Epidemiological Aspects of Transmission and Control of Porcine Reproductive and Respiratory Syndrome Virus Infection and Associated Diseases

by

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ABSTRACT

EPIDEMIOLOGICAL ASPECTS OF TRANSMISSION AND CONTROL OF PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS INFECTION AND ASSOCIATED DISEASES

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This thesis presents studies conducted to investigate an outbreak of porcine high fever disease (PHFD) in a small area of Vietnam, in terms of mortality, morbidity, spatial transmission between herds, and risk factors for the disease. This is a severe disease with very high mortality in all age groups which has been considered to be caused by highly pathogenic porcine reproductive and respiratory syndrome (PRRS) virus strains. The focus of the thesis then shifted; to the investigation of within-herd transmission of PRRS virus (PRRSV) infection in commercial herds typically present in Ontario; to the evaluation of commonly used control strategies; and to the estimation of sensitivity and specificity of the PCR test used in surveillance of PRRSV. During our investigation of a PHFD outbreak, it was found that 33.4% of households were cases, and the mortality in these cases was 24.3%, 22.8%, and 6.7% in sows, suckling-nursery pigs, and finishing pigs, respectively. The spatial spread of the disease in the area was very limited, whereas introduction of pigs into a farm before the outbreak was identified as a risk factor. Moreover, it was also found that raising ducks in proximity to pigs and feeding of water green crop to pigs increased the risk for PHFD. For within-herd dynamics of PRRSV infection, the basic reproductive number (R_0) for PRRSV and duration of detectable maternal antibodies (*m*) in suckling and nursery pigs was estimated. R_0 was found to be high (R_0=9.76) and *m* was short (*m*=3 weeks). The results of mathematical modeling suggested that it is possible to eliminate PRRSV infection from a

herd by using herd closure or mass immunization. However, duration of sow immunity, and efficacy of immunization could play a critical role in this result. Finally, our study found that the sensitivity of tissue PCR is higher than the sensitivity of serum PCR and the likelihood of detecting the virus in tissue was higher in pigs with dyspnea or rough hair coat, but lower in lame pigs. This finding can help to increase the sensitivity of risk-based surveillance programs.

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Chapter 1

Introduction, literature review, and objectives

Introduction

Porcine reproductive and respiratory syndrome (PRRS) is a highly contagious, economically devastating disease in swine production. It is characterized by reproductive problems that include reduced farrowing rate, late-term abortions, and increased numbers of stillbirths at farrowing. Respiratory problems include interstitial pneumonia and other severe respiratory tract lesions that occur due to the synergistic effect of PRRS virus (PRRSV) and other pathogens commonly circulating in swine herds. Infection with PRRSV alone, or in combination with other pathogens, leads to a decrease in productivity and increases in morbidity and mortality in infected pigs of all ages. This disease was first reported in the United States and Canada in the mid-1980's under the name “mystery swine disease,” and shortly afterwards in Europe (Reotutar, 1989; Baron, et al., 1992).

The virus causing this disease was isolated first in The Netherlands, and very soon afterwards, a similar virus was also isolated in the United States and Canada (Terpstra, et al., 1991; Collins, et al., 1992; Dea, et al., 1992). Many other countries have reported the presence of PRRSV, with only a few reporting swine populations free of it. The total annual cost of PRRS has been estimated at approximately $560 million for US swine producers (Neumann, et al., 2005) and $130 million for Canadian swine producers (Mussell, 2010). With such a high cost, it is not surprising that the disease has been considered one of the most significant problems of pig production. In addition, PRRSV has been linked with a recently emerged disease in South-East Asia named porcine high fever disease (PHFD), which is characterized by severe clinical signs, very high mortality, and large economic and social costs to farming communities of that region. The high costs associated with PRRSV and emergence of PHFD have led to PRRSV being increasingly recognized as an infectious agent that warrants better control and even elimination when this is feasible. This chapter is an overview of the disease and some novel approaches to investigating the epidemiology of this disease using observational studies. Finally, the objectives of the thesis will be presented at the end of the chapter.

Literature review

PRRSV

PRRSV belongs to the family *Arteriviridae* and is an enveloped RNA virus with a diameter of 48-83 nm (Benfield, et al., 1992). The genome of the PRRSV is 15kb in length including 9 open reading frames (ORFs). They are ORF1a, ORF1b, ORF2a, ORF2b, ORFs 3-7. Among them, ORF1a and ORF1b occupy 80% of the genome and

encode RNA replicase, an enzyme needed for virus replication. ORF2a, ORF2b, and ORFs 3 to 7 relate to viral structure proteins (GP: Glycoprotein). ORF5, relating to viral infectivity and neutralization, is most frequently used in molecular epidemiology to evaluate genetic variation among PRRSV strains. ORF1b encodes a non-structural protein, Nsp2, which has high genetic variation due to natural mutation (e.g., deletions and insertions) (Han, et al., 2006). ORF7 is also a target for demonstrating genetic variation (Murtaugh, et al., 1998).

Significant antigenic and molecular variation in PRRSV suggests that the virus consists of two distinct genotypes: type I (European genotype), and type II (North American genotype) (Wensvoort, et al., 1992).The homology of ORF5 between types 1 and 2 is about 55% (Murtaugh, et al., 1995). There is also a wide range of genetic variation within each type. Many areas in the world have now reported the presence of both types. Cross-protection between the 2 types and even between strains within each type is very limited. Recombination between PRRSV strains may occur and lead to PRRSV evolution. This recombination within type may be easier than recombination between types (van Vugt, et al., 2001).

Stability in the environment

The stability of PRRSV is rather low and the virus is quickly inactivated in the normal environment. In water, virus infectivity can remain for 1-6 days at 20-21°C, 3-24 hours at 37°C, and 6-20 minutes at 56°C. When stored at temperatures of –70° to –20°C; PRRSV can be detected in low titers for up to 30 days, but when kept at 4°C, 90% of its infectivity has been lost within one week (Zimmerman, et al., 2006). The virus can

survive at a pH of 6.0 to 7.5, and any change of pH out of this range can reduce the stability of the virus. Similarly, in serum or tissue, the virus is relatively easily inactivated at 25^{o}C. For example, when tested at the latter temperature only 47%, 14%, and 7% of the initially PRRSV-positive tissue samples had the PRRSV isolated at 24 hours, 48 hours, and 72 hours after the start of the experiment, respectively; however, when stored at 4^{o}C and freezing temperature (-20^{o}C) the isolation rates of the virus from these tissues was more than 85% after 72 hours (Bloemraad, et al., 1994). Pirtle and Beran (1996) reported that while the virus is stable in clean water for 9-11 days, it survives for just a few hours in swine saliva, urine, and fecal slurry.

The survival of PRRSV in the environment depends not only on temperature and pH, but also on other ambient materials and conditions. One study found that the viability of PRRSV in swine effluence is relatively short (1 day to 8 days) and infectivity is very limited (Dee, et al., 2005). The capacity of PRRSV in the air to be stable and infectious depends on temperature and relative humidity (RH). Aerosolized PRRSV was more stable at lower temperatures and/or lower RH, but temperature had a greater influence than RH on the half-life of aerosolized infectious PRRSV. For example, at 5^{o}C and 17% RH or 70% RH, the half-life of aerosolized infectious PRRSV was approximately 192 minutes and 118 minutes, respectively, while at 25^{o}C and 20% RH or 90% RH, the half-life was only 17 minutes and 19 minutes, respectively (Hermann, et al., 2007). The survival of PRRSV is related to the characteristics of the lipid envelope. Due to this envelope, lipid solvents (e.g., chloroform, ether, and detergents) can easily kill the virus. Conventional disinfectants can be used to inactivate the virus, eg., chlorine (0.03%) for

10 minutes, iodine (0.0075%) for one minute, and a quaternary ammonium compound (0.0063%) for one minute (Shirai, et al., 2000).

Pathogenesis

The PRRSV can invade the body via the respiratory system (e.g., airborne, nose-to-nose), blood (e.g., needles), reproductive system (e.g., contaminated semen, prenatal infection) (Zimmerman, et al., 2006), and digestive tract (Magar, et al., 1995). After penetrating into the body, PRRSV replicates in local macrophages or spreads to other lymphoid tissue via the blood stream. The primary target for replication is monocyte-derived cells with 220kDa glycoprotein receptors (Duan, et al., 1998). These cells include pulmonary alveolar macrophages, intravascular macrophages in the lung, macrophages in lymphoid tissue, subsets of macrophages in lymph nodes and spleen, and intravascular macrophages of the placenta and umbilical cord (Duan, et al., 1997). PRRSV replicates in macrophages and might induce lesions and clinical signs by the following mechanisms: (i) apoptosis of infected and nearby cells (Sirinarumitr, et al., 1998); (ii) induction of inflammatory cytokines resulting in increased levels of TNF-alpha, IL-1, and IL-6, which leads to activation of leukocytes and increasing microvascular permeability. This results in several lesions and clinical signs, including pulmonary edema, pyrexia, anorexia, and lethargy (Choi, et al., 2002). PRRSV infection also induces polyclonal B cell activation leading to lymphoid hyperplasia and reduced bacterial phagocytosis, which is related to increased susceptibility to secondary infections (Lamontagne, et al., 2001).

Viremia starts in the first 12h to 24h post-inoculation, with the highest titers occurring at 7-14 days. After reaching the maximum level in serum, virus titers decrease

rapidly and viremia may disappear by 28 days (Batista, et al., 2002a) or 56 days postinoculation (DPI) (Terpstra, et al., 1992). Tonsils are the most common tissue where the virus can be detected (Albina, et al., 1994; Albina, 1997; Horter, et al., 2002). This is primarily due to persistence of PRRSV in tonsils for up to 105 days (Horter, et al., 2002) or 225 days (Wills, et al., 2003) after infection. Persistence of PRRSV in serum, tonsils, or other tissues may depend on the strain of the virus and the age of the pig, i.e., the virus may persist longer in young pigs than in older pigs (Klinge, et al., 2009).

Lung, spleen, and thymus are also considered good sources of tissue for diagnosis (Van Alstine, et al., 1993). The virus remains detectable in lungs, lymph nodes, and spleen for 2-28 days (Rossow, et al., 1994). In some cases, virus can be isolated from heart, liver, and possibly kidney of infected pigs (Cheon and Chae, 2001) or in nasal, bronchial epithelium (Horter, et al., 2002) or spermatocytes (Swenson, et al., 1994). One study showed that meat from infected pigs does not retain detectable amounts of PRRSV (Larochelle and Magar, 1997). In sows, there is no evidence that PRRSV multiplies and causes damage in the ovaries (Sur, et al., 2001), but virus can access placenta and infect the fetal bloodstream (Prieto, et al., 1997). In boars, PRRSV was more often found in the epididymus than in the testes, and this fact explains shedding of virus in semen with consequent transmission of the disease through artificial insemination (Yaeger, et al., 1993). However, another study reported that PRRSV in semen did not only originate from infected testes, but also from the blood stream (Prieto, et al., 2003). The possibility of PRRSV detection in many tissues might also depend on the stage of infection, especially during the period of viremia (Klinge, et al., 2009).

The PRRSV may be present in many tissues, and many types of secretions and excretions from infected pigs may contain the virus. For example, PRRSV could be isolated from nasal secretions, saliva, urine, and sometimes feces (Wills, et al., 1997). Sows also shed virus via their milk (Wagstrom, et al., 2001). The periods of persistent shedding of virus differ greatly among different types of secretions and excretions, and may further vary between studies even for the same type of secretion or excretion. The most likely samples for detection of such a long-time carrier are oropharyngeal scrapings (Wills, et al., 1997; Batista, et al., 2002a).

Immunity

Different isotypes of antibodies against PRRSV have been reported. Immunoglobulin M was reported to appear at 5-7 DPI and reaches a peak at 14-21 days, but then rapidly waned to undetectable levels after 2-3 weeks. Immunoglobulin G directed against the nucleocapsid (N) protein appears from 7-10 DPI and remains for up to 300 DPI (Zimmerman, et al., 2006). Because of the higher quantity of IgG, it is the most common target for diagnostic tests. However, the duration of IgG production is very different among studies. For example, Yoon et al (1995) and Evan et al (2010) reported IgG production to last for approximately 36 weeks (Yoon, et al., 1995; Evans, et al., 2010), whereas Lager (Lager, et al., 1997) found antibodies persisted for up to 80 weeks.

