Evaluation of Potential Risk Factors of Porcine Reproductive and Respiratory Syndrome Virus Transmission

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Chapter 1:

Literature review of risk factors for porcine reproductive and respiratory syndrome virus transmission

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1.1 Introduction

Porcine reproductive and respiratory syndrome (PRRS) is the most economically significant disease to the US swine industry with annual losses estimated at approximately $560 million (Neumann et al. 2005). The etiologic agent of PRRS is a virus called porcine reproductive and respiratory syndrome virus (PRRSV) (Benfield et al. 1992). PRRS causes respiratory disease in all ages of pigs and reproductive disease in sows. The clinical signs associated with the disease include severe reproductive failure, post-weaning pneumonia, growth reduction, decreased performance and increased mortality (Cho et al. 2006). The objective of this literature review is to summarize the current state of knowledge of PRRSV diagnostics and risk factors for direct and indirect routes of transmission of the virus.

1.2 Etiology

PRRSV is an enveloped, single-stranded, positive-sense RNA virus that belongs in the family *Arteriviridae*, which include equine arteritis virus, lactate dehydrogenase-elevating virus of mice and simian hemorrhagic fever (Lunney et al. 2010). The viral genome is approximately 15 kb (kilobase pairs) in length and contains at least ten open reading frames (ORF). ORF1a and ORF1b constitute approximately 75% of the viral genome, which are translated and processed into 14 non-structural proteins (nsps). These non-structural proteins are mostly involved in viral genome replication and subgenomic mRNA transcription. ORF2-7 located at the 3’ end of the genome encode for the viral structural proteins. ORF2a, ORF2b, ORF3, ORF4, ORF5a, ORF5b, ORF6 and ORF7 encode for glycoprotein 2a (GP2a), GP2b (envelope protein), GP3, GP4, GP5a, GP5b, M

(matrix) and N (nucleocapsid protein), respectively (Johnson et al. 2006). GP5 and M proteins are the two major envelope proteins of PRRSV (Van Breedam et al. 2006).

1.3 History

PRRS emerged in North American and European swine herds in the late 1980s (Forsberg 2005). The first reported clinical case of PRRS in North America was in 1986 (Keffaber 1989) and the first reported clinical case of PRRS in Europe was in 1991 (OIE 1992). The etiological agent was not known at the time and there were several names for the disease, including "Mystery Swine Disease." After the virus was isolated in Europe in 1991 (Wensvoort et al. 1991) and North America in 1992 (Benfield et al. 1992) fulfilling Koch's postulate, there was a consensus made at the International Symposium on Swine Infertility and Respiratory Syndrome held in St. Paul, MN in 1992 to refer to the disease as porcine reproductive and respiratory syndrome and the etiologic agent as PRRSV. The European isolate and North American isolate are two distinct genotypes that share less than 60% homology (Stadejek et. al. 2006). The almost simultaneous emergence of two genetically distinct PRRSV genotypes remains a scientific mystery, but it has been speculated that a change in husbandry of pigs and increase movement of pig in the 1980s could have contributed to the emergence of PRRSV. During the 1980s, swine farms in North America became larger and more confined and there was increased movement between farms due to domestic and international trade. The aforementioned changes could have created a new niche for the virus to fill. PRRSV could have previously existed at a low prevalence and the changes in husbandry and trade in the 1980s allowed the virus to spread and exist at a high prevalence (Carmen et al. 1995).

1.4 Diagnostic methods of PRRSV

Polymerase chain reaction

Polymerase chain reaction (PCR) based assays are used to indentify the viral RNA in serum or tissues of an infected pig. Reverse transcriptase PCR (RT-PCR) converts viral RNA into DNA by reverse transcriptase and then amplifies the DNA to detectable levels. A popular qualitative RT-PCR among practitioners is NAEU PRRSV TaqMan® RT-PCR because it is sensitive, specific, rapid, and can differentiate between North American and European genotypes. Viral RNA can be detected in the serum less than 24 hours post-infection (Zimmerman et al. 2006). NAEU PRRSV TaqMan RT-PCR uses sequence specific DNA probes that are dual-labeled with a florescent reporter molecule on the 5' end and quencher molecule on the 3'end. The quencher molecule inhibits the florescent reporter from being detected by the real-time PCR thermocycler. During the annealing step of the reaction, the probe hybridizes to the complementary PRRSV DNA that has been recently reverse transcribed from RNA. The Taq polymerase has a 5' to 3' exonuclease activity in addition to its replication activity. As the Taq polymerase synthesizes the nascent strand of DNA, the 5' to 3' exonuclease activity of the polymerase degrades the probe. The degradation of the probe releases the florescent reporter molecule from the quencher molecule and allows the florescent reporter molecule to be detected by the real-time PCR thermocycler. The degradation of the DNA probe by the Taq polymerase reminded the researchers that developed this technology of the videogame "Pac Man" and thus called it TaqMan (Taq polymerase + PacMan=TaqMan) (Holland et al. 1991).

Sequencing

Sequencing is commonly requested by practitioners because it allows them to determine the relatedness between PRRSV strains. This type of analysis can be useful for a swine veterinarian to determine whether a PRRS infection is due to the reemergence of a previous strain or a new strain or to differentiate a vaccine strain from field strain of the virus. Diagnostic laboratories commonly sequence ORF5 PCR products because it is the least conserved area of the PRRSV genome and therefore easier to differentiate one strain from another. However, some researchers prefer to sequence ORF7 PCR products because it is a more conserved area of the PRRSV genome, which can be more convenient for analysis. Sequence results are often given as a three-digit code of restriction fragment length polymorphisms (RFLP) patterns. Before sequencing became commonly available, the relatedness between PRRSV strains was determined by digestion patterns of three restriction endonucleases. Today, computer programs can generate the RFLP patterns based on the sequence without being digested by restriction endonucleases. PRRSV sequences can be compiled together by a computer program to generate a phylogenetic tree or dendogram, which depicts similarities among genomic sequences (Christopher-Hennings et al. 2002).

Serology

PRRSV serological tests are used to indentify antibodies against PRRSV in the serum of an infected pig. There are a variety of serological tests used to diagnose the virus: indirect fluorescent antibody (IFA), enzyme-linked immunosorbent assay (ELISA), blocking ELISA, serum virus-neutralization (VN), and immunoperoxidase monolayer assay. The commercial ELISA (HerdChek® 2XR PRRS ELISA, IDEXX Laboratories Inc.,

Westbrook, Maine) is a popular assay among swine practitioners because it is specific, sensitive, standardized, rapid and inexpensive. The test detects antibodies against the nucleocapsid protein because it is the most abundant antibody in the serum. IgM antibodies against PRRSV are detectable 5-7 days post-infection (PI) and non-neutralizing IgG antibodies are detectable 7-10 days PI. ELISA results are interpreted as positive ($S/P \geq 0.4$) or negative ($S/P < 0.4$). PRRSV can be diagnosed using ELISA by demonstrating seroconversion or collecting acute samples that are negative and convalescent samples that are positive. The commercial ELISA does not differentiate between North American and European genotypes and 0.5% to 2% of reactions are false positive (Torremorell et al. 2002). IFA and blocking ELISA are used to confirm false-positive reaction and to differentiate between North American and European strains of PRRSV (Zimmerman et al. 2006).

1.5 Direct Routes of PRRSV Transmission

Direct contact

PRRSV transmission by direct contact is the most important risk factor for PRRSV transmission within a farm. PRRSV is highly infectious and an infected pig can potentially shed the virus in any secretion or excretion. Previous publications have demonstrated that infected pigs can shed virus in their urine (Willis et al. 1997) and feces (Christianson et al. 1993), which would contaminate the environment of a pen. PRRSV can be transmitted to a naïve pig by it simply being present in the same environment of an infected pig. Previous publications have also demonstrated that infected pigs can shed virus in their saliva (Willis et al. 1997) and nasal secretions (Benfield et al. 1994). Since

pigs are by nature curious and social, an infected pig could readily disseminate the virus by nose-to-nose contact with naïve pigs. There has also been a study demonstrating that PRRSV can be shed in mammary secretions (Wagstrom et al. 2004). An infected sow could potentially transmit the virus to her naïve piglet during nursing.

Vertical Transmission

Vertical transmission of PRRSV is an important route for direct transmission of the virus within a farm. PRRSV can be vertically transmitted from an infected sow to its offspring during mid to late gestation and can cause fetal death or infected pigs that are weak. PRRSV can replicate in the fetus in early stages of gestation, but the virus crosses the placenta more efficiently during mid to late gestation. It is speculated that the virus crosses the placenta more efficiently during late gestation because macrophages, which are the main target cell of PRRSV, develop in the fetal pig during this time (Van Breedam et al. 2010). Vertical transmission of PRRSV can be controlled by producing PRRSV negative piglets, which can be achieved by preventing PRRSV from entering a farm or acclimating replacement gilts. Gilt acclimation is exposing PRRSV negative gilts to the virus and allowing them to recover from the infection before they are introduced into the breeding herd. This method produces PRRSV-negative piglets with a passive-immunity to the virus. Gilts are exposed to PRRSV at an early age (2-4 months of age) and then introduced into the breeding herd when they are no longer shedding the virus. The incoming gilts can be inoculated with a modified live vaccine (MLV) or a field strain of the virus (Corzo et al. 2010).

Semen

Sexual transmission of PRRSV can be an important route for direct transmission of the virus. Infectious virus can be detected in semen for up to 42 days post-infection (Swenson et al.1994) and viral RNA can be detected up to 92 days post-infection (Christopher-Hennings et al. 1997). When PRRSV emerged in the late 1980s, shedding of virus in semen was probably the most important route of transmission because of the widespread use of artificial insemination. If a sow barn received semen from an infected boar, the virus could be transmitted to sows during artificial insemination. Today, the majority of boar stud farms in the US are PRRSV negative because of routine testing for the virus and improvements in preventing it from entering a farm (Corso et al. 2010).

