

**Epidemiology of PRRS virus in the United States: Monitoring,
Detection in Aerosols, and Risk Factors**

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Acknowledgments

My road to getting where I am today has taken me many directions through the years. As a veterinary student, my dream was to become a small animal veterinarian in a general practice. I remember the first course where I was introduced to the basics of bio-statistics and epidemiology. At the time, these courses seemed insignificant and unimportant as the board exam would include little on this subject. I also remember my first exposure to learning about swine health and population medicine. The idea of keeping a population of animals healthy and productive had been, until then, a completely foreign concept to me. Cattle, sheep, and especially pigs were merely objects of county and state fairs at that point.

The summer between my second and third year of veterinary school I spent time riding across the United States with Dr. Bob Morrison, looking at and learning about anaerobic manure digesters on swine farms. Unbeknownst to me, he would later go on to become one of the most important mentors in my life. Along the way that summer, there was plenty of discussion about the swine industry, veterinary medicine and graduate education. At this formative time in my career, I was drawn to a side of veterinary medicine that I suddenly found fascinating.

Much to the astonishment of my friends and family, I dove head first into the world of production animal medicine. During my third and fourth year as a veterinary student I intensively studied these concepts and directed all of my elective credits to this end. I was hooked. Drawn to the notion that I could apply my veterinary knowledge to improve the lives and well-being of food animals, the hard working farmers that depend on them, and be part of feeding a global population, I enthusiastically accepted the challenge.

I remember the day I was offered the position in the graduate program by Dr. Bob Morrison. The timing could not have been worse. Less than 24 hours prior, I had received notice that I had passed my veterinary board exam and had exuberantly declared that my formalized university training was soon going to be over! Beyond that, I was

beginning to set up interviews in practices and was looking forward to starting my career in a few months.

It still stuns me, at times, that I stopped to consider this opportunity for even a moment. I was promised at least another 4 years of late night studying for examinations in the fields of bio-statistics and epidemiology (now wishing I had paid better attention in veterinary school) as well as countless hours of proposal and manuscript writing, humbling committee meetings and frustration with unexpected results. Somehow, my wife and I had rationalized all of this, and decided this was the direction we wanted to go.

This portion of my life's journey has lived up to all of the expectations, if not far exceeded them in every way. I have learned more about science, the swine industry and people than I ever would have imagined. I've seen the good, the bad, and the ugly. Often times I have been left completely speechless and wondering what I have done with my life – for better and worse. This part of the journey was not easy, and it would not have been for the faint of heart. It was a decision that had to be carefully weighed after considering the options. That said, I would not trade it for anything.

I am grateful for my wife, Heidi. Through all of this you never questioned any decisions I have made. Instead, you celebrated the accomplishments with me, commiserated and agonized over the defeats, and even in the darkest of hours of these years, you always managed to somehow remind me that all of this is for a reason. You remind me that I would not have been truly happy had I not pursued this dream. As if life wasn't complicated enough, we added our beautiful son, Colton, into the equation about half way through. You two are my reasons why. You two are what keeps me grounded in all of the chaos that is the life as a parent, a husband, a veterinarian, and a graduate student. I look forward to the rest of life's adventures together. I know that I am the luckiest. I love you both.

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fine line that, at times, felt frustratingly unclear. I have now come to realize much of this was likely to have happened for a reason. I believe that I am better for it.

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I am grateful for the long list of graduate students I am proud to now call friends. The open sharing of ideas, as well as helping with studies, data analysis, homework and studying for exams was so incredibly helpful. It is my sincere hope that we continue to stay in touch through the years. Ideally, I would have opportunities to see all of your beautiful home countries one day.

I am grateful for everyone in the university that has supported me along the way. The list of names and what they have done for me could fill pages and pages, likely entailing a second volume of this thesis. You are the un-sung heroes of my tenure as a graduate student. Always working quietly behind the scenes to ensure my paper work is filled out, that I was making good progress, and that I had someone to talk to when things did not go according to plan. Your efforts never went unnoticed.

I am grateful for my colleagues at the Swine Vet Center in Saint Peter, Minnesota. Through the years they were always the first to stand up and volunteer to help with the projects I was working on. This often required them to put in extra hours at night and on weekends to organize data for me. For their effort, they usually only received an ‘honorable mention’ at the end of the manuscripts we wrote, if they were lucky. Let this stand publicly as my personal note of gratitude to all of you. I am truly humbled by your generosity of time and genuine interest in what I was doing. Two years ago they took a tremendous leap of faith and asked me – a relatively unproven scientist and veterinarian to join their team to help with research efforts and veterinary work. Again, as if life was not busy enough, I gladly accepted this position and have come to appreciate every moment of it.

I am grateful for all the support I have received from the entire industry. Without the kind contributions of many veterinarians at many companies and practices across the US, these projects never would have been possible. Without the kind financial support of the National Pork Board, United States Department of Agriculture, PRRS CAP II, National Pork Producers Council, American Association of Swine Veterinarians, and the College of Veterinary Medicine these projects never would have taken flight nor, as in the case of the Swine Health Monitoring Project, continued to grow well beyond my term as a graduate student. For this, thank you all.

Finally, I am grateful for my parents, Rob and Lori, who together raised me to be the person I am today. They taught me to work hard, be patient, be honest, treat others with respect, complain less, and relentlessly chase down my dreams and bring them home. They allowed me to explore my passions from a young age and given the number of finned, furred, and feathered friends I had as a child, it is no wonder I chose to pursue a career in veterinary medicine. They have always been supportive and have stood by quietly waiting to help keep me going in the right direction. If I can possibly be half the parent to Colton, and any of his future siblings, as you were to me, the world will be a better place. I hope I have made you proud.

Dedication

I dedicate this work to my loving, patient, and kind wife. Without you and your constant selfless sacrifices during these long years, this would not have been possible. I love you, very much.

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General Introduction

Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) is perhaps the most devastating disease of modern swine production in the world. Despite decades of dedicated scientific research, the annual financial losses incurred by the United States (US) industry due to production inefficiencies have been estimated between \$560 million (Neumann et al., 2005) and \$664 million (Holtkamp et al., 2013).

PRRSV is known to be spread into susceptible populations through contaminated transport and personnel, infected animals and a recently growing body of literature suggests risks of long distance infectious bio-aerosols. With this in mind, efforts in the US to control losses have focused on measures to reduce the incidence of new infections at the sow herd level. Area regional control and elimination projects have begun to lay the foundations of open communication and data sharing the likes of which have never been witnessed before in pig farming.

These collaborations, in addition to advancements in bio-security technologies such as bio-aerosol filtration and scientifically validated personnel and equipment decontamination procedures may have helped to reduce the overall disease pressure in the industry. That said, results appear to be variable across regions, years, farms and production companies and despite best efforts, the incidence of PRRSV infections is expected to increase in the fall of each year.

Driven by the frustration of this variability, it became clear there was a pressing need to begin accurately measuring the rate of new PRRSV infections ultimately leading to a better understanding of the epidemiology of PRRSV in the US. In the fall of 2011, we began a collaborative effort to develop a database of volunteer sow herds within the US into what is now known as the Swine Health Monitoring Project (SHMP). In the early days of the project, there were 371 sow herds housing 1.2 million sows (approximately 25% of the US sow population), from 14 different production companies (systems) in 15 states. Since then, the number of sows represented in the project has nearly tripled, and includes significant numbers of sows across most of the swine producing regions of the US.

Using the American Association of Swine Veterinarians PRRSV classification system (Holtkamp et al., 2011), the veterinarians or health managers working with these systems report the weekly status of each farm. Additionally, many of these systems have shared geographic locations of their farms allowing the first known spatial analysis of PRRSV in the US.

In 2013, Porcine Epidemic Diarrhea Virus (PEDV) was first detected in the US (Stevenson et al., 2013). This devastating virus moved quickly across the US swine industry and soon the participants in the SHMP began sharing PEDV incidence data. It was a testament to the flexibility of this monitoring system and the willingness of the participants to share additional data ultimately making this a more informative dissertation.

Given the lack of good epidemiological data regarding the incidence of PRRSV in the US, the objectives of this dissertation were to 1) document and describe spatial and temporal patterns of PRRSV in the US, 2) describe changes in these patterns over time, 3) investigate the frequency of aerosol PRRSV detection under field conditions as a potential means of transmission, 4) identify risk factors of PRRSV and PED infections and 5) identify factors associated with PRRSV introductions on filtered farms.

Chapter I: Literature Review

Sections of this chapter have been published in:

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History

In 1989, a new syndrome was being recognized in the southeastern United States (US) and was first reported in non-peer reviewed literature, characterized by reproductive disorders in sow herds and respiratory disease in growing pigs (Quaife, 1989; Loula, 1991). While several known pathogens were initially implicated in the pathogenesis of this disease, the major breakthrough came in 1991 when the first isolate of this virus was obtained in The Netherlands (Wensvoort et al., 1991).

Subsequently, Koch's postulates were fulfilled in 1991 when experimental aerosol exposure to sows with cell cultured virus, then known as the Lelystad virus, reproduced the clinical syndrome (Pol et al., 1991; Terpstra et al., 1991; Wensvoort et al., 1991).

In the US, efforts were under way to reproduce the clinical signs which led to the identification of a prototype virus known as VR2332 in 1992, which was isolated from continuous cell lines (Benfield et al., 1992; Christianson et al., 1992; Collins et al., 1992). While several names were originally applied to this syndrome including swine infertility and respiratory syndrome, the virus eventually became known as Porcine Reproductive and Respiratory Syndrome (PRRS) (Christianson et al., 1992).

Etiology

PRRS is a single-stranded, positive-sense enveloped RNA virus that is 50-65 nanometers in diameter and belongs to the order Nidovirales, family Arteriviridae, and genus Arterivirus (Benfield et al., 1992; Meulenbergh et al., 1993; Cavanagh, 1997). The genome of PRRS is approximately 15 Kb long, and consists of 7 open reading frames

(ORFs) The majority of the genome is comprised of ORF 1 (a and b), followed by 2 (a and b), 3, 4, 5, 6 and 7 (Meulenbergh et al., 1993; Snijder and Meulenbergh, 1998).

Structural proteins are coded for by ORFs 2-7 and are named GP2, 3, 4, 5, M and N proteins (Johnson et al., 2011). Whereas the M, N and GP5 proteins are the major structural proteins required from infectivity, the GP2, 3, and 4 are minor structural proteins also required for infectivity (Wissink et al., 2005).

Pathogenesis and Clinical Signs

PRRSV infects and reproduces in susceptible host macrophages and monocytes which produces a viremia lasting up to 5 weeks (Batista et al., 2002) as well as a prolonged infection of the lungs and lymphoid tissues up to 251 days (Allende et al., 2000; Wills et al., 2003). The virus is known to be transmitted through all bodily secretions including urine, feces, colostrum, milk, saliva, semen and nasal secretion (Rossow, 1998; Wagstrom et al., 2001). Additionally, the infectious dose appears to be low and variable based on virus isolate, route of entry, concurrent health challenges and age of the pig (Cho et al., 2006; Cho et al., 2007a; Cutler et al., 2011).

The disease can produce both a subclinical and clinical presentation, where the latter is a manifestation of cell death and apoptosis of infected cells leading to cytokine mediated inflammation, polyclonal B-cell induction and reduction of bacterial phagocytosis and killing by macrophages (Rossow, 1998).

In sows, the PRRSV causes abortions, still born piglets, mummified fetuses, irregular returns to estrus and mortality (Gordon, 1992; Hopper et al., 1992). In suckling and growing pigs, PRRSV causes pre-wean mortality, lethargy, respiratory disease, anorexia,

and reduction in daily weight gain (Loula, 1991; Gordon, 1992; Hopper et al., 1992). In boars of reproductive age, PRRSV causes acute respiratory illness, anorexia, lethargy, lack of libido, and reduced semen quality (de Jong, 1991; Loula, 1991; Feitsma H, 1992; Prieto C et al., 1994).

Diagnosis

Monitoring production parameters (weekly abortions, pre wean mortality) for dramatic shifts, or observation of clinical signs in pigs may indicate a problem such as PRRS. This is, however, a non-specific method, and laboratory confirmation is required. Antibodies can be detected in the serum via ELISA 1-3 weeks after infection and up to 12-24 months post-infection, however specificity is not 100% which requires the use of a confirmatory test such as indirect fluorescent antibody or immunoperoxidase monolayer assay in negative populations to rule out false positives (Torremorell et al., 2002).

Reverse-transcriptase polymerase chain reaction (RT-PCR) detects the presence of PRRS RNA in samples (Christopher-Hennings et al., 1995). These tests have been adapted to a multitude of diagnostic samples, allowing the clinician many options to detect the virus based on stage of infection and diagnostic objectives. Additionally, genetic sequencing of the ORF nucleotides allows for genetic analysis between isolates.

Viability and infectiousness of PRRS isolates is assessed by culturing in cell lines such as porcine alveolar macrophages or MARC-145 and then immunohistochemistry staining of the cells to detect antigens (Benfield et al., 1992). It has been suggested however, that swine bio-assay is the most sensitive method of determining viability and includes

injecting a sample into a pig and observing for development of viremia or seroconversion (Horter et al., 2002).

Differential diagnoses for PRRS includes parvovirus, porcine circovirus type 2, influenza virus, hemagglutinating encephalomyelitis virus, leptospirosis, cytomegalovirus, pseudorabies virus and classical swine fever virus (Zimmerman et al., 2006).

Epidemiology

A retrospective analysis of samples in the US suggested none or very little evidence of the virus in 1980, 1 in 26 herds positive in 1985 and nearly 63% of samples positive in 1988 (Zimmerman et al., 1997). In Canada, studies have suggested PRRS antibodies were present as early as 1979, however clinical signs were not first observed until the mid to late 1980's (Carman et al., 1995). While the majority of infections in the US are due to type II viruses (referred to as North American strains), the first type I virus (referred to as European strain) was identified in 1999 during routine diagnostics (Ropp et al., 2004). While present in the US sow herd, the type I viruses remain a relatively unimportant component of the US PRRS epidemics (Murtaugh, 2009).

PRRSV is distributed worldwide and is present in most Asian, North and South American and European countries with a few notable exceptions where it is not present such as Brazil, sporadic European countries and much of Oceania (Carman et al., 1995; Epizooties), 1997; Garner et al., 1997; Motha Mxj et al., 1997; Zimmerman et al., 1997; Ciacci Zanella et al., 2004; Corbellini, 2006; Carlsson et al., 2009).

While some progress has been made in attempting to understand the incidence of disease, until recently there was no large scale coordinated effort. Historically, accurate estimates

of the prevalence of infected herds have not been readily available, however, one study suggested 36% of herds sampled were seropositive for PRRS (Bautista et al., 1993).

These estimates of seroprevalence are complicated by vaccine use and differentiating from wild type exposure (Christopher Hennings et al., 1996; J. et al., 1997; Mengeling et al., 1998).

In 1995, the United States Department of Agriculture, National Animal Health Monitoring System (NAHMS) reported 47.7% and 58.7% of samples from non-vaccinated sow herds and finisher herds, respectively, were positive for PRRS (NAHMS, 1997). In 2000, 49.8% of non-vaccinated finisher herds sampled in the were reported to be positive in the NAHMS survey (Bush et al., 2003). In 2006, approximately 71.1% of all non-vaccinated herds sampled in the NAHMS survey were positive for PRRS (NAHMS, 2006).

Herd Classification

A classification system has been designed by a group in the American Association of Swine Veterinarians PRRS task force which outlines criteria for five different categories ranging from positive unstable, through positive stable, provisionally negative and negative (Holtkamp et al., 2011). Herds in the acute stage of PRRS infection are classified as positive unstable (category I), weaning a PRRS polymerase chain reaction (PCR) positive piglet. After four consecutive negative PCR tests from 30 individual piglets at least 30 days apart are obtained, a herd would then be classified as positive

stable¹. This category is further divided into herds with ongoing immune management strategies that do not include elimination (category II-A), such as gilt vaccination, or herds electing to return to negative status (category II-B). As negative gilts begin entering the herd, and remain negative for PRRS antibodies by Enzyme-linked Immunosorbent Assay (ELISA) for a period of 60 days, the herds is classified as provisionally negative (category III). Finally if the gilts test negative by ELISA for a period of 12 months, or the herd inventory is entirely replaced (through de-population, or culling and replacing) it is classified as negative (category IV).

Recently, an effort was undertaken to outline a decision making process to determine if a virus recovered from a population of animals is the same or different from historical strains which included frequency and duration of negative testing, previous herd immunity, clinical signs, location, sequence homology with historical strains, biosecurity and season (Yeske, 2013). These methods seek to standardize case definitions; however, the decision to declare if a virus is new can often be highly dependent on individual veterinarians, and production companies.

Transmission

Stability of PRRS virus has been evaluated. In general, is highly susceptible to inactivation by heat and drying, and at temperatures from 25-27°C infectious virus was not able to be recovered from a variety of common surfaces and materials beyond day zero (Pirtle and Beran, 1996). At temperatures between -20°C and -80°C PRRS can be

¹ Sample sizes derived from Cannon and Roe estimates to detect a disease with 10% prevalence with 95% confidence assuming a perfectly sensitive and specific test (Cannon, R., Roe, R., 1982. Livestock disease surveys: a field manual for veterinarians. Australian Govt. Pub. Service, Canberra.).

stable for months to years, but infectivity is quickly lost when the pH of the solution is below 6.0 or above 7.5 (Benfield et al., 1992; Bloemraad et al., 1994; Zimmerman et al., 2010). Further, stability has recently been evaluated in manure, and as expected survival time was dependent on time and temperature (Linhares et al., 2012a).

Infectious dose appears to vary by isolate and studies have suggested ranges from $1 \times 10^{0.26}$ TCID₅₀ for MN-184 to $1 \times 10^{3.1}$ TCID₅₀ for VR2332. (Hermann et al., 2009; Cutler et al., 2011) Additionally, route of entry seems to play a role and studies have suggested higher doses via oral and intranasal exposure ($1 \times 10^{5.3}$ TCID₅₀ and $1 \times 10^{4.0}$ TCID₅₀ respectively) and lower doses in intramuscular exposure, on the order of 20 virus particles (Yoon et al., 1999; Hermann et al., 2005).

The basic reproduction number, R_0 is a theoretical parameter influenced by the aforementioned features that models the number of secondary infections that occurs in a fully susceptible population given contact with one infectious individual. In general, basic reproduction numbers larger than 1 allows epidemics to spread, and larger numbers yield epidemics that are more difficult to control. For reference, the small pox epidemics have been estimated to have a R_0 between 5 and 7 meaning each infectious individual can cause 5 to 7 infections during the course of their infectious period. Several factors influence R_0 including duration of shedding in infectious individuals, infectiousness of the organism and the number of susceptible individuals in contact.

In one experimental study, R_0 was estimated to be 2.6 (1.8, 3.3) using a Spanish isolate of PRRS virus whereas another study estimated it to be 3.0 (1.5-6.0) (Nodelijk et al., 2000; Charpin et al., 2012). Most recently, these principles have been used to determine that

herd size was negatively associated with probability of achieving stabilized status and that repeated mass vaccination with gilt acclimatization was better than single exposure for control (Jeong et al., 2014).

The role of PRRSV transmission via various fomites has been studied and has been shown to be able to transmit viable virus (Otake et al., 2002a; Otake et al., 2002c; Pitkin et al., 2009a). Additionally, herd ownership, gilt source, and transportation were found to be important factors in transmission, whereas spatial proximity to other herds was not (Kwong et al., 2013). The first studies involving aerosol transmission of PRRSV dates back to 1997 (Torremorell et al., 1997; Wills et al., 1997; Lager and Mengeling, 2000). Results of these early trials were mixed, leading some to believe aerosol transmission was either rare or does not occur.

In 2002, Otake and colleagues reported aerosol transmission of PRRSV in a series of controlled trials at an experimental facility (Otake et al., 2002b). In an observational study on a large cohort of swine herds in Europe, it was concluded that aerosol transmission was a likely source of disease spread (Mortensen et al., 2002). It appears that the likelihood of transmission varies between strains (Cho et al., 2007b).

In a field experiment including several replicates over approximately one year, 20 of 190 (10.5%) positive aerosol samples were detected at a distance of 120 meters from the source farm (Pitkin et al., 2009b). Additionally, associations with meteorological conditions on positive transmission days were reported which included cool temperatures, high relative humidity, low wind velocity, rising barometric pressure, low sunlight (Dee et al., 2010b).

Because PRRS virus has been detected in air samples up to 9.1 km from an infected site (Dee et al., 2009a; Otake et al., 2010b), bio-aerosol filtration has been implemented on many swine farms and has shown significant reductions of PRRS introductions into populations of pigs (Dee et al., 2009c; Pitkin et al., 2009b; Dee et al., 2010b; Spronk et al., 2010; Alonso et al., 2013b). Additionally, the payback period for the investment ranged from 2 to 7 years depending on the ventilation type employed on the farm, weaned pig prices and premiums paid for negative pigs (Dee et al., 2010c; Spronk et al., 2010; Alonso et al., 2013a). One of these studies documented that the seasonal epidemic was no longer clearly evident in the post filtered period (Alonso et al., 2013b).

Elimination and Control

Economic cost of PRRS, due to increased reproductive and growth inefficiencies, has been discussed previously and estimates are as high as \$560 – \$640 million per year to the US industry (Neumann et al., 2005; Holtkamp et al., 2013). One study in The Netherlands estimated the loss to approximately 98 Euros per sow per year (Nieuwenhuis et al., 2012). Before implementation, it has been suggested that economic analysis of any control measure is of paramount importance and should be conducted prior to onset of the strategy (Fraile, 2012).

At the country level, eradication has only been successful in three circumstances including Chile, Sweden and South Africa (Pinilla et al., 2006; Buhrmann et al., 2008; Ramirez et al., 2008; Carlsson et al., 2009; Frossling et al., 2009). Each of these situations was perhaps ideal for elimination due to several factors, including relatively

low prevalence, clustered outbreaks, small swine industries, government producer veterinarian cooperation and perhaps attributes of the virus that favor eradication.