Virus neutralizing (VN) antibodies produced against glycoproteins GP4 and GP5, and protein M appear about 3 weeks PI and are maintained at low levels for a long time. Neutralizing antibody responses varied greatly between individual pigs infected with different PRRSV strains (Lager, et al., 1997). This class of antibody is believed to protect

the animal against viremia (Yoon, et al., 1996; Plagemann, 2006). In contrast, other studies did not find the correlation between VN antibodies and clearing of the virus from the circulation (Osorio, et al., 2002), possibly because the level of VN antibodies was insufficient to clear the virus from the circulation. Thus, more research is needed to elucidate the role of VN antibodies. A vaccine capable of inducing VN antibodies would have the potential to prevent clinical disease and could be a key tool in eradication of PRRSV (Mateu and Diaz, 2008).

Immune sows provide maternal protection to piglets via colostrum. The decay of maternal antibodies was reported from 4 weeks to 10 weeks of age (Houben, et al., 1995; Zimmerman, et al., 2006; Liu, et al., 2008). No specific study has described the relationship between maternal immunity and susceptibility of piglets to PRRSV infection. However, observational studies found that the proportion of infected pigs increased when maternal immunity declined (Nodelijk, et al., 1997; Mateu and Diaz, 2008).

The immunity to PRRS vaccines is not well understood. A key issue in disease prevention strategies related to vaccination is protection of vaccinated animals against field isolates as well as cross-protection among different strains. The degree of protective efficacy of homologous and heterologous vaccines may be related to genetic diversity of viruses. Early studies showed that attenuated live vaccines produced a high level of protection with homologous strains and reduced disease severity, duration of viremia, virus shedding, and incidence of heterologous PRRSV infection (Albina, et al., 1994; Houben, et al., 1995; Chung, et al., 1997). However, many studies found that current vaccines, based on a single PRRSV strain, are either ineffective or are only partially effective in protecting against infections with heterologous strains of PRRS field virus

(Zimmerman, et al., 2006). Protection against PRRSV infection is also believed to be more complex than the comparison of genetic similarity between viruses would suggest (Kimman, et al., 2009). The level of genetic homology between vaccine strains and field strains is not an accurate reflection of vaccine efficacy (Prieto, et al., 2008). It is also possible that vaccine efficacy is associated with an efficient cell-mediated response (Martelli, et al., 2009). However, the topic of cell-mediated immunity is beyond the scope of this review and will not be included here.

Clinical signs

Clinical signs of PRRS vary greatly from very mild to severe disease due to many factors: virus strain, host immune/susceptibility status, concurrent infections, and management factors. During an epidemic infection in a naïve herd**,** there are two phases at herd level. The first phase lasts for 2 or more weeks, with anorexia and lethargy in 5%-75% of animals in one or more production stages, and subsequently (within 7-10 days) observed in all production stages with some additional clinical signs such as high fever, hyperpnea, and cyanosis of extremities. The second phase is characterized by reproductive failure in the third trimester of pregnancy, with high preweaning mortality. This phase may last up to 4 months and continue as an endemic disease (Zimmerman, et al., 2006). In an endemically infected herd, PRRS is characterized by a variable abortion rate, irregular return-to-estrus, high preweaning mortality, and occasional acute outbreaks (Stevenson, et al., 1993)

In diseased sows, later-term reproduction failure with mummification of fetuses, small weak-born piglets, and sometimes live abnormal piglets are typically observed.

One to four percent mortality of infected sows related to pulmonary edema and/or nephritis can occur (Hopper, et al., 1992). Occasionally, abortion rates may reach 10%-50% and other signs, such as agalactia, atrophic rhinitis, sarcoptic mange, and nervous signs (including incoordination, ataxia, circling, and paresis) may be also observed (Halbur and Bush, 1997). Return-to-estrus may be delayed. In suckling pigs, preweaning mortality can reach 60% and clinical signs may include splay-legs, dyspnea, and sometimes paddling. In grower pigs, most cases relate to anorexia, lethargy, hyperpnea, and mortality of 10%-12% (Stevenson, et al., 1993).

Transmission

Direct routes include contact with infected pigs and infected semen, and vertical transmission from sows to offspring. Oral and nasal transmission have been proven under controlled field conditions (Magar, et al., 1995; Bierk, et al., 2001). Using the same needle or other tools for ear notching, tail docking, and teeth clipping are all potential methods of spreading PRRSV (Otake, et al., 2002b) . Naive sows can be infected if inseminated with infected semen (Benfield, et al., 2000). Vertical transmission during mid to late gestation has also been reported because the virus can cross the placenta (Prieto, et al., 1997).

Several routes of indirect transmission by fomites such as boots, coolers and containers, shipping parcels, and vehicles have been implicated (Otake, et al., 2002a). Between-herd transmission may occur with the introduction of infected pigs (i.e., gilt replacement). Other animals may also be mechanical vectors for PRRSV transmission. Flies and mosquitoes were identified as virus carriers in some preliminary studies (Otake,

et al., 2002c; Otake, et al., 2003). Mallard ducks were infected with PRRSV and it was believed that their migration might be involved in regional PRRS spread (Zimmerman, et al., 1997). However, this is still controversial (Trincado, et al., 2004).

Airborne transmission is inconsistent between studies. Experimental studies proved airborne transmission of PRRSV (Brockmeier and Lager, 2002; Kristensen, et al., 2004). In the field, airborne movement of the virus has been confirmed up to a distance of 9.1 km (Otake, et al., 2002). However, others failed to prove airborne transmission of PRRSV between farms over a shorter distance (Fang, et al., 2005). Airborne transmission may occur more readily with some strains of virus than others (Torremorell, et al., 1997). More specifically, a study showed that while PRRSV strain 1-8-4 can travel up to 9.1 km, strains 1-8-2 and 1-26-2 could not be detected at a distance of 2.1 km (Otake, et al., 2010). Despite some inconsistent findings, distance to infected farms is a major risk for the disease (Mortensen, et al., 2002). Studies show that using air-filtration systems can significantly reduce the risk of introducing the virus into a herd (Dee, et al., 2006; Dee, et al., 2010). Airborne transmission might also depend on weather conditions, e.g., temperature, humidity, wind, and precipitation. High temperature and high humidity can reduce infectivity of PRRSV in air by reducing the half-life of infectious virus (Hermann, et al., 2007). Thus, in practice, establishing PRRS-free herd sites in the winter time results in a higher risk of becoming infected than when herds are established during the summer.

Risk factors

Herd size could be considered a risk factor for many diseases. The larger farm has more chances to adopt practices which may introduce disease than small farms; for example, more sow replacements, number of workers, and sources of materials and equipment. In contrast, large farms often apply better biosecurity than small farms to prevent introducing pathogens. Thus, when analyzing risk factors, herd size should be taken into account. Studies examining the epidemiology of PRRSV differ in their assessment of the importance of herd size as a risk factor for PRRS. According to Mousing et al (1997) (Mousing, et al., 1997), herd size was not related to the risk of PRRSV seropositivity, while Holtkamp's study found that larger herd size increased the risk for PRRSV (Holtkamp, et al., 2010).

It is known that the movement of infected pigs, particularly the purchase of weaned pigs or replacement breeding animals, is the most important route of spread between herds (Mortensen, et al., 2002). Many studies have found the introduction of pigs from unknown or untested sources is a significant risk factor for PRRS (Mousing, et al., 1997; Zimmerman, et al., 2006). During transportation, transmission can also occur between infectious pigs and susceptible pigs, probably by nose-to-nose contact or by breaks in the skin of susceptible animals being contaminated with urine or feces of infected animals (Mortensen, et al., 2002).

Density of farms in an area or close proximity to other farms may be risk factors for PRRS (Mousing, et al., 1997). Infected boars can shed virus in semen and transmit to sows in other farms through artificial insemination. It is agreed that semen is one of the

most important routes of between-herd transmission of PRRSV (Mortensen, et al., 2002). For example, there was potential for rapid and widespread transmission of PRRS in Denmark in 1996 when the infection was introduced into artificial insemination (AI) centres (Mortensen, et al., 2002). In contrast, another study indicated that using artificial insemination with semen from PRRS-seropositive boars did not increase the risk of PRRS seropositivity for herds (Mousing, et al., 1997). The reason for these contrasting results might be that boars with antibodies to PRRSV, but that are not viremic, pose little danger, whereas an active outbreak in a boar stud with PRRSV shedding into semen is very likely to spread PRRSV to large numbers of herds.

Diagnostic tests

Diagnostic tests are used to detect virus or antibodies against the virus. Detection of PRRSV antibodies is the most common method because of the convenience, quick results, and low cost of serological tests. However, serological tests currently cannot distinguish between antibodies due to vaccination and antibodies induced by infection with field strains. Serological testing is thus very useful for monitoring herds that are presumably negative, and is best used when combined with testing of viral nucleic acid (Collins, et al., 1996).

Five serological tests to detect antibodies to PRRSV have been described: indirect fluorescent antibody (IFA), enzyme-linked immunosorbent assay (ELISA), blocking ELISA, serum neutralization (VN), and immunoperoxidase monolayer assay (IPMA). The most commonly used serological test for detection of PRRSV antibodies is ELISA. In North America, many diagnostic laboratories use a commercial ELISA (IDEXX

Laboratories) for detection of antibodies to both the US and European strains of PRRSV. A sample-to-positive (s/p) ratio of greater than or equal to 0.4 is considered a positive result, according to the manufacturer of the commercial test. The most recent release of the commercial ELISA (ELISA PRRS 3XR Test kit; IDEXX Laboratories, Inc, Westbrook, Maine) claims 99.9% specificity and the ability to detect eastern European strains.

PRRSV can be detected by isolation of the virus. Samples for virus detection should be submitted to the laboratory within 2 days after collection and kept at 4°C. Sensitivity of virus isolation appears low because not all PRRSV strains replicate in all cell types, and results depend on the type of sample used and the amount of virus in the sample. Serum, lung, lymph nodes, and tonsils collected between 4 and 28 DPI were found to be the most appropriate specimen types for isolation of virus. In addition, tissue samples collected from euthanized liveborn pigs of early farrowings, or from late-term abortions are more appropriate for virus detection than those from mummies and stillborn fetuses because of tissue autolysis (Zimmerman, et al., 2006). Oropharyngeal scrapings and lymph node samples are also more appropriate for detection of persistent PRRSV infection than serum and lung samples (Rowland, et al., 2003). Recently, molecular-based tools have been used to diagnose PRRSV infections by detecting specific viral RNA. These techniques are usually more sensitive than virus isolation. Reverse-transcription polymerase chain reaction (RT-PCR) is one of the most commonly used techniques to detect RNA virus in serum and in many types of tissues and secretions or excretion (Oleksiewicz, et al., 1998; Batista, 2005; Martínez, et al., 2008)

Prevention, control, and elimination

In general, strategies for prevention, control, and elimination of PRRSV have been published, but it is difficult to evaluate the effectiveness of each strategy under field conditions. For prevention, strategies such as a high level of external biosecurity can limit introduction of pathogens into a herd (Dee, et al., 2004). For control of the disease, strategies aimed to reduce spread of infection within a herd include the "McRebel" management system (management changes to reduce exposure to bacteria to eliminate losses), gilt acclimatization, and vaccination (McCaw, 2000; McCaw, et al., 2003). Finally, for elimination of PRRSV from a herd, test and removal, herd closure, depopulation, and rollover are considered (Corzo, et al., 2010).

External biosecurity is applied not only for prevention of PRRSV introduction but also to prevent other diseases. It is broadly understood as measures taken to limit the introduction of the pathogen into the herd. External biosecurity measures that could be applied to limit introduction of PRRSV into a herd rely on proper introduction of animals, equipment, and people, and control of other potential mechanical vectors, as well as filtration of incoming air (Dee, et al., 2004; Dee, et al., 2005). Of interest in this thesis is the issue of incoming breeding animals. Quarantine must be performed in an isolation barn for at least 30 days to clear pathogens and perhaps PRRSV before incoming animals are comingled with other pigs in the barn (Pitkin, et al., 2011).

