texas, and California, have
become more widely distributed in the U.S. in recent years. The USDA currently estimates
the current feral swine population to be around 3-5 million and are established in at least 32
states. These increases in distribution have resulted in increased risks for transmission of
disease agents between feral swine and commercial and transitional swine. Furthermore, the association of feral swine with commercial and transitional swine also presents a risk for transmission of foreign animal diseases. Modern swine confinement buildings have been quite successful in minimizing exposure of domestic hogs to wild hogs. Having a perimeter fence can also be helpful in preventing wildlife coming too close to your facilities. However, when feral swine do come in contact with commercial swine, producers should call their veterinarian who can contact USDA APHIS Wildlife Services. Surveillance for PRV and brucellosis is routinely performed on feral swine.

**Pets**

Dogs can spread swine dysentery and brucellosis pathogens. Dogs have been shown to harbor TGE for up to 14 days and *Brachyspira hyodysenteriae* for up to 13 days. Cats are a potential source of *Pasteurella*, leptospirosis and toxoplasmosis to pigs. Although some producers feel that cats can serve as a good rodent control program, if you do the simple math, it is difficult for them to eat that many mice. Dogs and cats can keep rodents out of sight, but the rodents are not necessarily gone. The risks of disease transmission from pets to pigs are probably much greater than the benefits in regards to rodent control.

**Birds**

Natural transmission of swine pathogens from birds to pigs has not been demonstrated. However, it has been determined that birds can carry *Bordetella* and tuberculosis. There is also evidence that birds can transmit the viruses that cause classical swine fever, PRRSV, IAV, and TGE to swine. Birds can transmit TGE (36 hour survival
and 25 mile range) and can carry erysipelas and *Salmonella*. All buildings need to be bird proof to prevent direct contact of pigs with any birds.

**Insects**

In some cases, insects can serve as a biosecurity risk. Houseflies may contribute to horizontal transmission of PRRSV among pigs within infected commercial farms. Flies (*Musca domestica*) will travel distances of up to 2 miles and can transmit *S. suis* serovar 2 (2-5 days in the crop), *Brachyspira hyodysenteriae* (4 hours) and TGEV (3 days), and may potentially transmit *Salmonella, Actinobacillus pleuropneumoniae*, and Pasteurella. However, mosquitoes and stable flies (*Stomoxys calcitrans*) are not likely to serve as biological vectors of PRRSV.

Integrated pest management is an effective way to manage fly populations. Monitoring should begin before fly season and continue every two weeks throughout the season. Cleaning up spilled feed, removing feces from pens and alleyways, spraying around facilities and keeping grass mowed are all environmental control methods to reduce fly populations. Fly bites on animals should also be treated. The easiest way to control mosquito populations is to control populations at the egg stage by removing breeding grounds. Mosquito control can also center on larvae and adult populations as well. Insect screens can be very effective in facilities to minimize exposure to outside insects.

**Zoonotic**

Zoonotic diseases are pathogens which are naturally transmissible from animals to humans. In pigs, these zoonotic pathogens can be divided into foodborne pathogens and
Foodborne pathogens from pork mainly include *Salmonella*, *Yersinia*, *Toxoplasma*, and *Campylobacter*. Influenza A virus, *Streptococcus suis*, brucellosis, colibacillosis, campylobacteriosis, erysipelas, and leptospirosis are all examples of diseases which could be spread to employees while they handle or care for their pigs. Therefore, these pathogens could be categorized as occupational zoonoses. A few pathogens have the potential to cause both foodborne and occupational zoonosis. For many of these pathogens, prevention of human infection is the same as with pigs - focus on hygiene.

Employers need to make sure employees have access to hand washing stations, and personnel protective equipment (gloves, coveralls, N95 masks, etc.) is properly stocked and functional. Employees should be informed about zoonotic diseases and prevention techniques which they should implement. Practicing several specific simple hygiene steps can prevent infections in employees.

**Steps to prevent disease transmission from pigs to man:**

1. Wear gloves whenever caring for pigs, handling their wastes (feces, urine and dirty bedding), or handling any body product such as: blood, meat, viscera, nasal discharges, or fluids draining from wounds. After removing your gloves, wash your hands with soap and water.

2. Never eat or drink in areas where pigs, their wastes, or body products are handled. Absolutely no eating, drinking, or smoking is allowed in areas where pigs are housed.

3. Report concerns over sick pigs to your veterinarian, so the
veterinarian may determine the cause of the illness and implement any additional protective steps. When the veterinarian does post signs, telling everyone to wear special, protective clothing such as masks, gowns, rubber boots or shoe covers, be sure to follow these procedures. If you don't, you increase your chances of getting sick, but also, you may expose other people by transmitting the disease pathogen on your clothes or hands!