In other endemically infected countries, such as the US, the continued rise in the cost of the disease perhaps indicates the inability to contain the virus. Country wide eradication of the virus would therefore seem difficult if not impossible with current methodologies. Instead, in these countries, the goal is often to exclude the virus from unaffected farms and regions with an emphasis on herd biosecurity.

Commonly, PRRS is eliminated from herds, and this can be accomplished in several ways. It is a complex decision making process involving long term goals of the farm, economics of the control strategy and ability to prevent re-introduction of virus. Several methods have been evaluated for efficacy at achieving homogenous immunity within a herd through planned exposure in breeding or replacement animal populations. The ultimate goals of these programs are; stopping transmission and producing PRRS negative weaned pigs within a herd (Ruen et al., 2007; Corzo et al., 2010).

Whole herd depopulation and repopulation appears to be an effective means of eliminating virus from a herd, however, the disruption in production comes at a dramatic financial cost (Marsh, 1988; Dial et al., 1992). Long term financial gains from this method are only realized if there are PRRS free replacement animals available and the ability of the farm to prevent reinfection.

Modifications of the whole herd depopulation strategy have been described including partial depopulation, test and remove and herd closure (Dee et al., 1997; Pejsak et al., 2000; Dee et al., 2001; Schaefer and Morrison, 2007). Arguably the most common of

these methods are the partial depopulation or herd closure methods. Test and removal methods have important financial and logistic considerations and are rarely applied in the swine industry except on small boar stud farms (Dee and Molitor, 1998; Reicks, 2001).

Herd closures have been evaluated previously (Schaefer and Morrison, 2007), and involve establishing a closed population of animals (either on site, or in offsite locations) that can be exposed to either the resident herd virus, commercial live-attenuated vaccine virus or some combination of both. Ideally, all susceptible animals will all become infected in a short period of time, and subsequently become resistant or immune to the virus.

An important piece of any herd closure program are the methods used to prevent the spread of virus throughout the farm (horizontal transmission), especially in the later stages of the elimination. Such management practices include all in, all out pig movement where entire rooms are filled and emptied at once, opposed to continuously moving animals in and out in addition to completely eliminating any animal movement as well as adoption of biosecurity protocols (McCaw, 2000; Pitkin et al., 2009c)

More recently a large prospective observational study was conducted to understand factors influencing the time to negative pig production and time to baseline production in herds infected with PRRS and concluded that exposure to field virus promoted a quicker return to negative pig production and exposure to vaccine virus promoted a quicker return to baseline production (Linhares et al., 2014). Overall, the median time to negative pig production was 27 weeks; however, there was considerable variation among herds in the study. Additionally, financial analysis of homogenizing immunity within herds with

vaccine versus wild type live virus showed a benefit to commercial vaccine use (Linhares, 2013).

Although infection seems to produce reasonable, long lasting immunity, the wide array of virus strains and the apparent limited cross-protection between strains make maintaining stable population immunity difficult (Lager et al., 1997; Cano et al., 2007). Because of this, there is a perception that vaccination with heterologous virus produces an inferior protection when compared to homologous vaccination. Ultimately, this may have led to the development of methods to expose animals to the wild type virus found within the herd. The majority of this work is based on empirical evidence, and includes direct inoculation with serum or deliberate contact with or exposure to ‘feedback’ material produced from tissues of infected individuals (Fano et al., 2005; Vashisht et al., 2008; Lambert et al., 2012b).

Exposure to live virus obtained from infected animals on farm raises the concern of the unintentional spread of other pathogens and experimental studies have been conducted to evaluate the safety and efficacy in growing pigs (Opriessnig et al., 2007). Unwilling to take risk of unintentional introduction of pathogens into breeding herds, many veterinarians are adopting the use of commercial vaccine as the preferred method of homogenizing immunity in breeding herds. While several commercially available killed vaccines are marketed around the world, current evidence suggests limited efficacy and have instead been suggested as a means of boosting immunity after exposure to live virus, yet this remains to be intensively studied (Zuckermann et al., 2007; Geldhof et al., 2012).

Summary

While the efforts to understand PRRS virus have come a long ways since first recognized in the US in 1989, there is still work to be considered. Longitudinal cohort studies such as the Swine Health Monitoring Project make up the frame work of surveillance systems. Carefully designed networks of participants, such as this, are quickly adaptable and might provide good insight into emergent disease such as Porcine Epidemic Diarrhea virus, Swine Delta Coronavirus, or any foreign animal disease.

Real-time surveillance is crucial to early response in disease epidemics. Additionally, these types of studies would allow for unique spatial and temporal analysis of epidemics. In the case of PRRS, such studies may help identify hot spots of infections or reservoirs of disease that would help direct control effort resources to these areas. These studies also allow for risk factor analysis and identification of most significant predictors of infection.

Chapter II – Temporal and spatial dynamics of porcine reproductive and respiratory syndrome virus infection in the United States

This chapter has been published in:

Tousignant, S.J.P., Perez, A.M., Lowe, J.F., Yeske, P.E., Morrison, R.B., 2014. Temporal and spatial dynamics of porcine reproductive and respiratory syndrome virus infection in the United States. *American Journal of Veterinary Research* 76, 70-76.

Summary

Objective – Measure incidence and estimate time-space dynamics of Porcine Reproductive and Respiratory Syndrome virus (PRRSV) in a voluntary cohort of US sow herds.

Design – Prospective longitudinal cohort study.

Animals – 372 sow farms in the United States representing 14 unique production companies.

Procedures – Exponentially Weighted Moving Average (EWMA) was used to monitor incident infections for onset of epidemic. The spatial scan statistic was used to identify areas at significant high risk of PRRS outbreaks. A chi square test was used to estimate whether there were significant differences in the quarter and annual PRRS incidence among time periods and a bivariable logistic regression model was fit to estimate whether infection on a given year increased the odds of being infected in the following year.

Results – During the four year period of this study, 29% (2009/'10), 33% (2010/'11), 38% (2011/'12), and 32% (2012/'13) of the herds reported new infections. Weekly incidence was low during the spring and summer and high during the fall and winter. The EWMA signaled the onset of the annual PRRSv epidemic during the middle two weeks of October each year. Disease incidence was spatially clustered. Infection in the previous year increased the odds of infection in 2010-2011 and in 2011-2012.

Conclusions and Clinical Relevance – Results here demonstrate a striking repeatability in annual PRRS spatial and temporal patterns across four years of data and between 14

unique production companies, suggesting that efforts to control the virus on a regional level should continue to be supported.

Abbreviation List

EWMA – Exponentially Weighted Moving Average

NASS – National Agricultural Statistics Service

OIE – Office International de Epizooties

PCR – Polymerase Chain Reaction

PRRSV – Porcine reproductive and respiratory syndrome virus

RMSD – Root Mean Square Deviation

US – United States

Introduction

Porcine Reproductive and Respiratory Syndrome virus (PRRSV) is a positive-stranded, enveloped, RNA virus that is 50-65 nm in diameter with a smooth surface, cuboidal nucleocapsid core with a diameter of 25-35 nm (*Benfield et al., 1992; Cavanagh, 1997*) As other members of the *Arteriviridae* family, the PRRSV is species-specific (infecting only swine) and highly variable. The virus is known to be transmitted through all bodily secretions including urine, feces, colostrum, milk, saliva, semen and nasal secretions. (*Rossow, 1998; Wagstrom et al., 2001*) The infectious dose appears to be low and variable based on virus isolate, route of entry, concurrent health challenges and age of the pig. (*Cho et al., 2006; Cho et al., 2007a; Cutler et al., 2011*)

Clinical manifestation of PRRSV infection was first documented in the United States (US) in North Carolina during the 1980s. (*Keffaber, 1989; Hill, 1990; Loula, 1991*) Since then, the PRRSV spread quickly through the US swine population and is now considered to be the most economically important disease of modern swine production with annual production losses estimated at \$560 million in 2005 to \$664 million in 2011. (*Neumann et al., 2005; Holtkamp et al., 2013*) Direct production losses due to PRRSV infection have been far-reaching. In sows, the PRRSV causes abortions, still born piglets, mummified fetuses, irregular returns to estrus and mortality. (*Gordon, 1992; Hopper et al., 1992*) In suckling and growing pigs, PRRSV causes pre-wean mortality, lethargy, respiratory disease, anorexia, and reduction in daily weight gain. (*Loula, 1991; Gordon, 1992; Hopper et al., 1992*) In boars of reproductive age, PRRSV causes acute respiratory illness, anorexia, lethargy, lack of libido, and reduced semen quality. (*de Jong, 1991; Loula, 1991; Feitsma H, 1992; Prieto C et al., 1994*)

Several studies have examined the relationships between risk factors and probability of reporting a new PRRSV infection including herd size, biosecurity score (discussed below), season in which a herd was established PRRSV negative, status of neighboring farms (Mortensen et al., 2002; Firkins and Weigel, 2004; Holtkamp et al., 2010; Holtkamp et al., 2012) The Production Animal Disease Risk Assessment Program is a commonly used survey in the swine industry that allows herds to compute an internal (practices done within farm) and external (conditions outside the farm) biosecurity score which can then be benchmarked herds in a national database.(Holtkamp et al., 2012) Studies have identified correlation between new PRRSV infection and season which a herd was established as negative, favoring summer months over the winter months.(Holtkamp et al., 2010) Additionally they have found that herds with low overall external biosecurity risk scores from the survey had a significantly higher probability of remaining PRRSV free longer than herds with high scores.(Holtkamp et al., 2010) Other known risk factors of PRRSV infection include distance to, size of and duration of infection of neighboring swine herds(Mortensen et al., 2002) and sow herd size.(Mortensen et al., 2002; Firkins and Weigel, 2004)

However, certain critical aspects of PRRSV epidemiology are yet to be elucidated. For example, the nature and extent of the time, space, and time-space clustering in the US has never been documented. The goal of the study presented here was to provide metrics that describe the apparent repeatability of the temporal and spatial patterns of PRRSV epidemics in the US using data collected over a four-year period on 372 sow farms. Results presented here are the first comprehensive assessment of time-space dynamics of PRRSV infection in the United States. In addition to allowing for the documentation of

PRRSV dynamics in the US, this project has developed a cooperative data sharing process which may help to direct timing and location of control efforts as well as lay the groundwork for previously non-existent, ongoing surveillance systems that will ultimately better prepare the US swine industry for emergent and potential foreign animal disease introductions.

Materials and Methods

Study population – At the onset of the project in 2011, the study population was the US sow herd and a convenience sample was enrolled based on awareness of the project through presentations at meetings such as the annual American Association of Swine Veterinarians (AASV) conference and follow-up contact. Participation was voluntary and anonymous and systems provided data retrospectively from July 2009 to date of enrollment. Thus, four full years of data (from July 2009 through June 2013) are presented and analyzed here. Within each system, eligible sow herds weaned piglets into off site locations and at the time of enrollment were requested to test for PRRSV using polymerase chain reaction (PCR) on at least 30 piglets of weaning age on a monthly basis. Since some systems have large multi-site breeding operations where animals live multiple barns during different life stages, the World Organization for Animal Health or Office International de Epizooties (OIE) definition of epidemiologic unit (Epizooties, 2013) was used to avoid counting a new case twice. Therefore, sites sharing animal or personnel movement without isolation or biosecurity practices were considered as one unit, regardless of geographic distance between them. Participants were required to sign participation and confidentiality agreements. This project was approved by the University of Minnesota Institutional Animal Care and Use Committee.

Case definition – New cases were reported on a weekly basis by the veterinarian or health manager associated with each participating system to the project personnel. A standard case definition was not used by all participating systems, however, new cases were typically defined using a combination of PRRSV open reading frame 5 sequence homology, clinical signs, history of PRRSV testing, herd immunity, location, biosecurity and veterinary professional judgment in a fashion similar to the decision making process adopted many US sow herds for many years, and more formally described by Yeske et al in 2013.(Yeske, 2013)

Temporal patterns

Incidence –Beginning July 1st of each year, annual and quarterly cumulative incidences were computed as the proportion of susceptible epidemiological units with reported new PRRSV cases on a given year(Dohoo et al., 2010)

Annual, as well as quarterly cumulative incidence, were compared using chi square tests.^a

The odds of an epidemiological unit reporting new PRRSV outbreaks in consecutive years was estimated using a logistic regression model in which, for each epidemiological unit j , infection status in year i (yes, no) and in year $i-1$ were used as response and explanatory variables, respectively.^b

Exponentially Weighted Moving Average (EWMA) – The weekly incidence of PRRSVV was monitored using an EWMA. The approximated EWMA (“smoothed”) cases per week (E) at time period (t) were calculated as:(Montgomery, 2005)

$$E = \lambda \times X_t + (1 - \lambda) \times E_{t-1}$$

A smoothing constant (λ) was chosen to minimize the root mean square deviation (RMSD) of the residuals between E and the observed number of cases (X). (Smith, 2001) The epidemic threshold was defined as the Upper Confidence Limit (UCL) of the data using the following equation. (Montgomery, 2005)

$$UCL = \mu + K \times \sigma \times \sqrt{\frac{\lambda}{(2-\lambda)} \times (1(1-\lambda)^{2t})}$$

The average (μ) and standard deviation (σ) of observed new infections during the summer months (July, August and September) of the previous years were also computed. K is a multiplier function that was arbitrarily chosen to minimize false signal during the summer months, yet not delay the epidemic signal and was the control for the tradeoff between the specificity and sensitivity of the algorithm. (Ivanov et al., 2003) The smoothing constant (λ) was set at 0.2787 and K was set at 2.2. Using this method, the week of the year in which the number of cases exceeded the epidemic threshold was identified, indicating the onset of the epidemic.

Cluster Analysis – The spatial scan statistic was described by Kulldorff in 1997 and has been used to study if processes (here, disease occurrence in a cohort of sow herds in the US) are purely random or if clusters in excess of baseline can be detected. (Kulldorff, 1997) This method applies a window of varying size across a study area and compares the number of observed cases of disease against the number of expected cases of disease, which are assumed under the null hypothesis to be randomly distributed. This method has been applied in veterinary medicine in several studies including investigations into clustering of bovine tuberculosis in Argentina, leptospirosis among dogs in the US and Canada, and to identify associations between soil type and Johnes disease in US cattle herds. (Perez et al., 2002; Ward, 2002; Ward and Perez, 2004) Here, a purely spatial

analysis Bernoulli model (where epidemiological units were considered cases if they reported a new infection and controls otherwise) was fitted separately for each year of analysis. The model was set up to scan for areas with high rates, with 50% of the population at risk, using a circular spatial window, and no geographical overlap. Clusters were said to be significant if their probability of occurrence by chance (P) was lower than 5% ($P < 0.05$), as estimated by the comparison of the observed results with 999 random scenarios generated using Monte Carlo simulation.^c

Results

Approximately 21% of the US sow population (NASS, March 2013) from 14 unique production companies, representing 372 farms with approximately 1.2 million sows, in 15 states were enrolled in the project from July 2009 through June 2013. The project began in early 2011 with four companies, and five more were added later that year. In 2012, four more companies were added, and in 2013 the last company was added. The total number of herds increased from 316 in July 2009 to 371 in May 2013. There was an average of 27 herds per system with a range of 7 to 68. Average herd size was approximately 3,100 and ranged from 500 to 12,500. 300 (81%) farms were identified as commercial sow production, 49 (13%) as multiplier production (herds that produce replacement sows for commercial herds) and 23 (6%) were identified as genetic nucleus production (herds of pure bred animals that produce sows for multiplier herds). Additionally, 78 (21%) herds were employing bio-aerosol filtration at the end of the June 2013. (Pitkin et al., 2009b)

The cumulative incidence by year between 2009 and 2013 shows a similar pattern of incidence across the four years of this study (Figure 1). Incidence was lowest in the spring and summer months (February through September) with a dramatic increase in the fall and winter (October through January). Annual cumulative incidence was 29%, 32%, 38% and 31% for year 2009 through 2013, respectively. No significant differences ($P > 0.05$) were detected in cumulative incidence during each quarter across all four years of data (Table 1). In herds reporting an infection the previous year, the odds of infection were 2.45 times higher in 2010-2011 ($p = 0.0005$) and 1.88 times higher in 2011-2012 ($p = 0.0085$), however it did not significantly increase odds of infection in 2012-2013 ($p = 0.0523$). The week in which the PRRSV epidemic began in this cohort was the same for the four years assessed here (Figure 2).

One significant cluster ($P < 0.05$) for each year was identified. (Table 2, Fig. 3a – 3d). Centroids of each cluster were located in Iowa with radii ranging from 162.8 to 302.1 km. In each cluster, 1.97, 1.34, 1.88 and 2.07 times the expected number of cases were observed each year from 2009 to 2013 respectively.

Discussion

The study presented here is the first systematic description of the time, space, and time-space dynamics of PRRSV in the US. Findings suggest a highly consistent, repeatable pattern of infection observed across four years of data. Herds reporting an infection in the previous years were at increased odds of reporting an infection in the following year. Additionally, the EWMA suggests a narrow window of time where the onset of the

PRRSV epidemic occurs each fall. Finally, the spatial scan statistics indicate a repeatable pattern of disease clustering in the upper Midwest region of the US.

Spatial scan statistics have been used previously to identify regions of higher than expected disease clustering which could provide a scientific basis for planning control programs.(Perez et al., 2002) These data support the need for continued efforts to understand why the disease occurs most frequently in this region and subsequently what actions may be taken to mitigate such impact. The results of the spatial scan in this study might suggest this region has unique characteristics that drive this finding. For example, it is possible this region simply has the highest swine density, the most traffic moving pigs to slaughter plants in the region, or they are dominated by production companies with different view on PRRSV management. In the US, Canada and Mexico, regional control and elimination programs aimed at controlling and potentially eliminating PRRSV virus have been undertaken.(Batista et al., 2010; Corzo et al., 2010; Morrison, 2010; Waddell, 2010; Morrison, 2011; Loula et al., 2012; Becton and Morrison, 2013) While the majority of the work remains largely beyond the realm of peer reviewed literature, the growing body of empirical evidence begins to suggest that with the right leadership and cooperation, these projects might be successful tool for reducing the impact of PRRSV virus in some regions of the US. The results of this study corroborate the continued need for ongoing development of these types of regional control in swine dense regions in the US, where at the time of this study, there have been very few of these efforts undertaken. Vaccination has been another strategy to help manage PRRSV, however, use of heterologous vaccine strains of PRRSV virus seems to produce incomplete immunity.(Lager et al., 1997; Cano et al., 2007) That said, there are a

limited number of studies suggesting that vaccination may help reduce shedding at the individual and population level and the simultaneous mass application of commercial vaccine within these regional control programs could prove useful.(Linhares et al., 2012b; Samson et al., 2012)

While regional control programs have gained some early success, it is likely they will only be one piece of the solution to PRRSV. Ongoing research efforts should seek to quantify risk factor of infections in cohorts such as this. Application of rigorous biosecurity measures and aerosol filtration(Pitkin et al., 2009b) have shown promise as effective means of reducing the frequency of PRRSV introductions into many swine herds(Dee et al., 2010d; Spronk et al., 2010; Alonso et al., 2013b) and should continue to be studied. Finally, much has been learned in the areas of vaccinology, immunology and host genetics in recent years, yet the role each plays in the control of PRRSV is still under investigation.

Results here may have been affected by issues related with representativeness and recall biases. First, the herds represented here are a volunteer cohort and not a random sample of the US sow herd and, therefore, it is possible for some unmeasured factor to have influenced the results of this study. Of particular importance, are that some areas of swine production are underrepresented in the spatial scan statistics including regions of the south and south east including areas around Oklahoma and North Carolina and these data may not truly reflect the impact of PRRSV in those areas. Additionally, we have not included growing sites, which could be important factors in lateral spread of this virus. While this project started in 2011, participants were required to provide retrospective data from date of enrollment back to 2009 potentially imparting recall bias into the study.

Furthermore, diagnostic rigor employed on farms may have differed between pre and post-enrollment periods and in systems of smaller size and fewer herds. While no formal study was done to understand these potential differences, because of familiarity with the participating systems it is speculated that these differences are relatively minor. Finally, no formal case definition was used in this study which could have imparted bias into the results, especially when trying to define new infections in herds with resident or persistent, stable PRRSV infections potentially resulting in underreporting in these circumstances. Despite of those potential sources of bias, the large number of analyzed systems and farms, accounting for more than one-fifth of the sow population of the US, suggests that the results presented here are an important population to understand the dynamics of, arguably, one of the most important diseases affecting the swine industry of the country.

In conclusion, this project described the highly repeatable PRRSV epidemic in the U.S. from 2009 to 2013. It corroborates the need for ongoing support of regional control and elimination projects. Additionally, this project paves the way for ongoing, coordinated, producer driven surveillance projects at a national level.

Acknowledgments

The authors of this study would like to acknowledge the National Pork Board, the PRRSV Coordinated Agriculture Project (PRRSV CAP), and the University of Minnesota College of Veterinary Medicine for providing funding and support for this study. Finally, the authors would like to thank the participating veterinarians and

production companies involved in this project for their generous contributions of time and data.

Conflict of Interest

The authors report Drs. Yeske and Lowe provide veterinary consulting services for two of the production companies involved in this project.

Footnotes

^a Win Episcopo, Universidad de Zaragoza. Available at <http://www.winepi.net>. Accessed April 6, 2014.

^b Statistix 9.0, Analytical Software, Tallahassee, FL 32317

^c Kulldorff M. and Information Management Services, Inc. SaTScan™ v8.0: Software for the spatial and space-time scan statistics. [www.satscan.org], 2009. Accessed April 6, 2014

Tables and figures

Table 1 – Number of herds newly infected with PRRSV and cumulative count of PRRSV-infected herds during each quarter of each project year (July 1 through June 30) from 2009 to 2013.

Quarter	2009-2010		2010-2011		2011-2012		2012-2013		χ^2 Test result	P value
	No. of newly infected herds	Cumulative count								
July–September	11	11	7	7	6	6	9	9	1.998	0.573
October–December	40	51	65	72	57	63	77	86	5.145	0.162
January–March	28	79	20	92	50	113	31	108	2.292	0.514
April–June	12	91	14	106	22	135	9	117	4.143	0.247

The project included 371 US sow herds (epidemiological units) from 14 production companies, which represented approximately 21% of the US sow population.