The concept of the McRebel system has been introduced to control spread of pathogens in suckling pigs (McCaw, et al., 2003). This includes measures such as decreasing cross-fostering, culling poor-growing pigs, and changing needles between

litters or pens (McCaw, 2000). Although this strategy may not have a clear effect in eliminating PRRS, it should be used continuously to avoid re-infection after use of other elimination strategies or to ensure the success of other elimination strategies (Zimmerman, et al., 2006).

Gilt acclimatization is a practice used to expose replacement gilts to an endemic PRRSV strain to induce specific PRRSV antibody before introduction into the herd (Batista, et al., 2002b). Under ideal conditions, acclimatized gilts would get infected with PRRSV, produce antibody and are islolated for a period of time until they recover and do not shed the virus before entering the herd. Acclimatization can be performed by inoculating negative gilts with serum or tonsillar scrapings obtained from PRRSV viremic nursery pigs (McCaw, et al., 2003). These gilts rapidly become PRRSV-positive with a high rate of success. However, this method is costly and labor intensive because of the large amount of positive serum or tissue required to treat all gilts (Batista, et al., 2002b). Another less costly method of acclimatization is to inoculate only a proportion of gilts ("seeders") and allow the remaining gilts to mingle with the seeders (nose-to-nose contact), becoming infected. However , the success rate of this method is unknown. In fact, gilts that are infected late could be introduced into the herd while still viremic and this might cause outbreaks in the recipient herd (McCaw, et al., 2003).

The rationale for acclimatization is appropriate in the context of infection control in the recipient herd, but potential problems can be introduced if sufficient immunity in gilts was not mounted or if gilts with active infection are introduced into the herd. Thus, timing is very important to overcome this weakness. Acclimatization should be started as soon as possible after gilts arrive. It is recommended that the period of acclimatization

should be 30 days, and that this period should be followed by another 30 days for proper development of immunity and to avoid introduction of gilts with active infection (McCaw, et al., 2003). Virological and serological testing should also be used to determine the length of the acclimatization period. However, it is also advised that each farm develop its own criteria for acclimatization.

Vaccination against PRRSV, particularly with attenuated live vaccines, is commonly applied in swine populations, but the effect of vaccination is difficult to evaluate. This is in part because vaccination is used in a variety of different ways and for different purposes. However, one of the more frequently quoted reasons is the large diversity of PRRSV. Although there are 2 main types of PRRSV, cross-protection between types or within types is very limited. Thus, using the PRRS vaccine with homologous strain circulating in the farm is the key for a successful vaccination program. Modified live attenuated vaccine (MLV) showed some effect on reducing clinical disease and viremia under experimental conditions (Cano, et al., 2007b). However, the results of applying MLV in a herd infected with a heterologous PRRSV strain is not effective in terms of protection and reducing clinical disease and viral load in tissues (Cano, et al., 2007a). In addition, a PRRS vaccine strain can circulate in the herd, resulting in a persistently infected herd, or may revert to a virulent strain and cause disease (Kimman, et al., 2009). Mass vaccination – also called whole-herd vaccination – can be used to achieve stable immunity of the entire herd, and this is the first required step for a PRRS eradication plan. Mass vaccination with herd closure has been used as an effective strategy for control and elimination of PRRSV from sow and finishing populations (Philips and Dee, 2003; Gillespie and Carroll, 2003)

Test and removal is a method to eliminate PRRSV in a herd based on culling virus carrier animals. The principle of test and removal is to test for PRRSV antibodies using ELISA and for viral RNA in blood samples using PCR. Animals that are positive by either ELISA or PCR or both are immediately removed (Dee, S.A., et al., 2000). This is a highly efficacious, rapid method to eliminate PRRSV in a herd, but may be costly due to extensive testing. In addition, correct identification of the PRRS status of removed pigs is very important to avoid false-negatives or false-positives, and demands highly specific and sensitive diagnostic tests. During test and removal, the herd should be maintained at a high level of biosecurity, because transmission of PRRSV between infectious animals and the susceptible population would be very rapid. Farms should have enough facilities and buildings to separate the negative herd from the other animals.

Herd closure refers to a period of time during which no gilt replacements are introduced into the herd. This method is based on the idea that during closure time, sows become infective and resistant gradually and the infection dies out over time because no new susceptible sows are introduced. In order to ensure that all sows become infected and recover, closure time should be at least 6 months, depending on the production characteristics of the farm (Torremorell, et al., 2003). Herd closure is less expensive than the test-and-removal strategy, but its success varies between herds (Sandri, et al., 2010). Herd closure is frequently used in combination with other strategies such as immunization in order to eliminate infection from sow herds over a period of time. Such a strategy is called “rollover” and is frequently applied to sow herds managed under North American conditions (Corzo, et al., 2010)

Depopulation/repopulation is a strategy that can eliminate not only PRRS but also other diseases (Blanquefort and Benoit, 2000). This method is effective but very costly. As a part of this strategy, thorough cleaning and disinfection should be performed after depopulation, the barn should be empty for at least 1 month, and gilts should be introduced from a herd that is free of PRRSV infection (Zimmerman, et al., 2006).

On a larger scale, in order to control, eliminate, and prevent a disease in a geographical region, a surveillance program should be used to detect the presence of that disease in each herd in the areas of interest (Stark, et al., 2006). For example, Chile has reported the successful elimination of PRRSV from the country after a national surveillance program had been initiated in 2000 (Osorio, 2010). Regulation of surveillance (regarding routine testing, numbers of animals to sample, type of samples to test, and specific diagnostic tests) is the main issue for this program for early detection of disease in an area. More studies are needed to contribute technical information to construct an effective surveillance program.

PRRSV-related disease: porcine high fever disease (PHFD)

Since June 2006, many pigs in 6 provinces of China have died of a disease characterized by high fever, redness of the skin, and dyspnea. Between June and September of 2006, total morbidity due to the disease was approximately 2 million pigs, with at least 400,000 deaths (Normile, 2007). Because the causative agent was unknown, this outbreak was called “porcine high fever disease” (PHFD). In 2007, PHFD continued to spread to the west and the south of the country and included all types of production

(backyard to large intensive farms). As a result, pig production in China dramatically declined and the price of pigs and pork increased during 2007 (McOrist and Done, 2007).

The main characteristics of PHFD are as follows. All ages of pigs are affected, but generally the disease starts with sows and spreads to other ages (Tong, et al., 2007). The disease transmits quickly from one farm to another in a large area. In an affected farm, the outbreak might last for 1-3 weeks. Morbidity ranges from 50%-100% and mortality is approximately 20% in sows and finishing pigs and 70%-100% in nursing and nursery pigs. Clinical signs in sows have been characterized by abortions at different stages of gestation. Affected animals of all ages show depression, anorexia, lethargy, and rubefaction of skin. Respiratory signs include sneezing, coughing, and dyspnea. Other signs that may be observed include conjunctivitis, diarrhea or constipation, neural signs, and cyanosis of the extremities (e.g., ears) (Zhou and Yang, 2010).

Epidemiological investigations and laboratory testing have shown that this disease may be related to a combination of many pathogens, such as infection with classical swine fever virus, PRRSV, porcine circovirus type 2, and bacteria including *Streptococcus suis*; *Actinobacillus pleuronemoniae*, or *Pasteurella multocida* (Tian, et al., 2007). Laboratory results based on virus isolation from dead pigs confirmed that PRRSV was closely associated with the disease (Tian, et al., 2007; Zhou, et al., 2008). However, challenge trials were inconsistent in causing deaths (Zhou, et al., 2008; Wu, et al., 2009). Thus, the cause of PHFD is still unclear. There were at least 56 variants of PRRSV isolated from different outbreaks. All viral isolates belonged to the distinct US genotype. In particular, the 2 discontinued deletions in NSP2 encoded by a region in the PRRSV genome were identical in all variants (Tian, et al., 2007; Feng, et al., 2008).

However, these deletions were proven not to be associated with virulence of the PRRSV, thus, they have been used as a marker for PRRSV associated with PHFD (Zhou, et al., 2009). Due to high mortality and the close relationship with PRRSV, PHFD is sometimes called highly pathogenic PRRS or atypical PRRS. In 2007, PHFD spread extensively in China and to other neighboring countries, including Vietnam, with the PRRSV rapidly evolving (Normile, 2007).

In Vietnam, the initial outbreak of PHFD (locally called blue ear disease) occurred in Hai Duong (a northern province of Vietnam) in March 2007 with approximately 580 deaths (Pham and Dam, 2007). Subsequently, PHFD spread to many provinces in the north and center of the country. During 2008, PHFD spread across the country and killed at least 300,000 pigs (Department of Animal Health of Vietnam, 2009). Clinical signs and lesions of the disease were similar to the descriptions in Chinese outbreaks. Moreover, studies confirmed that PRRSV strains in the outbreaks in Vietnam were 99% identical to the reported strains in China at the genomic level (Metwally, et al., 2010).

From the beginning of the outbreak, the national veterinary service cooperated with the World Organization for Animal Health to diagnose, confirm, and control the outbreak. Quarantine of animals, disinfection of outbreak areas, movement control, and destruction of diseased animals were all applied (Department of Animal Health of Vietnam, 2009). Vaccination was cautiously recommended for producers. However, PHFD continued to occur during 2008 and 2009. In 2010, the first 200,000 doses of a PRRS attenuated vaccine based on a Chinese strain, JAX1, were officially recommended (Department of Animal Health of Vietnam, http://www.cucthuy.gov.vn/). However, until

2010, the outbreak appeared to be continuing (provincial veterinary service, personal communication).

Many issues relating to the failure of control measures in the country are the following ones. (i) Most of the farms in the country (70%) are small households which raise less than 20 pigs (Huynh, et al., 2006). This makes application of all control measures very difficult. (ii) Although pig production is on a small scale, close proximity of households results in many high-density pig regions. (iii) It is difficult to supervise pig movement. Gilt and post-weaning pigs can be purchased and moved between farms without certification of disease status. Laboratory service is not available everywhere for farmers to test for disease, or farmers are not aware of the importance of testing. In addition, breeding farms or certified farms do not supply enough pigs to meet the demand. (iv) The compensation policy has not been sufficient. There was a tendency for farms to market diseased pigs because of the low compensation price. This made control strategies more difficult. (v) Many other major swine diseases are prevalent in the country, e.g., classical swine fever, foot-and-mouth disease (FMD), and leptosirosis, making identification of PHFD more complicated. (vi) Biosecurity is not well applied in small and large farms. (vii) Destruction of pigs and their disposal during outbreaks has not been well organized, leading to soil and water pollution and posssibly to the distribution of virus in the water source or conservation of the virus in the soil. (viii) Not many studies were performed on the genetic variation of PRRSV in the country. If more studies were conducted, researchers would have a better understanding of the virus. (ix) Most reports from the outbreaks were the results of monitoring and surveillance activities. There have been no targeted epidemiological studies. Thus, major risk factors

have not been identified. Better understanding of the risk factors would help with development of control strategies.

Overview of novel methods applied to study the epidemiology of PRRS

Spatial epidemiology methods

Geographical information systems (GIS) have become widely used as tools for management, display, and analysis of spatial data. In addition, availability of spatial data, advances in development of spatial statistical methods, and increased availability of such methods in commonly applied statistical software have all contributed to the increased use of such methods. In general, spatial epidemiology is defined as "*the description and analysis of the geographic, or spatial, variations in disease with respect to demographic, environmental, behavioral, socioeconomic, genetic, and infectious risk factors*" (Elliott and Wartenberg, 2004). In the field of veterinary medicine, spatial epidemiology has been used widely in investigating diseases such as equine grass sickness, infectious bursal disease, and especially foot-and-mouth disease (French, et al., 2005; Sanchez, et al., 2005; Picado, et al., 2007). For PRRS, Goldberg (2009) explored the spatial autocorrelation of genetic variation in PRRSV in Illinois and Iowa, and Mondaca-Fernandez et al (2007) used spatial analysis in a program to control the disease in the United States. However, application of quantitative spatial epidemiological techniques in studying PRRS spread and control has generally been limited. In this review, we will mention some useful techniques used in spatial epidemiology, including disease mapping, disease clustering, and disease cluster detection.