Prolonged Infection

The prolonged infection caused by PRRSV is an important risk factor for direct transmission of the virus. PRRSV, like other viruses in the family *Arteriviridae,* can cause a prolonged infection in its respective host. During a PRRSV infection, there are initially high levels of virus which eventually decrease to low or "smoldering" levels (Cho et al. 2006). PRRSV RNA can be detected up to 120 days in pigs post-natally infected and up to 210 days in pig congenitally infected (Van Breedam et al. 2010). PRRSV has been transmitted to a naïve sentinel group of pigs from an infectious group that was infected 99 days earlier (Zimmerman et al. 1992). The mechanism or mechanisms of PRRSV ability to cause a prolonged infection remains unknown. It has been proposed that the virus induces T-regulatory cell populations, which would inhibit an anti-viral immune response, allow the virus to evade the immune system and thus cause a prolonged infection (Wongyanin et al. 2009). The prolonged infection caused by PRRSV determines the long time period required for herd closure. Herd closure is a

pathogen elimination method where no replacement pigs are introduced into the barn for a period of time. If no susceptible pigs are in the population, then virus cannot persist in the herd. Herd closure for PRRSV requires longer time period than for other porcine viruses like transmissible gastro-enteritis virus. A period of 6 months is usually recommended for PRRSV, but depending on the status of the barn and pig flow, a longer time period may be required (Torremorell et al. 2003).

1.6 Indirect routes of PRRSV Transmission

Fomites

Fomites (inanimate objects capable of transmitting infectious microbes) are important risk factors for indirect transmission of PRRSV. Any object in a farm could potentially act as a fomite. Many farm owners require visitors and employees to comply with strict sanitation protocols, or biosecurity protocols, because all routes of PRRSV transmission are not known at this time. Boots, coverall, needles, and transport vehicles are all objects that have been experimentally shown to act as fomites. If boots or coveralls worn by an individual come into contact with an infected pig, then these objects can act as a fomite for PRRSV. This risk can be eliminated by farm personnel changing boots and coveralls between farms or buildings (Otake et al. 2002a). If a needle that was used on an infected pig is injected into a naïve pig, then the needle can act as a fomite for PRRSV. This risk can be eliminated by changing needles between each pig (Otake et al. 2002b). Any truck, trailer, or other vehicle that comes into contacted with an infected pig can act as a fomite for PRRSV. This fomite is especially a concern during winter months because the virus can survive for longer periods below 0°C. The risk can be eliminated by washing,

disinfecting and drying all vehicle that come into contact with pigs (Dee et al. 2004). Many other objects have been known to act as fomites under field conditions. Therefore, it is important to treat all objects that are entering a farm as fomites and properly disinfect them.

Aerosol

According to a recent survey of experienced swine veterinarians, aerosol secretions are thought to be the most important risk factor for transmission of PRRSV (Desrosiers 2011). The virus has been detected up to 9.1 km from a PRRSV source population (Otake et al. 2010). Aerosol transmission of PRRSV is influenced by isolate pathogenecity. Pigs that were experimentally inoculated with a PRRSV isolate of high pathogenecity had a significantly higher frequency of shedding and transmission in their aerosol secretions than pigs inoculated with an isolate of low pathogenecity (Cho et al. 2007). Aerosol transmission is also influenced by the weather conditions on the day of transmission. Winds of low velocity along with periodic gusts that are blown from a PRRSV source population towards a naïve population are a risk factor for aerosol transmission. Cool temperature, higher relative humidity and pressure, low sunlight levels are protective factors for a virus that is susceptible to ultraviolet radiation, heat, and drying. (Dee et al. 2010). The risk of aerosol transmission of PRRSV can be reduced by using high efficiency particulate air (HEPA) filtration system to filter the incoming air into a barn (Dee et al. 2006).

Mechanical vectors

PRRSV only replicates in pigs and there is no experimental evidence that it replicates in any other species of animals (Willis et al. 2000). It was originally thought that PRRSV

could replicate in mallard ducks (Zimmerman et al.1997), but subsequent studies have challenged this conclusion (Trincado et al. 2004). However, other species of animals can act as mechanical vectors of PRRSV. A mechanical vector is defined as an animal that is capable of transmitting an infectious agent. Unlike biological vectors, the infectious agent does not replicate in the mechanical vector. Under experimental conditions, arthropods and humans can act as short-term mechanical vectors for PRRSV. PRRSV has been collected from the intestinal tract of house flies (*Musca domestica)* and mosquitoes (*Aedex vexans)* 12 and 6 hours post-feeding, respectively (Otake et al. 2003 & Otake et al. 2002c). After contact with an infected pig, PRRSV has been detected 2.4 km away from the PRRSV source population. The risk of transmission of PRRSV by house flies and mosquitoes can be reduced by using screens on side-wall inlets insecticides (Schurrer et al. 2006). PRRSV has been detected on the hands of farm personnel immediately after exposure with an infected pig. The risk of transmission of PRRSV by farm personnel can be reduced by washing their hands and showering before entering a farm (Otake et al. 2002a).

Pork products

Pork products are a risk factor for indirect transmission of PRRSV, albeit a less important one when compared to the other risk factors. The virus can be detected in several porcine secretions and excretion, including muscle transudate, "meat juice" (Bloemraad et al. 1994). A pork product could be a risk factor if an employee brought a pork product from a pig that was infected with PRRSV into a barn. Employees of pig barns frequently eat their breakfasts and lunches in a kitchen area located at the farm. If an employee handled an infected pork product and then re-entered a barn without washing their hands, the

employee could act as a mechanical vector for PRRSV. In one study, viable virus could be detected in meat refrigerated at 4° C for 7 days from pigs that were experimentally infected with PRRSV (Cano et al. 2007). The risk of pork products transmitting the virus can be eliminated by not bringing pork products into a swine barn, especially uncooked products.

1.7 Conclusions

At the 2010 Pork Forum, producer delegates approved a resolution that the National Pork Board Council should develop a voluntary program for the elimination of PRRSV from the US herd. Therefore, as the swine industry begins to develop area/regions control and elimination programs, it is essential that all transmission routes for PRRSV are evaluated. There has been much progress in our understanding of risk factors for PRRSV transmission, but there are new risk factors that need to be evaluated and known risk factors that need to be reevaluated. Needle-free injection device (NFID) is a new risk factor for direct transmission of PRRSV within a barn that is assessed in chapter 2. Feral pig populations are risk factors for indirect transmission of PRRSV between farms. Previous studies have demonstrated a low seroprevalence of PRRSV in feral (Saliki et al. 1998). Feral pig populations were previously restricted to southern states with low commercial pig populations, but their populations and distribution has expanded in the past decade (USDA 2005). A concern for the swine industry is feral pig populations have been found in states with large commercial pig populations, like Iowa and North Carolina. Could a larger and expanded distribution of feral pig populations facilitate the transmission of PRRSV between commercial and feral pig populations? To

address this concern, feral pig populations as a risk factor for indirect transmission of PRRSV should be revaluated. In chapter 3, this risk factor was reassessed because of the expanding population of feral pig populations in the United States (USDA 2005).

NFID are a relatively new technology to the swine industry and have not been assessed as risk factors for PRRSV transmission. NFID injects a vaccine or medicine directly through the skin and has the potential to transmit the virus via an intramuscular route of exposure. Any object in a farm can act as a fomite for PRRSV, but depending on the route of exposure some objects pose a greater risk for PRRSV transmission than others. Infectious dose for a pathogen is commonly measured in 50% tissue culture infectious dose ($TCID_{50}$), which is the amount of a pathogen that will produce pathological change in 50% of cell cultures The infectious dose of PRRSV via an oral route of exposure is $10^{5.3}$ $TCID_{50}$, a nasal route of exposure is $10^{4.0}$ $TCID_{50}$ (Herman et. al 2005), and vaginal route of exposure is $10^{4.5}$ $TCID_{50}$ (Benfield et al. 2000). The infectious dose of PRRSV via an intramuscular route of exposure is less than 20 virus particles (Yoon et al. 1999), which is much less than an oral, nasal, or vaginal route of exposure. A fomite that transmits the virus via an intramuscular route can pose a greater risk for PRRSV transmission than a fomite that transmits the virus via an oral, nasal or vaginal route. As previously mentioned, several objects have acted as fomites for PRRSV under experimental conditions. Other objects in farms should be evaluated under experimental conditions as potential fomites, especially objects that can transmit the virus via an intramuscular route of exposure, like NFID. In chapter 4, the risk of NFID is evaluated.

1.8 References

1. Benfield DA, Nelson E, Collins JE, Harris L, Goyal SM, Robison D, Christianson WT, Morrison RB, Gorcyca D, Chladek D. (1992) Characterization of swine infertility and respiratory syndrome (SIRS) virus (isolate ATCC VR-2332). J *Vet Diagn Invest.* 4(2):127-33.
2. Benfield DA, Yaeger MJ, Collins JE. (1994) Experimental studies on the transmission and persistence of swine infertility and respiratory syndrome virus (Mystery Swine Disease). Research Investment Report, National Pork Producers Council, De Monies, Iowa, pp. 5-14
3. Benfield DA, Nelson C, Steffen M, Rowland RRR. (2000). Transmission of PRRSV by artificial insemination using extended semen seeded with different concentration of PRRSV. *Proc Annu Meet Am Assoc Swine Pract.* 405-408
4. Bloemraad M, de Kluijver EP, Petersen A, Burkhardt GE, Wensvoort G. (1994) Porcine reproductive and respiratory syndrome: temperature and pH stability of Lelystad virus and its survival in tissue specimens from viraemic pigs. *Vet Microbiol.* 42(4):361-71.
5. Cano JP, Murtaugh MP, Dee SA. (2007) Evaluation of the survival of porcine reproductive and respiratory syndrome virus in non-processed pig meat. *Vet Rec.* 160(26):907-8.
6. Carman S, Sanford SE, Dea S. (1995) Assessment of seropositivity to porcine reproductive and respiratory syndrome (PRRS) virus in swine herds in Ontario--1978 to 1982. *Canadian Veterinary Journal*. 36(12):776-7
7. Cho JG, Dee SA. (2006) Porcine reproductive and respiratory syndrome virus. *Theriogenology*. 66(3):655-62.