The definition of a new case of PRRSV infection was not standardized among the participating herds, but typically included professional veterinary judgement, a positive

PCR test result from a piglet on the farm as well as PRRSV open reading frame 5 sequence heterology from historical strains (if any), and clinical signs consistent with PRRSV infection.

Table 2 – Results of spatial scan statistic to detect clusters of PRRSV-infected herds during the period of July 2009 through June 2013.

Project year	Center coordinates	Radius (km)	No. of herds	No. observed cases	No. expected cases	Ratio of observed to expected cases	P value
2009-2010	42.8 N, 94.3W	198.6	107	63	31.98	1.97	< 0.0001
2010-2011	42.6 N, 91.8 W	302.1	146	63	46.86	1.34	0.039
2011-2012	43.4 N, 94.6 W	162.8	113	81	43.08	1.88	< 0.0001
2012-2013	40.9 N, 94.4 W	280.8	77	45	21.76	2.07	< 0.0001

See Table 1 for key.

Figure 1 – Annual cumulative incidence of PRRSV-infected sow herds (epidemiological units) for project years 2009 to 2010 (solid gray line), 2010 to 2011 (dashed gray line), 2011 to 2012 (dashed black line), and 2012 to 2013 (solid black line). The project included 371 US sow herds from 14 production companies, and a project year was defined as July 1 through June 30. A PRRSV-infected herd was a herd in which at least 1 new case of PRRSV infection was diagnosed during that project year. The definition of a new case of PRRSV infection was not standardized among the participating herds, but typically included professional veterinary judgement, a positive PCR test result from a piglet on the farm as well as PRRSV open reading frame 5 sequence heterology from historical strains (if any), and clinical signs consistent with PRRSV infection. (next page)

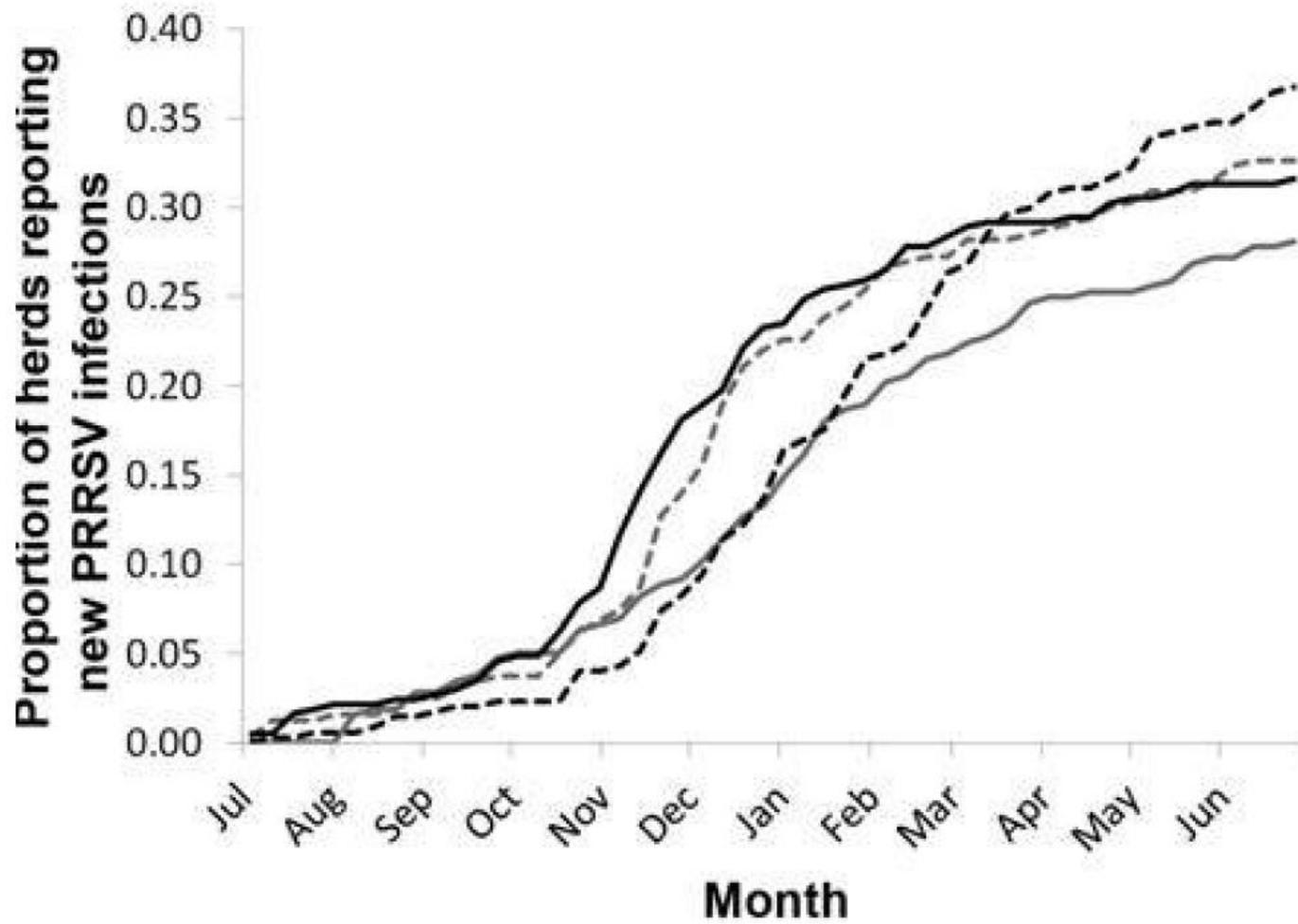


Figure 2 – Exponentially weighted moving average number of new cases of PRRSV infection per week for the herds of Figure 1 from July 1, 2009, through June 30, 2013. The black line represents the EWMA smoothed value. The gray line represents the epidemic threshold. The onset of a PRRSV epidemic was identified (dates in squares) when the EWMA exceeded the epidemic threshold. See Figure 1 for remainder of key.

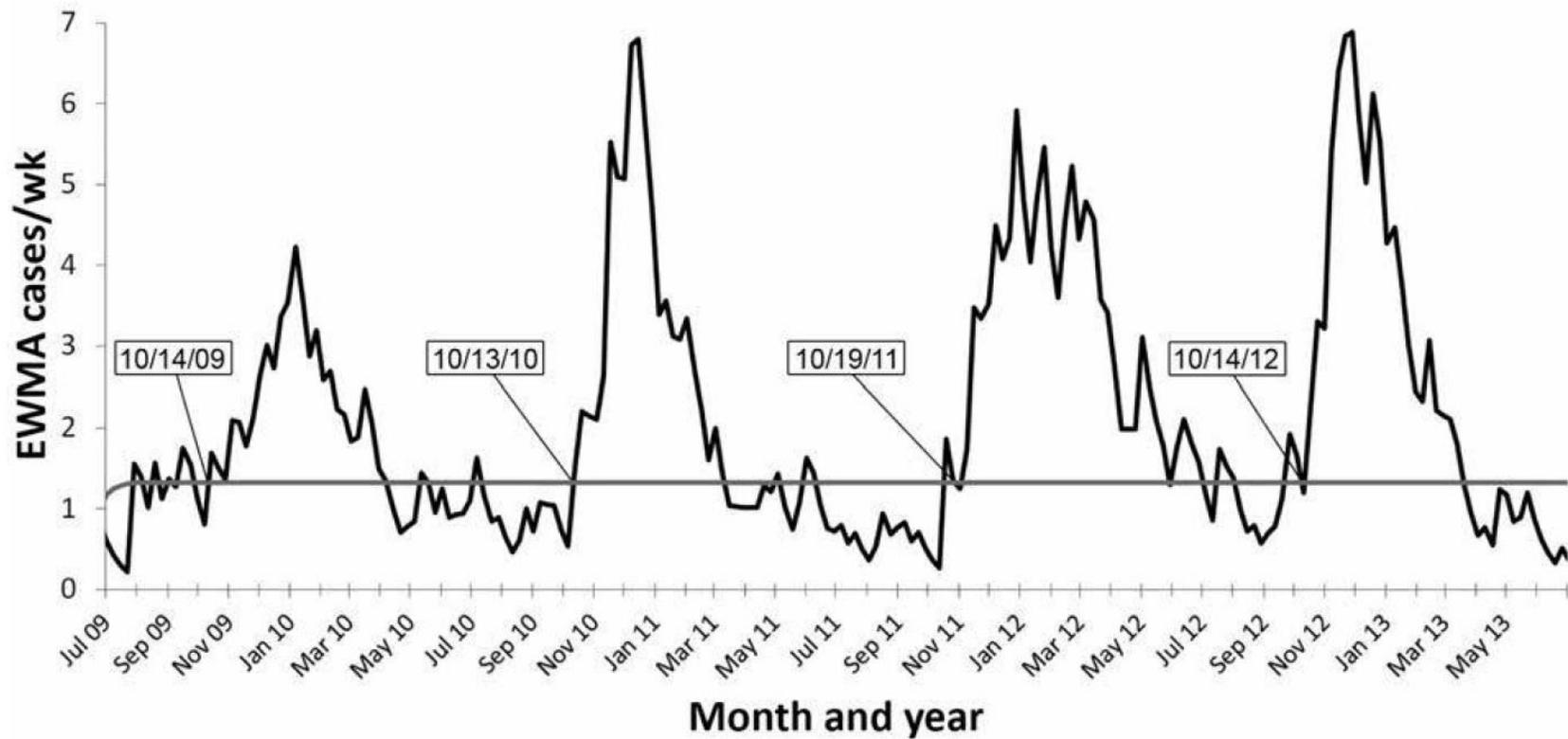
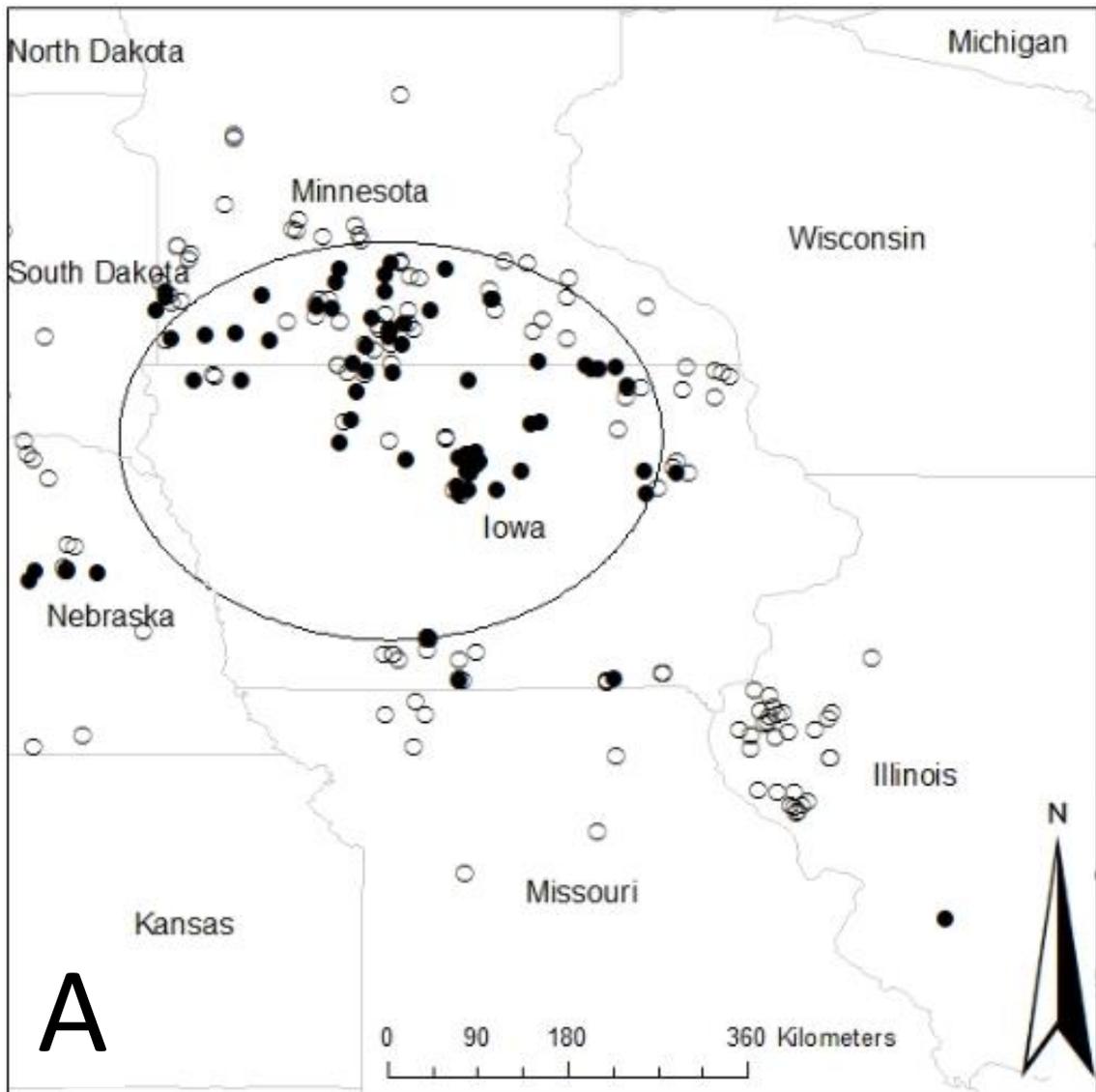
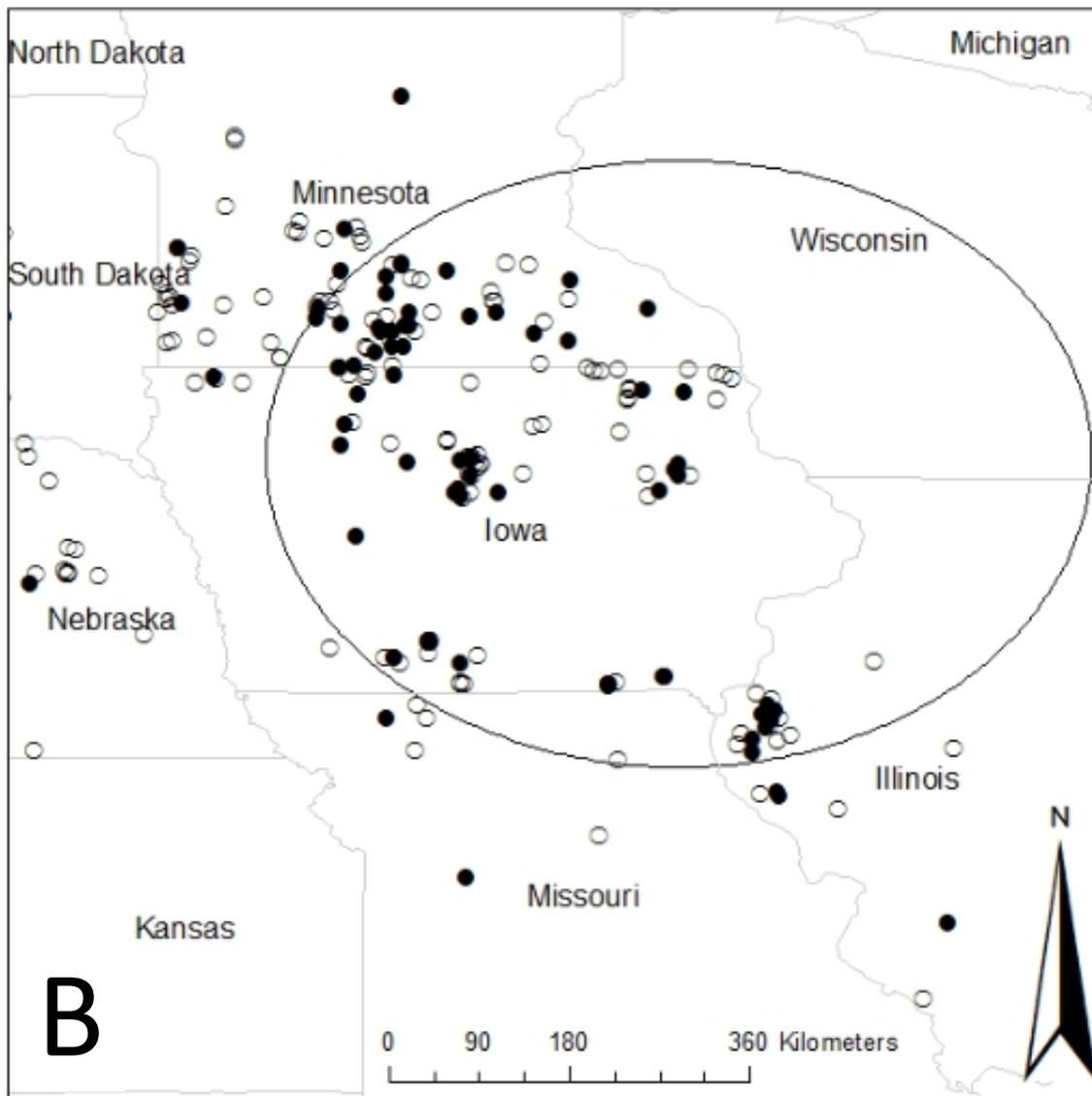
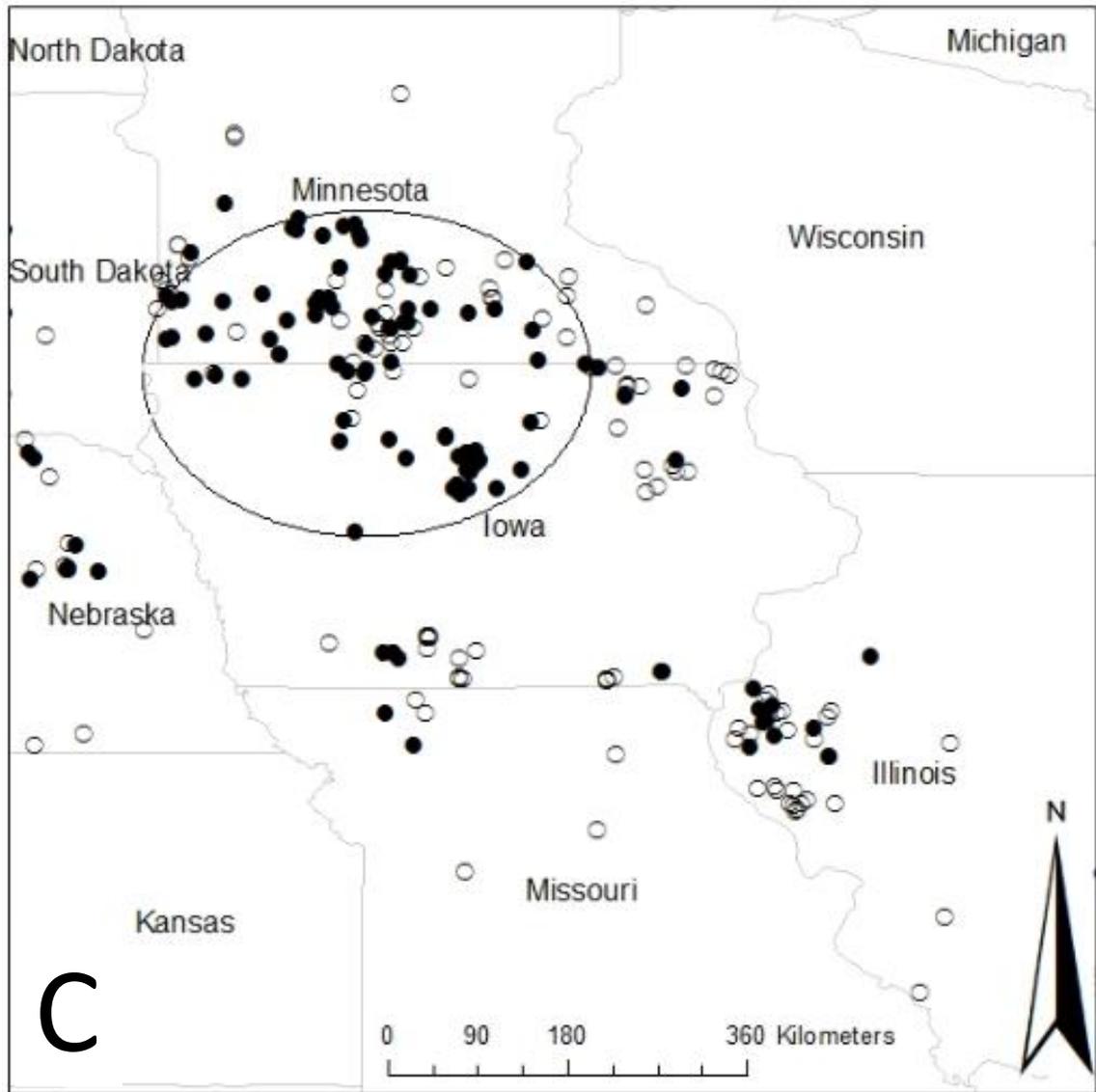
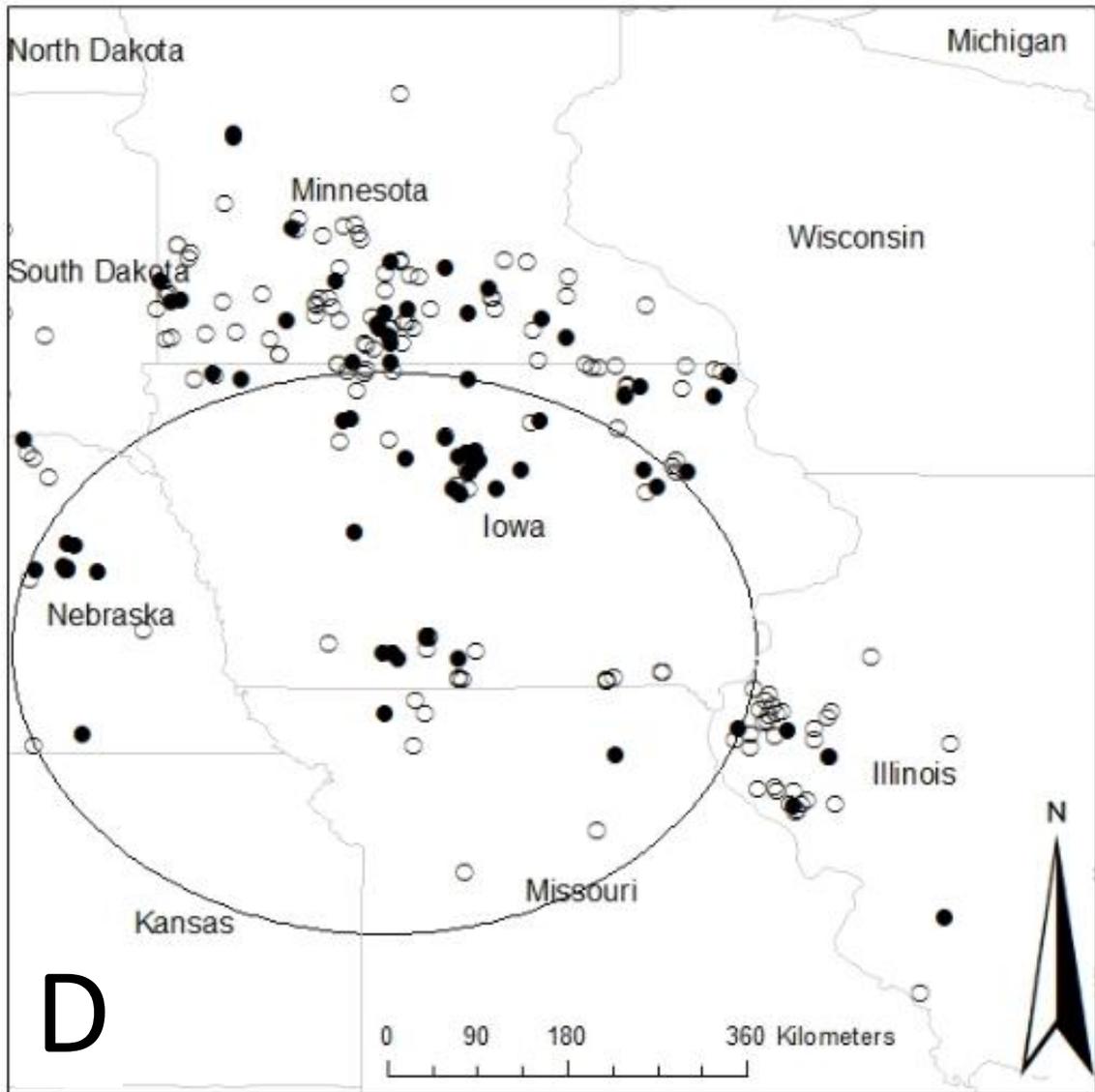


Figure 3—Results of the spatial scan statistic to detect clusters of PRRSV-infected herds during project years 2009 to 2010 (A), 2010 to 2011 (B), 2011 to 2012 (C), and 2012 to 2013 (D). Small black circles represent the location of PRRSV-infected herds, and small white circles represent the location of PRRSV-negative herds. The large circle demarcates the centroid of the epidemic cluster. See Figure 1 for remainder of key.