Disease mapping is the act of visualizing the spatial distribution of health outcome data on a map to summarize and visualize spatial variation in the occurrence of outcome, to generate hypotheses about disease etiology, and to highlight areas of higher risk (Pfeiffer, et al., 2008). The product of disease mapping is called a disease map, and depends on characteristics of the data. There are many types of disease maps that can be produced. For point data (e.g., cases or outbreak locations), dot or spot maps are commonly used. For areal data (e.g., number of cases in given areas or regions) choropleth maps are commonly used. Isopleth maps are used to display continuous data (e.g., prevalence, risk, or relative risk), and are frequently based on the application of special techniques to extrapolate unsampled locations and smooth the observed values between neighboring areas.

Disease clustering is the tendency of observations to be situated closer to one another than what would be expected (Berke, 2005). This tendency can suggest the presence of an infectious agent (i.e., the disease is transmissible). Disease clustering can be tested by many methods according to the characteristics of the data. For example, Cuzick and Edward's test for point data compares the number of observed cases in each of the k-nearest neighbors of the case to the number of cases in each of the k-nearest neighbors of cases that are based on randomizations of disease labels for observed locations (Cuzick and Edwards, 1990). Spatial clustering has also been evaluated using the difference between the K-function for diseased and non-diseased locations (D-function) (Diggle and Chetwynd, 1991).

A disease cluster is a collection of cases in a high risk area. More specifically, it is defined as "a geographically bounded group of occurrences of sufficient size and

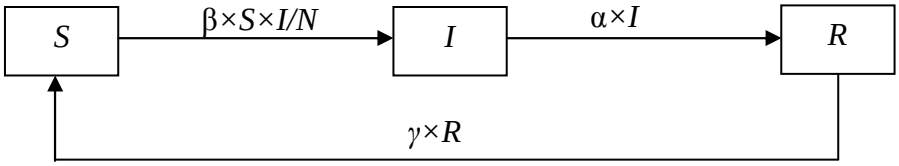
concentration to be unlikely to have occurred by chance" (Knox, 1989). The value in detecting disease clusters is to suggest the presence of an environmental risk factor (e.g., stronger environmental exposure of subpopulation, that the disease is not communicable, or that a single outbreak happened). One of the more common methods for the detection of spatial clusters is spatial scan statistics. For point data, a common approach is to use a purely spatial scan test based on the Bernoulli model (Kulldorff and Nagarwalla, 1995). The basis of this spatial scan statistic can be simplified in that for each specified location, a series of windows varying in size is constructed until the windows include a fixed percentage of the total population. For each window, a test statistic value is calculated (T_K) and the alternative hypothesis is that there is an elevated risk of disease within the window, compared to that outside the window. Monte Carlo simulation is performed to compare (T_K) with the distribution of values generated under the null hypothesis.

Another relatively new method to detect disease clusters is the spatial relative risk surface. This method uses a ratio of disease density and non-disease density (Waller and Gotway, 2004; Davies and Hazelton, 2010). Specifically, kernel smoothing, using quartic kernels and a fixed band-width, is used to estimate the density of cases and noncases on a grid map. Then the spatial relative risk surface is constructed using a ratio of case density to non-case density. This observed spatial relative risk surface is compared with relative risk surfaces on the basis of Monte Carlo simulation of case labelling. When the observed spatial relative risks rank higher than relative risk of 95% of randomly labelled datasets, that is considered to be a high-risk area and to represent a significant spatial cluster.

Mathematical Modeling

A mathematical model is a description of a system using mathematical concepts and language used in many fields of sciences such as physics, biology, earth science, and meteorology. In medicine, mathematical models have been widely used to understand infectious disease transmission of outbreaks to extrapolate from current information about the state and progress of the outbreak, to predict the future, and, most importantly, to quantify the uncertainty in these predictions. In veterinary medicine, many diseases have been investigated using mathematical models, such as FMD and avian influenza (Kitching, et al., 2006; Tracht, et al., 2010). For PRRSV, two such studies were both based on European data (Nodelijk, et al., 2000; Evans, et al., 2010). Among mathematical models, the deterministic susceptible-infectious-resistant-susceptible (*SIRS*) compartmental model is one of the most commonly used and will be reviewed here.

Here, a deterministic compartmental model is considered to analyze the transmission, spread, and effect of contagious pathogens. In brief, a population (*N*) can be divided into susceptible (*S*), infectious (*I*), and resistant (*R*) compartments. Susceptible individuals become infectious immediately after sufficient contact with an infectious individual. Once infectious, an *I* individual can infect others, the number depending on the frequency of effective contact with susceptible individuals and the length of time the individual remains infectious. At the end of the infectious period, the individual becomes resistant (R) for a period of time, later losing this immunity and becoming susceptible. Differential equations are used to explain the movements of individuals in N between compartments at time t (Vynnycky and White, 2010)



where:

$\frac{dS}{dt} = \beta SI/N$ defines the flow from *S* to *I*; β is called the transmission parameter and is defined as the rate of becoming infected, given the proportions of *S* and *I*.

$\frac{dI}{dt} = \alpha I$ is the flow from *I* to *R*; α is called the recovery rate. It is defined as the rate of leaving the infectious compartment and is equal to the inverse of the duration of the infectious period (1/α).

$\frac{dR}{dt} = \gamma R$ is the flow from *R* to *S*; γ is called the immunity decay rate, which is defined as the rate of leaving the resistant compartment and is equal to the inverse of the duration of the immune period (1/γ).

In addition, a key parameter in this modeling method is the basic reproduction number (R_o=β/α), which is defined as the number of secondary infections from a single infectious case in a completely susceptible population (Vynnycky and White, 2010). This value depends on many factors, including contact rate, transmission probability, and duration of the infectious period. When R>1, an outbreak will occur in a herd, and when R<1, the disease will fade out and disappear. When R=1, disease becomes endemic in the population.

Several studies have tried to estimate R_o for PRRS with inconsistent results. Assuming that the duration of the infectious period is 56 days, R_o of sows in a breeding herd was calculated to be 3 (1.5-6) (Nodelijk, et al., 2000). In nursing and nursery pigs, two studies estimated R_o with different results. One study found that the mean R_o for a particular strain was approximately 0.463 (0.124-1.656), and there was no difference between vaccinated and nonvaccinated pigs on R_o (Mondaca-Fernandez, et al., 2007). The other study found that R_o was > 4.9 in vaccinated pigs (Nodelijk, et al., 2001).

Using mathematical models, many questions related to disease control can be foreseen. For example, a small farm (approximately 100 sows) can eliminate PRRS in 6 years (Nodelijk, et al., 2000). Or, comparing R_o in vaccinated and nonvaccinated herds, researchers failed to prove the effect of PRRS vaccination in disease control (Nodelijk, et al., 2001). Mathematical models have been underutilized for studying infectious diseases in the field of veterinary epidemiology and should be used to support disease control programs.

Objectives

Several issues of PRRS have not been well studied, especially transmission, control, and elimination of the disease. In addition, the emergence of PHFD related to novel PRRSV strains with a high clinical impact could be considered a threat to pig production around the world. There is a need to understand the spread of PHDF under field conditions. This understanding, combined with knowledge concerning the dynamics of PRRSV infection, can form the basis for selecting control and elimination programs at herd level, for PHFD specifically, or for PRRS generally. Mathematical modeling would be an appropriate method to estimate the effectiveness of these control and elimination

programs. Finally, for PRRS control on a regional scale, disease surveillance is very important. Two important issues for disease surveillance are which pigs to sample, and what values of sensitivity and specificity for the diagnostic test should be considered. Thus the specific objectives of this thesis will be to:

(1) Describe an outbreak of PHFD in an area of Vietnam in order to understand the frequency of mortality and morbidity, and to report clinical signs observed during the outbreak (*Chapter 2*).

(2) Explore some epidemiological aspects of PHFD with respect to spatial and spatio-temporal spread and risk factors for its emergence (*Chapter3*).

(3) Understand the dynamics of PRRSV infection in a herd (*Chapter 4*).

(4) Evaluate strategies to control and eliminate PRRSV in a herd using mathematical modeling (*Chapter 4*).

(5) Contribute to surveillance of PRRSV by estimating sensitivity and specificity of diagnostic tests for PRRSV (*Chapter 5*).

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Chapter 2

Investigation of mortality and morbidity during an outbreak of "Porcine High Fever Disease" in a small area of Vietnam

Written in the style of "Research in Veterinary Science"

Abstract

Outbreaks of porcine high fever disease (PHFD) involving variants of porcine reproductive and respiratory syndrome virus and other pathogens have occurred in East Asia since 2006, at huge economic cost. A retrospective cross-sectional study was performed in a small area in southern Vietnam to describe an outbreak of PHFD in terms of clinical signs and mortality. Case definitions were based on clinical signs. Analysis of mortality and morbidity were performed using descriptive statistics, Poisson, negative binomial regression models, and random effect logistic regression: 33.4% of households were classified as PHFD cases. Case households exhibited clinical signs consistent with the multifactorial nature of PHFD. The negative binomial model best described the data. The random effect model showed that household contributed more to mortality variation

than hamlet, suggesting that household-level practices had been driving the emergence of PHFD

Introduction

An outbreak of unusual swine disease characterized by severe clinical signs and high mortality across all age groups was initially described in 2006 in China (McOrist and Done, 2007; Tong et al., 2007). Clinical signs of this outbreak included high fever, high proportion of abortions, premature delivery, inappetence, coughing, respiratory distress, and death in severe cases (Li et al., 2007; Tian et al., 2007). Within months, the disease appeared to spread across large geographic areas resulting in considerable economic loss to the swine industry and social impact on rural communities. At that time, the causation of this condition was uncertain, therefore, it was called porcine high fever disease (PHFD).

Results from outbreak investigations and laboratory testing have linked this disease to porcine reproductive and respiratory syndrome (PRRS) virus which causes numerous clinical signs associated with the reproductive and respiratory systems. However, unlike earlier outbreaks of PRRS, this outbreak caused very high morbidity (50%–100%) and mortality (20%–100%) in all production classes, and thus, the disease is also referred to as atypical PRRS (Tian et al., 2007). Variants of PRRS virus (PRRSV) isolated from PHFD outbreaks in China shared a 30-amino acid discontinued deletion in the Nsp2 coding region, unlike typical type-2 PRRSV, and these variants were considered to cause PHFD. Several challenge trials confirmed their virulence, although mortality varied (Zhou et al., 2008; Wu et al., 2009). Later, this deletion was reported not to be

related to the pathogenicity of the virus, but to act as a useful marker for PRRS variants linked with PHFD (Zhou et al., 2009). The true mechanism of PHFD is not well understood. It was believed that the disease was caused by a combination of PRRSV and other viral pathogens, including porcine circovirus type 2, classical swine fever (CSF) virus, pseudorabies virus, and several bacterial species, such as *Pasteurella multocida*, *Actinobacillus pleuropneumoniae*, and *Streptococcus suis* (Li et al., 2007).

Subsequently, the outbreak of PHFD spread to neighboring countries during 2007 and 2008, especially to Vietnam, where it caused enormous losses and was called "blue ear disease," because of transient cyanosis of the ears in some pigs (Normile, 2007). Total deaths, including animals culled for disease control purposes, exceeded 300,000, and 26/60 provinces were affected during 2008 (Department of Animal Health of Vietnam, 2009). The national veterinary service made efforts to control the outbreak, but the disease continued to occur across the country (Nguyen, 2010).

Previous studies described the disease at the individual animal level in terms of clinical signs present in PHDF cases (Le, 2007; Li et al., 2007; Department of Animal Health of Vietnam, 2007). In contrast, national reports described the disease as a large-scale outbreak (provincial or national basis) in which mortality was reported as a key parameter. Most commonly, these mortalities were calculated without taking into account either the variability of swine herds in different areas or the time periods during which the disease occurred. Thus, this study was conducted in a small area to describe the outbreak with two specific objectives: (1) to identify clinical signs related to occurrence of PHFD at the herd level; and (2) to estimate mortality during this outbreak of PHFD while accounting for herd membership.