8. Cho JG, Deen J, Dee SA. (2007) Influence of isolate pathogenicity on the aerosol transmission of Porcine reproductive and respiratory syndrome virus. *Can J Vet Res.* 71(1):23-7
9. Christianson WT, Choi CS, Collins JE, Molitor TW, Morrison RB, Joo HS. (1993) Pathogenesis of porcine reproductive and respiratory syndrome virus infection in mid-gestation sows and fetuses. *Can J Vet Res*. 57(4):262-8
10. Christopher-Hennings J, Nelson EA, Nelson JK, Benfield DA. (1997) Effects of a modified-live virus vaccine against porcine reproductive and respiratory syndrome in boars *Am J Vet Res*. 58(1):40-5.
11. Christopher-Hennings J, Faaberg KS, Murtaugh MP, Nelson EA, Roof MB, Vaughn EM, Yoon KJ, DVM, Zimmerman JJ. (2002). Porcine reproductive and respiratory syndrome (PRRS) diagnostics: Interpretation and limitations. *J Swine Health Prod.* 10(5):213-218
12. Corzo CA, Mondaca E, Wayne S, Torremorell M, Dee S, Davies P, Morrison RB. (2010) Control and elimination of porcine reproductive and respiratory syndrome virus. *Virus Res.* 154(1-2):185-92
13. Dee SA, Deen J, Otake S, Pijoan C. (2004) An experimental model to evaluate the role of transport vehicles as a source of transmission of porcine reproductive and respiratory syndrome virus to susceptible pigs. *Can J Vet Res*. 68(2):128-33.
14. Dee SA, Deen J, Cano JP, Batista L, Pijoan C. (2006) Further evaluation of alternative air-filtration systems for reducing the transmission of Porcine reproductive and respiratory syndrome virus by aerosol. *Can J Vet Res*. 70(3):168-75

15. Dee S, Otake S, Deen J. (2010)Use of a production region model to assess the efficacy of various air filtration systems for preventing airborne transmission of porcine reproductive and respiratory syndrome virus and *Mycoplasma hyopneumoniae:* results from a 2-year study. *Virus Res*. 154(1-2):177-84.
16. Desrosiers R. (2011) Transmission of swine pathogens: different means, different needs. *Anim Health Res Rev*.
17. Forsberg R (2005).Divergence time of porcine reproductive and respiratory syndrome virus subtypes. *Mol Biol Evol*. 22(11):2131-4
18. Hermann JR, Muñoz-Zanzi CA, Roof MB, Burkhart K, Zimmerman JJ. (2005) Probability of porcine reproductive and respiratory syndrome (PRRS) virus infection as a function of exposure route and dose. *Vet Microbiol*. 30;110(1-2):7-16.
19. Holland PM, Abramson RD, Watson R, Gelfand DH. (1991) Detection of specific polymerase chain reaction product by utilizing the 5'----3' exonuclease activity of *Thermus aquaticus* DNA polymerase. *Proc Natl Acad Sci USA*. 88(16): 7276–7280.
20. Johnson CR, Griggs TF, Gnanandarajah JS, Murtaugh MP. (2011) Novel Structural Protein in Porcine Reproductive and Respiratory Syndrome Virus Encoded in an Alternative Open Reading Frame 5 Present in All Arteriviruses. *J Gen Virol.*
21. Keffaber KK. (1989) Reproductive failure of unknown etiology. *Am Assoc Swine Pract Newsletter* 1:1-10

22. Lunney JK, Benfield DA, Rowland RR. (2010) Porcine reproductive and respiratory syndrome virus: an update on an emerging and re-emerging viral disease of swine. *Virus Res.* 154(1-2):1-6.

23. Neumann E, Kliebenstein JB, Johnson CD, Mabry JW, Bush EJ, Seitzinger AH, Green AL, Zimmerman JJ (2005). Assessment of the economic impact of porcine reproductive and respiratory syndrome on swine production in the United States. *J Am Vet Med Assoc*. 227,385-92.

24. OIE (Office international des Épizooties) World Animal Health 1992. Volume VII. Number 2. Animal Health Status and Disease Control Methods (Part One: Reports) p. 126

25. Otake S, Dee SA, Rossow KD, Deen J, Joo HS, Molitor, Pijoan C. (2002a) Transmission of porcine reproductive and respiratory syndrome virus by fomites (boots and coveralls). *J Swine Health Prod.* 10(2):59-65

26. Otake S, Dee SA, Rossow KD, Joo HS, Deen J, Molitor TW, Pijoan C. (2002b) Transmission of porcine reproductive and respiratory syndrome virus by needles. *Vet Rec*. 150(4):114-5

27. Otake S, Dee SA, Rossow KD, Moon RD, Pijoan C. (2002c) Mechanical transmission of porcine reproductive and respiratory syndrome virus by mosquitoes, *Aedes vexans* (Meigen). *Can J Vet Res.* 66(3):191-5.

28. Otake S, Dee SA, Rossow KD, Moon RD, Trincado C, Pijoan C. (2003) Transmission of porcine reproductive and respiratory syndrome virus by houseflies (*Musca domestica*).*Vet Rec*.152(3):73-6.

29. Otake S, Dee S, Corzo C, Oliveira S, Deen J.(2010) Long-distance airborne transport of infectious PRRSV and Mycoplasma hyopneumoniae from a swine population infected with multiple viral variants. *Vet Microbiol*. 26;145(3-4):198-208.
30. Saliki JT, Rodgers SJ, Eskew G (1998). Serosurvey of selected viral and bacterial diseases in wild swine from Oklahoma. *J Wildl Dis*. 34(4):834-8.
31. Schurrer JA, Dee SA, Moon RD, Deen J, Pijoan C. (2006) Evaluation of three strategies for insect control on a commercial swine farm. *J Swine Health.* 14(2):76-81.
32. Stadejek T, Oleksiewicz MB, Potapchuk D, Podgórska K. (2006) Porcine reproductive and respiratory syndrome virus strains of exceptional diversity in Eastern Europe support the definition of new genetic subtypes. *Journal of General Virology*. 87(7):1835-41.
33. Swenson SL, Hill HT, Zimmerman JJ, Evans LE, Landgraf JG, Wills RW, Sanderson TP, McGinley MJ, Brevik AK, Ciszewski DK, Frey ML. (1994) Excretion of porcine reproductive and respiratory syndrome virus in semen after experimentally induced infection in boars. *J Am Vet Med Assoc*.15;204(12):1943-8.
34. Torremorell M, Polson D, Henry SC, Morrison RB (2002). Specificity of the PRRSV IDEXX ELISA test in negative farms. *Proc Intl Pig Vet Congr* 1:209
35. Torremorell M, Henry S, Christianson WT. (2003) Eradication using herd closure. In the PRRS Compendium (2nd Edition). J. Zimmerman, K-J Yoon, eds. Des Moines, Iowa: National Pork Board, pp. 157-161.

36. Trincado C, Dee S, Rossow K, Halvorson D, Pijoan C (2004). Evaluation of the role of mallard ducks as vectors of porcine reproductive and respiratory syndrome virus. *Vet Rec*. 154(8):233-7

37. United States Department of Agriculture/Animal and Plant Health Inspection Services (2005) Feral/Wild Pigs: Potential problem for Farmers and Hunters. Agriculture Information Bulletin No. 799

38. Van Breedam W, Delputte PL, Van Gorp H, Misinzo G, Vanderheijden N, Duan X, Nauwynck HJ. (2010) Porcine reproductive and respiratory syndrome virus entry into the porcine macrophage. *J Gen Virol*. 91(Pt 7):1659-67

39. Wagstrom EA, Chang CC, Yoon KJ, Zimmerman JJ. (2001)Shedding of porcine reproductive and respiratory syndrome virus in mammary gland secretions of sows. *Am J Vet Res.* (12):1876-80.

40. Wensvoort G, Terpstra J, Pol EA, ter LaakM, Bloemraad E P, de Kluyver C, Kragten L, van Buiten A, den Besten F, Wagenaar JM, Broekhuijsen P, Moonen T, Zetstra E, de Boer H J, Tibben MF, de Jong P, van't Veld G, Groenland, J A, van Gennep MT, Voets, JHM, Verheijden, and Braamskamp. (1991) Mystery swine disease in the Netherlands: the isolation of Lelystad virus. *Veterinary Quarterly.* 13:121-130

41. Wills RW, Zimmerman JJ, Yoon KJ, Swenson SL, Hoffman LJ, McGinley MJ, Hill HT, Platt KB. (1997) Porcine reproductive and respiratory syndrome virus: routes of excretion. *Vet Microbiol*. 57(1):69-81.

42. Willis RW, Osorio FA, Doster AR. (2000) Susceptibility of selected non-swine species to infection with PRRS virus. *Proc Annu Meet Am Assoc Swine Pract,* 411-413

43. Wongyanin P, Buranapraditkun S, Chokeshai-Usaha K, Thanawonguwech R, Suradhat S. (2010). Induction of inducible CD4+CD25+Foxp3+ regulatory T lymphocytes by porcine reproductive and respiratory syndrome virus (PRRSV). *Vet Immunol Immunopathol.* 133(2-4):170-82.

44. Yoon KJ, Zimmerman JJ, Chang CC, Cancel-Tirado S, Harmon KM, McGinley MJ (1999). Effect of challenge dose and route on porcine reproductive and respiratory syndrome virus (PRRSV) infection in young swine. *Vet Res*. 30(6):629-38

45. Zimmerman J, Sanderson T, Eernisse KA, Hill HT, Frey ML. (1992) Transmission of SIRS virus from convalescent animals to comingled penmates under experimental conditions. *Am Assoc Swine Practitioners Newsletter* 4(4):25

46. Zimmerman J, Yoon KJ, Pirtle EC, Wills RW, Sanderson TJ, McGinley MJ. (1997) Studies of porcine reproductive and respiratory syndrome (PRRS) virus infection in avian species. *Vet Microbiol.* 55(1-4):329-36.

47. Zimmerman J, Benfield DA, Murtaugh, MP, Osorio F, Stevenson GW, Torremorell M. (2006) Porcine Reproductive and Respiratory Syndrome Virus. Diseases of Swine 9th Edition. Blackwell Publishing

Chapter 2:

Evaluation of a needle-free injection device (AcuShot™) for reduction of hematogenous transmission of PRRS virus

Seth Baker, Enrique Mondaca, Dale Polson, Scott Dee

2.1 Summary

Objective: To evaluate the ability of a needle-free injection device (NFID), AcuShot™, to prevent hematogenous transmission of porcine reproductive and respiratory syndrome virus (PRRSV)

Materials & Methods: Eight-eight, 5-week-old gilts from a PRRSV-negative source were organized into 5 groups and individually housed in isolation rooms. On Day 1, pigs in Group 1 (PRRSV source population) were inoculated with PRRSV isolate MN-184, and pigs in Group 4 (sham-inoculated group) were inoculated with virus-free media. On Days 5, 6 and 7 post-inoculation, pigs from Groups #1, #2 and #3 were vaccinated with a *Mycoplasma hyopneumoniae* bacterin using the needle/syringe and AcuShot. The same needle/syringe and AcuShot used to vaccinate Group #1 were used to vaccinate pigs in Group #2 (Needle/Syringe) and Group #3 (AcuShot), respectively. Serum samples were collected from all pigs and tested for PRRSV by PCR and ELISA.