**Chapter III – A comparison between the 2013-2014 and 2009-2012
annual PRRSV epidemics in a cohort of sow herds in the United States**

This chapter has been accepted for publication as a short communication in

The Canadian Veterinary Journal

Tousignant, S.J.P., Perez, A.M., Morrison, R.B., *In press*. A comparison between the 2013-2014 and 2009-2012 annual PRRSV epidemics in a cohort of sow herds in the United States. The Canadian Veterinary Journal.

Summary

The purpose of this study was to describe the 2013/14 porcine reproductive and respiratory syndrome virus (PRRSV) epidemic in the United States and compare that with the previous 4 years of data from 2009 – 2012. A total of 371 herds participated in the study, representing nearly 1.2 million sows in 15 states. There were significantly fewer PRRSV cases during this study period and the onset of the annual epidemic was delayed approximately 3 weeks. Interestingly, cluster analysis revealed a pattern similar to previous years. Reasons for these observations are speculated including the role of spurious observations, increased awareness of PRRSV epidemics, and the role of porcine epidemic diarrhea virus detection in the United States swine herd.

Study design, results and discussion

Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) continues to cause major production losses in the United States with recent cost estimate of \$640 million annually (Holtkamp et al., 2013). Despite efforts from the veterinary community to control the virus between 2009 and 2012 the onset of the annual epidemics showed: a consistent date of onset, no significant change in the annual cumulative incidence of new cases, a repeatable location of clustered disease distribution, and a pattern of infection in herds with previous infection (Tousignant et al., 2014). The same study showed the onset of the PRRSV epidemic was in the middle week of October and annual cumulative incidence ranged from 29 – 38%. In the fall of 2013, the same cohort of sow herds failed to signal the epidemic during this time period for the first time in four years, instead signaling the onset approximately 3 weeks later. Additionally, the annual cumulative incidence was lower than expected based on the previous four years of data. Therefore, the objective of this paper was to report and describe apparent differences in the onset of the epidemic in 2013 compared to previous years, including estimates of annual cumulative incidence, associations between reporting PRRSV cases in consecutive years, and the identification of any changes in the spatial distribution of incidence in the 2013/14 PRRSV monitoring season. These results will contribute to understanding the dynamics of PRRSV transmission, which ultimately may help to mitigate the impact of the disease in the U.S.

The study cohort consisted of a convenience sample of 371 farms in 14 unique production companies representing 1.2 million sows in 15 states. Herds were enrolled in the study between 2011 and 2013. PRRSV status data were provided retrospectively

from 2009 until the date of enrollment, and then weekly thereafter. New cases were reported weekly via email by the veterinarian or health manager. New cases were diagnosed based primarily on positive results of Polymerase Chain Reaction testing of at least 30 weaning age piglets on the sow farm. Veterinary professional judgment was used to determine if detection of PRRSV within a previously infected herd incursion was new based on sequence heterology (commonly a cut off of 2% or greater difference was used) from historical strains (if any), as well as clinical signs in sows or piglets. Producers were responsible for costs associated with all diagnostic testing. The median number of herds per system was 27 and of the 371 herds, 300 (81%) produced piglets for commercial production, 48 (13%) were genetic multiplication, and 23 (6%) were involved in genetic nucleus production.

Annual and quarterly cumulative incidence were calculated from July 1st, 2013 to June 30th, 2014 at the aggregated level (all 14 systems combined) and at the system level (each 14 systems considered separately) and compared with the average cumulative incidence between 2009 and 2012 using a Chi square test (and Fisher's test when needed).^a The association between reporting new PRRSV cases in consecutive years was estimated using logistic regression, where, for each farm j , the response and explanatory variables were infection status in year i (yes, no) and year $i-1$ (yes, no), respectively.^b

An Exponentially Weighted Moving Average (EWMA) was used to monitor the weekly incidence of PRRSV cases. An upper confidence limit was calculated such that when the EWMA was greater than this limit, the PRRSV epidemic was signaled.

Spatial scan statistic (Kulldorff, 1997) was used to identify clusters of PRRSV cases in excess of a random process using a Bernouli model. The model was fitted for the observation period scanning for areas with high rates that would include a maximum of 50% of the population at risk, using a circular spatial window and no geographical overlap. Significant clusters were identified at $p < 0.05$ as estimated by the comparison of the observed results with 999 random scenarios generated using Monte Carlo simulation.^c

The results of this analysis showed that overall there was a significant decrease in the incidence of new PRRSV cases during the 2013/14 year (Figure 1). The average number of weeks between July 1st and the onset of the PRRSV epidemic was 15.3 weeks for the years 2009 to 2012 as opposed to 18.3 weeks for 2013 (Table 1). There was no significant difference in the cumulative incidence for the first quarter of the year ($p = 0.3284$). There were 32 fewer cases at the end of the second quarter ($p = 0.0045$), 47 fewer at the end of the third quarter ($p = 0.0018$), and 50 fewer at the end of the fourth quarter (Table 1). Additionally, there were 29 fewer cases reported during the second quarter ($p = 0.0046$), 15 fewer during the third quarter ($p = .0221$), however, the same number of cases were reported in the fourth quarter ($p = 0.7702$) (Table 1). These data suggest the PRRSV epidemic was similar to the previous four years during the summer months (Q1), but different in fall and winter months (Q2, and Q3 respectively). The number of cases reported in the spring and early summer was the same.

Of the 14 production companies represented in the database, 12 reported numerically fewer new PRRSV cases during the 2013/14 year compared to the previous 4 year average, and in 4 of them, such decrease was significant. One system reported the same number of infections, and one reported 3 additional infections. Reasons for these

observations may be due, in part to geographic location and regional PRRSV risk, as well as management factors that may have influenced the results.

The odds of reporting a PRRSV case in 2013/14 was not significantly associated with reporting a case in the previous year (OR: 1.7, CI: .93 – 3.13, $p = 0.0848$). This is different from the first four years of the study where having a case in the current year (i), was significantly associated with having a case in the previous year ($i - 1$) (Tousignant et al., 2014).

A significant cluster of cases was identified in 2013/14, with the centroid in a location similar to the previous 4 years of data in Iowa (Tousignant et al., 2014). Also similar to the previous 4 years of data, the cluster had a radius of 164.93 km and 2.08 times the number of expected cases was observed.

There was a decrease in the number of new PRRSV infection in 2013/2014 in the cohort of sow herds and a delay in the onset of the epidemic. Interestingly, PRRSV cases were still spatially clustered in the same geographical region in which they were clustered in previous years and 13/14 systems reported a low incidence of the disease. Those features suggest that the decrease in PRRSV incidence observed in 2013/2014 was associated with a background decrease in risk in the region, rather than with a decrease in PRRS virus frequency in highly incident areas or systems.

There are a number of factors that may explain, at least in part, the findings reported here. Arguably, the most important change in the epidemiological conditions of the region in 2013/2014, compared to previous years, was the introduction of Porcine Epidemic Diarrhea Virus (PEDV) into the U.S. swine herd. For that reason, epidemiological

features that may explain the differences in PRRS virus incidence in 2013/2014, compared to previous years, may be divided into PEDV-related and non-PEDV-related factors. For example, due to the fear of PEDV, many producers increased biosecurity measures on their farms aimed at preventing lateral transmission of PEDV. Those biosecurity practices, primarily intended to prevent PEDV introduction, may have also helped to reduce introduction of PRRSV into susceptible farms. Additionally, it might also be possible that PRRSV-infected herds were more likely to become infected with PEDV than PRRSV-uninfected farms, which ultimately may have resulted in fewer PRRSV-infected growing pigs and a consequent decrease in lateral transmission of PRRSV. Several non-PEDV factors may have contributed to the reduction of PRRSV incidence. As data from the first years of monitoring became available, this may have increased the awareness of the annual mid-October PRRSV epidemics. This may have resulted in better bio-security preparation aimed at reducing the introduction of PRRSV into susceptible herds during this time. It has also been speculated that there may have been an increase in the application of PRRSV vaccine which could have affected transmission dynamics within the U.S. sow herd. In support of this, the percentage of herds in this database choosing to maintain immunity in their population through ongoing management programs significantly increased during the same time period. Additionally, the application of bio-aerosol control measures (filtration), which has been shown to significantly reduce the number of new PRRSV infections (Alonso et al., 2013b), has increased dramatically in the past years. If this technology was applied to farms with high probability of infecting other farms or to a large number of PRRSV-uninfected farms, then transmission may have been mitigated due to either or both reduction in the

infectiousness of infected farms or in the number of susceptible farms. If this ultimately resulted in fewer sow farms becoming infected, then it may have led to fewer PRRSV infected pigs entering the growing pig population, which could have decreased lateral transmission of the virus to other farms. Another potential explanation is that the decrease in incidence was the consequence of secular cycles, which are fluctuations in disease incidence that occur over a one year period. Secular cycles may be associated with fluctuations in factors such as immunity, contact rates, or virus virulence and have been studied in foot and mouth disease epidemics in Paraguay (Peralta et al., 1982) and *Neospora* associated abortions in dairy cattle (Thurmond et al., 1995). If the decrease here is associated with occurrence of a secular cycle, compared to a decreasing trend, then one may expect incidence to increase and return to values similar to those observed before, within the next few years.

It may also be possible that the association reported here was spurious and that PRRSV incidence has not truly decreased in the U.S. swine population. Potential causes of a spurious association might include changes in case definition, changes in diagnostic rigor or changes in ability to detect PRRSV in PEDV- infected herds. Clinical signs of PEDV are often severe on the sow farm, with mortality in piglets often nearing 100%, which may result in having no piglets to test during the peak of the infection. Additionally, some herds may have elected to temporarily cease PRRSV testing during the PEDV case. Fortunately, as herds affected with PEDV have recovered and resumed testing for PRRSV, the incidence of PRRSV remained unchanged. Additionally, the participant group remained the same between the two study periods of this project, so it is unlikely changes in case definition occurred. Finally, the decrease in PRRSV incidence was

observed in 13 of the 14 systems from which data were collected. Together, these observations support the hypothesis that the reduction in PRRSV incidence in 2013/2014 reported here was true and represent the conditions observed in the field during the study period.

There are important limitations of this project that should be considered. First, this was a voluntary cohort which may have imparted some selection bias into the results.

Additionally, there was no strict case definition, which may have imparted misclassification bias if some systems reported new breaks differently than others.

Spatial scan statistics force a circular shape to the data set, which may not accurately reflect the true nature of irregularly shaped disease clusters

In conclusion, there was significantly less PRRS reported in 2013/14 and a delayed epidemic was signaled. That decrease was evident in 13 of the 14 systems and the persistence of a spatial cluster of high incidence, similar to previous years. This report is important because it demonstrates changes in the epidemiological conditions of the disease, which may be related, or not, to the emergence of PEDV in the country.

Evaluation of disease evolution in future years will ultimately help to elucidate whether the decrease reported here was due to a true decreasing trend in virus incidence, to a secular cycle, or a false association, shedding light on the nature and extent at which PEDV-related and -unrelated factors may have affected the results. Nevertheless, these results will contribute to the understanding of the epidemiology of PRRS in the U.S, and, ultimately, to enhance the effectiveness of prevention and control programs in the country.

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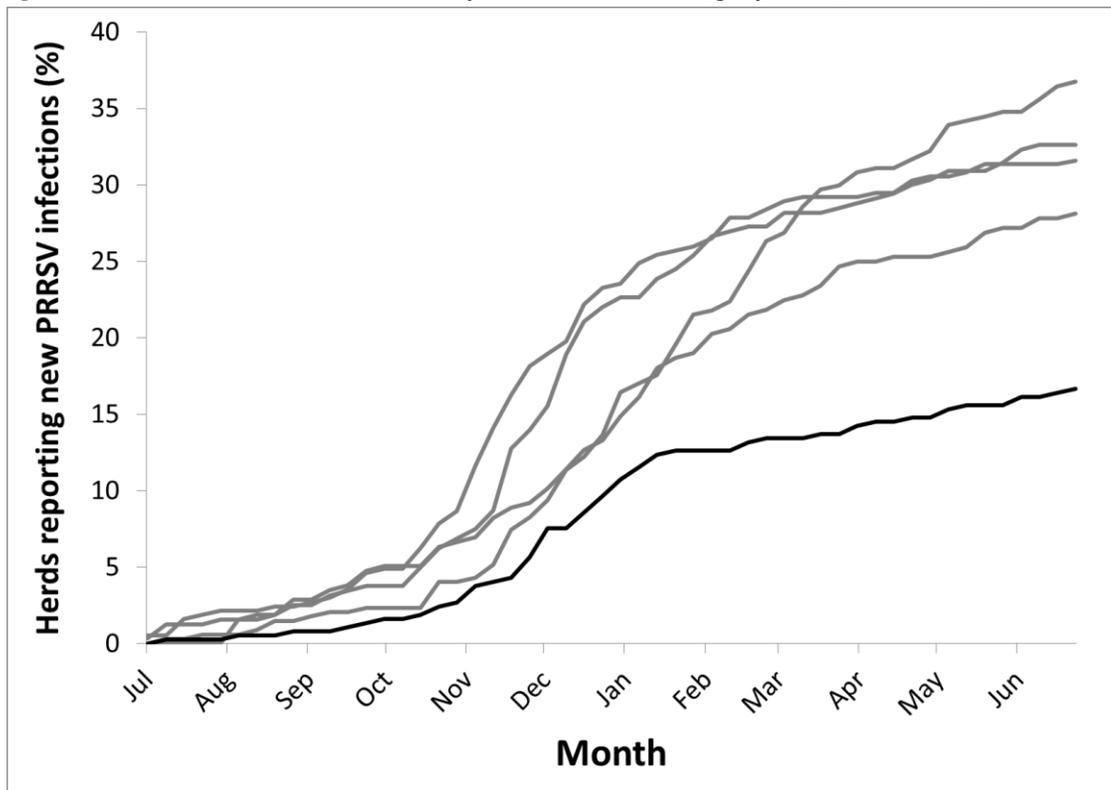
- a. Win Episcopo, version 2.0, Universidad de Zaragoza, Zaragoza, Spain.
- b. Statistix, version 9.0, Analytical Software Inc, Tallahassee, Fla.
- c. SaTScan, version 8.0, Information Management Services Inc, Calverton, Md.

Table and figure

Table 1 – Weeks to epidemic onset (starting from July 1) and cumulative incidence (with 95% confidence interval) comparison between the average of the first four monitoring years (2009-2013) and the 2013/14 monitoring year.

	4 year average (95% CI)	2013/14	P value
# weeks until epidemic:	15.3	18.3	--
Cumulative incidence (count of cases)			
July – Sept (Q1)	8 (7.74 – 8.26)	5	0.3284
Oct – Dec (Q2)	60 (58.25 – 61.75)	31	0.0046
Jan-Mar (Q3)	30 (28.30 – 31.70)	15	0.0221
Apr – Jun (Q4)	14 (13.35 – 14.65)	11	0.7702
July – Dec (Q1 – Q2)	68 (66.24 – 69.76)	36	0.0045
July – Mar (Q1 – Q3)	98 (96.15 – 99.85)	51	0.0018
July – Jun (Q1 – Q4)	112 (109.79 – 114.21)	62	0.0039

Figure 1 – Cumulative incidence for years 2009 - 2012 (gray lines) and 2013 (black line)



**Chapter IV – Frequency of Porcine Reproductive and Respiratory
Syndrome Virus detected outside eight sow farms in swine dense regions
of Minnesota**

This chapter is in preparation to be submitted to The Veterinary Journal

S.J.P. Tousignant, P. Davies, P. C. Raynor, A. Rovira, R. Morrison. Frequency of Porcine Reproductive and Respiratory Syndrome Virus detected outside eight sow farms in swine dense regions of Minnesota. [in prep, to be submitted to The Veterinary Journal]

Summary

Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) causes devastating economic losses due to production inefficiency around the world. Aerosol transmission has been implicated as a possible source of infection into many sow herds, and bio-aerosol filtration has been employed to reduce the frequency of introduction via this route. What remains unknown is the frequency at which sow herds in swine dense regions are exposed to aerosolized PRRSV. Therefore, the objective of this study was to determine the detection frequency of aerosolized PRRSV exposure outside eight sow farms in swine dense regions.

Utilizing previously published methods, 0 of 241 samples tested positive for PRRSV by polymerase chain reaction (PCR) during what was the height of the 2012 PRRSV epidemic in the United States. Because all farms were employing bio-aerosol filtration, they were presumed to be at risk of aerosolized transmission. 6 of the 8 farms (75%) in the present study, and 3 of the 4 farms (75%) in a previous study, did not have a neighborhood structure that would have supported epidemic spread of disease using algorithms developed during Highly Pathogenic Avian Influenza outbreaks in Denmark. Additionally, there were no instances during the study when climatic conditions aligned with previously reported ranges that favor aerosol transmission of PRRSV. However, there were times when conditions were similar to when PRRSV was identified in air samples in more recent work. Therefore, it appears that aerosol exposure to PRRSV virus is an infrequent, but high consequence, event in sow farms in hog dense areas.

Introduction

For almost three decades Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) has been the most devastating virus afflicting US swine production with economic losses due to production inefficiencies estimated to be \$664 million annually (Holtkamp et al., 2013). Belonging to the family *Arteriviridae*, the virus was first described in Europe in 1991 (Wensvoort et al., 1991). It is a small (50-60nm), positive-stranded, enveloped, RNA virus (Benfield et al., 1992; Cavanagh, 1997). PRRSV is highly susceptible to inactivation by heat and drying. At temperatures between -20°C and -80°C, PRRSV can be stable for months to years (Zimmerman et al., 2010). However, at temperatures from 25-27°C infectious virus could not be recovered from a variety of common surfaces and materials after more than 24 hours (Pirtle and Beran, 1996). The role of PRRSV transmission via fomites has been studied and contaminated items such as clothing, blood collection equipment, snout snares and feed bags were associated with previously negative pigs becoming infected (Otake et al., 2002a; Otake et al., 2002c; Pitkin et al., 2009a). More recently herd ownership, gilt source, and transportation were found to be important factors in transmission, whereas spatial proximity to other herds was not (Kwong et al., 2013).

The first study involving aerosol transmission of PRRSV dates back to 1997 (Torremorell et al., 1997). Shortly thereafter, several groups began trying to understand the role of aerosol transmission of PRRSV (Wills et al., 1997; Lager and Mengeling, 2000). Results of these early trials were mixed, leading some to believe aerosol transmission was either rare or does not occur. From 2002, Otake et al., reported aerosol

transmission of PRRSV in a series of controlled trials at an experimental facility (Otake et al., 2002b). The likelihood of aerosol transmission was suggested from an observational study on a large cohort of swine herds in Europe (Mortensen et al., 2002), and experimental studies indicated that the potential for aerosol transmission of PRRSV varied between strains (Cho et al., 2007b). In another field experiment including several replicates over approximately one year, 20 of 190 (10.5%) positive aerosol samples were detected at a distance of 120 meters and associations with meteorological conditions on positive transmission days were reported (Pitkin et al., 2009b). A narrow range of weather conditions favoring long distance aerosolized transmission of PRRSV have been reported for temperature (-2.6 – 4.8°C), relative humidity (77 – 82%), wind velocity (1.4 – 1.9 m/s), gust velocity (2.8 – 3.7 m/s), barometric pressure (979 – 984 hPa), precipitation (0.0008 – 0.006 mm), sunlight (114 – 164 W/m²) and sunlight power (330 – 476 micromol/m²/s) (Dee et al., 2010b). More recently, the same group detected one positive PRRSV sample 9.1 km downwind from an acutely PRRSV infected finishing herd during a 21 day period (Otake et al., 2010a).