Materials and Methods

Study design

A 10x10-km rectangular region was selected from a province in the south of Vietnam where local veterinary services reported an outbreak of PHFD in 2008. This study area included 5 communes with 37 hamlets and is considered one of the most pig-dense production areas of the province. An investigation was performed by a group of trained veterinary students in conjunction with local veterinarians in the study area from July to August 2009. Almost all households that raised pigs in this area during 2008 were visited to be interviewed. The responder for the interview was the person responsible for raising pigs in the household.

The questionaire for this investigation was initially developed in English by epidemiologists working in the field of swine health. It was translated into Vietnamese, then field-tested, and further modified by local experts in animal health. The final version of the questionnaire contained 3 sections: a general information section (such as farm identification, number of pigs in each production class), a section related to occurrence of clinical signs suggestive of PHFD, and a section related to management practice (see Appendix 1). The second section was designed to determine if health problems had occurred and began with a key question asking producers whether they had had “unusual disease” occurring in their pigs during 2008. If the answer was “yes” (first response), producers would be asked to identify clinical signs they observed during this unusual disease, the calendar time of its onset, and duration. In the questionaire, possible clinical signs were entered into a list including all clinical signs of PHFD reported in other areas

of Vietnam, as well as other clinical signs suggestive of other diseases (Table 2.1). In total, 21 clinical signs were listed for each pig production class including sows, young pigs (less than 60 days old), and finishing pigs. Additional space was provided for other clinical signs not in the list. All technical terms for clinical signs were offered as informal words that were expected to be easily understood by farmers (Table 2.1). If the answer for the key question was "no," the instrument asked the producer to indicate the presence or absence of any clinical signs (with calendar time and duration) in the same list in the data collection instrument (second response) in 2008. If interviewees reported no usual disease and no clinical signs, they were asked to report the number of pigs that had died during June of 2008 (third response). For each response category, producers were asked to indicate the number of pigs in each production class that had died, so that all herds would have data concerning mortality, inventory, and a time period over which mortality occurred. If households were not included in any scenario, their herds were recorded as "not any disease." Spatial analysis of data based on this questionnaire has been previously published (Le et al., 2011).

Case definition

Because of the retrospective nature of the study, no samples were available for laboratory investigation. However, before this period, PHFD outbreaks were officially declared in three neighboring provinces with typical clinical signs and high mortality (Department of Animal Health of Vietnam, 2007). In this study, the final case definition of PHFD was described at the household level and was based on a combination of reported clinical signs. The combination of clinical signs constructed was based on reports in the literature (Le, 2007; Li et al., 2007; Tian et al., 2007; Zhou et al., 2008),

and further validated by epidemiologists working in swine health and experienced veterinarians in the study area. For herds that contained sows, death or abortion accompanied by one of the suspected signs, including high fever, stillbirth, cyanosis of the ears (blue ears), respiratory problems, or mortality of young pigs over 20% was considered PHFD. For herds that had other production classes, a death rate of more than 10% with fever and respiratory signs was considered PHFD.

Data analysis

Descriptive statistics and clinical signs

Questionnaire data were entered into Epidata software (version 3.1, EpiData Association, Odense, Denmark, 2005), checked for validity, and then exported to Stata software (Stata Intercooled, version 10; Stata Corporation, College Station, Texas, USA) for data management. The overall mortality of each pig production class in each of the five communes in the study area was calculated. The order of responses in which producers reported "unusual disease" was used as an additional stratum. Durations reported in the first and second responses were compared using the Mann-Whitney non-parametric test. The numbers of case and non-case households in the first and second response were used to calculate odds ratio (OR) in order to evaluate association between response order and case status. For each production class, a simple logistic regression was used to investigate association between PHFD status and each clinical sign that was not included in the case definition.

Estimating mortality rate and transformed mortality risk

Reported durations of unusual disease were considered as the outbreak periods for each household. Mortality rate (number of deaths per pig-week) was calculated for each household. The mean and 95% confidence interval of mortality rate were constructed using standard Wald asymptotic confidence limits. Then, mortality risk in one week of the outbreak was calculated from the mortality rate by using the transformation equation (Dohoo et al., 2003).

Estimating mortality risk during the outbreak

The following approaches were used to estimate mortality risk during the outbreak period. In order to distinguish among these mortalities calculated by different approaches, we will refer them as *overall, raw, Poisson, negative binomial*, and *Poisson random effect mortality* risks. Specifically, these measures are as follows. (1) The *overall mortality* risk was calculated by dividing the total number of deaths by the total number of pigs in the area (i.e., herd membership not accounted for); the 95% confidence interval was based on the Bernoulli distribution. (2) *Raw mortality* risk was calculated as an arithmetic mean of individual household mortality risks based on the Gaussian distribution. The following regression models were used to estimate mortality: (3) *Poisson*, (4) *Negative binomial*, and (5) *Poisson random effect* with hamlet as a random intercept. All of these regression models used the number of pig deaths in the household as the outcome and inventory as the offset, with no other covariates. The Akaike information criterion (AIC) values from these models were compared to identify the model that best fit the mortality data. The mortality risk from all of the above approaches was calculated separately for case households and non-case households.

Variability of mortality at different hierarchical levels

The data were expanded to the individual pig level for each household to fit a random effect logistic regression with three hierarchical levels, i.e., individual pig, household, and hamlet. This model had only an intercept (no covariates) and used all household data ("full data" scenario):

$$\text{Logit}(p_i) = \beta_0 + u_j + v_k$$

Where u, v are the random effect of household *j* and hamlet *k*, respectively, containing individual pig *i* with possibility of death p_i, and it is assumed that $u_j \sim N(0, \sigma_u^2)$ and $v_k \sim N(0, \sigma_v^2)$. Variance components (VC) and intra-cluster correlation coefficient (ICC) were calculated using the latent response variable method (Snijders and Bosker, 1999) as follows:

$$VC_{\text{household}}(\%) = \sigma_u^2 \times 100 / (\sigma_v^2 + \sigma_u^2 + \pi^2/3)$$

$$VC_{\text{hamlet}}(\%) = \sigma_v^2 \times 100 / (\sigma_v^2 + \sigma_u^2 + \pi^2/3)$$

$$ICC_{\text{household}} = (\sigma_v^2 + \sigma_u^2)/(\sigma_v^2 + \sigma_u^2 + \pi^2/3)$$

$$ICC_{\text{hamlet}} = \sigma_v^2/(\sigma_v^2 + \sigma_u^2 + \pi^2/3)$$

Due to the concern that very small herds might have a disproportionate influence on VC and ICC, this process was repeated by including only households with more than 3 sows, or 30 young pigs, or 10 finishing pigs (this refers as the "reduced data" scenario). The analysis was performed using pseudo-likelihood estimation with proc GLIMMIX in SAS 9.2 (SAS Institute Inc., Cary, NC).

Results

General production information in the study area, including the total number of investigated households and the numbers of pigs in each production class in five communes, is shown in Table 2.2. Out of the 955 investigated households, 885 (92.7%), 786 (82.3%), and 487 (51%) households had sows, young pigs, and finishing pigs, respectively. Most of these households were small scale farms. The number of sows varied greatly from 1 to 350 sows with a median of 3. The median numbers of young pigs and finishing pigs in each household were 22 (range 2 to 800) and 20 (range 1 to 700), respectively. The percentages of dead pigs in the whole study area during unusual disease periods were 7.9%, 10.3%, and 2.8% for sows, young pigs, and finishing pigs, respectively.

Table 2.3 shows that 670 (70%) households reported health problems with a median duration of 5 days (range 1 to 60 days). Among those, 576 households (86%) reported unusual disease in their pigs (first response), while 94 households (14%) had to be asked indirect questions to explore these health problems (second response). No households reported unusual disease and mortality without any clinical signs (third response). The Mann-Whitney test results showed that first responders reported a longer duration of disease than second responders (p= 0.011). In total, 319 households (33.4%) were classified as case households, with 290 case households (91%) classified from the first response. Similarly, among 670 households reporting health problems, 50% of the first response households, and 30.85% of the second response households, were eventually classified as case households. A first response household was more likely to

be a case household than was a second response household, with the OR = 2.27 (95% confidence interval: 1.43 – 3.61).

Clinical signs reported in case and non-case households for each type of pig are shown in Tables 4, 5, and 6. Ignoring the clinical signs in the case definition, all clinical signs in sows except epistaxis and neurological signs were significantly associated with case status at herd level (Table 2.4). In young pigs, weakness at birth, inappetence, depression, erythema, lameness, neurological signs, and petechiae on skin were significantly associated with case status (Table 2.5). In finishing pigs, diarrhea, inappetence, erythema, depression, petechiae, cyanosis of ears, eyelid edema, neurological signs, conjunctivitis, and constipation were related to case status (Table 2.6).

Figure 2.1 depicts the variable distribution of raw mortality risk levels in case households. A high proportion of households had no pig deaths reported, and then the proportion of affected households decreased in the later mortality categories. However, the highest category, 80%-100% mortality, showed a higher proportion than expected from the declining trend . Among the approaches to calculate mortality risk, the negative binominal method showed the best-statistical fit to the data, with the lowest AIC values in each case status and production class (Table 2.7). Mortality risk in case households was always higher for sows than for other production classes. Mortality risk estimated from the *negative binomial* estimation was very similar to the *raw* mortality risk but with wider confidence intervals. Both the *raw* and *negative binomial* approaches reported larger point estimates than those based on *overall* calculation, *Poisson*, and *Poisson random effect* models. Mortality rates and transformed mortality risk in one week of health problem for each production class and case status are shown in Table 2.8. Logistic

random effect regression showed that household level contributed more to variability in mortality than hamlet level (VC = 34.2% and 16.6% in sows, respectively) (Table 2.9). VC calculated ignoring very small households resulted in similar values of VC calculated from full data.

Discussion

In the study area, the emergence of this new disease was characterized by a mortality risk that was higher in sows and lower in finishing pigs. Unfortunately, typical pig mortality in the local area had not been published previously, but mortality in these outbreaks could be compared to standard productivity in modern commercial herds. Ignoring herd membership, the percentage of dead pigs in all production classes was higher than usual for commercial herds. The percentage of death in sows in the whole area during periods of outbreak (7.9%) was higher than on standard commercial pig herds in a year i.e., 3.3% to 5.7% (Chagnon et al., 1991; Duran, 2001). For young pigs, the percentage of dead pigs (10.3%) was high (Lemke et al., 2006), but might be acceptable if comparing to preweaning mortality (11.8%) in commercial herds (Cutler et al., 2007). However this is much higher than the accepted level of 6.8% in nursery pigs (de Grau et al., 2005). Mortality in finishing pigs in the study area (2.7%) was also higher than the standard mortality (less than 2.0% during the finishing phase) (Losinger et al., 1998). This high percentage of dead pigs, especially sows, confirms the occurrence of an unusual disease in this area. It was particularly unusual that mortality in sows was much higher than in finishing pigs. This indicates that the disease had a major effect on sows, in

concordance with reports from other outbreak areas (Department of Animal Health of Vietnam, 2007).

When household effect was taken into consideration using negative binomial regression, the mean sow mortality risk of case households was estimated to be 24.3% (20%-29.4%). This result is higher than that reported from earlier PRRS outbreaks in which sow mortality was approximately 5% to 10% during outbreaks of a 1- to 3-month period (Halbur and Bush, 1997), but might be less serious than PHFD outbreaks in China with 20% to 100% mortality (Zhou et al., 2008). This mortality risk appears, however, similar to other outbreaks in other provinces in Vietnam reporting sow mortality of approximately 23% (Department of Animal Health of Vietnam, 2007). However, these reports did not mention clearly the duration of the outbreaks.

Expected mortality risk in case and non-case households obtained from negative binomial regression was found to be much higher than the *overall* mortality risk and the risk calculated from the Poisson and Poisson random effect models. This can be explained by exploring the distribution of raw mortality risk (Figure 2.1). We found that the observed values were more dispersed than expected on the basis of a Poisson distribution. Thus, it was not surprising that negative binomial regression models were the best to estimate mortality, on the basis of negative binomial distribution (Biggeri, 2005) and the statistical criteria (e.g., AIC) from this data. This could be particularly relevant for study areas with small households. The use of negative binomial regression models to calculate mortality in smokers has been reported previously (Preston et al., 2010), while in veterinary medicine this regression was used to estimate the number of cases of disease on farms, the number of parasites in each animal or mastitis prevalence

in dairy herds (Nodtvedt et al., 2002; Schukken et al., 1991). The result of high variation in expected mortality estimated by different approaches suggested that, in reports of an outbreak at regional level, it would be highly recommended to report how mortality was calculated, so that meaningful comparisons could be made to other studies.