Results: On Day 12, all the pigs in Group 2 (needle/syringe) tested positive for PRRSV RNA (100% transmission) suggesting that transmission of PRRSV occurred between Groups 1 and 2 by repeated use of the same needle. On Day 22, all pigs in 1 replicate of Group 3 (AcuShot) (25% transmission) tested positive for PRRSV RNA suggesting that transmission of PRRSV occurred between Group 1 and 3 by repeated use of the same AcuShot.

Implications:

- Hematogenous transmission of PRRSV can occur from infected pigs to susceptible pigs via repeated use of the same needle.

- Needle-free injection devices reduce, but do not prevent the hematogenous transmission of PRRSV.

Keywords: Swine, porcine reproductive and respiratory syndrome virus, needle-free injection devices

2.2 Introduction

Porcine reproductive and respiratory syndrome (PRRS) is the most economically significant disease for the US swine industry with annual losses estimated at approximately $560 million (Neumann et al. 2005). The etiologic agent of PRRS is a virus known as porcine reproductive and respiratory syndrome virus (PRRSV) (Benfield et al. 1992). Controlling the disease with conventional methods such as vaccination and pig flow has had limited success due in part to the multiple direct and indirect routes of transmission. PRRSV has been known to be directly transmitted from infected pigs to naïve pig horizontally via multiple porcine secretions, like blood and semen (Cho et al. 2006). PRRSV has also been known to be indirectly transmitted from an infected pig to a naïve pig by fomites. Boots and coveralls (Otake et al. 2002a), needles (Otake et al. 2002b), containers, and vehicles (Dee et al. 2004) are objects at farms that have been known to act as fomites for PRRSV. The risk of other objects at farms to act as fomites needs to be evaluated to indentify other indirect routes of transmission. At the 2010 Pork Forum, producer delegates approved a resolution that the National Pork Board should strive to develop a voluntary program of the elimination of PRRSV from the US herd. Therefore, as our industry begins to develop area/regions control and elimination programs, it is essential that risk factors for PRRSV transmission within herds are evaluated.

The risk of needle free injections devices (NFID) to act as a fomite for PRRSV transmission has not been evaluated. There has been a renewed interest in NFID for delivering vaccine or medicine in the swine industry because they offer some advantages over conventional needle and syringe methods: elimination of broken needles, consistent vaccine delivery, reduction of injuries to on-farm personnel, elimination of needle disposal, and reduced pain and stress to pigs (Chase et al. 2008). NFID deliver vaccine or medicine directly through the skin or trans-dermally by forcing it out of the device at such a high velocity (> 100m/sec) that it creates a small hole in the skin. The hole is estimated to be about 1/7 the size of an 18 Gauge needle. The NFID used is the study was the gas-powered jet injector, AcuShot™ (AcuShot™ Needle-Free, Winnipeg, MA, Canada).

Since it has been demonstrated that hematogenous transmission of PRRSV can occur from infected pigs to susceptible pigs via repeated use of the same needle (Otake et al. 2002b) another potential advantage of needle-free technology is the reduction of transmission of PRRSV. Therefore, the objective of this study was to evaluate the ability of the NFID, AcuShot, to reduce or eliminate the hematogenous transmission of PRRSV. The study was based on the hypothesis that the risk of hematogenous transmission of PRRSV from infected pigs to susceptible pigs is prevented through the use of AcuShot.

2.3 Materials and Methods

All animal care and handling was approved by the University of Minnesota Institutional Animal Care and Usage committee.

Pigs and housing

Four-week-old gilts were purchased from a farm that was known to be PRRSV-naïve on the basis of 6 years of diagnostic data and absence of clinical signs. The pigs were divided into four replicates of 5 groups. Each replicate was housed in a separate room in an isolation facility at the University of Minnesota. The isolation rooms were separately ventilated and contained an individual slurry pit. Strict bio-security protocols were followed when entering each room to minimize the risk of contamination between groups. After exposure to a given group of pigs, the investigator would shower, change scrubs, gloves, hairnet and boots before entering the next room (Dee et al. 2006).

Experimental Design

A total number of 88 pigs were divided into four replicates of five groups. Group 1 was the PRRSV infected group and consisted of ten pigs per replicate. Groups 2, 3, 4, and 5 consisted of three pigs per replicate. Group 2 was the needle/syringe group, Group 3 was the AcuShot group, Group 4 was the sham-inoculated group and Group 5 was the negative control.

Study Timeline

On Day 1, pigs in Group 1 (infected group) were inoculated intramuscularly with 2 ml of PRRSV isolate MN-184 at a concentration of 1×10^5 $TCID_{50}$ (Figure 1). PRRSV isolate MN-184 was used for this project because it is classified as being of high pathogenecity based on high levels of viral shedding, a high viral load in blood and severe clinical signs (Cho et al. 2007). All pigs in Group 4 (sham-inoculated group) were intramuscularly inoculated with 2 ml of virus-free media minimal essential medium (MEM).

On Day 5, 6, and 7 post-inoculation (PI), pigs in Group 1 (infected group) were vaccinated with 4 ml of *M. hyopneumoniae* bacterin (Ingelvac MycoFLEX®, Boehringer

Ingelheim Vetmedica, Inc., St. Joseph, MO) via the traditional needle & syringe system (2 ml adjustable bottle-mount Vaccinator Prima Tech, Kenansville, NC) and AcuShot. Vaccinating with *M. hyopneumoniae* bacterin was used in this study because nursery pigs are commonly vaccinated against this bacterium. On the right side of their neck, all pigs in Group 1 were vaccinated with 2 ml of vaccine intramuscularly via the same needle and syringe. On their left side of their neck, the same pigs in Group 1 were vaccinated with 2 ml of vaccine trans-dermally via the same AcuShot. Swabs were then taken of the surfaces of the needle and syringe and the AcuShot, excluding the tip, to validate the absence of PRRSV (Pitkin et al. 2009). Swabs from surfaces were put into individual sterile tubes (Falcon, Franklin Lakes, NJ) containing 2 ml of PBS. The surfaces of the needle and syringe and AcuShot, excluding the tip, were wiped with an iodine solution. The needle and syringe and AcuShot were placed into sealed containers and transferred to their respective rooms. The same needle and syringe and AcuShot that were used to vaccinate the infectious group were used to vaccinate Group 2 (needle/syringe) and Group 3 (AcuShot), respectively. On Day 22 PI, all pigs in each group were euthanized.

Pig Sampling

Bloods samples were collected from all pigs on day 0 to ensure that they were PRRSV-negative when they arrived. Samples were collected from all pigs in Group 1 (infected group) on Day 2 and Day 5 PI to determine if the inoculation of PRRSV was successful. Samples were collected from all pigs in Group 4 (sham-inoculated group) to ensure that the pigs were PRRSV-negative. Samples were collected from all pigs in Group 2 (needle/syringe) and Group3 (AcuShot) on day 7, 12, 19 and 22 to determine if PRRSV was transmitted from the infectious group to the Group 2 and 3 via the needle and syringe

and AcuShot, respectively. Samples were collected from all pigs in Group 4 (sham-inoculated group) and Group 5 (negative control) on day 22. Sera were separated from blood samples by centrifugation.

Diagnostic Analysis

Serum samples were tested for the presence of PRRSV RNA by polymerase chain reaction (PCR) using the TaqMan PCR assay (Perkin-Elmer Applied Biosystems, Foster City, California) (Molitor et al 1997). Sera were tested for PRRSV antibodies by IDEXX 2XR enzyme-linked immunosorbent assay (ELISA) (IDEXX laboratories, Westbrook, Maine USA) (Snyder et al. 1995). An ELISA S:P ratio ≥ 0.4 was considered positive. All PRRSV ELISA positive results were confirmed with PRRSV Immunofluorescent assay (IFA). A positive PRRSV PCR from each replicate was submitted to the University of Minnesota Veterinary Diagnostic Laboratory for sequencing of the ORF5 region. The sequence results were compared to the ORF5 from the original inoculum (PRRSV wild-type isolate MN-184). A swine bioassay was performed to confirm if two PRRSV-PCR positive results were infectious (Swenson 1994). PRRSV-naïve pigs (sentinel) were injected intramuscularly with 2 ml of the PCR-positive sample for the swine bioassay. Sentinel pigs were housed in isolated rooms to prevent transmission between pigs. The pigs were bled 7 and 14 days post inoculation and the sera were tested for PRRSV by PCR.

Statistical Analysis

The statistical analysis was run with Minitab® software (Minitab Inc., State College PA) applying Fisher's exact test.

2.4 Results

Pig samples

All pigs were PRRSV-PCR negative (Table 1) and sero-negative by ELISA (S:P ratio <0.4) (Table 2) on arrival. All pigs in Group 4 (sham-inoculated group) and Group 5 (negative control) remained PRRSV-PCR negative and sero-negative.

Group 1 (Infected Group): All the pigs in each replicate were PRRSV PCR-positive on Day 2 PI (Table 1) and sero-positive on Day 22 PI (Table 2). Clinical signs of anorexia and lethargy were observed in several pigs. Ten pigs died or were euthanized due to respiratory distress during the experiment. The nucleic acid in the ORF5 region of 4 representative samples was 99.8% homologous with the PRRSV inoculum isolate.

Group 2 (Needle/Syringe Group): All pigs in each replicate tested PRRSV PCR-positive on Day 12 PI or Day 7 post-exposure (PE) (Table 1) and were sero-positive on Day 22 PI or Day 17 PE (Table 2). The nucleic acid in the ORF5 region of 4 representative samples was 99.8% homologous with the PRRSV inoculum isolate.

Group 3 (AcuShot Group): All pigs in one replicate tested PRRSV PCR-positive on Day 19 PI or Day 12 PE (Table 1) and were sero-positive on Day 22 PI or Day 17 PE (Table 2). The nucleic acid in the ORF5 region of 1 representative sample was 99.2% homologous with the PRRSV inoculum isolate. One sample from the PCR-positive replicate tested positive for infectious PRRSV by Swine Bioassay.

Data analysis

The proportion of PRRSV PCR-positive pigs was significantly lower in the AcuShot group compared with the needle/syringe group ($p \leq 0.05$).