Collectively, these studies suggest PRRSV can be transmitted with some repeatability over short distances, but transmission may be less frequent over long distances. Strong seasonality of PRRSV epidemics was documented over a four year period in a large cohort of sow herds (Tousignant et al., 2014) suggesting factors that favor transmission might occur more frequently during late fall and winter. In the past decade, bio-aerosol filtration (Pitkin et al., 2009b) has been implemented to reduce the frequency with which aerosolized PRRSV enters the farms. Several observational studies,

in limited cohorts of sow herds, have reported significant reductions in new PRRSV infections following filtration (Spronk et al., 2010; Dee et al., 2012; Alonso et al., 2013b). One of these studies documented that the seasonal epidemic was no longer clearly evident in the post filtered period (Alonso et al., 2013b).

Local thresholds for geographical spread of infectious diseases have been studied in the case of avian influenza epidemics in The Netherlands (Boender et al., 2007b). Here, the epidemiologic principles of reproduction ratios are used to identify areas with the potential for epidemic spread of disease as a function of Euclidean distance between farms within an area and probability of infection. A local reproduction ratio (R_{hi}) is greater than a critical reproduction ratio (R_c), suggests the likelihood of epidemic spread of disease within an area via aerosolized spread.

While many sow farms are implementing bio-aerosol filtration control measures, very little is known regarding the frequency and diversity of aerosolized PRRSV strains to which they are exposed. Therefore, the objective of this study was to describe the frequency of PRRSV detected in air samples collected outside eight filtered sow farms in swine dense regions of Minnesota during the height of a PRRSV epidemic season.

Materials and Methods

Study Population

Two groups of four farms employing bio-aerosol filtration (Dee et al., 2009c; Pitkin et al., 2009b; Dee et al., 2012) were selected for this trial. All farms were required

to have at least 1 other pig site within a 3 mile radius, and to be weaning piglets into off-site facilities. To facilitate sample collection within each group, farms were required to be within 20 miles of each other, but not closer than 3 miles. Group one was located Blue Earth, Wagonwan, and Martin counties in south central Minnesota. Group two was located in Renville and Kandiyohi counties in west central Minnesota. At enrollment, farms were required to be designated as stable, vaccinated stable, provisionally negative, or negative using the American Association of Swine Veterinarians PRRSV classification (Holtkamp et al., 2011). Farms were required to share PRRSV status changes during the study.

Study design

Samples were collected approximately every two weeks between October 1st, 2012 and March 30th, 2013. At each collection, wind direction was established and collectors were placed approximately 10 meters from the upwind side of the barn and at least 10 meters from any object that could interfere with wind flow (ie. liquid propane tanks, other buildings, tree lines). When it was not possible to avoid these objects, collections were made at a pre-determined location on the farm site away from these structures. When weather conditions were hazardous (freezing rain and ice, or more than 6 inches of snow) collection dates were adjusted by one or two days to allow for safe travel.

Air samples

Two cyclonic collectors (Midwest Microtek, Brookings, South Dakota, USA) capable of moving an estimated 200 liters of air per minute were placed approximately 0.5 meter off the ground and at least 3 meters from each other situated in a line perpendicular to the wind direction. During collections when the temperature was below

freezing, collectors were placed inside the cab of the vehicle parked perpendicular to the wind direction with the heater running and collectors were elevated to the height of the windows adjusted to allow air to pass through the vehicle yet prevent the liquid collection media from freezing (Otake et al., 2010a). Collection cups and collector fan blades were thoroughly swabbed prior to each collection using a Stuart's Media (Becton, Dickenson and Company, Franklin Lakes, New Jersey, USA) swab pre-moistened in the transport tube, and immediately placed onto dry ice. Collection vessels were then filled with 11.0 ml of Dulbecco's Modified Eagle Medium (Gibco Life Technologies, Grand Island, New York, USA) containing: 0.02% Bovine Serum Albumin Fraction V (7.5%), (Gibco Life Technologies, Grand Island, New York, USA), 0.01% Anti-Anti (100X) (Gibco Life Technologies, Grand Island, New York, USA), 0.0015% Trypsin-TPCK, (Sigma-Aldrich, Saint Louis, Missouri, USA) and 0.001% Gentamicin sulfate (Lonza, Allendale, New Jersey, USA). Collectors were operated for 30 minutes and the remaining liquid media was poured into a sterile 10.0 ml Falcon Tube (Becton, Dickenson and Company, Franklin Lakes, New Jersey, USA) and placed immediately onto dry ice. After each collection, the collectors were disassembled and cleaned in the field using a warm water rinse, followed by saturation of all surfaces with 70% ethanol solution allowed at least two minutes contact time, rinsed again with warm water and wiped with clean paper towels and allowed to air dry. Collectors were placed into individual plastic storage containers for transport and storage.

On each collection date, a group of four farms was visited twice yielding a total of 16 samples on each day. A rotating schedule was set so that first samples of the day were

collected at a different farm each week. First samples were always collected between the times of 0500 and 0600. Equipment was cleaned, packed for transport and then moved to the next location. The remaining samples for that day were collected between the times of 0630 and 0730; 0800 and 0900; 0930 and 1030; 1100 and 1200; 1230 and 1330; and 1400 and 1430. The last samples were collected between 1500 and 1600.

Liquid samples from the collection vessel were stored at -80°C in duplicate, and a 1.0 ml aliquot of each sample was sent to the University of Minnesota Veterinary Diagnostic Laboratory and tested for the presence of European and North American PRRSV ribonucleic acid by polymerase chain reaction (PCR). Pre collection swabs were stored at -80°C to be tested by PCR in the event of a positive test from the liquid media. Samples were considered positive if PCR cycle time values were less than 40.

Weather data

Weather data of wind speed, temperature, relative humidity, barometric pressure and sky conditions were obtained from the National Oceanographic and Atmospheric Administration's (NOAA) National Climactic Data Center Quality Controlled Local Climatological Data website from the Fairmont and Olivia Minnesota regional/municipal airports for the study period. Data were imported into an Excel spreadsheet and analyzed to determine the frequency of events where climactic conditions corresponded with those previously reported as associated with PRRSV airborne transmission (Dee et al., 2010b). Additionally, retrospective weather data were collected in a similar manner from weather stations nearest the sow farms in a previous study in Northwest Iowa (Sheldon,

Iowa), Northeast Iowa (Waterloo, Iowa), Eastern South Dakota (Pipestone, MN) and Southwest Minnesota (Pipestone, MN) (Brito et al., 2014).

Threshold for geographical spread

A unitless local reproduction ratio, R_{hi} , (Boender et al., 2007b) was calculated for each farm by identifying all swine facilities within a 3 mile radius ($r_s = 3$) using high resolution satellite imagery (google earth), and local area knowledge. First, R_{hi} was estimated for each farm i with non-homogeneous farm density

$$R_{hi} = \sum_{j \neq i} p(r_{ij})$$

where j and i are unitless farm indices, p is the spatial kernel and r_{ij} is the distance between farms i and j . The first step of estimating the unitless critical reproduction ratio, R_c , was to approximate the value in terms of $p(r)$ using the moments

$$0: \bar{p} = \int_0^{\infty} p(r') dr'$$

$$1: \langle r \rangle = \int_0^{\infty} \frac{p(r')}{\bar{p}} r' dr'$$

$$2: \langle \frac{1}{2} r^2 \rangle = \int_0^{\infty} \frac{p(r')}{\bar{p}} \frac{1}{2} r'^2 dr'$$

in which \bar{p} is the intensity, $\langle r \rangle$ is the spatial dispersion, and $\langle \frac{1}{2} r^2 \rangle$ the second moment

$p(r)$, where $\bar{p}\langle r \rangle$ is a measure of the infectivity. Next, the spatial kernel was described as

$$p(r) = \begin{cases} p_0 \left(1 - \left(\frac{r}{r_s}\right)^2\right)^2 & r \leq r_s \\ 0 & r > r_s \end{cases}$$

where r_s is the scaling and p_0 is the probability of transmission at $r = 0$. Finally, R_c is estimated using the equation

$$R_c = 1 + \left(\frac{\langle r \rangle^2}{\langle \frac{1}{2} r^2 \rangle} \right)^z \left(\frac{\bar{p}}{\langle r \rangle} \right)^y$$

where z and y are constants that have been previously estimated as 1.7 and 0.6, respectively (Boender et al., 2007b).

Results

Air samples

A total of 241 air samples, representing 482 hours of air sampling, were collected. No samples were positive for PRRSV (PCR cycle time >40).

Weather

In group one, the average distance between the farms and the weather station in Fairmont, MN was 14.9 miles (range: 7.5 – 18.4 miles). In group 2, the average distance between the farms and the weather station in Olivia, MN was 13.4 miles (range: 7.7 – 16.4 miles). A total of 24,020 observations were recorded approximately every 15 minutes from the two NOAA weather stations during the study period. There were zero observations at which temperature, relative humidity, and wind speed were all simultaneously within the reference ranges that were previously associated with airborne transmission in an experimental setting (Dee et al., 2010b). Even after the ranges for each variable were expanded by 20%, there were still no instances when all variables were simultaneously within the respective ranges.

The average distance between the farms and weather stations in the Brito study was 13.5 miles (range: 7.8 – 22.0). On the dates and times where PRRSV positive air samples had been collected in Southwest Minnesota and Eastern South Dakota, the

average conditions were: temperature 0.9 degrees Celsius (range: -20.0 – 7.8); relative humidity 85.6% (range: 60.0 -100.0); wind speed 4.3 meters per second (range 0 – 10.3); and barometric pressure 28.0 mmHg (range: 711.2 – 721.4). Likewise, from the station in Northwest Iowa, average condition were: temperature 1.7 degrees Celsius (range: -7.7 – 12.2); relative humidity 89.9% degrees Celsius (range 62.0 – 100.0), wind speed 4.9 meters per second (range: 0 – 11.6); and barometric pressure 721.4 mmHg (range: 706.1 – 731.5). The data station in Northeast Iowa was not regularly recording data during the study period and therefore, results are not reported.

The ranges of each weather parameter were then used to identify the frequency of similar patterns during collection times at the Fairmont and Olivia weather stations. Approximately 20 – 25% of the time during which samples were being collected in the two groups of the present study had weather patterns that were similar to the conditions present in the Brito study.

Threshold for geographical spread

The average number of farms within three miles of farms in group one was 11.25 (range 10-15), and in group two was 2.5 (range 1-4). At a maximal probability of transmission ($p_0=1$), R_c was 3.35. Average values of R_{hi} (when $p_0 = 1$) for the farms were 4.2 (range 3.0-6.4) in group one and 0.8 (range 0.3-1.2) in group two. Two farms in group one, and zero farms in group had instances where R_{hi} was greater than R_c at any value of p_0 (figure 1).

Discussion

Aerosol transmission of PRRSV is thought to be important and requires a significant economic investment at the farm level to address (Alonso et al., 2013a).

Among farms employing bio-aerosol filtration, a significant reduction of PRRSV introductions into populations of pigs has been a consistent finding in observational and experimental field studies. (Dee et al., 2009c; Pitkin et al., 2009b; Dee et al., 2010b; Spronk et al., 2010; Alonso et al., 2013b).

The inability to detect PRRSV in air samples in the swine dense regions of this study using previously published materials and methods was unexpected. This is inconsistent with a recent report describing 37% PRRSV positive air samples collected outside of four swine farms in a densely populated region of the from October 15th, 2012 to December 15th, 2012 (Brito et al., 2014). In the present study, a large number of air samples were collected across an extended period during the height of the 2012 PRRSV epidemic (Tousignant et al., 2014). Additionally, these samples were collected over a wide range of time points, and climatic conditions within swine dense regions outside farms presumed to be at high risk of aerosolized PRRSV transmission. Two weeks after the study was initiated, one site in group one reported a PRRSV infection with a virulent virus that caused significant reproductive losses. PRRSV RNA was not detected in air collected outside this location before or during this outbreak.

Recent observational studies found no significant relationships between new PRRSV infections and proximity to other swine herds in a Canadian cohort, instead reporting stronger associations with ownership, gilt source and market trucks (Kwong et al., 2013). Another Canadian study reported similar findings where there was little evidence of aerosol spread, but rather reported links to animal source and herd ownership

suggesting the networks within swine production play a more prominent role in the transmission of PRRSV (Rosendal et al., 2014). These studies are unique because they estimate the relative impact of these factors on risk of PRRSV infections on farms.

Previously, ranges of weather conditions that favor the transmission of PRRSV have been reported (Dee et al., 2010b). These data, however, are limited to 16 positive observations over a two year period at one location. Under the conditions of a recently completed trial (Brito et al., 2014), the previous estimates may have been somewhat conservative. Interestingly, during the Brito study, positive air samples were collected on days and times at multiple locations when there was potentially no air movement, as indicated by a weather station approximately 13.5 miles away. It could be argued that the weather conditions locally on the farm differed slightly than at the weather stations, and that the weather stations might not be sensitive enough to pick up very slight wind movement that could still be capable of carrying virus. On the days when virus was found in the air at site 'D', the virus recovered did not match the resident vaccine or wild type viruses on the farm, whereas the other farms enrolled in the study were considered negative for PRRSV (Brito et al., 2014).

The number and distance to neighboring farms within a region (neighborhood structure) could be important to consider for aerosolized spread of disease (Boender et al., 2007b). The scaling factor (r_s) of 3 miles was chosen somewhat arbitrarily under the presumption (due to lack of compelling evidence to suggest otherwise) that the majority of viable virus transmission would occur within this distance. Additionally, limiting the

scaling factor aided in the collection of accurate data regarding the types of animal production in surrounding farm sites. In a sensitivity analysis, using data from previous avian influenza epidemics, it was determined that the algorithms were relatively insensitive to the uncertainties of the spatial kernels used, suggesting the robustness of this method (Boender et al., 2007b). Additionally, these methods have been applied in outbreaks of Classical Swine Fever in The Netherlands from 1997 – 1998 to study the role of farm size on infectiousness and susceptibility (Boender et al., 2014). We did not include farm size in this model due to the voluntary nature of this project, and inability to accurately collect this information. Therefore, the results of this model should be interpreted cautiously.

Because all farms were filtered, it was believed they were at high risk of aerosolized transmission of PRRSV. With this in mind, when these algorithms were applied to the herds in this study, 6 of the 8 farms in this study did not appear to have a neighborhood structure that would support epidemic transmission of PRRSV even with a hypothetical virus that had a 100% probability of transmission. This suggests the importance of other biosecurity factors on these farms and may, in part, provide insight into the historical route of entry for PRRSV into these farms.

Cyclonic collectors have been used frequently to study PRRSV aerobiology (Pitkin et al., 2009b; Otake et al., 2010a), and until recently, little was known about their sensitivity or consistency for detecting PRRSV. When multiple cyclonic collectors were used to collect air samples in air chambers containing artificially generated PRRSV

aerosols, considerable variability in detection rates among collectors were seen at low concentrations of virus (Wedel et al., 2014). The ability of various types of collection devices to collect aerosolized pathogens has been studied, and under highly controlled laboratory conditions, recovery was an infrequent event for some bacterial pathogens, and did not occur for 2 strains of PRRSV (Hermann et al., 2008). Unpublished measurements by the authors of the present study suggest that the cyclonic collectors have limited collection efficiency for particles smaller than 1 μm in diameter and may lose some particles larger than 8 μm in diameter if they are solid enough to bounce off of interior surfaces. The analytical sensitivity and collection efficiency of impingers and cyclonic collectors has been described, and may in part explain the inability to detect pathogens if the airborne virus concentrations were below the limit of detection (Hermann and Zimmerman, 2008). Undetectable virus concentrations might be biologically significant and able to produce infection in exposed pigs. Additionally, the collection media and collection duration used may impact pathogen recovery and should therefore be validated for each collection method and pathogen (Hermann et al., 2006). Recently, impactor type collectors have been used to determine what particle size and collector substrates influenced viability of human viruses, and may provide the framework for similar studies using swine pathogens (Appert et al., 2011). Cyclonic collectors rely on inertia and the ability of particles to be captured in a liquid media. The particles on which PRRSV is transmitted over long distances may not have the appropriate size to be captured regularly with these methods, and additional studies should examine relationships between PRRSV and particle size.

Limitations of previous studies should be considered, including small source populations of infected animals (in the case of the experimental field conditions studies), and small databases of herds representing a fraction of the industry (in the case of the commercial field conditions studies) which could introduce bias and decrease the external validity of the results. Additionally, an implicit assumption of the observational studies that indicated reduced risk in filtered farms is that all other biosecurity measures were unaltered between the periods before and after the addition of filters (Alonso et al., 2013b). It is difficult to know with certainty if farm staff behavior and compliance with other biosecurity practices changed after the large investment into filters was made. It is common practice for filtered herds to receive at least an annual audit that regularly includes a comprehensive biosecurity review of the farm, staff, and external visitors. The effect of these annual reviews has not been assessed. While it is possible to use observational studies to compare pre and post-filtration periods, caution should be used when interpreting the results in the context of potentially confounding variables.

There have been no randomized controlled trials conducted on filtration where farms have been randomly assigned a placebo filter. Controlled studies are required to reduce potential confounding and validate the effect of interventions; however, the logistics and costs of conducting such trials will likely prevent them from being conducted. With this in mind, however, no other control measures until this point have been documented to consistently, and significantly reduce the incidence of PRRSV resulting in a positive return on investment (Alonso et al., 2013a). Therefore, bio-aerosol

filtration and the potential behavior changes associated with them should continue to be studied, improved and applied at the farm level.

To ensure proper function of the collectors, they were subsequently used to sample air within a relatively small swine nursery facility that was confirmed to be PRRSV positive by PCR on 30 of 30 individual serum samples (approximately 20% of the entire population) and oral fluids collected from ropes hung in 2 of 2 pens (25% of the total number of pens). Additionally, clinical signs including lethargy, dyspnea, and anorexia in a substantial number of the pigs were observed. Utilizing the methods described in this study, 29 of 98 (29.6%) samples tested positive for PRRSV by PCR across 7 sampling events during a 16 day period, suggesting the validity of the methods used. When the next group of pigs was delivered into the nursery approximately 2 months later, samples were collected outside the facility using the same collection devices and methods. The diagnostic data and clinical description were similar to before and 0 of 24 samples tested positive for PRRSV by PCR collected at 5, 10 and 20 meters from the barn.

It has also been suggested that a potentially significant difference between the collection methods utilized in this study and the Brito study is the fact that samples were collected from inside a vehicle. In this study, as in previous studies where positive air samples have been successfully collected (Otake et al., 2010a), the vehicle was parked perpendicular to the prevailing wind direction, windows opened to allow air flow and the vehicles heater turned on just high enough to prevent the liquid media from freezing. It

has been speculated that this method may cause changes in the dynamics of aerosolized PRRSV due to slight positive pressure situation, abrupt change from cold to warm temperatures, etc. In the Brito study, samples were collected outside the barn facility. It is not clear, however, how samples were prevented from freezing in the collector (thus rendering the collector inoperational) on days where temperature was several degrees below zero Celsius. Additionally, it is not known how any other methods that could be employed to prevent freezing (ie adding propylene glycol to the liquid collection media, or heating collection chamber) would affect the sensitivity of the collection device or specificity of the diagnostics performed at the University of Minnesota.

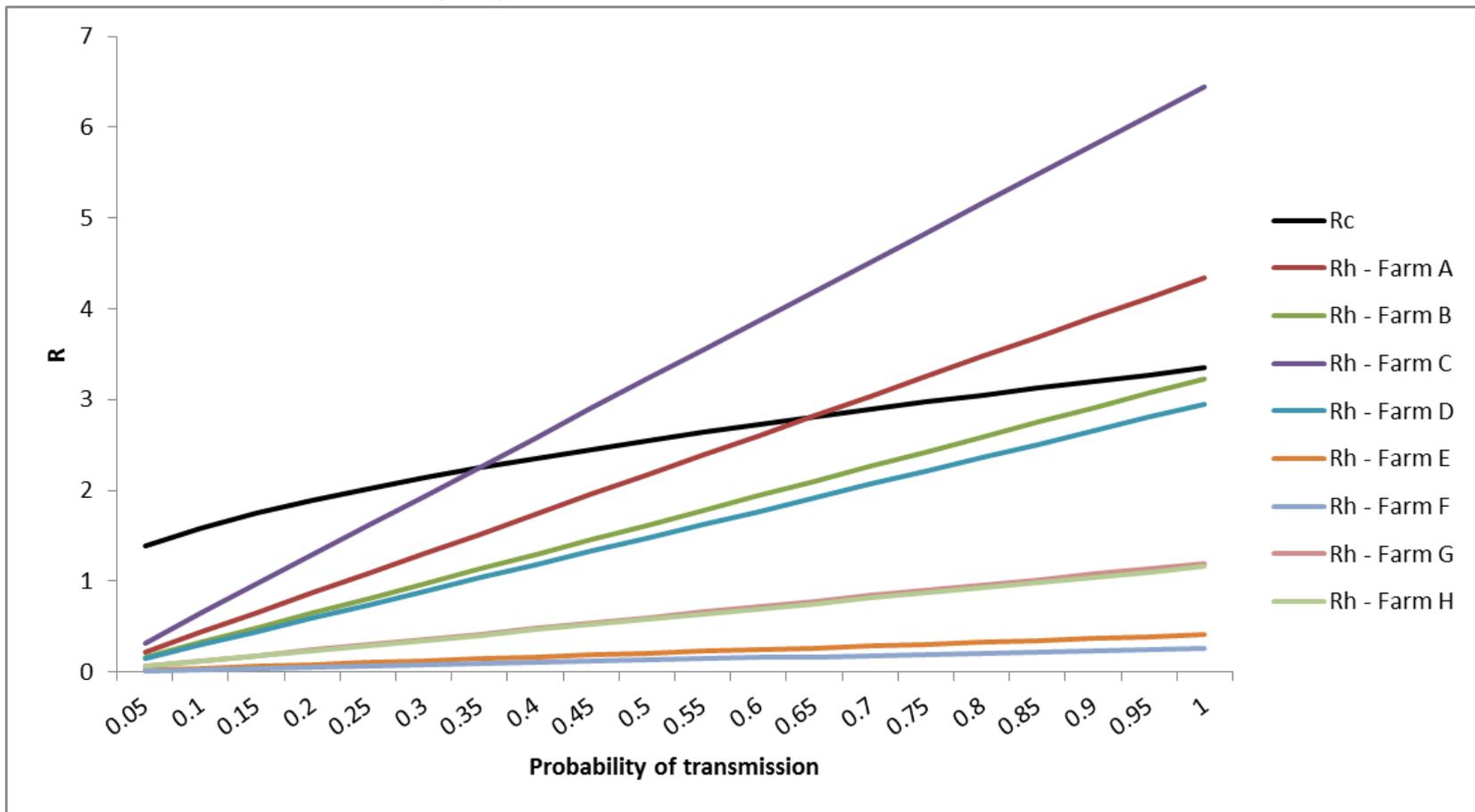
In conclusion, the results of this study suggest that detection of aerosolized PRRSV in a swine dense region may be a very infrequent event. Additionally based on recent data by Brito, it appears as though PRRSV transmission might occur under a wider range of climatic conditions than previously reported. Furthermore, frequency of PRRSV aerosol detection may have localized, regional differences beyond density, spatial structure of the neighboring swine farms, and weather influencing aerosolized PRRSV patterns. In general, detection of aerosolized PRRSV seems to differ greatly by region and by study. Regardless, an infrequent event may still be sufficient to infect sow herds and have major economic consequences. Future studies could be directed at validating more sensitive collection devices and trying to understand the factors that influence the frequency of aerosolized PRRSV detection frequency within and across regions.