Mortality rate is another parameter that can be used to describe the severity of an outbreak, but to our knowledge, no report on PHFD mortality rate had been published previously. This is due to challenges of determining exact onset and duration of disease during field investigations, a necessary component of rate calculation. In our study, the median disease duration was observed to be 5 days, which was similar to other outbreaks in Vietnam (Pham and Dam, 2007), but shorter than in China with a range in duration of 5-20 days (Tian et al., 2007). The short duration in Vietnam might be due to small scale pig production. However, the time period of unusual disease in the questionnaire was difficult to obtain and might be subject to recall bias. The duration of clinical disease outbreaks should be recorded with as much accuracy as possible to have complete and useful measurements in terms of rates and risks.

The fact that most of the variability in mortality resided at the household level implies that emergence of disease was driven primarily by household-level factors, with relatively little impact of the higher organization levels (e.g., hamlets). This is in close concordance with the nature of risk factors for herd-level PHFD identified in a previously reported part of this study, where all significant risk factors identified were at the household level; neither hamlet-level factors nor the contextual variables at the hamlet-level could be identified as being associated with case status, and little spatial or space-time clustering was detected (Le et al., 2011). Thus, at least in this study area, a feasible

method of helping to prevent disease could be the use of compaigns to influence behavioral changes of individual producers (e.g., educational campaigns).

Besides the clinical signs listed in the case definition, many others were associated with case status, suggesting an association between PRRSV and concurrent infections in PHFD development. In sows, petechiae on the skin and conjunctivitis might be clinical signs associated with many diseases, including CSF or salmonellosis, while dermatitis and constipation might be associated with other diseases as well (Jackson and Cockcroft, 2007). Eyelid edema, with a very high associated OR, but few reports, might have a less reliable association with PHFD. In both young pigs and finishing pigs, the significant clinical signs, including diarrhea, neurological signs, erythema, petechiae, and conjunctivitis, might be related to several diseases, such as salmonellosis, CSF, pseudorabies, or streptococcal infection (Jackson and Cockcroft, 2007). All these mentioned diseases were known to be endemic in the Mekong Delta, which is where the study area is located (Kamakawa et al., 2006). However, wasting and dermatitis were not significantly associated with PHFD in young and finishing pigs. This might not support the possibility that porcine circovirus (PCV) is involved in PHFD development, at least not in this study. All these signs had also been observed in other PHFD outbreaks (Le, 2007; Pham and Dam, 2007; Tian et al., 2007). According to Department of Animal Health of Vietnam (2007), CSF, salmonellosis, pasteurellosis, streptococcal and mycoplasmal infection were concurrent with the outbreak, while outbreaks in China found PCV type 2, *Haemophilus parasuis*, *Streptococcus suis*, and *Pasteurella multocida* infections associated with PHFD cases (Li et al., 2007). Some clinical signs, such as epistaxis, were included in the list because they were easily observed and were expected

to be easily recalled, although they were not previously documented as important during PHFD outbreaks. The frequency of reporting this sign in either cases or control herds was low, and this improved validity of recalling clinical signs that existed there, because it is possible that swine producers did not just acknowledge presence of all clinical signs, but were trying to remember relevant clinical signs.

Case definition based on clinical signs was one of the limitations of our study. Because this was a retrospective study and data from the local veterinary service were unavailable, using case definition was the only means to achieve the purpose of the study. This case definition was validated from reports in the literature and from specialists' input as described in the materials and methods. Other reasons to ensure this definition were that PHFD was also reported in some other provinces during the time of interest (Department of Animal Health of Vietnam, 2009). These clinical signs were very characteristic and easy to record, and during the investigation, the veterinarians working in the study area joined the interview groups and helped us to communicate with farmers, and to clarify the clinical signs in each household. In addition, the method used to gain the first and second responses in our study was used to increase the sensitivity of the case definition. Specifically, if we had not included the second response, we might have lost 30.8% of cases in the area. This approach may provide a good model for other surveys in this area. After the collection of data with high sensitivity, we defined cases using serial interpretation of clinical signs – an approach that would increase specificity.

In conclusion, PHFD in this region was a severe outbreak that occurred during 2008 with high mortality in sows. Most of the variability in mortality resided at the household level, suggesting that interventions at this level would lead to the most

effective preventive measures. Concurrent clinical signs of PHFD revealed that the disease might be a combination of many endemic pathogens. There were inconsistent results between different methods of mortality calculation, and this was mostly due to the high variability among herds and large over-dispersion. Probably as a result of this variability in mortality, negative binomial regression was the method that best fit the data. Time component of disease occurences should be used to calculate mortality rate and mortality risk to describe future outbreaks.

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Table 2.1.

Description of clinical signs at the household level reported by producers

Clinical signs	Description
Abortion	Any kind of abortion in the late pregnancy period
Agalactia	No milk in lactation sows
Cyanosis of ears	Cyanosis or blue discoloration of the ears
Constipation	Very firm stools
Coughing	Prolonged coughing
Depression	Depression, or lethargy
Dermatitis	Any kind of infection on skin
Diarrhea	Watery stools, any kind of diarrhea
Conjunctivitis	Acute eye discharge
Dyspnea	Difficult to breath
High fever	Producers' assessment of elevated body temperature, typically by palpation on animals
Lameness	Difficulty in moving, walking or abnormal gait in walking
Epistaxis	Blood discharged from nose
Inappetence	Did not want to eat, lack of appetite, off-feed
Petechia on skin	Ecchymotic Hemorrhages, small red spots on the skin
Erythema	Producing redness on the skin, rubefaction.
Stillbirth	Piglet died at birth or some mummification
Eyelid edema	Swelling on eyelids
Wasting	Excessive and progressive weight loss
Neurological signs	Any kind of neurological signs such as ataxia, trembling or convulsion
Weak at birth	Very weak piglet at birth

Table 2.2.

Number of pigs raised and number of pigs that died during the PHFD outbreak in 2008

	Commune					Total
	1	**2**	**3**	**4**	**5**	
Investigated hh*	377	131	195	246	6	955
SOWS						
No. of hh*	360	115	176	229	5	885
Total sows	1503	1148	529	1265	14	4459
No. of sows/hh* (mean)	3.99	8.76	2.71	5.14	2.33	4.67
Total dead sows	57	90	72	132	0	351
% dead sows in the area	3.79	7.84	13.61	10.43	0.00	7.87
% hh. having dead sows	8.89	25.22	21.02	20.09	0.00	16.27
YOUNG PIGS						
No. of hh*	306	107	162	206	5	786
Total pigs	11593	4215	4078	7201	133	27220
No. of pigs/hh* (mean)	37.89	39.39	25.17	34.96	26.60	34.63
Total dead pigs	787	476	432	1093	6	2794
% dead pig in the area	6.79	11.29	10.59	15.18	4.51	10.26
% hh. having dead pigs	24.51	40.19	43.21	37.38	40.00	33.97
FINISHING PIGS						
No. of hh*	155	73	112	142	5	487
Total pigs	4658	2077	2176	6024	82	15017
No. of pigs/hh* (mean)	30.05	28.45	19.43	42.42	16.40	30.84
Total dead pigs	78	64	46	218	2	408
% dead pigs in the area	1.67	3.08	2.11	3.62	2.44	2.72
% hh. having dead pigs	8.39	12.33	4.46	11.97	20.00	9.24

*household(s)

Table 2.3.

Number of households raising pigs during 2008 and reporting health problems for the first or second response with duration of these problems

		Commune					**Total**
		1	**2**	**3**	**4**	**5**	
TOTAL	No. of hh*	377	131	195	246	6	**955**
Not any disease	No. of hh*	126	36	53	69	1	**285**
Health problems	No. of hh*	251	95	142	177	5	670
	Duration (days)	10.41	10.57	4.61	6.82	3	8.21
	Median	7	7	3	5	3	5
	Min-Max	[1-60]	[1-60]	[1-45]	[1-60]	[3-3]	[1-60]
1st response	No. of hh*	193	93	126	159	5	**576**
	Duration (days)	11.07	10.66	4.81	7.14	3	**8.48**
	Median	7	7	3	5	3	**5**
	Min-Max	[1-60]	[1-60]	[1-45]	[1-60]	[3-3]	**[1-60]**
2nd response	No. of hh*	58	2	16	18	0	**94**
	Duration (days)	8.27	7	2.92	4.13	0	**6.59**
	Median	5	7	3	3		**5**
	Min-Max	[1-60]	[7-7]	[1-7]	[1-7]	0	**[1-60]**
3rd response	No. of hh.	0	0	0	0	0	**0**

*household(s)

Table 2.4.
Number and percentage of households in total, case ,and non-case groups reporting specific clinical signs in sows

	Total hh[§] N=885	CASE N=308		NON-CASE N=577		OR	p
Clinical signs	No.	No.	%	No.	%		
Abortion*	242	220	71.4	22	3.8	-	-
High fever*	271	178	57.8	93	16.1	-	-
Inappetence	202	123	39.9	79	13.7	4.19	<0.001
Agalactia	118	69	22.4	49	8.5	3.11	<0.001
Dyspnea *	77	58	18.8	19	3.3	-	-
Lameness	104	52	16.9	52	9.0	2.05	0.001
Coughing*	86	50	16.2	36	6.2	-	-
Diarrhea	99	45	14.6	54	9.4	1.66	0.019
Erythema	56	44	14.3	12	2.1	7.85	<0.001
Depression	44	34	11.0	10	1.7	7.04	<0.001
Cyanosis of ear*	35	27	8.8	8	1.4	-	-
Petechiae on skin	28	21	6.8	7	1.2	5.96	<0.001
Conjunctivitis	32	20	6.5	12	2.1	3.27	0.001
Dermatitis	27	18	5.8	9	1.6	3.92	0.001
Constipation	22	13	4.2	9	1.6	2.78	0.020
Eyelid edema	13	11	3.6	2	0.3	10.65	0.002
Neurological signs	4	3	1.0	1	0.2	5.67	0.134
Epistaxis	1	1	0.3	0	0.0	-	-

[§] household(s)
*clinical signs in the case definition

Table 2.5.
Number and percentage of households in total, case, and non-case groups reporting specific clinical signs in young pigs

	Total hh[§] N= 786	**CASE N=284**		**NON-CASE N=502**		**OR**	**p**
Clinical signs	No.	No.	%	No.	%		
Diarrhea	464	196	69.0	268	53.4	2.09	<0.001
Stillbirth*	200	141	49.6	59	11.8	-	-
Coughing*	141	60	21.1	81	16.1	-	-
Inappetence	100	44	15.5	56	11.2	1.72	0.017
Neurological signs	70	41	14.4	29	5.8	2.78	<0.001
Wasting	92	40	14.1	52	10.4	1.48	0.092
Weak at birth	69	40	14.1	29	5.8	2.80	<0.001
Erythema	54	31	10.9	23	4.6	3.11	<0.001
Lameness	61	28	9.9	33	6.6	1.72	0.048
High fever *	54	26	9.2	28	5.6	-	-
Dyspnea *	38	21	7.4	17	3.4	-	-
Eyelid edema	44	21	7.4	23	4.6	1.74	0.078
Conjunctivitis	26	20	7.0	6	1.2	6.26	<0.001
Red spot	26	18	6.3	8	1.6	4.18	0.001
Cyanosis of ear	26	13	4.6	13	2.6	1.80	0.156
Depression	18	12	4.2	6	1.2	3.65	0.011
Dermatitis	21	10	3.5	11	2.2	1.63	0.271
Epistaxis	2	1	0.4	1	0.2	1.77	0.687
Constipation	6	1	0.4	5	1.0	0.35	0.341

[§] household(s)
*clinical signs in the case definition

Table 2.6.
Number and percentage of households in total, case, and non-case groups reporting specific clinical signs in finishing pigs

Clinical signs	Total hh. N=487	CASE N=178		NON-CASE N=309		OR	p
	No.	No.	%	No.	%		
Coughing*	93	50	28.1	43	13.9	-	-
Diarrhea	93	47	26.4	46	14.9	2.21	0.001
Inappetence	84	41	23.0	43	13.9	1.79	0.016
High fever*	74	38	21.3	36	11.7	-	-
Erythema	42	27	15.2	15	4.9	3.60	<0.001
Dyspnea *	27	21	11.8	6	1.9	-	-
Depression	22	17	9.6	5	1.6	5.60	0.001
Petechiae on skin	24	15	8.4	9	2.9	3.07	0.010
Cyanosis of ear	19	13	7.3	6	1.9	3.65	0.011
Eyelid edema	18	11	6.2	7	2.3	2.84	0.034
Neurological signs	15	10	5.6	5	1.6	3.59	0.039
Dermatitis	17	9	5.1	8	2.6	2.00	0.161
Lameness	18	9	5.1	9	2.9	1.57	0.363
Conjunctivitis	11	8	4.5	3	1.0	4.80	0.022
Constipation	8	7	3.9	1	0.3	12.61	0.018
Wasting	8	6	3.4	2	0.6	4.44	0.077
Epistaxis	2	2	1.1	0	0.0	-	-

hh.: household(s)
*: clinical signs in the case definition

Table 2.7.