4. Although a normal, healthy adult person may have only mild symptoms when they become infected with a zoonotic disease, that person may spread the disease to others. Cases of animal handlers "carrying home" zoonotic diseases to their infants, with serious consequences can occur. Therefore, good hygiene is not only to protect the person working directly with pigs, but all persons with whom they have contact.

5. For personnel at the farms, wear a designated pair of shoes and jeans, or coveralls, while working at the farm and don't wear these clothes anywhere else! Wash the designated clothing separate from the family wash, or have the farm unit do the washing to reduce chance of contamination.

6. Before leaving the farm, either change to another clean pair of shoes, or clean the "farm" shoes before getting into your vehicle. Use a brush to remove manure and mud, then spray with a commercial disinfectant.

**Reporting Suspect Foreign Animal Diseases**

As discussed previously in this document, the U.S. swine industry is at a continuous
risk a foreign animal disease (FAD) introduction. If a producer or veterinarian observes clinical signs that could resemble a FAD, call your State Animal Health Official (SAHO) or Area Veterinarian-In-Charge (AVIC) to report your concerns. Contact information for the SAHO and the local AVIC can be obtained by calling (866) 536-7593. You can also call the USDA APHIS Veterinary Services National Center for Animal Health Emergency Management at (800) 940-6524 (24 hours) for assistance. The SAHO or AVIC will let you know if or approximately when the Foreign Animal Disease Diagnostician (FADD) will conduct a site visit. Precautions to take concerning people movement and contact with animals should be discussed while waiting for the FADD to arrive. They will also want to start gathering information from you and the producer. Discuss the next steps to follow with the SAHO or the AVIC you have contacted. Information will be held confidential to prevent unwarranted sharing of information.

Some of the concerns to be discussed over the phone or when the FADD arrives include the following:

- When were the first lesions evident?
- When were animals last transported from the farm and what was their destination?
- When were these animals delivered to the farm and where did they come from?
- Does this producer care for other livestock?
- How many employees work at this site?
- Do the employees have livestock at home?
- Is equipment shared between sites or with neighbors?
- Does the producer grind his own feed or when was the last delivery of feed?
- Have there been any foreign visitors to the farm?
- Have any employees recently visited a foreign country?
- Are employees permitted to consume meat in the livestock buildings?

When the FADD arrives, communication will continue between the FADD, the veterinarian, and the producer. Many questions will need to be answered during the investigation. Be assured that there will be a constant stream of communication to keep those involved informed of the procedures and timeframe for sample testing.

**Risk Communication**

Risk communication is a two-way, interactive process that has been occurring throughout the risk assessment between the facility owner, risk assessor (veterinarian), the employees and other interested parties. Information has been collected, the analysis has occurred, and now information needs to be delivered to those affected by the risk assessment.
and risk management plan.

One of the major barriers to effective risk communication is inadequate planning and preparation. Before designing an educational program, it is important to consider who is best suited to communicate the message, what message will be most effective, and when and where the information should be communicated.

In large operations, the biological risk management plan may be formulated by upper management, and some employees may not understand the importance of the plan. Risk management plans must be understood, supported, and adopted by every employee for effective implementation. Because many employees may not understand disease transmission routes and the chain of events involved in disease spread, this communication can be difficult and employees may not fully appreciate the significance of the measures they are asked to follow.

Characteristics of effective risk communication:

- It must be adapted to meet the needs of the audience. If bilingual information is required, make sure it is provided;
- It should present the important information in more than one way (appeal to both visual and auditory learners);
- Keep sessions focused to a maximum of three main points and 45 minutes maximum;
• Sessions are more valuable if they are timely and the participants can apply the new information immediately;

• Sessions should cover what, when, where, how, by whom, and why;

• Give participants the opportunity to take ownership of the production process and the ramifications of decisions that impact their area. They should be actively engaged in the question at hand so that they share information, and most importantly provide input so that decisions become a collective agreement.

• Schedule meetings earlier in the day. Meetings at the end of the working day are less effective.

Educational programs that inform employees and other affected individuals of the risk assessment and management plan can take many forms, and may include:

• Face to face/group meetings (one of the best communication forms if the presenter and participants have open dialogue);

• Newsletter, fliers or bulletin;

• Videos, CD’s, PowerPoint presentations or web-based instruction;

• Posted signs or information panels placed at key locations on the farm (break rooms, shower /changing rooms);
• Employee questions and suggestions (question/answer board, suggestion box, question period during meetings, etc.);

• Mentoring of new employees by experienced employees;

• Recognition or incentive program that rewards employees when BRM goals are reached (this has been used on some farms focused on farrowing rates, and preweaning mortality).

Educational programs should not be limited to one form. Facility owners may incorporate many of the above mentioned education forms to create a program that fits the needs of their facility.