Personnel and fomite samples

All the swabs taken from the surface of the needle and syringe between transferring it from Group 1 (infected group) to Group 2 (needle/syringe) tested PRRSV PCR-negative on Day 6 PI. There was one swab taken from the surface of the syringe that tested positive for PRRSV on day 7 PI. All swabs taken from the surface of the AcuShot between transferring it from Group 1 (infected group) to Group 3 (AcuShot) tested PRRSV PCR-negative on Day 6 and 7 PI.

2.5 Discussion

The study was based on the hypothesis that the risk of hematogenous transmission of PRRSV from infected pigs to susceptible pigs is prevented through the use of AcuShot. The results indicated that AcuShot reduced but did not prevent the hematogenous transmission of PRRSV. A possible explanation is that blood was occasionally observed at the site of vaccination. If the high velocity of the vaccine delivery by the AcuShot ruptured blood vessels near site of injection, blood from PRRSV infected pig could have contacted the tip of the AcuShot and then been injected into PRRSV-negative pig. Therefore, NFID, like AcuShot, have the potential to acts as fomites for PRRSV transmission.

Overall, the study had several strengths. Each study group had multiple replicates, multiple diagnostic methods (PCR, ELISA, Sequencing, IFA and swine bioassay) to validate the results, and multiple control groups. The first control group was the needle and syringe group which was the positive control. This study supports a previously published study which reported that hematogenous transmission of PRRSV can occur from infected pigs to susceptible pigs via repeated use of the same needle (Otake et al.

2002b).The sham-inoculated group was a negative control for inoculation. The negative control group demonstrated that PRRSV was not transmitted between rooms via personnel or air.

Besides its strengths, the study has two acknowledged limitations. First, the mechanism for PRRSV transmission by AcuShot was not identified. Future studies are needed to identify the mechanisms of PRRSV transmission by NFID. Another limitation of this study was that this experiment was performed at isolation barns. Further evaluation of needleless devices under field conditions is warranted to ensure their suitability for use in swine production.

Despite these limitations, it was possible to demonstrate NFID are a new technology that could reduce the hematogenous transmission of PRRSV within a pig herd during vaccination. However, there is a risk, albeit a smaller risk, of NFID acting as fomites for PRRSV transmission. The results of this study are important to the swine industry because it evaluated the risk of NFID as fomites for PRRSV. A swine veterinarian can explain to a producer that NFID reduce but does not eliminate the hematogenous transmission of PRRSV.

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2.7 Implications

- Hematogenous transmission of PRRSV can occur from infected pigs to susceptible pigs via repeated use of the same needle.
- Needle-Free Injection Devices reduce but do not prevent the hematogenous transmission of PRRSV.

2.8 References

1. Benfield DA, Nelson E, Collins JE, Harris L, Goyal SM, Robison D, Christianson WT, Morrison RB, Gorcyca D, Chladek D. (1992). Characterization of swine infertility and respiratory syndrome (SIRS) virus (isolate ATCC VR-2332). J *Vet Diagn Invest.* 4(2),127-33.
2. Chase CL, Daniels CS, Garcia R, Milward F, Nation T. (2008) Needle-free injection technology in swine: Progress toward vaccine efficacy and pork quality. *J Swine Health Prod.* 16,254–26
3. Cho JG, Dee SA. (2006) Porcine reproductive and respiratory syndrome virus. *Theriogenology* 66,655-662
4. Cho JG, Dee, SA, Deen, J, Murtaugh MP, Joo HS. (2007) An evaluation of isolate pathogenicity on the transmission of porcine reproductive and respiratory syndrome virus by aerosols. *Can J Vet Res.*71,23-27.
5. Dee SA, Deen J, Otake S, Pijoan C. (2004) An experimental model to evaluate the role of transport vehicles as a source of transmission of porcine reproductive and respiratory syndrome virus to susceptible pigs. *Can J Vet Res*. 2004;68(2),128-33.
6. Neumann E, Kleibenstein JB, Johnson CD, Mabry JW, Bush EJ, Seitzinger AH, Green AL, Zimmerman JJ. (2005) Assessment of the economic impact of porcine

reproductive and respiratory syndrome on swine production in the United States. *J Am Vet Med Assoc*. 227,385-92.

7. Otake S, Dee SA, Rossow KD, Deen J, Joo HS, Molitor TW, Pijoan C. (2002a) Transmission of porcine reproductive and respiratory syndrome virus by fomites (boots and coveralls). *J Swine Health Prod*.10(2),59-65.
8. Otake S, Dee SA, Rossow KD, Joo HS, Deen J, Molitor TW, Pijoan C. (2002b) Transmission of porcine reproductive and respiratory syndrome virus by needles. *Vet Rec.* 2002;150(4),114-115
9. Pitkin A, Deen J, Dee SA. Further assessment of fomites and personnel as vehicles for mechanical transport and transmission of porcine reproductive and respiratory syndrome virus. *Can J Vet Res.* 2009;73, 298-302
10. Molitor TW, Tune KA, Shin J, Collins J, Kapur V. Applications of TaqMan™ PCR in the detection of porcine reproductive and respiratory syndrome virus. *Proc AD Leman Swine Conf.* 1997;173–175
11. Snyder ML, Mermer B, Anderson PR, Wensvoort G, Hill HT. Evaluation data for an immunological ELISA for porcine reproductive and respiratory syndrome virus. *Proc 2nd Int Symp PRRSV*. 1995;15
12. Swenson SL, Hill HT, Zimmerman JJ, Evans LE, Landgraf JG, Wills RW, Sanderson TP, McGinley MJ, Brevik AK, Ciszewski DF, Frey ML. Excretion of porcine reproductive and respiratory syndrome virus in semen after experimentally induced infection in boars. *JAVMA.* 1994; 204,1943–1948

Table 2.1: PRRSV PCR results of groups where transmission of PRRSV was detected from the Infected Group (Group 1) to the Needle/Syringe (Group 2) and AcuShot groups (Group 3)

Groups		Day 0	Day 2	Day 5	Day 7	Day 12	Day 19	Day 22
Group #1 Infected	Pigs	0/40[a]	39/39[b]	39/39[b]	NT	NT	NT	NT
Group #2 Needle/ Syringe	Pigs	0/12[a]	NT	NT	9/12[b]	12/12[b]	12/12[b]	NT
	Replicates	0/4	NT	NT	4/4	4/4	4/4	NT
Group #3 AcuShot	Pigs	0/12[a]	NT	NT	0/12[a]	2/12[a]	3/12[a]	3/12[a]
	Replicates	0/4	NT	NT	0/4	1/4	1/4	1/4
Group #4 Sham Inoculation	Pigs	0/12[a]	NT	NT	NT	NT	NT	0/12[a]
Group #5 Negative Control	Pigs	0/12[a]	NT	NT	NT	NT	NT	0/12[a]

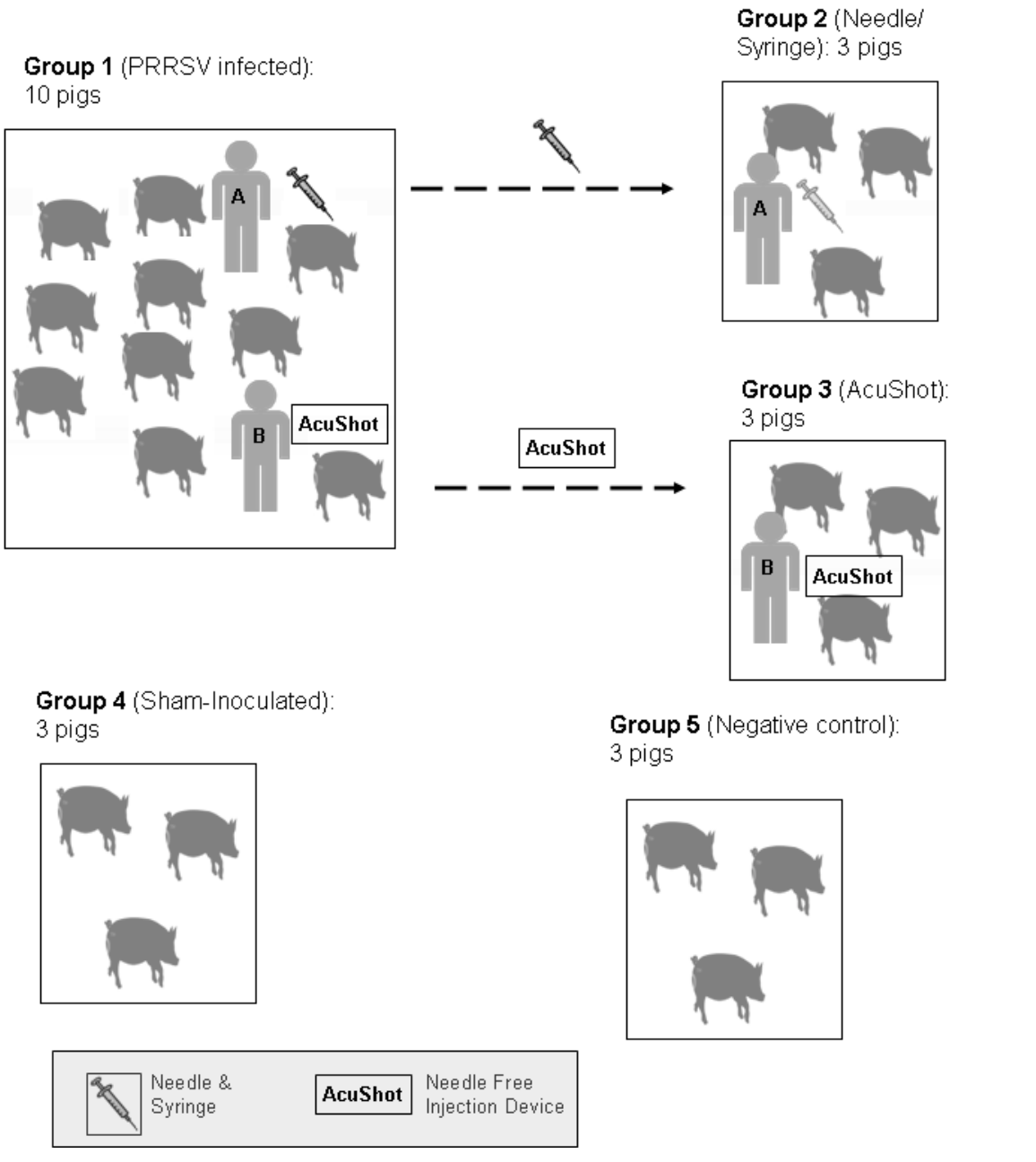
#/# Number of PRRSV PCR positives/total number of pigs tested
Different superscripts within columns indicate significant differences at $p \leq 0.05$
Shaded areas of the table are PRRSV PCR-positive
NT: "Not Tested"