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Figure

Figure 1 – Fig. 1. Calculated R_c (black line) and R_h values for farms in group one (Farms A – D), and group two (Farms E – H). Three of the twelve farms analyzed have $R_h > R_c$ suggesting a spatial structure of neighboring farms with the potential for epidemic spread of disease within a 3 mile radius ($r_s = 3$).



**Chapter V – Porcine Reproductive and Respiratory Syndrome virus
and Porcine Epidemic Diarrhea virus Infection Patterns and Risk
Factors in Sow Farms in the United States**

This chapter is in preparation to be submitted to PLOS ONE

Steven J.P. Tousignant, Pablo Valdez-Donoso, Andres M. Perez, Robert B. Morrison.
Porcine Reproductive and Respiratory Syndrome virus and Porcine Epidemic Diarrhea
virus Infection Patterns and Risk Factors in a Convenience Sample of Sow Farms in the
United States between 2013 and 2014. [in prep, to be submitted to PLOS ONE].

Summary

Porcine Epidemic Diarrhea virus and Porcine Reproductive and Respiratory Syndrome virus are two economically important diseases of swine production in the United States of America. The objective of this study was to assess patterns of co-infection of these two diseases using data from a group of voluntary participants enrolled in the Swine Health Monitoring Project in the Midwest region of the United States of America. First, disease clusters were identified using a multinomial space-time scan statistic. Then, a multinomial, multivariate regression model was fit to quantify associations between farm level management factors and disease outcomes including if the farm was located in a disease cluster. Five significant space-time clusters with two or more herds were identified as being co-infected, infected with either Porcine Epidemic Diarrhea virus or Porcine Reproductive and Respiratory Syndrome virus only, or infected with neither virus. The type of cluster a farm was located in and county density significantly affected the odds of being infected with Porcine Epidemic Diarrhea virus alone or with Porcine Reproductive and Respiratory Syndrome virus, whereas high biosecurity including bio-aerosol filtration significantly decreased the odds of being in any disease category. These results suggest that these diseases clustered in time and space between the fall and winter of 2013 through the spring of 2014. At the farm level, being in high swine dense areas increased a farm's risk of becoming infected with either disease, and high biosecurity including bio-aerosol filtration reduced the risks. Efforts could be directed at identifying better ways of implementing strict biosecurity practices that are common among filtered farms as a means mitigating these pathogens in the United States of America.

Introduction

Porcine Reproductive and Respiratory Syndrome virus (PRRSV) is an enveloped, RNA virus belonging to the family *Arteriviridae* causing both reproductive failures and respiratory disease (Rossow, 1998). Since its emergence in the US in the 1980's, it has been an economically important disease of swine herds with losses estimated to be \$664 million annually (Holtkamp et al., 2013). Porcine Epidemic Diarrhea virus (PEDV) is an enveloped, single stranded alpha coronavirus that is capable of causing up to 100% morbidity and mortality in suckling piglets in naive herds (Saif et al., 2012). Clinical signs are characterized by profuse watery diarrhea and vomiting leading to dehydration and death, especially in suckling piglets (Stevenson et al., 2013). In the spring of 2013, PEDV was first detected in the swine population of the United States of America (USA) and after 12 months, approximately 50% of the national sow population became infected (Goede and Morrison, 2013).

Data from the voluntary Swine Health Monitoring Project have shown striking repeatability in the onset and clustering of PRRSV epidemics since 2009 (Tousignant et al., 2014). Additionally, it has been reported that both viruses can be detected over long distances between farms in aerosols (Otake et al., 2010b; Alonso et al., 2014). Herd size, season that a herd was established, low biosecurity practices, distance to infected neighboring herds, as well as pig and semen movement have been associated with PRRSV outbreaks (Mortensen et al., 2002; Firkins and Weigel, 2004; Holtkamp et al., 2010; Holtkamp et al., 2012). In one study, it was reported that 5.2% of swine transport trailers were contaminated with PEDV at slaughter plants suggesting the potential for

transmission via trucking (Lowe et al., 2014). Studies have reported associations between PRRS infections and herd ownership, replacement female source, and transportation of animals and vehicles as well (Kwong et al., 2013). In addition to herd size, another study also found significant associations between PRRS and distance to closest pig site, no shower at entrance, direct access to site by rendering truck and lower overall biosecurity practices (Lambert et al., 2012a) Contaminated feed has been implicated in outbreaks of PEDV at the farm level (Stevenson et al., 2013; Bowman et al., 2015; Dee et al., 2015) and hazard analysis has assessed risk at various points throughout the manufacturing process which included to the potential for cross contamination at all stages of manufacturing (Sampedro et al., 2015; Snider et al., 2015).

In 2013, the overall magnitude of the annual PRRSV epidemic was significantly less when compared to the previous four years and the onset was delayed, suggesting a potential link to the introduction of PEDV (Tousignant et al., *In press*). Until now, patterns of co-infection for these two viruses and associated farm level management factors have not been studied. Therefore, the objectives of this study were to first, identify time-space clusters of PRRSV and PEDV co-infections, and second, to quantify associations between these pathogen outcomes and various farm management factors including feed milling, trucking, manure pumping, and facility ownership. Identification of high risk regions and the associations with various disease risk factors would provide a scientific basis for control measures aimed at mitigating the impact of one or both of these viruses on sow farms and within regions.

Materials and Methods

Data source and case definition

A codified data set was obtained from the Swine Health Monitoring Project (SHMP) at the University of Minnesota via informed consent from the project participants. All participating herds from one veterinary practice were included in the survey. This corresponded to a convenience sample of 109 (29.3%) of the 371 sow herds and 5 (35.7%) of the 14 systems enrolled in the project at the end of 2014. On a weekly basis, the veterinarian or health manager for each herd reported to the SHMP both PRRSV and PEDV status from July 1st 2013, through June 30th, 2014. PRRSV infections were defined using the American Association of Swine Veterinarians (AASV) guidelines (Holtkamp et al., 2011). Briefly, these definitions specify a herd in the acute stage of an outbreak to be category I, moving to a positive stable (with or without ongoing vaccination programs) classification (category II-A or B) after achieving negative testing over time, then provisionally negative (category III) with more time, and eventually into a negative category IV when there is no longer serologic evidence of PRRSV in the herd. In the case of deciding between recirculation of resident PRRSV or new incursion, some professional veterinary judgement was used as described by Yeske in 2013 (Yeske, 2013) which included time of year, virus homology, herd history, etc.

Case definition for PEDV infection was more simplistic as all herds were previously negative, and were declared positive after diagnostic laboratory confirmation by fecal or tissue Polymerase Chain Reaction (PCR). PEDV monitoring was not usually in place, as the clinical presentation of the disease is dramatic enough to lend substantial confidence

to the date of onset. As such, herds were then classified into four categories: PRRSV- /PEDV- (0), PRRSV-/PEDV+ (1), PRRSV+/PEDV- (2), or PRRSV+/PEDV+ (3).

Space-time analysis

A space-time multinomial probability model was fit to identify clusters of the four disease co-infection categories using the likelihood function given by the following equation:

$$L(Z, p_1, \dots, p_k, q_1, \dots, q_k) \propto \prod_{k=1}^K \left(\prod_{i \in Z} p_k^{C_{ik}} \prod_{i \notin Z} q_k^{C_{ik}} \right)$$

where C_{ik} is the number of observations in each k (0, 1, 2 or 3) category for each i farm, p_k and q_k are the probability of being in a k category within and outside of a cylindrical window Z respectively. 999 Monte Carlo simulation was performed to identify significant clusters (Jung et al., 2010). A previous study of PRRSV epidemics in this cohort since 2009 indicated an average cluster radius of 235 km of PRRSV over the previous four years (Tousignant et al., 2014), and therefore, a maximal cluster radius of 235 km and a temporal window lasting a maximum of 50% of the study period were chosen. Observed cluster patterns that had a P value of ≤ 0.05 were considered significant. Space-time analysis was conducted in SaTScan version 9.3 (Kulldorf M. and Information Management Services, www.satscan.org), and maps were constructed in ArcMap 9.2 (Environmental Systems Research Institute, Redlands CA, USA).

Farm Characteristics and Management factors

A survey was distributed to collect individual herd level data on demographic data including if a farm was located in a disease cluster, average inventory, type of production, and management factors including production company, production type (commercial or genetic), filtration status, frequency of previous PRRSv infections and PRRSv vaccine use in the past 4 years, feed mill type (toll or owned and size), carcass disposal (external rendering or onsite method) and whether a particular herd owned or contracted (exclusively or not) any trucking, manure pumping, or facilities. County level estimates of swine density were obtained through the National Agricultural Statistics Service and stated as number of operations with swine within the county (Service, 2015).

Farm level risk analysis

Using the farm level disease category, a multinomial, multivariate logistic model with backward selection was used to estimate the odds of being in one of the three diseased combinations with the reference group being negative for both diseases.

$$\ln \frac{p(Y = j)}{p(Y = 1)} = \beta_0^j + \beta_1^j X$$

Coefficients β_0 and β_1 were estimated for farm level disease type j and were compared to the reference level (category 0). Independent predictor variables were mentioned above.

Regression modeling was conducted in SAS University Edition (SAS Institute, Cary, NC, USA). P-values < 0.05 were considered significant.

Results

Space-time analysis

The multinomial space-time analysis identified six significant clusters. However, only five had at least two herds, and as such were considered for analysis (Figure 1). Four of the five corresponded to a high risk for one or both pathogens only one low risk (PRRSV-/PEDV-) cluster was detected (Table 1). The number of herds per cluster ranged from 2 to 34 (mean = 11; median =7; Table 1). The most likely cluster was situated in south-western Minnesota and north-western Iowa, had a radius of 116 km, and occurred between January 2014 and June 2014 (Table 1, figure 1). Three of the five clusters occurred during the winter of 2014 through June 2014 whereas cluster 5 occurred between July 2013 and December 2013, and cluster 2 (PRRSV+/PEDV-) occurred during the fall in conjunction with previously documented PRRSV epidemic seasons (Tousignant et al., 2014) (Table 1).

Farm level risk analysis

The final backwards selection, multivariate assessment showed that county density was significantly associated ($p < 0.05$) with a farm being infected with either PEDV or PRRSV, but not both and similarly increased the odds of being infected with one or the other disease by 1.03 (95% CI = 1.01 – 1.05) for each additional swine site in the county (Table 3). The odds of being infected with PEDV or both diseases was significantly lower in herds with high biosecurity and filtration ($p < 0.05$). Specifically, in herds with PEDV only, the OR was 0.12 (95% CI = 0.02-0.67), in herds with both diseases the OR

was 0.07 (95% CI = 0.01-0.71) and in herds with PRRSV only, the OR was 0.18 (95% CI = 0.03-1.32) (Table 2).

Discussion

PRRSV and PEDV are two devastating viral diseases of swine in the US (Holtkamp et al., 2013; Alonso et al., 2014). While the temporal and spatial dynamics of PRRS has been previously described between 2009 and 2013 (Tousignant et al., 2014), little knowledge about factors associated with being identified as a herd within a high risk clusters of PRRSV and/or PEDV has been generated. If specific farm level management factors, or regional traits could be identified as being significantly associated with disease outcome, measures could be implemented to more carefully control, reduce or potentially eliminate them. This is a novel study that reports factors associated with either increasing or decreasing odds of infection of one or both of these viruses.

In this study, a space-time analysis detected five significant clusters indicating spatial aggregation of disease. These patterns are similar to PRRSV epidemics previously described, both in timing and location (Tousignant et al., 2014). To account for this spatial aggregation of the disease outcomes, the type of cluster was included in the farm level models to prevent bias (Keitt et al., 2002). Being in a co-infected cluster was significantly associated with being infected with PEDV with or without PRRSV, but not PRRSV alone. This was due to the fact that there were a substantial number of herds infected with PRRSV only that were located outside of the disease clusters.

The number of farms and distance between them has been a consistently important predictor of risk of infections in many animal species and for many diseases and suggest a method of identifying areas at high risk of epidemic spread of diseases (Mortensen et al., 2002; Boender et al., 2007a; Boender et al., 2007b; Boender et al., 2008; Boender et al., 2014). Higher density increases the probability of spreading the pathogen to neighboring farms regardless of route of transmission (ie aerosol, personnel, equipment or animal movement). The data of this study suggest a similar association using county level density as a measure of number of farms within a defined geographic region. It should be noted, however, that the distribution of farms across these counties is not likely to be homogeneous, therefore, potentially biasing these associations toward the null hypothesis that states risk does not change as county density increases. Regardless, these data may support the widely held belief that as the number of farms in an area increases, the importance of good biosecurity practices aimed at preventing the spread of infectious organisms becomes evident

In a previous study, it was shown that contamination of at least 5.2% of all trucks at slaughter plants could be possible for PEDV (Lowe et al., 2014) and was likely due to the numbers of animals and trucks arriving from different sites passing through common areas at the slaughter plant. Biosecurity and strict hygiene are difficult to manage in these environments, and therefore it is reasonable to expect a percentage of clean trucks at arrival to the slaughter facility will become contaminated while unloading animals. In another study, market trucks (along with gilt source and common herd ownership) were associated with the spread of a 1-18-4 PRRSV virus in Ontario, Canada (Kwong et al.,

2013). When compared to trucking owned by a production company, contracted trucking companies may have different protocols for how personnel move into and out of the truck at the slaughter facility, strictness of washing and disinfecting protocols, and sequence of farms visited. These differences may, in part, explain this relationship. While this data alone does not establish a causal relationship between trucking and pathogen clusters,

With this in mind, additional caution should be exercised by production companies when moving market pigs, especially if employing contracted trucking. A prudent recommendation would be to work together to establish mutually beneficial biosecurity protocols that strive for the highest level bio-security practices possible. Washing and disinfecting have previously been described (Pitkin et al., 2009c) as means of reducing pathogens. Establishing clean/dirty lines at the point of contact with the truck and trailer on any loading and unloading structures and preventing the movement of personnel from either the farm, or the truck over this line may help minimize the spread of pathogens. Likewise, pigs should not be allowed to cross back over this line (accidentally or intentionally) once they have been on the trailer. Washing, disinfecting and completely drying any other structures (i.e. loading chutes) or equipment (i.e. sort boards) used to aid in the loading or unloading of pigs after each use may also help to reduce the risk of transmitting diseases (Dee et al., 2004; Pitkin et al., 2009c).

Bio-aerosol filtration has been implemented on many sow farms across the swine dense regions of the upper Midwest in the US and has reported to be associated with reduced incidence of PRRSV incursions on these farms (Spronk et al., 2010; Dee et al., 2012;

Alonso et al., 2013b). On many, but perhaps not all of these farms, the installation of bio-aerosol filtration also involves substantial upgrades to other biosecurity practices on these farms. Because of this, studying the effect of filtration alone is therefore difficult, and other improved biosecurity measures must be considered when interpreting this data. That said, the financial return on investment still seems to favor these interventions (Alonso et al., 2013a) and therefore, filtration and high levels of bio-security should continue to be explored and may prove to be an important piece of mitigating the effects of these and other pathogens in the US swine herd.

For the sow farms in this study, manure is generally stored on farm either in deep pits or lagoons. Annually, or semi-annually, this manure is removed from storage and applied to agricultural fields after crop harvest in the fall or before seed planting in the spring. There are many challenges associated with pumping manure, namely the effective cleaning and disinfecting the large and complex equipment required for this job. It is known that both viruses are shed in feces or other fluids including urine, nasal secretions and saliva (Christianson et al., 1992; Christianson et al., 1993; Rossow et al., 1994).

Additionally, PRRSV can survive in manure for up to 4.5 days at cold temperatures (Linhares et al., 2012a) and some data has suggested that PEDV can survive in manure slurry up to 4 months after infected pigs were in a barn (Tousignant, 2014). It would then stand to follow that equipment could be contaminated with viable pathogen that could be transported to other sites. While the data of this study failed to find differences between owned and contracted manure pumping equipment, efforts aimed at sequencing order of

operations when working with manure pumping equipment should be recommended, For example, pumping first at herds free of PRRSV and or PEDV disease and pumping last at herds with disease as well as careful consideration about placing manure on fields in close proximity to non-infected sites may help mitigate some of these risks and subsequent spread of these pathogens. Additional studies could attempt to validate cleaning, disinfecting and down time protocols for this type equipment.

In these data, rendering was not associated with PRRSV or PEDV infections. This was somewhat surprising as rendering has been documented to increase odds of PRRSV due to the fact that rendering vehicles potentially contact a high percentage of infected farms every day across a wide geographic region (Bates et al., 2001; Lambert et al., 2012a). Additionally, these data did not support associations with toll mills even though contaminated feed has been implicated as a route of transmission of PEDV (Stevenson et al., 2013; Bowman et al., 2015; Dee et al., 2015) and supported by numerous anecdotal reports from veterinarians in the field. This may be due to the limited number of farms within PRRSV and PEDV negative clusters that were manufacturing their own feed in an owned mill.

The primary limitation of this study is the small, convenience sample of herds. Additionally, veterinary oversight is managed by one veterinary practice, and therefore the consistent biosecurity recommendations and training on these farms may have biased these results. These data represent only a small portion of the sow herds within the US, and even more specifically within the upper Midwest. Caution should be used when

extrapolating these results to other swine producing regions of the US, or into herds that have influence from other veterinary practices. Given the cross sectional nature of this study, it cannot be known if the exposure to the risk factor occurred before or after the disease outcomes were detected and therefore the causal nature of these relationships cannot be established. Additionally, unmeasured confounding variables may have impacted these results. An important consideration when studying the impact of disease within a region is how the disease status of unknown farms might potentially influence the outcomes on these farms. Finally, the limited sample size reduced the power of this study to detect risk factors with smaller effects. Future studies could be directed at obtaining data from the remainder of the other herds in the SHMP, as well as attempting to improve participation in the project across the entire industry.

Conclusions

At the farm level, being in areas of high disease pressure as well as higher swine farm density increased the odds of being infected with PRRSV, PEDV or both viruses. High levels of biosecurity along with bio-aerosol filtration were associated with decreased odds of disease. These findings continue to support the need for additional biosecurity considerations at the regional and farm level as a means of reducing the effect of these, and potentially other, diseases especially in swine dense regions where there is high disease pressure. These data reinforce current recommendations to farmers and it is hoped that these efforts will cumulatively reduce disease incidence within regions.

Acknowledgments

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Tables and figures

Table 1 – Clusters of Porcine Respiratory and Reproductive Syndrome virus (PRRSV) and Porcine Epidemic Diarrhea virus

(PEDV) detected in and convenience sample of 109 of the 371 herds enrolled in the Swine Health Monitoring Project in the United States between July 1, 2013 and June 30, 2014.