To help the veterinarian facilitate communication, there are handouts about each of the routes of transmission with various applicable diseases provided on the Center for Food Security and Public Health website (www.cfsph.iastate.edu) to educate producers about the risk of zoonotic, endemic and foreign animal diseases. The reports that can be printed based on the answers to the assessment question provide a visual tool to the strengths and weaknesses for the various routes of transmission on a swine farm. The final report graphs that are generated are meant as a visual aid to illustrate potential areas of action. The various risk factors identified have not been quantified or prioritized. It should not be interpreted as an arbitrary number which is required for a facility or veterinarian to “pass,” or even that comparable scores for two different facilities mean they face equal risk. The reports should
be used to identify if a particular area seems to represent a disproportionate risk and help track progress over time through continued assessments. The management recommendations are made to minimize circumstances that could potentially result in the spread of infectious diseases.

Proper communication of the risk management plan is of utmost importance for effective infectious disease control. When communication is effective and efficient, disease spread can often be minimized and controlled. However, few management plans are successful if records are not kept or some form of biosecurity audit performed so that progress can be measured. Part of the risk communication process should include helping to ensure that a monitoring system is put in place to measure progress.

**Conclusion**

Biological risk management (BRM) is an essential part of all swine operations regardless of their size or mode of operation. Disease risk can never be completely eliminated, but BRM is a great approach to minimizing the possibility, as well as the consequences, of a new disease introduction or the spread of disease within a farm. Each operation has different strengths and weakness, but while keeping in mind the different routes of transmission, you can work with your veterinarian in figuring out the prioritization for your particular operation. Being aware of the different routes of transmission will also serve as a reminder that there are more diseases out there than just PRRSV. A full awareness of all risks is critical in mitigating threats of endemic, emerging, and foreign animal diseases. Submitting surveillance samples which test for diseases also raises our awareness to a
national level. For example, 14,666 samples were tested for CSF during 2010 as part of the CSF Surveillance Program. Testing included samples from both domestic and feral swine (personal communication). Surveillance of the U.S. swine herd is just as important as surveillance performed on the farm. While surveillance will help to identify a disease affecting one or more herds, practicing good BRM will give each producer the best chance of keeping that disease out of their herd. As we continue to learn about transmission routes of diseases, the BRM practices on each operation will need to be adjusted. The BRM is a working document that needs to be adjusted as new information is provided.

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CHAPTER 6. GENERAL CONCLUSIONS

Data from my influenza study suggests that swine confinement workers are at increased risk for zoonotic influenza infection. Our sample size was small and was restricted to a small population which most likely does not represent all swine workers. The serologic tests we used have not been validated, but they serve as a guide for future research in this area. I believe my data was the first evidence that smoking also increases the risk of IAV even more for swine workers. At least in Iowa, this is becoming less of an issue today as new state laws restrict smoking in the workplace. Probably even more important from a practical standpoint is the evidence that suggests the use of gloves during swine confinement work noticeably decreases the risk of swine influenza virus infection. Thus a rather simple personal protective measure might do much to reduce potential zoonotic infection transmission. Worker education along with access to properly stocked hand washing stations is critical in helping mitigate possible public health concerns.

As the U.S. swine industry participates in the new Influenza Virus Surveillance in Pigs program hopefully we will have a better understanding of the disease and pathogen ecology. This is critical as it will provide us a better understanding of IAV transmission especially within a herd. This better understanding can then help us plan for better monitoring as well as specific timing and areas where interventions can be instituted to mitigate the transmission and effects of IAV between pigs as well as between people and pigs and pigs and people. By having veterinary and swine industry involvement in this
research we can be better assured the right decisions are being made for all involved (pigs, people, and industry).

As we addressed the possibilities of zoonotic disease transmission in this first paper we also needed to focus on disease transmission between pigs. For this part, currently the number one disease agent of concern in the swine industry is porcine reproductive and respiratory syndrome virus (PRRSV). This is a disease that has been affecting the industry for over 22 years and we still have not figure out how to best prevent transmission between pigs. We are fortunate this disease is not zoonotic so we can focus our concerns strictly on pigs.

To mitigate PRRSV disease transmission we need to better understand viral transmission especially within a herd. There has been quite a bit of information working on the breeding herd, but limited data has been available regarding the wean through finishing period. Data from this study supports PRRSV as not being a highly contagious virus (contagious was defined in our study as having a short time to seroconversion) and goes even further to quantify the rate of transmission. Overall our data suggest it took just over 5 weeks (39 days, 95% CI 35.3 to 43.2) for all pigs in a cohort of 20 to seroconvert. This is quite an impressive number, and short confidence interval, considering all cohorts housed the 20 pigs together in a single pen so contact time between pigs was frequent.