Mortality proportion and their 95% of confidence intervals in all households, case, and non-case households

Production class	Group	Approach to calculate mortality	Estimate	95% C.I.		AIC
Sows	Case	Overall	0.144	0.129	0.158	-
		Raw	0.250	0.212	0.289	-
		Poisson	0.144	0.129	0.160	1213.46
		Negative binomial	0.243	0.200	0.294	847.01
		Poisson random effect	0.122	0.082	0.181	1010.16
	Non-case	Overall	0.008	0.004	0.012	-
		Raw	0.011	0.004	0.018	-
		Poisson	0.008	0.005	0.013	177.53
		Negative binomial	0.011	0.005	0.023	145.61
		Poisson random effect	0.002	0.000	0.010	163.68
Young pigs	Case	Overall	0.178	0.171	0.185	-
		Raw	0.231	0.194	0.268	-
		Poisson	0.178	0.171	0.186	4983.65
		Negative binomial	0.228	0.184	0.284	1519.86
		Poisson random effect	0.140	0.106	0.185	4012.87
	Non-case	Overall	0.044	0.041	0.047	-
		Raw	0.051	0.038	0.065	-
		Poisson	0.044	0.041	0.047	2968.54
		Negative binomial	0.050	0.037	0.068	1080.13
		Poisson random effect	0.026	0.017	0.041	2489.16
Finishing pigs	Case	Overall	0.043	0.038	0.048	-
		Raw	0.067	0.034	0.010	-
		Poisson	0.043	0.038	0.048	1423.81
		Negative binomial	0.067	0.037	0.124	366.08
		Poisson random effect	0.015	0.006	0.036	1033.54
	Non-case	Overall	0.015	0.012	0.018	-
		Raw	0.012	0.002	0.022	-
		Poisson	0.015	0.013	0.018	813.09
		Negative binomial	0.012	0.005	0.029	202.49
		Poisson random effect	0.002	0.000	0.007	561.41

Table 2.8.

Mortality rate and transformed mortality proportion with their 95% confidence intervals in all households, case, and non-case households

	Mortality rate (deaths/pig-week)			Transformed mortality risk per week		
	Estimate	**(95% C.I.)**		**Estimate**	**(95% C.I.)**	
Sows						
All	0.131	0.103	0.159	0.074	0.061	0.087
Case	0.367	0.291	0.444	0.205	0.173	0.237
Non-case	0.013	0.004	0.021	0.008	0.003	0.014
Young pigs						
All	0.154	0.124	0.183	0.091	0.078	0.104
Case	0.317	0.244	0.389	0.182	0.154	0.211
Non-case	0.072	0.049	0.095	0.045	0.034	0.057
Finishing pigs						
All	0.038	0.023	0.053	0.028	0.018	0.038
Case	0.066	0.036	0.095	0.049	0.028	0.069
Non-case	0.017	0.003	0.031	0.013	0.003	0.022

Table 2.9.

Intra-cluster correlation coefficients and proportion of variance in mortality at household level and hamlet level in all households by logistic regression from full data and reduced data

Production class	**Scenario**	**Intra-cluster correlation coefficient (ICC)**		**Variance components (VC) (%)**	
		Hamlets	Households	Hamlets	Households
Sows	Full data	0.166	0.508	16.6	34.2
	Reduced data	0.213	0.582	21.3	36.9
Young pigs	Full data	0.071	0.658	7.1	58.7
	Reduced data	0.116	0.676	11.6	56.0
Finishing pigs	Full data	0.015	0.668	0.015	65.3
	Reduced data	<0.001	0.679	<0.001	67.9

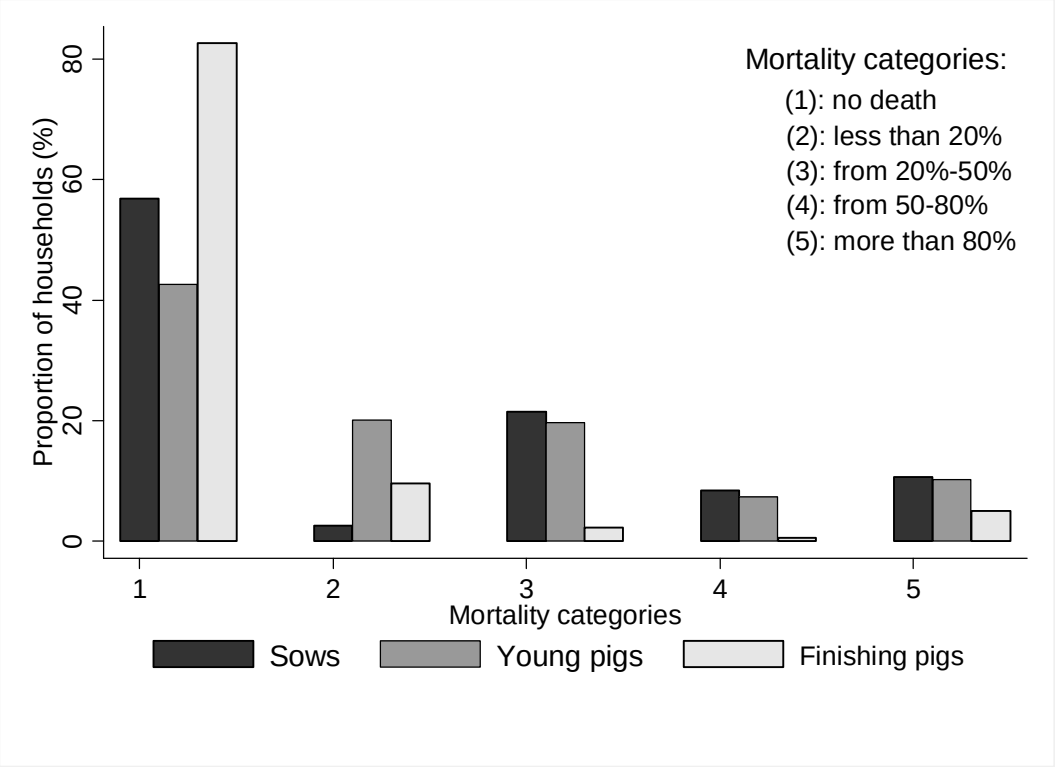

Figure 2.1.

Distribution of mortality categories in sows, young pigs, and finishing pigs in PHFD households

Chapter 3

Clustering of and risk factors

for the porcine high fever disease in a region of Vietnam

Written in the style of "Transboundary and Emerging Diseases"

Abstract

Porcine high fever disease (PHFD) emerged in 2006 in China and spread to Vietnam. Little work has been done to investigate PHFD risk factors and space-time dynamics. To fill this gap we investigated probable cases of PHFD at household level as the outcome. A study area, approximately 100 sq. km, was selected from a province of southern Vietnam that had reported the outbreak of PHFD in 2008. A survey was conducted in the study area to collect information about swine health problems during 2008. The questionnaire included three sections: general information, clinical signs of disease in pigs, and production factors believed to be risk factors. Cases were defined at the household level and included interpretation of clinical signs in series. Logistic

regression with a random intercept at the hamlet level was used to assess risk factors for PHFD at the household level. Spatial clustering was investigated using the D-function and a Cuzick-Edward's test. Spatial clusters were evaluated using a spatial relative risk surface and the spatial scan statistic using a Bernoulli model. Space-time clustering was explored using a space-time K-function and Knox's test. Space-time clusters were evaluated using a space-time permutation model in SaTScan. Of 955 households with questionnaire data, 33.4% were classified as cases. The statistical significance of space and space-time clustering differed between methods employed. The risk factors associated with occurrence of cases were higher numbers of sows and finishing pigs (log 2 transformed), receiving pigs from an external source, and the interaction between using "water green crop" (WGC) as pig feed and owning ducks with or without direct contact with pigs. The interaction between presence of ducks and feeding WGC to pigs suggested involvement of pathogens that might be present in water (environment), and could further replicate in or on ducks.

Introduction

The porcine high fever disease (PHFD) was initially described in China in 2006 as an epidemic in pigs, affecting all ages and production classes. The most likely causative agent of this disease was porcine reproductive and respiratory syndrome (PRRS) virus with a unique genetic marker (Zhou, et al., 2008). The disease was characterized by unexpectedly high morbidity and mortality, with clinical features such as fever and abortion in sows, high piglet mortality before and after weaning, and respiratory distress in pigs of different ages (Li, et al., 2007). Economically, it had a

devastating impact on the livestock industry as well as on some rural communities (Rowland, 2007). Following the initially detected outbreak in China, the disease continued to spread regionally and to neighboring countries where it also had a marked negative economic impact on swine production. In Vietnam, PHFD was initially reported in northern provinces during 2007, with subsequent outbreaks reported from multiple southern provinces during 2008 (Normile, 2007; Feng, et al., 2008). According to a report from the Department of Animal Health (DAH) in Vietnam, total mortality, including animals culled for disease control purposes, was more than 300,000 in 26 of the 60 provinces during 2008 (DAH, 2009).

So far, the exact nature of the causative agent and the pathogenesis of PHFD is not fully understood. Initial work has shown that the infectious agent associated with PHFD was a variant of PRRS virus (PRRSV) (Tong, et al., 2007). This is an RNA virus belonging to the family *Arterividae,* which has been broadly classified into two major genotypes (North American and European types) first identified in the late 1980s (Murtaugh, et al., 1995). The North American genotype has previously been reported as having generally greater pathogenicity and more genetic variability than the European type (Nelsen, et al., 1999). Although initially detected in North America, the North American genotype has been associated with PRRS outbreaks worldwide (Zimmerman, et al., 2006). A variant of the American-like lineage with unique 30-amino-acid discontinuous deletions in the NSP2 region of PRRSV has been associated with the PHFD outbreaks in China and Vietnam between 2006-2008 (Tian, et al., 2007; Feng, et al., 2008). However, it has recently been reported that this 30-amino-acid deletion was not related to the virulence of the highly pathogenic PRRSV emerging in China, but

could be used as a genetic marker of the PRRSV associated with the outbreaks of PHFD (Zhou, et al., 2009). In addition to PRRSV, other endemic pathogens such as virulent classical swine fever (CSF) virus, porcine circovirus type 2 genotype 2b, and secondary bacteria were thought to be associated with and contribute to the severity of the disease (Tian, et al., 2007; McOrist and Done, 2007). Uncertainties about the exact causative agent, and of the existence of other component causes, contributed to poor case definition that has led to difficulties in disease control.

Devastating losses due to the PHFD epidemic occurred, and this prompted a considerable interest of government veterinary services in the disease. Because of the occurrence of PRRSV as a pathogen in the early 1990s, several studies have focused on investigation of risk factors for PRRS infection and clinical PRRS (Mortensen, et al., 2002; Firkins and Weigel, 2004; Rovelo Celorio, et al., 2010). Moreover, since the emergence of PHFD, several virological studies have focused on PRRSV as the likely causative pathogen of PHFD (Han, et al., 2006; Tian, et al., 2007; Tong, et al., 2007; Wu, et al., 2009), while several epidemiological studies have described large-scale trends (Tian, et al., 2007; Zhou, et al., 2008) or case description in some farms (Li, et al., 2007). However, information about patterns of disease emergence and factors associated with occurrence of PHFD in swine herds is scarce. This paucity of understanding may be associated with unsuccessful strategies to control the outbreak. As a result, the disease still occurred in many places during 2009, and continues to occur in 2010 (Nguyen Hung, 2010). Thus, the objectives of this study were to describe spread of a suspected PHFD outbreak in a small geographical area in the south of Vietnam, and to identify potential risk factors associated with disease status at household level.