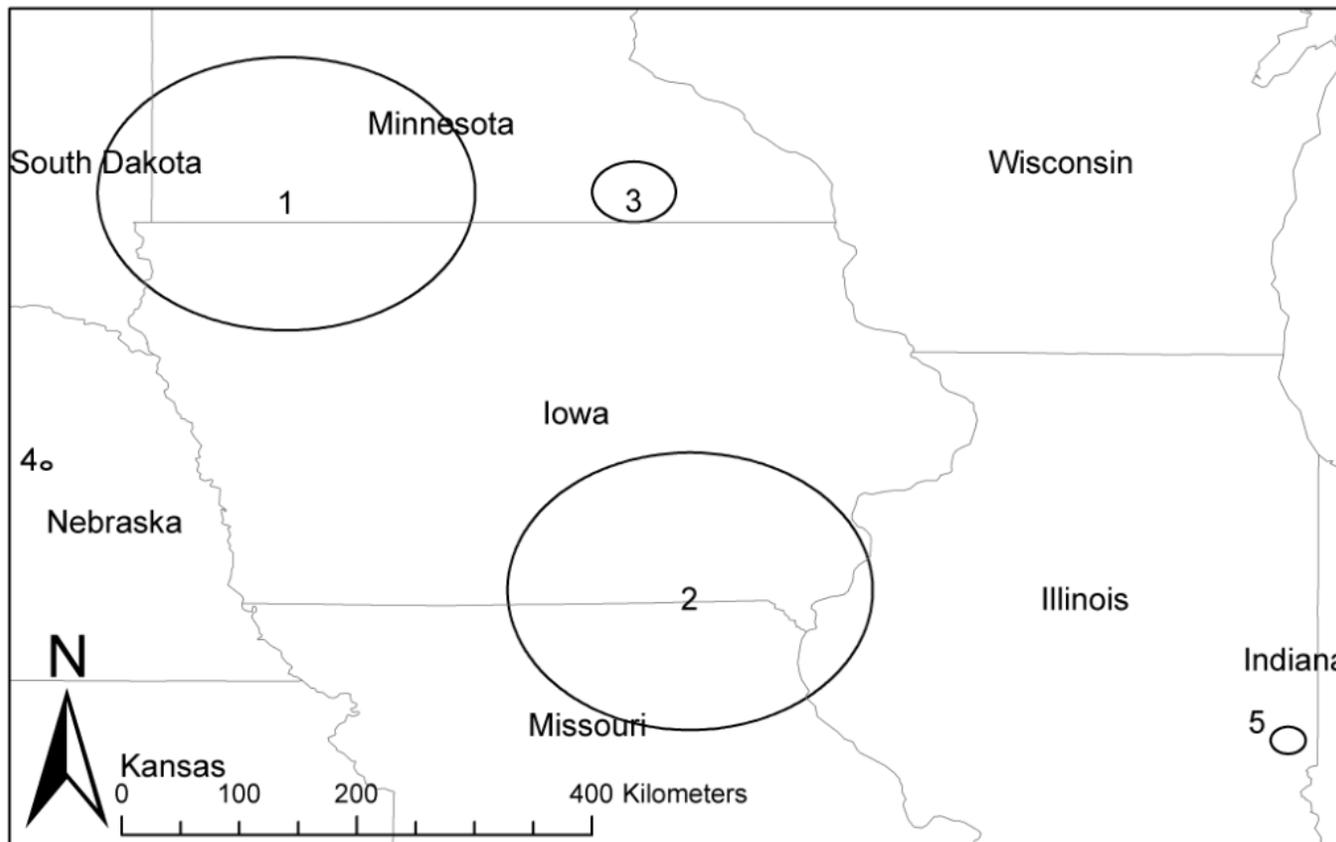
Category	Cluster ID	No of herds	Temporal Distribution			Spatial Distribution			Obs/Exp	P value
			Start	End	No. Months	Latitude	Longitude	Radius (km)		
PRRSV+/PEDV+	1*	34	Jan-14	Jun-14	6	43.7181	95.4285	116.0	4.65	0.001
PRRSV+/PEDV-	3	2	Oct-13	Apr-14	7	43.7314	92.7681	25.73	20.88	0.001
PRRSV-/PEDV+	2	7	Jan-14	Jun-14	6	40.6802	92.3391	117.76	8.29	0.001
	4	3	Feb-14	Jun-14	5	41.6407	97.2654	3.14	9.67	0.001
PRRSV-/PEDV-	5	10	Jul-13	Dec-13	6	39.5412	87.762	11.45	1.22	0.001

* most likely cluster

Table 2 – Results of a multinomial logistic regression analysis with backward selection to estimate the associations between different Porcine Reproductive and Respiratory Syndrome virus (PRRSV) and Porcine Epidemic Diarrhea virus (PEDV) status and the hypothesized farm level risk factors for infection in a convenience sample of 109 of the 371 herds enrolled in the Swine Health Monitoring Project in the United States between July 1, 2013 and June 30, 2014 compared to a base line category of herds identified as not being within any cluster or being within a PRRS-/PED- cluster

Category	Variable	Final Model		
		OR	p-value	95% CI
PRRSV-/PEDV+	Cluster Type (PRRSV+/PEDV+)	12.92	< 0.001	2.81 - 59.53
	County Density	1.03	0.013	1.01 - 1.05
	High biosecurity with filtration	0.12	0.016	0.02 - 0.67
PRRSV+/PEDV-	Cluster Type (PRRSV+/PEDV+)	6.04	0.051	0.99 - 36.85
	County Density	1.03	0.012	1.01 - 1.06
	High biosecurity with filtration	0.18	0.092	0.03 - 1.32
PRRSV+/PEDV+	Cluster Type (PRRSV+/PEDV+)	48.05	0.001	4.56 - 506.09
	County Density	1.01	0.358	0.99 - 1.04
	High biosecurity with filtration	0.07	0.025	0.01 - 0.71

Figure 1 – The upper Midwest region of the United States and clusters of Porcine Reproductive and Respiratory Syndrome virus (PRRSV) and Porcine Epidemic Diarrhea virus (PEDV) detected in a convenience sample of 109 of the 371 herds enrolled in the Swine Health Monitoring Project between July 1, 2013 and June 30, 2014. Clusters are identified by number, and Table 1 provides specific details.



Chapter VI – PRRS Aerobiology: Experimental evidence and 10 years of and field experiences with 119 Filtered Breeding Farms in the United States.

This chapter is in preparation to be submitted to Journal of Swine Health and Production

Steven J.P. Tousignant, Darwin L. Reicks, Peter. R Davies, Andres M. Perez, Robert B. Morrison. The History of PRRS Aerobiology Science and Experiences with 119 Filtered Breeding Farms Since 2005 in the United States.. [in prep, to be submitted to Journal of Swine Health and Production].

Introduction

Veterinarians became suspicious of the role of aerosol transmission of Porcine Reproductive and Respiratory Syndrome (PRRS) due to the apparent rapid area spread between farms in during the first outbreaks in the USA and Europe (Quaife, 1989; Loula, 1991; Mortensen et al., 2002). In 1997, a study of aerosol transmission of two strains of PRRS between experimentally infected and sentinel pigs was published. Pigs were housed in separate chambers connected by a 1 meter long tube and it was concluded that aerosol transmission can occur, but may be dependent on the strain of virus (Torremorell et al., 1997).

Two other studies in experimentally infected pigs were conducted during approximately the same time period with inconsistent results. Aerosol transmission was apparent in 1 of 2 trials in the first study, (Lager and Mengeling, 2000) and in 2 of 5 trials in the second study where the authors concluded: “Airborne transmission may be less likely than previously believed” (Wills et al., 1997). It is important to note these were small experimental studies conducted in relatively confined air spaces of research isolation units.

A few years later in 2002, another research group published their attempts to reproduce these early experiments in a commercial swine setting while also attempting to show transmission of PRRS to sentinel pigs housed outside the infected barn. They were unable to demonstrate transmission of live virus to the pigs outside the barn, reporting

that aerosol transmission could not be readily reproduced experimentally (Otake et al., 2002b).

Around the same time, a group in Denmark was attempting to explain the rapid spread of PRRS virus in a large observational study of 73 case herds and 146 control herds. They concluded that infected neighbors and movements of infected pigs and semen were significant routes of transmission. Additionally, they did not find that high levels of personnel and trucking biosecurity prevented infection and, therefore, concluded that aerosol transmission of virus must be a frequent event (Mortensen et al., 2002)

In 2004, Kristensen et al., evaluated the amount of air that needs to be exhausted from an infected population to infect a negative neighboring population in an experimental setting.(Kristensen et al., 2004) The authors reported that transmission, assessed with positive (>50) Immunoperoxidase Monolayer Assay – European (IPMA-EU) titers, could occur between two populations of pigs (n= 25 or 26) when 70, 10 and 1% of the exhaust air was transferred directly to uninfected pigs. The authors stated that concluded that up to 2% of inlet air in one barn originated as exhaust air from a neighboring barn, however no details were provided about the distance between the barns in these models.

In 2004, Trincado et al., attempted to transmit aerosolized virus under controlled field conditions from a source population of 150 experimentally infected pigs through a 15 meter long, 5 cm diameter, tube that directed exhaust air from the source population into a trailer containing sentinel pigs (Trincado et al., 2004). After 7 days of exposure, the

sentinel pigs remained negative by PCR and air samples collected using an all glass impinger were negative by PCR, virus isolation and bioassay. The authors concluded that aerosol transmission remains uncertain and recommended that producers follow sound biosecurity practices to prevent the entry of pathogens by established routes. Furthermore, they stated that veterinarians should conduct thorough investigations into new PRRS infections and not simply admit defeat and use aerosol transmission as the default explanation.

In 2005, Dee et al. conducted studies of the effect of air filtration in reducing the frequency of transmission events in a small-scale field experimental setting (Dee et al., 2005a). Experimentally infected pigs were housed in a chamber connected via a 1.3 meter long duct to a second chamber housing PRRS naïve recipient pigs. Apparent aerosol transmission was observed (recipient pigs become positive by serum PCR and ELISA) in 6 of 20 replicates when no filter was used in the duct compared to 0 of 20 when a filter was used. Thus the authors concluded that under these experimental conditions, the filters appeared to be effective at reducing aerosol transmission of PRRS.

In a second publication in 2005, Dee et al., described the first attempts to recover aerosolized PRRSv over distances greater than 1 meter (Dee et al., 2005b). In this study, a pipe of 150 meters in length was filled with artificially generated PRRSv aerosols that were moved down the length via a fan at the opposite end. Samples were collected at a variety of points down the length of the tube and tested by PCR and virus isolation. While this scenario is very artificial (high concentration of virus, very fast air speed, little

environmental interference), under these experimental conditions viable virus was recovered at a distance of 150 meters.

In 2006, the same authors compared alternative filtration methods due to the high cost of the HEPA filters (Dee et al., 2006). They concluded that HEPA filters (used in the first study) were superior to low cost furnace filters, or ultra violet light exposure through the 1.3 meter long duct. A limitation was that virus concentration to which the different treatments were exposed is likely to differ from concentrations generated by a barn of naturally infected pigs, or entering nearby barns.

In 2005, Desrosiers described a review of 16 publications spanning 35 years of literature on Foot and Mouth Disease (FMD), 12 publications spanning nearly 20 years of literature on *Mycoplasma hyopneumoniae* transmission, as well as anecdotal case reports in which explanations for PRRS virus movement between herds other than aerosol were not apparent (Desrosiers, 2005). He argued that the weight of evidence supporting aerosol transmission of FMD and *M. hyopneumoniae* lends plausibility to the likelihood of aerosol transmission of other pathogens, such as PRRS. He also encouraged the scientific community to begin to elucidate the frequency, distance over which transmission might occur and climatic conditions that are favorable to it.

2008 marks the beginning of a period in aerosolized PRRS research when early adopters of the aerosol hypothesis and control measures begin to report some ‘success.’ Most of

these reports are anecdotal and began to mold the direction of more controlled research and wider industry adoption.

In 2008, at an industry conference, the experiences and lessons learned from installing filtration systems on 25 farms were reported, including choice of filters, costs, maintenance and common trouble shooting issues (Reicks, 2008). This early success raised expectations that use of filtration technology would expand in commercial swine production settings.

In 2009, perhaps the most influential study on the interventions against aerosolized PRRS virus was published by Pitkin et al. in 2009. This replicated study utilized a 'production region' model with an infected source population of pigs and two naïve recipient populations of pigs housed in separate buildings located 120 meters away (Pitkin et al., 2009b). The two recipient barns employed identical biosecurity protocols apart from the installation of bio-aerosol filtration in one barn. Non-filtered populations became infected in 8 of 26 replicates over a one year period, while none of the 26 recipient replicates in filtered barns were infected. Strict bio-security measures alone did not appear to be sufficient at preventing introduction of PRRS into the non-filtered populations. For the first time, the effect of bio-aerosol filtration was being studied under semi-controlled conditions.

This study marked a change from previous experimental work where efforts to reproduce aerosol transmission between infected and non-infected populations had been

unsuccessful. It was also the first study in which the novel use of a mechanical air sampling device, the cyclonic collector, was employed to detect aerosolized PRRS virus. However the sensitivity and specificity of the mechanical air collection device were unknown.

In 2009, Dee et al., designed a study to evaluate the efficacy of different means of reducing aerosol transmission of PRRSv, again using artificially generated aerosols and a mechanical air collection device (Dee et al., 2009c). While this allowed the authors to test the filters against a range of virus concentrations, there was no attempt to classify particle size, or try to match it to bioaerosol particles generated naturally by infected swine. The authors concluded that the most expensive, electrostatic type filters were most effective at preventing the transmission of PRRS in artificially generated aerosols.

In 2010, Dee et al., described a production region model using a source population infected concurrently with PRRSv and *Mycoplasma hyopneumoniae* (Dee et al., 2010a). They compared the effects of various types of filters, and also described the climatic conditions associated with the detection of aerosolized PRRS. As before, strict biosecurity measures alone were not enough to prevent the transmission of virus. Several filters showed promise for preventing the transmission of PRRS and *M. hyopneumoniae* between the two populations. Additionally, the study suggested that certain climatic conditions favored the detection of aerosolized PRRSv. Not surprisingly, these corresponded with the conditions proposed to be favorable to the transmission of FMD and *M. hyopneumoniae* outlined in the review by Desrosier described above.

In a short communication in 2010, Dee et al., described a study where they followed 10 filtered and 26 non filtered herds for a period of 24 months, and found significantly fewer new PRRS introductions on filtered farms (0.2 cases per farm) than on non-filtered (1.4 cases per farm)(Dee et al., 2010d). Both cases on the filtered farms were traced back to contaminated transport and documented breaches in biosecurity protocols. It was concluded that although aerosol transmission could not be completely eliminated as a route of entry, it was deemed less likely given the results of the outbreak investigation.

In 2010, Spronk et al., reported the results of a similar study, where they followed filtered (n = 2) and non-filtered (n = 5) herds over a 12 month period and recorded new PRRS introductions (Spronk et al., 2010). Neither of the filtered herds had a new PRRS introduction, while all the non-filtered herds reported introduction of a previously undetected PRRS virus variant. Additionally, the authors collected air samples outside of one of the filtered farms during a 42 day period. Out of a total of 73 air samples, 2 were found to be positive for PRRSv by PCR, but no bioassay or cell culture was performed to determine if the viruses were viable.

Otake et al.,(2010) reported a 2 year study in which aerosol samples were collected downwind of the same source population herd as used previously. In this barn, PRRS negative pigs were regularly introduced into a population of pigs co-infected with PRRS virus and *M. hyopneumoniae* to create a sustained epidemic.(Otake et al., 2010b) On days where the pathogens were found in exhaust air of the facility, they were also found

downwind of the facility to a distance of 9.1 km for PRRS virus (a total of 5 of 114 samples positive) and 9.2 km for *M. hyopneumoniae* (a total of 6 of 114 samples positive). All of the PRRS virus positive samples and 50% of the *M. hyopneumoniae* positive samples were shown to be infectious by bioassay.

In 2011, Desrosiers discussed some of the early ‘before-and-after filtration’ studies (Desrosiers, 2011). In contrast with earlier work that indicated a low probability of aerosol transmission, the more recent observational data suggested it may be a relatively important route of PRRS spread between herds.

In 2012, Dee et al., compared the number of breaks after implementation of filtration with the number of breaks prior to filtration in a cohort of 38 sow herds in the US and concluded that filtration significantly reduced the odds of infection and increased the time to infection in this group of herds (Dee et al., 2012).

A thorough follow-up study on this group of herds was conducted by Alonso et al., in 2013 and they reported that filtered herds experienced fewer PRRS introductions than the non-filtered herds and that under most conditions, the return on investment made the decision to filter favorable in high risk regions (Alonso et al., 2013a; Alonso et al., 2013b).

In 2014, Brito et al., reported that depending on site (Southeast MN, Eastern SD, Northwest IA, and Northeast IA), 29% - 42% of all air samples were positive during the

peak of the annual PRRS epidemic in late fall (Brito et al., 2014) as described by Tousignant et al., in 2014 (Tousignant et al., 2014). Additionally, 12 -25 unique PRRS viruses were sequenced at the various locations. However, during approximately the same period, using similar methods, Tousignant et al., found no positive samples out of 241 samples outside of 8 filtered sow farms in swine dense regions of South central and West central Minnesota (Tousignant, 2015).

As more experience was gained, it became evident that under the negative pressure ventilation conditions in the swine industry, it is possible for air to enter the barn through unfiltered openings (Alonso et al., 2012). In 2012, Alonso et al., tested the effect of several preventive measures under laboratory conditions, and concluded that plastic shutters commonly used on fans were insufficient and modifications of collapsing nylon tubes or double shutters were required to prevent artificial PRRS aerosols from being detected inside the model facility (Alonso et al., 2012).

With this in mind, the objectives of this study were to describe the trends in additional improvements made and filter brands used on breeding farms during the past decade. Additionally, this study attempted to quantify the effects of these various improvements at further reducing the risk of PRRS infections. These data will provide a historical account of field application of filtration on breeding herds in the upper Midwest region of the US.

Study population and definition of infection

A convenience sample of 119 filtered breeding farms was enrolled through solicitation of veterinary practices participating in the Swine Health Monitoring Project (SHMP).

Herds were defined as being filtered if the operation of the filters began prior to October 1st of any year. PRRS infections that occurred on partially filtered herds between June and September or (when a herd was not under filtration) were not included in this analysis. If herds discontinued filter use, they were not included after that point.

New cases of PRRS were identified using a combination of clinical signs and sequence homology as previously described (Yeske, 2013).

Survey

A survey was used to obtain information regarding incident PRRS infections, facility upgrades including sealed pits, pressurized office, loadouts, back drafting prevention, as well as filter operation, brand and age. Additionally, data regarding farm construction type (new or retrofit), and biosecurity auditing were also collected for each year between 2005 and 2014 in which a farm was filtered as well as if that farm reported a PRRS break that year between October 1st and March 31st.

Results

Study population and survey results

The total number of herds increased from 10 in 2005, to 119 at the end of 2014 (figure 1). Of the ten filtered farms in the study in 2005, eight were boar studs, and three were sow farms. Among the 119 filtered farms in 2014, there were a total of 21 boar studs and 98

sow farms. Among the 98 sow farms, 33 were genetic multipliers or nucleus and 65 were commercial production. Of the filtered herds in the study, 89 belonged to 11 production companies with more than two breeding herds whereas the remaining 30 belonged to independent producers with one or three breeding herds. On average, each of the larger production companies had 9.7 filtered herds each (range = 1 – 23).

During the ten year study period (2005 – 2014), a total of 41 PRRS cases were reported on the filtered farms. Incidence of PRRS cases across all farms in the data set averaged 0.06 cases per year (range = 0.00 – 0.17) (figure 1) with an average of 0.35 PRRS cases per farm (range = 0 – 4). A total of 90 herds did not report any cases during the study period.

Six farms were filtered for the entire ten year study period, and 14 were filtered for one year with an average of 4.7 years filtered across all farms. The highest incidence of PRRS infections was reported in the 2011 – 2012 season, which was the year with the most newly filtered herds added to the database (figure 1). An average of 11.5 new herds were filtered each year (range = 0 – 23) (figure 1).

There was a general increase in the percentage of farms employing additional biosecurity measures including continuous filter operation, sealed pit pump outs, some or all shutterwalls, pressurized offices and load outs, and internal biosecurity auditors with (figure 2). Additionally, there were nearly stable percentages of farms that never had third party auditing or had less than or equal to one third party audits per year (figure 2).

At the beginning of the study in 2005, there were two filter options (A and C) used in swine breeding herds. In 2008, a third filter option (B) became available in the market and in 2010, some herds began using a combination of these two options (A and B). In 2013, a fourth option (D) entered the market, and with the increase application of this option, there has been relatively similar decreases in option C whereas A, B, and the combination of the two has remained relatively stable over the past two years (figure 3).

Discussion

Since 2007, there have been numerous studies undertaken by a handful of research groups. At times, the data is conflicting and inconsistent between and even within some groups. Regardless, as a few early adopters of aerosol filtration began to gain experience, the industry quickly began to notice the apparent success. Since then, aerosol filtration on swine breeding herds has proven to be a cost effective means of reducing the incidence of PRRS at the farm level (Spronk et al., 2010; Alonso et al., 2013a; Alonso et al., 2013b). Unfortunately, a number of filtered herds report new PRRS breaks every year. With this in mind, the intent of this study was to document changes in the modifications made on filtered farms during the past decade.

As anticipated in a report from the Swine Disease Eradication Center in a 2009, (Dee et al., 2009b) there has been an increase in the number of commercial sow herds using this technology. This is likely due, in part, to the early success of many farms to remain PRRS free after the implementation of filtration. Additionally, as experience has

increased, so has the ability to retrofit facilities at a lower cost, therefore making the capital investment easier to justify for many swine farmers.

The PRRS incidence data in this study are well correlated with the annual incidence reported in the SHMP since 2009 ($R^2 = 0.81$, figure 4a) and fairly well correlated with the number of newly filtered herds each year ($R^2 = 0.46$, figure 4b). These data suggest similar PRRS pressure to other herds in the US at the same time, and that perhaps some of the incidence recorded in 2011 (highest in this study) may be explained by the number of newly filtered farms. Intuitively, this seems plausible as during the first year of filtration, there may be unresolved issues with biosecurity or construction that an internal or third party auditor may not have had the opportunity to correct, which in turn may explain the PRRS break.

Breaks occurring during the summer months (June – September) on partially filtered herds were not included in the analysis because during this time, the barn is not operating under filtered conditions. This is a deliberate decision by farmers based on the belief that risk of aerosolized PRRS transmission is lowest in the hot summer months. This tactic is then used to reduce quantity of filters on the barn that would have otherwise been required to handle the increased air flow demands of summer ventilation, which can produce considerable cost savings to the farm.

Additional modifications to farms have been implemented in attempts to reduce the number of new PRRS infections. Some of these improvements have been made along

fan walls on many breeding farms known as shutter walls. A shutter wall consists of a second wall and roof in front of existing exhaust fans, effectively creating an air space outside and adjacent to the animal airspace. This air space is maintained under negative pressure so that any air that would back draft into this space through idle fans is therefore drawn back out. Alternatively, the fans are moved to the outside wall, leaving the louvers on the animal airspace wall effectively creating the same situation.

Other vulnerable areas, such as offices and entry ways and animal loading areas, (or anywhere doors are frequently opened to the outside) have been modified with positive pressure ventilation using filtered air, thus preventing unfiltered air from entering.

Additionally, there are also several cases where improvements have been made at manure pump outs to prevent unfiltered air from entering barns. Such modifications are designed with some type of a baffle system maintained below the level of the manure slurry so that manure is pumped out without letting unfiltered air into the farm.