Table 2.2: PRRSV ELISA results of groups where transmission of PRRSV was detected from the Infected group (Group 1) to the Needle/Syringe (Group 2) and the AcuShot groups (Group 3)

Groups		Day 0	Day 22
Group #1 Infected	**Pigs**	0/40[a]	30/30[b]
Group #2 Needle/ Syringe	**Pigs**	0/12[a]	12/12[b]
	Replicates	0/4	4/4
Group #3 AcuShot	**Pigs**	0/12[a]	3/12[a]
	Replicates	0/4	1/4
Group #4 Sham Inoculation	**Pigs**	0/12[a]	0/12[a]
Group #5 Negative Control	**Pigs**	0/12[a]	0/12[a]

#/# Number of PRRSV PCR positives/total number of pigs tested
Different superscripts within columns indicate significant differences at $p \leq 0.05$
Shaded areas of the table are sero-positives
NT: "Not Tested"

Figure 1: Designation of groups and personnel movement between a group of pigs (Group 1) infected with porcine reproductive and respiratory syndrome virus (PRRSV) and two groups of PRRSV naïve sentinel pigs (Groups 2 & 3).Group 4 pigs, sham-inoculated group, were inoculated with virus-free media. Group 5 pigs were PRRSV-Naïve controls. Each of the five groups was housed in isolation. Days 5, 6, and 7 post-inoculation of Group 1, Person A vaccinated the pigs in Group 1 with a needle and syringe and Person B vaccinated the same pigs with AcuShot. The needle and syringe was transferred to a room that housed pigs in Group 2 and the AcuShot was transferred to a room that housed pigs in Group 3. Person A vaccinated pigs in Group 2 with the same needle and syringe used to vaccinate Group 1. Person B vaccinated pigs in Group 3 with the same AcuShot used to vaccinate pigs in Group 1. There were four replicates of this experimental design.



Chapter 3:

Pilot Study: An estimate of seroprevalence of production-limiting diseases in feral pigs

Seth Baker, Kevin O'Neal, Marie Gramer, Scott Dee

Introduction 3.1

Wild boars (*Sus scrofa*) and their semi-domestic relatives, feral pigs, are distributed throughout many countries on all continents except Antarctica. Feral pig populations have recently expanded, particularly in the United States (USDA 2005) and Europe (Sáez-Royuela et al. 1986) due to the development of a commercial hunting industry (Acevedo et al. 2006). As this trend continues, there is a growing concern regarding the ability of feral pigs to serve as reservoirs of pathogens for domestic pig populations. Therefore, as the risk of contact between feral and domestic pig populations increases, it becomes important to determining the health status of feral pig populations.

In the literature today, the majority of publications on the prevalence of infectious agents in feral pigs have focused on regulatory diseases, i.e. Aujesky's disease and Brucellosis; however, a few papers have been published on the prevalence of production-limiting diseases in feral pigs. For example, in regards to surveys conducted in Europe, the serostatus of porcine circovirus type 2 (PCV2) in feral pig populations was approximately 35 percent in Belgium (Sanchez et al 2001) and 48 percent in Spain (Vincente et al. 2004), while the serostatus of *Mycoplasma hyopneumoniae* was 21 percent in Slovenian feral pigs (Vengust et al. 2006). In regards to the United States the seroprevalence of porcine reproductive and respiratory syndrome virus (PPRSV) in feral pig populations has been reported to be 1.7% in the state of Oklahoma (Saliki et. al 1998) and 0 percent and 0.8 percent in the states of South Carolina and North Carolina, respectively (Corn et al. 2009). In addition, the serostatus of swine influenza virus A (SIV) subtype H1N1 (endemic) and subtype H3N2 in feral pig populations was 3 percent and 14 percent respectively, in the state of Texas (Hall et al. 2008).

In order to better approximate the risk that feral pigs may pose to domestic populations, a better understanding of the seroprevalence of production-limiting diseases in feral populations is required. Therefore, the objective of this study was to expand this database by testing additional feral pigs originating from the United States for antibodies to PRRSV, PCV2, *M. hyopneumoniae*, and SIV subtypes H1 and H3.

3.2 Materials and Methods

To conduct this survey, sera were collected from feral pigs at exsanguination in an abattoir. Pigs originated from Texas, Oklahoma, Arkansas and Louisiana. Based on an assumed 2 percent seroprevalence (Saliki et al. 1998), 50 serum samples were required for a 95 percent level of confidence with a ± 3 percent accuracy. Following collection and processing, sera were tested for antibodies against PRRSV by ELISA (IDEXX laboratories, Westbrook, Maine USA); for antibodies against PCV2 by ELISA (Synbiotics Kansas City, Missouri USA); for antibodies against *M. hyopneumoniae* by ELISA (IDEXX); for antibodies against SIV H1 (A/Sw/NC/2001 H1N1) by hemagglutination inhibition assay; and for antibodies against SIV H3 (A/Sw/TX/1/98/H3N2) by hemagglutination inhibition assay.

3.3 Results and Discussion

Overall, our data were similar to those previously published in regards to PRRSV, PCV2, *M. hyopneumoniae* and SwIV H1 (Saliki et al.1998, Corn et al. 2009, Sanchez et al. 2001, Vincente et al. 2005, Vengust et al. 2006, Hall et al. 2008) (Table 1). The low seroprevalence of PRRSV in this study and previous publications suggest that feral pigs may be a low risk reservoir factor for the transmission of this virus to domestic pigs (Ruiz-Fons et al. 2008); however, further studies are required before any conclusions can

be drawn. In contrast, feral pigs may serve as a risk factor for the spread of PCV2, *M. hyopneumoniae* and SIV to domestic pigs, secondary to the high level of antibodies to these pathogens observed in this and previous studies. Surprisingly, the serostatus of SIV H3 in our study was 40 percent which was substantially higher than previously reported. One explanation for this difference may be that the serostatus of SIV H3N2 in feral pig populations is higher than previously estimated; however, a larger sample size and further studies would be needed to validate this conclusion.

In closing, information from this paper helped to expand the database regarding the seroprevalence of production-limiting pathogens in feral pig populations. There were several strengths to this study. First, the sample selection of pigs in this study was broad as feral pigs were collected from several states in the United States. Secondly, its findings regarding the seroprevalence of PRRSV, PCV2, *M. hyopneumoniae*, SIV H1 supported that of other publications. Finally, new information was provided in regards to SIV H3N2. Future studies should involve larger samples sizes across varying geographic regions and seasonal periods, as well as determine how frequently feral pigs can transmit these agents to domestic pigs.

3.4 References:

1. Acevedo P, Escudero MA, Muñoz R, Gortázar C. (2006) Factors affecting wild boar abundance across an environmental gradient in Spain. *Acta Theriol*. 51, 327–336.
2. Corn JL, Cumbee JC, Barfoot R, Erickson GA. (2009) Pathogen exposure in feral swine populations geographically associated with high densities of transitional

swine premises and commercial swine production. *Journal of Wildlife Diseases* 45, 713-21

3. Hall JS, Minnis RB, Campbell TA, Barras S, Deyoung RW, Pabilonia K, Avery ML, Sullivan H, Clark L, McLean RG. (2008) Influenza exposure in United States feral swine populations. *Journal of Wildlife Diseases* 44, 362-8.
4. Ruiz-Fons F, Segalés J, Gortázar CA. (2008) A review of viral diseases of the European wild boar: effects of population dynamics and reservoir rôle. *Vet J.*176(2),158-69
5. Sáez-Royuela C, Tellería JL. (1986) The increased population of wild boar (*Sus scrofa*) *Europe. Mammal Review*. 16 (2), 97–101
6. Saliki JT, Rodgers SJ, Eskew G. (1998) Serosurvey of selected viral and bacterial diseases in wild swine from Oklahoma. *Journal of Wildlife Diseases* 34, 834-8.
7. Sanchez R, Nauwynck H, Pensaert M. (2001) Serological survey of porcine cirocovirus 2 antibodies in domestic and feral pig populations in Belgium. *Proceedings of the ssDNA viruses plants, birds, pigs and primates meetings. St. Malo, France, September* 24-27, 122
8. United States Department of Agriculture/Animal and Plant Health Inspection Services. (2005) Feral/Wild Pigs: Potential problem for Farmers and Hunters. *Agriculture Information Bulletin No. 799*
9. Vengust G, Valencak Z, Bidovec A. (2006) A serological survey of selected pathogens in wild boar in Slovenia. *Journal of Veterinary Medicine. B, Infectious Diseases and Veterinary Public Health.* 53, 24-7.

10. Vicente J, Segalés J, Höfle U, Balasch M, Plana-Durán J, Domingo M, Gortázar C. (2004) Epidemiological study on porcine circovirus type 2 (PCV2) infection in the European wild boar (*Sus scrofa*).*Vet Res.* 35(2), 243-53.

Table 3.1: Results of serological assays to test for antibodies against PRRSV, PCV2, *M. hyopneumoniae (M. hyo),* SIV subtype H1 and H3

	PRRSV	**PCV2**	***M. hyo***	**SIV H1**	**SIV H3**
Positives	1/50	21/50	16/50	1/50	20/50
Percentage	2%	42%	32%	2%	40%

#/# Number of positives/total number of pigs tested
Percentage is the estimated seroprevalence for each of the agents tested

Chapter 4:
General Discussion

Seth Baker

Biosecurity protocols are preventative measures designed to decrease the risk of transmission of an infectious agent. An effective biosecurity protocol for a pig farm should specifically address experimentally, identified risk factors for PRRSV transmission. As mentioned in the introduction, vertical transmission, sexual transmission via semen, prolonged infection of the virus, fomites, aerosols, mechanical vectors and pork products are risk factors that have been experimentally evaluated. Swine farms with effective biosecurity protocols specifically address these aforementioned risk factors. Despite the gains in our understanding of PRRSV transmission, there still remain several unidentified risk factors. The National Pork Board proposed eliminating PRRSV from US swine herd last year. Indentifying other potential risk factors is imperative for implementing more effective biosecurity protocols and thus achieving the goal of the National Pork Board. Therefore, the objective of the thesis was to evaluate potentially new risk factors for PRRSV transmission.