The most significant risk factor identified by our study contributing to a quicker time-to-seroconversion (defined as the hazard) was the pig’s PRRSV polymerase chain reaction (PCR) result at enrollment. Being PRRSV PCR positive at enrollment had a HR=34.00
(95%CI, 8.06 to 143.44). Besides the individual pig itself being viremic, for every PCR positive pig in the cohort at enrollment time the HR of 1.66 (95%CI, 1.23 to 2.23) would increase exponentially. So having three more pigs would provide a mean HR of 1.663 or 4.57. The effect of both of these variables decreased over time.

Obviously this was expected and makes biological sense. What our data does is confirm that this is true as well as provide supporting evidence that being a small pig within a group or having a high or low PPRSV ELISA at enrollment was not a good predictor for the pig’s PRRSV PCR status at that time. Unfortunately many in the industry still look at pig size as being a “disease.” This was propagated even more with the reported work by Cano et al in which smallest male piglets are purposely being selected for PRRV PCR monitoring of the sow herd. Their study as well as our study suggests that weaning time pig size is not correlated with viremia.

Our data also showed that the low contagiousness of the PRRSV is creating an environment of rising and lowering antibody titers over time. This is significant as serologic homogeneity was dynamic and difficult to achieve in such a small cohort with constant exposure to each other. It is challenging to even think about the dynamics in a large sow population.

Things are complicated enough when looking at a single agent like PRRSV but in reality animals are exposed to more than one pathogen over time. In our oral fluid surveillance study, we identified that of 600 samples collected overall, 508 (85%) samples were positive for PCV2, 73 (12%) for PRRSV, 46 (8%) for IAV, 483 (81%) for Torque teno
genogroup 2 (TTV2), and 155 (26%) for TTV1. There were only 15 (3%) samples that tested negative for all 5 agents. Repeated sampling over time showed that a variety of pathogens circulate both intermittently and continuously in pig population. We also found that barn “health” is highly variable, even among barns in the same production system. It was also interesting to find that PCV2 shedding occurs throughout the entire growing period of the pig even in these barns with pigs that had been vaccinated. Again it is not a matter of is it there or not, trying to quantify how much is there as well as what else is there at the same time is critical in allowing us to make better decisions that hopefully will maximize pig health.

In our study, when analyzing the different pathogens for their association with mortality (time-to-death) our results varied significantly between the univariate and multivariate modeling supporting the idea that we must look at things from a multi-agent interaction perspective rather than a single agent at a time. The univariate analysis identified statistically significant hazard ratios (HR) for TTV1 (HR = 1.089; $p = 0.0217$) and IAV (HR = 1.160; $p < 0.0001$) with PRRSV (HR = 1.075; $p = 0.0522$) being close to statistically significant. Multivariate hazard ratios for TTV2 (HR = 0.805; $p = 0.0075$) and IAV (HR = 1.203; $p < 0.0001$) were the only two identified as statistically significant.

Our “proof-of-concept” study also provides a look at a simple, practical, and cost effective means to collect more timely data more often. Many assays are still being refined and validated for use with oral fluids, but the future is looking promising especially as we can introduce selective antibody class detection (IgM vs IgG vs IgA).
The facility of multi-agent, multi-farm, oral fluid data collection over time will result in large, complex datasets. This development will mandate better training in more sophisticated statistical and analytical methods. We have the tool, now we need to get our knowledge caught up. The use of data, whether collected by farm personnel or a veterinarian, does not eliminate the need for veterinarians to “walk the barns”. Clinical skills and the powers of observation will continue to remain important tools in the armamentarium.

The last chapter of this dissertation focuses on biological risk management (BRM). The term BRM has been specifically selected rather than simply biosecurity. Biosecurity implies a yes or no approach to things whereas BRM takes the approach that it is all a matter of managing risk as most risks cannot be totally eliminated. For many thesis and dissertations, the literature review serves as a way to be “introduced” into a topic. It lays the foundation for the studies to follow. In this case, my literature review work will serve as a guide on how to work to prevent disease transmission (animal-to-animal as well as animal-to-human and human-to-animal) in the swine industry. It is a comprehensive document that tries to move away from specific pathogens and focus on disease transmission (focus of this dissertation) from the perspective of routes. This is important because once again, many times we are focused on only one pathogen and ignore many others. This is especially true when looking at a new or re-emerging disease as well as a foreign animal disease (FAD). Focusing on routes of transmission is the core to all BRM practices.

If one is to fully focus on BRM, then why do we need to research pathogen transmission? The key is that we have limited resources and time (personnel) thus the
approach to BRM is to focus on the greater risks first. For example, one needs to evaluate the risk for disease transmission from re-using needles for injections (fomites) compared to installing a complex and expensive air filtration system (aerosol). The ultimate goal is to maximize our investment in time and money through better health.

This dissertation has been focused on swine pathogen transmission. We need to be able to recognize pathogens of concern, identify their significance, develop monitoring plans so that the effect of our interventions can be better quantified. The key to success lies in science based Preventive Veterinary medicine.
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