Many production companies conduct audits of filters and biosecurity protocols on an ongoing basis. These can either be accomplished through a third party, or an internal auditor or biosecurity officer. The goals of audits are to identify malfunctions with filters (ie damage, improper installation, need for replacement, etc.), air leaks and improper protocol compliance. The intention is to identify potential problems before an incursion of PRRS virus occurs. These personnel are usually responsible for sealing cracks in ceilings, along J channels where walls and ceilings meet as well as performing other general maintenance issues that arise.

Several filter options are available to the swine industry which are classified by the type of technology used, and air flow ratings. As time passes, the number of filter options continues to expand, and the choice of which type to install, and after what period filters should be replaced has been largely based on opinion and anecdotal observation. Additionally, the decision to use filters continuously (12 months of the year) or seasonally (typically during fall, winter and spring months) is frequently based on the economics of purchasing the quantity of filters required to ventilate a barn during summer months as well as the presumed risk of PRRS infection at that farm.

Unfortunately, this study was not able to identify any associations between these additional modifications and PRRS infections. For now, these decisions will likely continue to be based largely on opinion, anecdotal observation and likely some economics.

The history of these herds may be very different from the history on the herds represented by the other veterinary practices that were not included in this study. It is important to note that cause of the PRRS break was not considered in this analysis. It could be argued that at least some of these cases were due to factors other than aerosol transmission including the movement of positive animals or unintentional lapses in biosecurity protocols. Had these cases been censored from the dataset, the sample size would have been further reduced thus limiting the power of the statistical models even more.

While observational data can be fraught with measured and unmeasured confounding and biases, conducting side by side randomized trials in the field to eliminate these sources of error would not seem feasible. Therefore, these data must always be analyzed carefully using appropriate statistical tests, and interpreted with caution with consideration given to differences between the study and target populations of interest.

In conclusion, much has been learned about how to effectively filter swine breeding farms over the past decade. While little scientific evidence to support the implementation of these modifications exists, they will likely continue to be implemented. Filtering a farm is a dynamic, ongoing process that requires daily input. Certainly, the foundation of any filtered farm is a set of strong biosecurity protocols that are designed to prevent the introduction of PRRS via animals, people or equipment. Once those possible routes of entry are rigorously controlled, adding filtration to a farm may prove to be the final step in reducing the incidence of PRRS.

Tables and Figures

Figure 1 – Number of newly filtered herds (gray bars), cumulative number of filtered farms (solid black line) and incidence of PRRS (dashed black line) in a voluntary sample of 119 filtered breeding herds from the Midwest region of the US from 2005 – 2014.

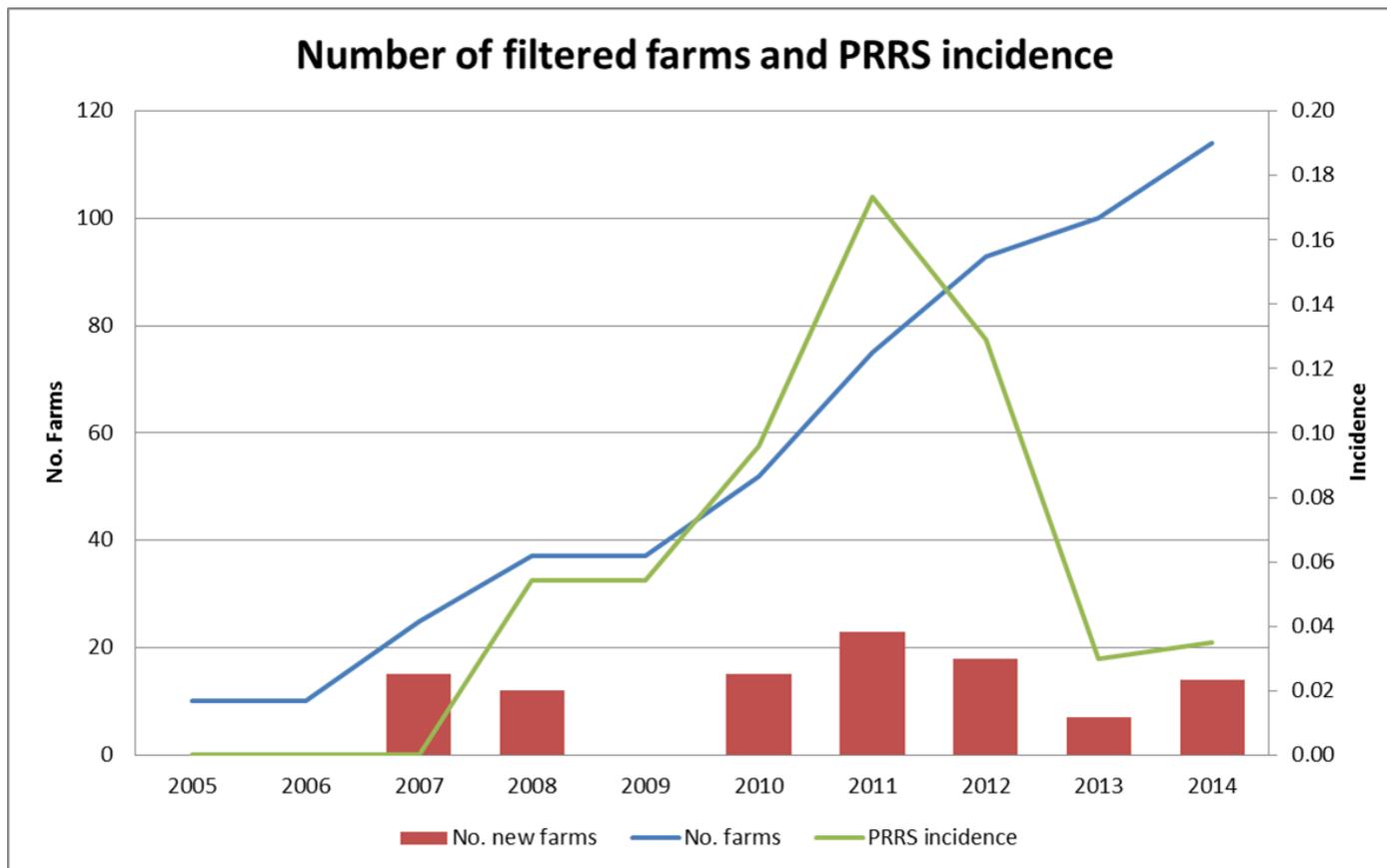


Figure 2 – Changes in the relative frequency of various facility modifications in a voluntary sample of 119 filtered breeding herds from the Midwest region of the US from 2005 – 2014

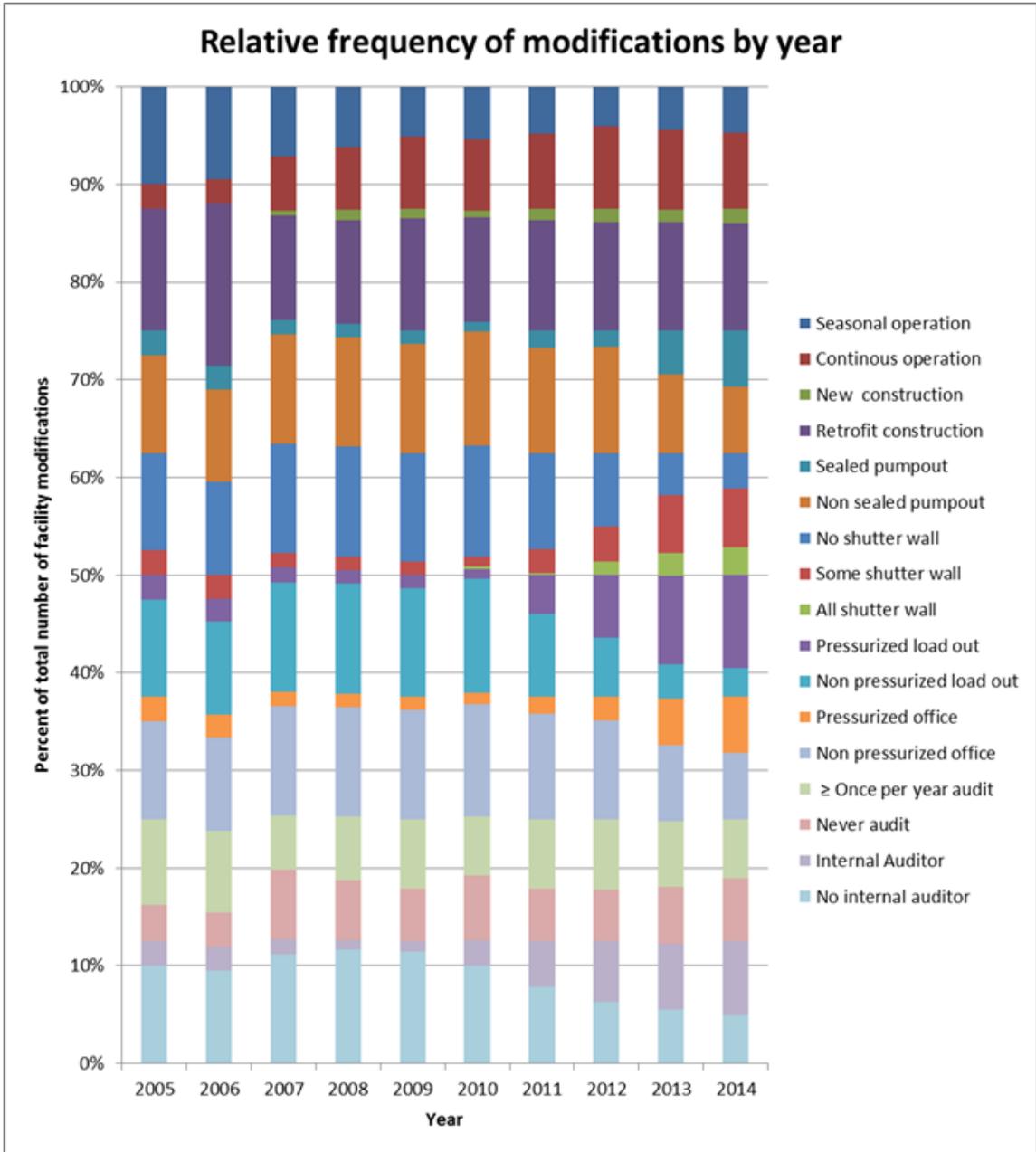


Figure 3 – Changes in the relative frequency of filter options in a voluntary sample of 119 filtered breeding herds from the Midwest region of the US from 2005 – 2014

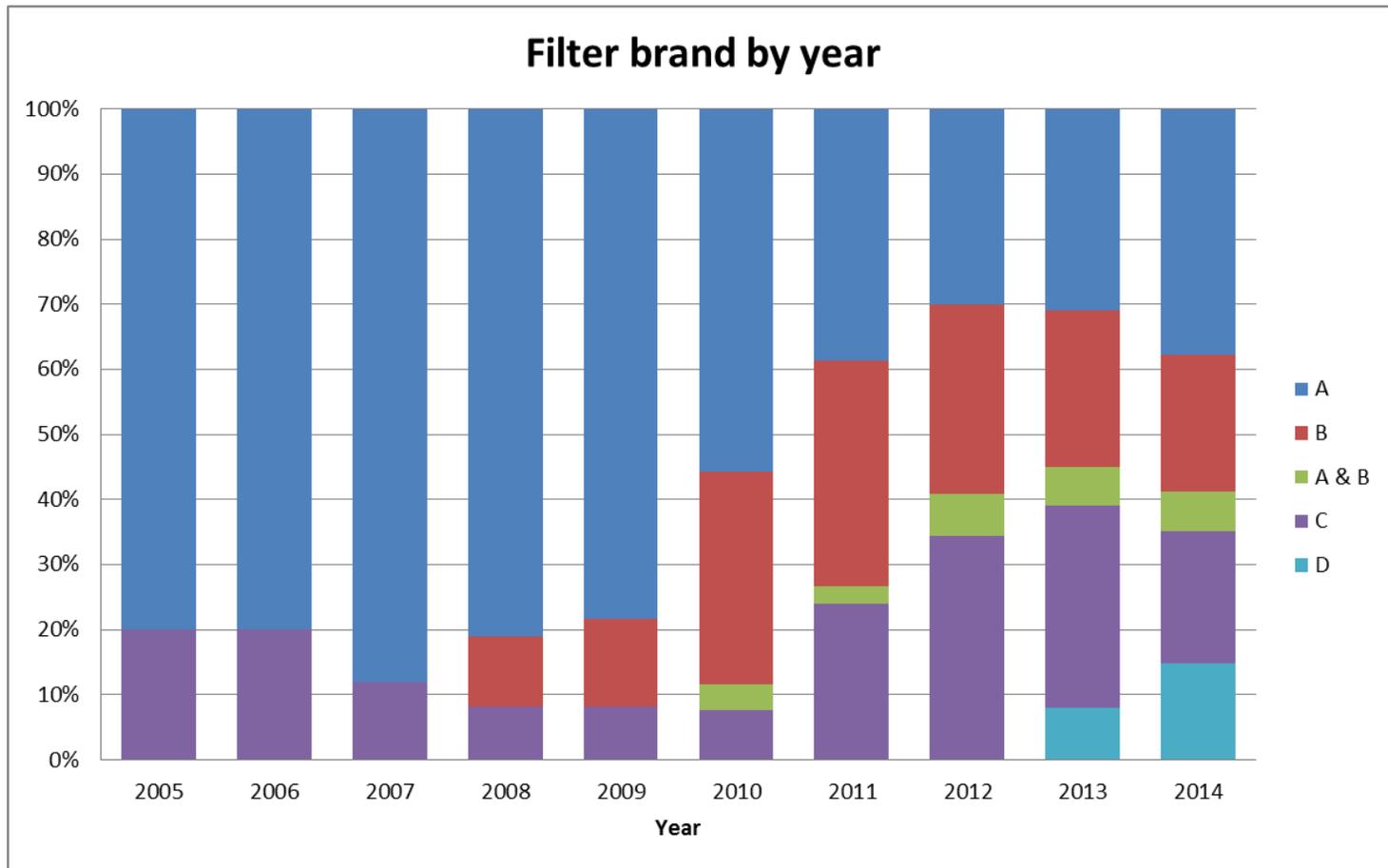


Figure 4a – Correlation between SHMP incidence and filter farm incidence suggesting the two populations experienced similar PRRS pressure through the years.

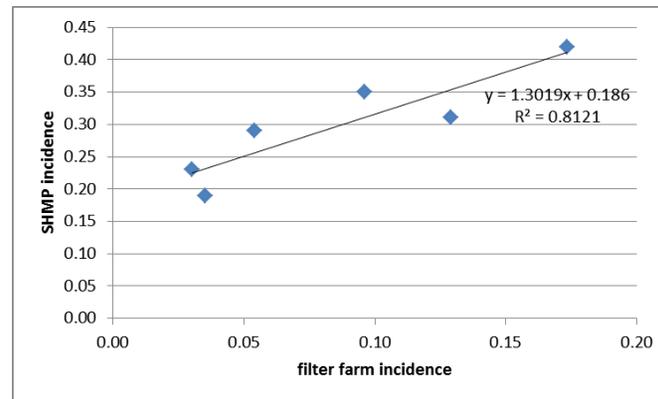
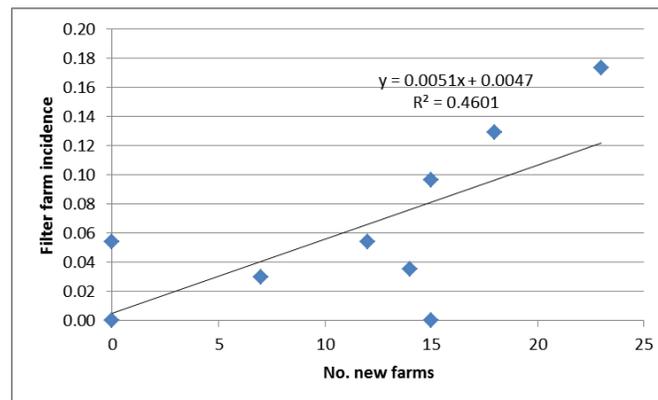


Figure 4b – Correlation between filter farm incidence and the number of newly filtered farms each year suggesting incidence is moderately well correlated to the number of newly filtered farms.



Chapter VII – General Discussion and Conclusions

General Discussion and Conclusions

While much progress has been made in the past few decades regarding the control of PRRS virus, the incidence was expected to increase during the fall of each year despite the best efforts of the veterinarians and pig farmers. This thesis set out to describe what had only been, until this point, anecdotal observations. Additionally, this thesis laid the ground work for what has become the most successful prospective, longitudinal cohort of sow farms in the United States, and likely the world.

The introduction and literature review dove into the current understanding of the virus, and the epidemiology of PRRS. It was clear that there was a lack of good epidemiologic studies and monitoring efforts that described not only the incidence of disease, but also the patterns of epidemics.

Chapter 2 described the first four years of what has become known as the Swine Health Monitoring Project (SHMP). Conceived by a handful of forward thinking academics and practitioners, the project soon identified a highly repeatable pattern of PRRS across a large number of sow herds in the swine producing regions of the upper Midwest. Specifically, the incidence was expected to increase in the fall, and decrease in the summer. Using an Exponentially Weighted Moving Average, these data suggest the onset of the annual PRRS epidemic falls in approximately the same two week window in the middle of October. Additionally, these data suggest an area in South West Minnesota and North West Iowa is a hot spot of PRRS activity. These areas may prove to be a crucial piece of the puzzle of trying to control PRRS on a larger, perhaps national level.

Chapter 3 identified a dramatic change in the incidence of PRRS during the 2013/14 year. Whereas the previous years of the SHMP suggest 30 – 40% of the sow herds in the US become infected with PRRS virus, the incidence dropped to approximately 24%. Several potential explanations for this exist, most notably was the introduction of Porcine Epidemic Diarrhea (PED) virus into the US swine herd. This devastating virus destroyed a considerable amount of piglet production, which in turn, may have reduced the transmission of PRRS. Other possible explanations in addition to a simple spurious observation, included increased use of vaccine, and bio-aerosol filters, in addition to better biosecurity measures to prevent PED and a better awareness of the annual PRRS epidemics resulting in better preparedness. Interestingly, at the time of compiling this thesis and writing this conclusion, the 2014/15 monitoring year in the SHMP revealed a similar decreased incidence to the 2013/14 year.

It is also important to note that the introduction of PED proved the flexibility and adaptability of a voluntary based monitoring program. Within a matter of weeks to months, the participants of the SHMP were reporting PED incidence on a weekly basis and an extraordinary amount of data was being collected. It also stands as a testament of the industry to be willing to take a tremendous step in the right direction for collaboration.

Due to the commonly held belief that PRRS virus can be transmitted via aerosols, chapter 4 attempted to describe the frequency of detection outside of presumed high risk

sow farms across the swine dense regions of Minnesota. While this study failed to find any positive samples out of 241 collected over a 6 month period, a similar study was concurrently conducted (and reported) during the same time in which approximately 37% of samples were positive. These data suggest potential differences in specific areas and the risk of aerosolized PRRS virus. While aerosolization of PRRS virus may be infrequent, PRRS virus transmission to a susceptible population is still a highly consequential event, and therefore pig farmers will likely continue to adopt the use of aerosol filtration on their farms.

In an effort to better understand potential relationships between PRRS and PED, chapter 5 studied the coinfection patterns between these diseases in a convenience sample of sow herds in the SHMP. This study suggests that being in areas with increased amounts of disease, as well as higher swine density increased the risk of being infected with one or both of these viruses. Additionally, these data suggest high biosecurity and air filtration reduce the risk of becoming infected. With this in mind, prudent recommendations are to strive for the highest level of biosecurity possible on all farms especially when considering the movement of pigs and dealing with the removal of manure.

Chapter 6 focused on the history of PRRS aerosol research in the swine industry as well as documenting the changes seen in the past decade on filtered farms. The history of the research is long and complicated involving a variety of small pseudo-experimental trials and relatively uncontrolled field trials. That notwithstanding, recent observational studies consistently report reduced PRRS incidence on herds after the implementation of

filtration and a very favorable return on investment. Given these data, there has been a rapid increase in the number of filtered herds in the US.

This chapter also tried to indentify what additional facility modifications might further reduce the risk of PRRS breaks on filtered farms. Because filtered farms break so infrequently, the appropriate statistical models were not able to be utilized. Therefore, the decisions to implement additional facility modifications will be based largely on opinion, experience and an increased awarenenss on how to limit the amount of unfiltered air from entering a sow farm. Studying the effect of filtration on sow farms will continue to be a challenge in the future.

The studies of this thesis confirm some basic assumptions within the swine industry regarding the epidemiology of PRRS. Additionally, this thesis laid the foundation of one of the most powerful epidemiological databases in swine industry anywhere in the world. Given the quick and early adoption of many production companies, this project grew and over time, additional companies have joined bringing the total number of sow farms to over three times the number enrolled at the beginning of this work. The participant base eagerly anticipate a weekly report from the new group of scientisits managing this project which has expanded to include an informative research summary (from related fields) each week.

This unprecedented database likely holds additional answers to unlocking the mysteries of PRRS virus. Additional studies have already been implemented using this database including an economic analysis of a regional control and elimination project in

Minnesota, as well as follow up studies to the well known “Time to Stable” study by Linhares in 2014 (Linhares et al., 2014). Additionally, this thesis suggests the possibility of ongoing analyses of filtered farm data, risk factor analyses and benchmarking among companies. Further work could also be directed at attempting to elucidate reasons for the decreased incidence of PRRS over the past several years in the US swine industry.

Given the success of this project at this point, there have been discussions of a coordinated national PRRS elimination project. While still a completely hypothetical discussion, the coordinated sharing of data like this would be of paramount importance to the success of such a project. Such a project will require discussion at a larger, national level in order to be successful. Most would consider it advantageous if such a project could be undertaken without regulatory influence from the national government.

Finally, the ultimate outcome of the monitoring project developed in this thesis may be to serve as a crucial piece of response to the introduction of one or more foreign animal diseases. Given the recent introduction of Porcine Epidemic Diarrhea virus into the US swine herd, and the Highly Pathogenic Avian Influenza introduction into the US poultry flock, it would seem to be only a matter of time before yet another, potentially more damaging disease is introduced. The frame work established here would potentially allow for rapid collaboration of governmental agencies to quickly contain and minimize the effect of such an outbreak.

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