The projects of this thesis were conducted to address reasonable hypotheses predicted about two potential risk factors for PRRSV transmission. As mentioned in the introduction, Needle Free Injection Devices (NFIDs) are a relatively new technology to the swine industry. Since NFIDs by definition do not require needles to administer vaccination or medicine, it has been hypothesized that NFIDs could eliminate the hematogenous spread of PRRSV. According to this hypothesis, NFIDs would be a protective factor against viral transmission within a barn. Therefore, the objective of chapter 3 was to evaluate AcuShot™ (the NFID used in this study) as a risk factor for PRRSV transmission within a herd. As mentioned in the introduction, feral pig populations have recently increased their population and expanded their distribution in

the United States. Since pigs (*Sus scrofa)* are the only host for PRRSV, it has been hypothesized that feral pig populations could readily transmit the virus between farms. Therefore, the objective of chapter 3 was to evaluate feral pig populations as a risk factor for PRRSV transmission between herds.

The two main conclusions of this thesis are that NFIDs reduce but do not eliminate the risk of PRRSV transmission and that there is a low seroprevalence of PRRSV in feral pig populations. The former of the aforementioned conclusions contradicts the hypothesis that NFIDs can eliminate the hematogenous spread of PRRSV within a herd. This study demonstrated that AcuShot reduced but did not eliminate the transmission of the virus. Therefore, NFIDs are a risk factor for PRRSV transmission, albeit a less important risk factor when compared needles. This conclusion is a novel finding in relation to previous publications on risk factors of PRRSV transmission. The latter of the aforementioned conclusions contradicts the hypothesis that feral pig populations readily transmit the virus between farms. While further studies would need to be conducted specifically addressing transmission of PRRSV between feral pig populations and commercial pig populations, the low seroprevalence of PRRSV found in this study and previous studies suggest that this is not a likely event. Therefore, feral pig populations are most likely a low risk factor for PRRSV transmission.

Other conclusions were derived from the studies of this thesis. In the AcuShot study, it was concluded that needles can act as a risk factor for PRRSV transmission, which supports a previous publication. In fact, PRRSV was transmitted more frequently in this study compared to the previous study probably because we used a more virulent strain of PRRSV (MN 1-8-4) and the other study used a less virulent strain (VR2332) (Satochi et

al 2002). In the feral pig study, the seroprevalence of swine influenza virus (SIV) subtype H1N1, porcine circovirus type 2 (PCV2) and *Mycoplasma hyopneumoniae* were similar to previous publications. The high seroprevalence of PCV2 and *M. hyopneumoniae* suggest that feral pig populations could be an important risk factor for transmission of these pathogens between farms. Also, there was a higher seroprevalence of SIV subtype H3N2 in our study compared to other studies, which suggest that prevalence of this subtype is higher than expected.

There are three acknowledged limitations to the AcuShot portion of this thesis. The first and probable the most important limitation was the mechanism of transmission of PRRSV by AcuShot was not identified. It was presumed that the virus was transmitted hematogenously by AcuShot because blood was observed at the site of injection, but this was not validated. It would be useful to indentify the specific mechanism of transmission in future projects because maybe NFIDs could be modified to prevent the transmission of PRRSV. The second limitation was that only one strain of PRRSV (MN 1-8-4) was tested. It would be useful to test other strains of PRRSV in future projects because some strains of virus might be more transmissible than other strains. The final limitation was that AcuShot was the only type of NFID used in the study. Other types of NFID, like Pulse NeedleFree Systems™ (Pulse NeedleFree Systems™, Lenexa KS, USA) should be evaluated to fully address NFID as a risk factor for PRRSV transmission.

There are four acknowledged limitations to the feral pig portion of this thesis. The first limitation is that all the pigs tested were less than 50 kg (personal observation). A cross-sectional analysis of different sizes of feral pigs could address this limitation. The second limitation is that sera were collected at one time period, which does not account

for the seasonality of certain infectious agents, like PRRSV and SIV. Future studies could collect sera at different times of the years to account for the seasonality of these pathogens. The third limitation is the serological assays performed in study do not measure active shedding of infectious agents. Performing antigenic assays, like polymerase chain reaction, of the pathogens tested in this study would address this limitation. The fourth limitation is the sample size was too small to accurately estimated the seroprevalence of several infectious agents tested in this study. The sample size was determined by the estimated seroprevalence of PRRSV and not the estimated seroprevalence of other infectious agents. A larger sample size would necessary to accurately estimate the seroprevalence of PCV2, *M. hyopneumoniae,* and SIV subtype H3N2.

Despite the acknowledged limitations, this thesis has contributed to our understanding of PRRSV transmission by evaluating potentially new risk factors. The AcuShot study is the first study evaluating NFIDs as a risk factor for PRRSV transmission. When developing a biosecurity protocol for a farm, veterinarians should consider that NFIDs reduce but do not eliminate the transmission of PRRSV. Demonstrating that feral pigs have a low seroprevalence of PRRSV supports previous publications. When developing a biosecurity protocol for a farm, veterinarians should consider there is variation in degree of risk between risk factors. A measurement that considers this variation between risk factor is relative risk, which is the risk of developing disease relative to exposure to the infectious agent. Although relative risk was not measured in this study, the relative risk for PRRSV transmission by feral pig populations is probably much lower compared to the relative risk of other risk factors, like aerosols. Biosecurity protocols should consider

all risk factors for PRRSV transmission, but should concentrate on risk factors with a high degree of relative risk.

Reference

1. Otake S, Dee SA, Rossow KD, Joo HS, Deen J, Molitor TW, Pijoan C. (2002) Transmission of porcine reproductive and respiratory syndrome virus by needles. *Vet Rec*. 150(4):114-5

General Conclusions

1. Needle-free systems reduce, but do not eliminate the hematogenous transmission of PRRSV (Chapter 2)
2. Hematogenous transmission of PRRSV can occur from infected pigs to susceptible pigs via repeated use of the same needle (Chapter 2)
3. US feral pig populations have a low seroprevalence of PRRSV (Chapter 3)

Comprehensive Reference:

1. Acevedo P, Escudero MA, Muñoz R, Gortázar C. (2006) Factors affecting wild boar abundance across an environmental gradient in Spain. *Acta Theriol*. 51, 327–336.
2. Benfield DA, Nelson E, Collins JE, Harris L, Goyal SM, Robison D, Christianson WT, Morrison RB, Gorcyca D, Chladek D. (1992) Characterization of swine infertility and respiratory syndrome (SIRS) virus (isolate ATCC VR-2332). J *Vet Diagn Invest.* 4(2):127-33.
3. Benfield DA, Yaeger MJ, Collins JE. (1994) Experimental studies on the transmission and persistence of swine infertility and respiratory syndrome virus (Mystery Swine Disease). Research Investment Report, National Pork Producers Council, De Monies, Iowa, pp. 5-14
4. Benfield DA, Nelson C, Steffen M, Rowland RRR. (2000). Transmission of PRRSV by artificial insemination using extended semen seeded with different concentration of PRRSV. *Proc Annu Meet Am Assoc Swine Pract.* 405-408
5. Bloemraad M, de Kluijver EP, Petersen A, Burkhardt GE, Wensvoort G. (1994) Porcine reproductive and respiratory syndrome: temperature and pH stability of Lelystad virus and its survival in tissue specimens from viraemic pigs. *Vet Microbiol.* 42(4):361-71.
6. Cano JP, Murtaugh MP, Dee SA. (2007) Evaluation of the survival of porcine reproductive and respiratory syndrome virus in non-processed pig meat. *Vet Rec*. 160(26):907-8.

7. Carman S, Sanford SE, Dea S. (1995) Assessment of seropositivity to porcine reproductive and respiratory syndrome (PRRS) virus in swine herds in Ontario--1978 to 1982. *Canadian Veterinary Journal*. 36(12):776-7
8. Chase CL, Daniels CS, Garcia R, Milward F, Nation T. (2008) Needle-free injection technology in swine: Progress toward vaccine efficacy and pork quality. *J Swine Health Prod.* 16,254–26
9. Cho JG, Dee SA. (2006) Porcine reproductive and respiratory syndrome virus. *Theriogenology*. 66(3):655-62.
10. Cho JG, Deen J, Dee SA. (2007) Influence of isolate pathogenicity on the aerosol transmission of Porcine reproductive and respiratory syndrome virus. *Can J Vet Res.* 71(1):23-7
11. Christianson WT, Choi CS, Collins JE, Molitor TW, Morrison RB, Joo HS. (1993) Pathogenesis of porcine reproductive and respiratory syndrome virus infection in mid-gestation sows and fetuses. *Can J Vet Res*. 57(4):262-8
12. Christopher-Hennings J, Nelson EA, Nelson JK, Benfield DA. (1997) Effects of a modified-live virus vaccine against porcine reproductive and respiratory syndrome in boars *Am J Vet Res*. 58(1):40-5.
13. Christopher-Hennings J, Faaberg KS, Murtaugh MP, Nelson EA, Roof MB, Vaughn EM, Yoon KJ, DVM, Zimmerman JJ. (2002). Porcine reproductive and respiratory syndrome (PRRS) diagnostics: Interpretation and limitations. *J Swine Health Prod.* 10(5):213-218
14. Corn JL, Cumbee JC, Barfoot R, Erickson GA. (2009) Pathogen exposure in feral swine populations geographically associated with high densities of transitional

swine premises and commercial swine production. *Journal of Wildlife Diseases* 45, 713-21

15. Corzo CA, Mondaca E, Wayne S, Torremorell M, Dee S, Davies P, Morrison RB. (2010) Control and elimination of porcine reproductive and respiratory syndrome virus. *Virus Res.* 154(1-2):185-92
16. Dee SA, Deen J, Otake S, Pijoan C. (2004) An experimental model to evaluate the role of transport vehicles as a source of transmission of porcine reproductive and respiratory syndrome virus to susceptible pigs. *Can J Vet Res*. 68(2):128-33.
17. Dee SA, Deen J, Cano JP, Batista L, Pijoan C. (2006) Further evaluation of alternative air-filtration systems for reducing the transmission of Porcine reproductive and respiratory syndrome virus by aerosol. *Can J Vet Res*. 70(3):168-75
18. Dee S, Otake S, Deen J. (2010)Use of a production region model to assess the efficacy of various air filtration systems for preventing airborne transmission of porcine reproductive and respiratory syndrome virus and *Mycoplasma hyopneumoniae:* results from a 2-year study. *Virus Res*. 154(1-2):177-84.
19. Desrosiers R. (2011) Transmission of swine pathogens: different means, different needs. *Anim Health Res Rev*.
20. Forsberg R (2005).Divergence time of porcine reproductive and respiratory syndrome virus subtypes. *Mol Biol Evol*. 22(11):2131-4
21. Hall JS, Minnis RB, Campbell TA, Barras S, Deyoung RW, Pabilonia K, Avery ML, Sullivan H, Clark L, McLean RG. (2008) Influenza exposure in United States feral swine populations. *Journal of Wildlife Diseases* 44, 362-8.

22. Hermann JR, Muñoz-Zanzi CA, Roof MB, Burkhart K, Zimmerman JJ. (2005) Probability of porcine reproductive and respiratory syndrome (PRRS) virus infection as a function of exposure route and dose. *Vet Microbiol*. 30;110(1-2):7-16.

23. Holland PM, Abramson RD, Watson R, Gelfand DH. (1991) Detection of specific polymerase chain reaction product by utilizing the 5'----3' exonuclease activity of *Thermus aquaticus* DNA polymerase. *Proc Natl Acad Sci USA*. 88(16): 7276–7280.

24. Johnson CR, Griggs TF, Gnanandarajah JS, Murtaugh MP. (2011) Novel Structural Protein in Porcine Reproductive and Respiratory Syndrome Virus Encoded in an Alternative Open Reading Frame 5 Present in All Arteriviruses. *J Gen Virol.*

25. Keffaber KK. (1989) Reproductive failure of unknown etiology. *Am Assoc Swine Pract Newsletter* 1:1-10

26. Lunney JK, Benfield DA, Rowland RR. (2010) Porcine reproductive and respiratory syndrome virus: an update on an emerging and re-emerging viral disease of swine. *Virus Res.* 154(1-2):1-6.

27. Molitor TW, Tune KA, Shin J, Collins J, Kapur V. Applications of TaqMan™ PCR in the detection of porcine reproductive and respiratory syndrome virus. *Proc AD Leman Swine Conf.* 1997;173–175

28. Neumann E, Kliebenstein JB, Johnson CD, Mabry JW, Bush EJ, Seitzinger AH, Green AL, Zimmerman JJ (2005). Assessment of the economic impact of porcine

reproductive and respiratory syndrome on swine production in the United States. *J Am Vet Med Assoc*. 227,385-92.

29. OIE (Office international des Épizooties) World Animal Health 1992. Volume VII. Number 2. Animal Health Status and Disease Control Methods (Part One: Reports) p. 126
30. Otake S, Dee SA, Rossow KD, Deen J, Joo HS, Molitor, Pijoan C. (2002a) Transmission of porcine reproductive and respiratory syndrome virus by fomites (boots and coveralls). *J Swine Health Prod.* 10(2):59-65
31. Otake S, Dee SA, Rossow KD, Joo HS, Deen J, Molitor TW, Pijoan C. (2002b) Transmission of porcine reproductive and respiratory syndrome virus by needles. *Vet Rec*. 150(4):114-5
32. Otake S, Dee SA, Rossow KD, Moon RD, Pijoan C. (2002c) Mechanical transmission of porcine reproductive and respiratory syndrome virus by mosquitoes, *Aedes vexans* (Meigen). *Can J Vet Res.* 66(3):191-5.
33. Otake S, Dee SA, Rossow KD, Moon RD, Trincado C, Pijoan C. (2003) Transmission of porcine reproductive and respiratory syndrome virus by houseflies (*Musca domestica*).*Vet Rec*.152(3):73-6.
34. Otake S, Dee S, Corzo C, Oliveira S, Deen J.(2010) Long-distance airborne transport of infectious PRRSV and *Mycoplasma hyopneumoniae* from a swine population infected with multiple viral variants. *Vet Microbiol*. 26;145(3-4):198-208.

35. Pitkin A, Deen J, Dee SA. Further assessment of fomites and personnel as vehicles for mechanical transport and transmission of porcine reproductive and respiratory syndrome virus. *Can J Vet Res.* 2009;73, 298-302

36. Ruiz-Fons F, Segalés J, Gortázar CA. (2008) A review of viral diseases of the European wild boar: effects of population dynamics and reservoir role. *Vet J.*176(2),158-69

37. Sáez-Royuela C, Tellería JL. (1986) The increased population of wild boar (*Sus scrofa*) *Europe. Mammal Review*. 16 (2), 97–101

38. Saliki JT, Rodgers SJ, Eskew G (1998). Serosurvey of selected viral and bacterial diseases in wild swine from Oklahoma. *J Wildl Dis*. 34(4):834-8.

39. Sanchez R, Nauwynck H, Pensaert M. (2001) Serological survey of porcine cirocovirus 2 antibodies in domestic and feral pig populations in Belgium. *Proceedings of the ssDNA viruses plants, birds, pigs and primates meetings. St. Malo, France, September* 24-27, 122

40. Schurrer JA, Dee SA, Moon RD, Deen J, Pijoan C. (2006) Evaluation of three strategies for insect control on a commercial swine farm. *J Swine Health.* 14(2):76-81.

41. Snyder ML, Mermer B, Anderson PR, Wensvoort G, Hill HT. Evaluation data for an immunological ELISA for porcine reproductive and respiratory syndrome virus. *Proc 2nd Int Symp PRRSV*. 1995;15

42. Stadejek T, Oleksiewicz MB, Potapchuk D, Podgórska K. (2006) Porcine reproductive and respiratory syndrome virus strains of exceptional diversity in

Eastern Europe support the definition of new genetic subtypes. *Journal of General Virology*. 87(7):1835-41.

43. Swenson SL, Hill HT, Zimmerman JJ, Evans LE, Landgraf JG, Wills RW, Sanderson TP, McGinley MJ, Brevik AK, Ciszewski DK, Frey ML. (1994) Excretion of porcine reproductive and respiratory syndrome virus in semen after experimentally induced infection in boars. *J Am Vet Med Assoc*.15;204(12):1943-8.
44. Torremorell M, Polson D, Henry SC, Morrison RB (2002). Specificity of the PRRSV IDEXX ELISA test in negative farms. *Proc Intl Pig Vet Congr* 1:209
45. Torremorell M, Henry S, Christianson WT. (2003) Eradication using herd closure. In the PRRS Compendium (2nd Edition). J. Zimmerman, K-J Yoon, eds. Des Moines, Iowa: National Pork Board, pp. 157-161.
46. Trincado C, Dee S, Rossow K, Halvorson D, Pijoan C (2004). Evaluation of the role of mallard ducks as vectors of porcine reproductive and respiratory syndrome virus. *Vet Rec*. 154(8):233-7
47. United States Department of Agriculture/Animal and Plant Health Inspection Services (2005) Feral/Wild Pigs: Potential problem for Farmers and Hunters. Agriculture Information Bulletin No. 799
48. Van Breedam W, Delputte PL, Van Gorp H, Misinzo G, Vanderheijden N, Duan X, Nauwynck HJ. (2010) Porcine reproductive and respiratory syndrome virus entry into the porcine macrophage. *J Gen Virol*. 91(Pt 7):1659-67

49. Vengust G, Valencak Z, Bidovec A. (2006) A serological survey of selected pathogens in wild boar in Slovenia. *Journal of Veterinary Medicine. B, Infectious Diseases and Veterinary Public Health.* 53, 24-7.

50. Vicente J, Segalés J, Höfle U, Balasch M, Plana-Durán J, Domingo M, Gortázar C. (2004) Epidemiological study on porcine circovirus type 2 (PCV2) infection in the European wild boar (*Sus scrofa*).*Vet Res.* 35(2), 243-53.

51. Wagstrom EA, Chang CC, Yoon KJ, Zimmerman JJ. (2001) Shedding of porcine reproductive and respiratory syndrome virus in mammary gland secretions of sows. *Am J Vet Res.* (12):1876-80.

52. Wensvoort G, Terpstra J, Pol EA, ter LaakM, Bloemraad E P, de Kluyver C, Kragten L, van Buiten A, den Besten F, Wagenaar JM, Broekhuijsen P, Moonen T, Zetstra E, de Boer H J, Tibben MF, de Jong P, van't Veld G, Groenland, J A, van Gennep MT, Voets, JHM, Verheijden, and Braamskamp. (1991) Mystery swine disease in the Netherlands: the isolation of Lelystad virus. *Veterinary Quarterly.* 13:121-130

53. Willis RW, Zimmerman JJ, Yoon KJ, Swenson SL, Hoffman LJ, McGinley MJ, Hill HT, Platt KB. (1997) Porcine reproductive and respiratory syndrome virus: routes of excretion. *Vet Microbiol*. 57(1):69-81.

54. Willis RW, Osorio FA, Doster AR. (2000) Susceptibility of selected non-swine species to infection with PRRS virus. *Proc Annu Meet Am Assoc Swine Pract,* 411-413

55. Wongyanin P, Buranapraditkun S, Chokeshai-Usaha K, Thanawonguwech R, Suradhat S. (2010). Induction of inducible CD4+CD25+Foxp3+ regulatory T

lymphocytes by porcine reproductive and respiratory syndrome virus (PRRSV). *Vet Immunol Immunopathol.* 133(2-4):170-82.

56. Yoon KJ, Zimmerman JJ, Chang CC, Cancel-Tirado S, Harmon KM, McGinley MJ (1999). Effect of challenge dose and route on porcine reproductive and respiratory syndrome virus (PRRSV) infection in young swine. *Vet Res.* 30(6):629-38
57. Zimmerman J, Sanderson T, Eernisse KA, Hill HT, Frey ML. (1992) Transmission of SIRS virus from convalescent animals to comingled penmates under experimental conditions. *Am Assoc Swine Practitioners Newsletter* 4(4):25
58. Zimmerman J, Yoon KJ, Pirtle EC, Wills RW, Sanderson TJ, McGinley MJ. (1997) Studies of porcine reproductive and respiratory syndrome (PRRS) virus infection in avian species. *Vet Microbiol.* 55(1-4):329-36.
59. Zimmerman J, Benfield DA, Murtaugh, MP, Osorio F, Stevenson GW, Torremorell M. (2006) Porcine Reproductive and Respiratory Syndrome Virus. Diseases of Swine 9th Edition. Blackwell Publishing