Materials and Methods

Study area

The area selected for inclusion into the study was a 235-km^2 district of a province in the south of Vietnam. This district had been identified by local veterinary services as having experienced the outbreak of PHFD during the year 2008. This outbreak was less severe than those reported in northern provinces during 2007, and no specific disease-control measures by veterinary services had been implemented to control the outbreak. Because of the lack of control measures, the disease continued to occur and spread, and thus this district was an appropriate region for an investigation of the "natural" course of an outbreak. The study area was selected in cooperation with local veterinary services. In the district, an approximate 10 × 10-km rectangular area was selected in one of the densest pig production parts of this province. This study area included five communes with a total of 37 hamlets, each comprising hundreds of households. Members of most households in the area worked as farmers who planted rice or fruit on their lands, and some raised pigs for extra income. Pig production was usually small scale, even though there are also some large farms with more than 100 sows. In this study, a swine herd was defined as a household with at least 1 sow or more than 5 pigs other than sows, including large commercial farms.

Data collection

A data collection instrument was initially developed by English-speaking epidemiologists with expertise in swine diseases. It was further modified by experts in local culture and production systems. The translated instrument was reviewed by officials

in local veterinary services and was then field tested. The final version of the instrument contained 50 questions related to general information concerned with household identification, occurrence of clinical signs suggestive of PHFD, and management practices prior to unusual clinical signs during 2008 (see Appendix 1). A copy of the questionnaire is available upon request.

Expected clinical signs were listed in a table. These included all clinical signs of PHFD observed in other areas of Vietnam plus other signs that might have been recorded in any pig farms. In total, 21 clinical signs were listed for each production class of pig, including sows, young pigs, and finishing pigs. Young pigs were used as a category since weaning age was suspected to vary much between households and was not always obtained easily. Additional space was provided for other clinical signs which were not in the list. Questions about clinical signs were asked in two ways. First, the instrument asked about the presence of an unusual disease during 2008. If the answer was “yes” (scenario 1), the producer would be further asked to indicate particular clinical signs that were observed in this unusual disease and about the calendar time of its onset and its duration. If the answer for the question on occurrence of an unusual disease was “no” (scenario 2), the instrument asked the producer to simply indicate the presence or absence of clinical signs from the list of each production classes. Furthermore, producers were asked to recall the calendar time of the onset and duration of clinical signs indicated on the list. Once the duration of the period was identified, producers were asked to indicate the number of pigs in each production class that were present in the herd and the number of pigs that died with unusual disease (scenario 1) or during the period when specific clinical signs had been indicated by the producer (scenario 2). In the theoretical case in

which no clinical signs were identified, the data collection instrument had a question about the number of pigs in each production class that were present in the herd and the number of pigs that died during June of 2008 (scenario 3). This month was thought to be the fairest to select, since it is the middle of year, and this is consistent with common epidemiological principles (i.e., a common assumption when calculating the denominator for risk). Once the time of interest was established, producers were asked to recall management practices occurring before the onset of the period of interest (unusual disease for scenario 1, observed clinical signs for scenario 2, and June of 2008 for scenario 3).

A survey based on the final instrument was conducted in the study area by a group of trained veterinary students between July and August of 2009. Almost all households who raised pigs in this area during 2008 were visited to be interviewed. Approximately 30 to 50 small scale or isolated households were not visited. Local veterinarians guided the group of interviewers, and introduced each interviewer to a representative member of the household. These interviewers explained the aim of the project, what information would be collected, and how data would be used, and then asked if producers wished to participate in the study. The inclusion criterion was that the responder should be the person in charge of raising pigs for the farm. Households' locations were recorded during the survey by using a hand-held global positioning system (GPS), Etrex (Garmin Ltd, Olathe, KS, USA). The boundary shape-files of the area were acquired from the local government veterinary department.

Case definition

Due to the retrospective nature of the study, no samples were available for laboratory confirmation. Thus, the case definition at herd level was based on simultaneous occurrence of clinical signs (recorded during the interview) in either sows, or growing pigs, or both. The combination of clinical signs for a case was validated by the literature (Le Van Nam, 2007; Li, et al., 2007; Tian, et al., 2007; Zhou, et al., 2008), by experts in swine diseases, and by experienced veterinarians in the area. For herds containing sows, a case was defined as death of a sow or abortion accompanied by at least one of the following signs: high fever in sows, stillbirth, blue ear in sows, respiratory problems (dyspnea or coughing) in sows, pre-weaning mortality, or post-weaning mortality over 20%. For herds containing only other production classes, or sows without any health problems, a case was defined as mortality greater than 10% in these other pigs, with fever and respiratory signs.

Data processing

Questionnaire data were entered into Epidata software (version 3.1, EpiData Association, Odense, Denmark, 2005), checked for validity, and then exported to Stata software (Stata Intercooled, version 10; Stata Corporation, College Station, Texas, USA) for management. For spatial analysis, all coordinates were projected into WGS-1984 UTM Zone 48N and analyzed using the R software (version 2.9.0) (R Development Core Team, 2009).

Description of disease distribution

Data were first examined descriptively by using a point map and a histogram based on monthly counts. Distribution of disease was assessed in terms of time and space. For time, temporal clustering was explored to identify the most likely period of the outbreak. For space, several exploratory techniques were used for accessing spatial trend or clustering and to detect potential clusters. Then the interaction between time and space was also considered for these assessments.

Temporal clustering

Dates of recognized cases were used for temporal scan tests in which maximum temporal cluster size was 25% of the population and time was aggregated into 7 days. Discrete Poisson models were applied for this test to detect a period with a high rate of occurrence. The analysis was performed in SaTScan software (version 8.0.1) with 9999 Monte Carlo replications to obtain a *P*-value under the null hypothesis of no temporal clustering (Kulldorff and Nagarwalla, 1995; Kulldorff, 2009).

Spatial trend, spatial clusters and clustering (spatial autocorrelation)

Kernel smoothing using quartic kernels and a fixed bandwidth of 1000 meters was used to estimate the density of PHFD-positive herds and the density of all herds in the study area using the SPLANCS library in the R software. The ratio of two kernels was used to represent a smoothed surface of the cumulative risk of PHFD in 2008 over the entire study area (Berke, 2005). Then, existence of global spatial clustering was evaluated using Cuzick and Edward's test (Cuzick and Edwards, 1990) in commercial software (ClusterSeer version 2.3.2, TerraSeer Inc). Briefly, the number of observed case households in each of the k-nearest neighbors of a case were counted and compared to

the number of case households in each of the k-nearest neighbors of cases based on 9999 Monte Carlo randomizations of case labels to observed locations. Existence of spatial clustering at each of the first 10 neighborhood levels was evaluated and the overall *P*-value was adjusted for multiple comparisons using the Simes approach. Global spatial clustering was additionally evaluated using the difference between the K-function for PHFD-positive and PHFD-negative herds (D-function) (Diggle and Chetwynd, 1991; Bivand, et al., 2008). Then the D-function was estimated over a distance of 300 m in steps of 10 m using data from the entire study area. The 95% simulation limits were estimated on the basis of 999 random labeling of cases, and 95% confidence limits were additionally calculated using a normal approximation. This analysis was performed using the R software (SPLANCS).

Existence and location of purely spatial clusters of cases were analyzed using two approaches. Firstly, a spatial scan test based on the Bernoulli model (Kulldorff and Nagarwalla, 1995) and circular windows with a maximum size of up to 50% of the population at risk and 9999 randomizations was used in SaTScan (version 8.0.1) (Kulldorff, 2009) to detect clusters with unexpectedly high risk of PHFD. Secondly, the spatial relative risk surface was constructed using a ratio of case density and non-case density. This observed spatial relative risk surface was compared to relative risk surfaces on the basis of 999 random labeling of cases as described earlier (Waller and Gotway, 2004; Davies and Hazelton, 2010; Bivand, et al., 2008). Briefly, the ratio of densities based on observed PHFD-positive and PHFD-negative herds (spatial relative risk) was estimated using quartic kernels with a bandwidth of 1000 m on a regular grid (50 m × 50 m). Then, PHFD-positive labels were randomly allocated to observed locations in 999

iterations, and the spatial relative risk was estimated for each iteration using the same parameters previously used for observed data. Then for each cell on a grid, the spatial relative risk based on observed data was compared to randomly labeled data, and areas with cells of a regular grid where observed spatial relative risks ranked higher than relative risk of 95% of randomly labeled datasets were considered to be the high risk area and to represent a significant spatial cluster of PHFD-positive cases. Areas (cells in a grid) with a relative risk >1 and $P < 0.05$ were considered significant clusters.

Space-time clusters and spatio-temporal clustering

First, Knox's test was applied to examine the excess of cases that were close both in space and time to a PHFD case, using commercial software (ClusterSeer software, version 2.3.20, TerraSeer Inc) (Knox and Bartlett, 1964; Kulldorff and Hjalmars, 1999). The cut-offs for determining spatial closeness were 50 m, 200 m, and 500 m, and for temporal closeness cut-offs were 7, 14, and 30 days. Each combination of spatial and temporal distances was used to obtain 9 comparisons. The *P*-values were then adjusted for multiple comparisons using the Bonferroni approach. Specifically, a $P \leq 0.0055$ was needed on the Knox's test in order to reach a Bonferroni-adjusted *P*-value ≤ 0.05, which was considered statistically significant. Secondly, the space-time K-function was executed to further evaluate space-time clustering (Diggle, et al., 1995). The space-time K-function has been previously used to examine the nature of local spread during a foot-and-mouth disease (FMD) outbreak in the UK and for other diseases in animal population (French, et al., 2005; Sanchez, et al., 2005; Picado, et al., 2007). In this study, a temporal vector (t) between 7 and 90 days with sequential steps of 7 days, and a spatial vector (s) between 10 and 300 m with sequential steps of 10 m, were used to estimate component

processes of K-function. Then, a proportional increase in the number of cases above expectations in a distance and during a period of time ($D_0(s,t)$) was constructed to interpret the interaction between time and space. The value of $D_0(s,t) >1$ was considered a biologically significant interaction. The $D_0(s,t)$ plot was inspected visually. The overall significance of clustering was evaluated using 999 randomizations. These processes were performed in R with SPLANCS library.

Finally, the space-time permutation scan test was performed to detect specific clusters in space and time. This test utilized a large number of overlapping cylinders as scanning windows, in which the base was the geographical area and the height was the time scale of the outbreak area during 2008. The scan windows were defined such that maximum spatial size was 50% of the population at risk and maximum time was 50% of the outbreak period, which was aggregated into 14-day periods. Based on 9999 Monte Carlo simulations, the likelihood ratio test statistic and *P*-value for each cylinder were calculated (Kulldorff, et al., 2005). These process were performed in SaTScan software (version 8.0.1) (Kulldorff, 2009). The most likely and secondary clusters were reported without geographical overlapping.

Risk-factor analysis

Data entered were checked for validity, in terms of correctly recorded reasonable and reliable data. All possible questionnaire variables for inclusion are defined in Table 3.1. First, a univariable analysis was performed using logistic regression with hamlet as a random effect. Parameters were estimated via maximum likelihood estimation. Variables collected at the household level in the questionnaire were examined first. Second,

contextual variables, defined as hamlet-level means of each continuous and binary variable, were also used. Then, multivariable models were built using logistic regression with the random effect of hamlets on the intercept. Variables with $P < 0.2$ in univariable analysis were entered into the full model and then each non-significant variable was dropped step by step. The criteria for dropping a variable were the largest *P*-value of Wald's test and confirmation of the dropping process by a likelihood ratio test. All possible two-way interactions were also examined after identifying what seemed to be the variables in the final model. The final model was checked by considering deviance residuals and outliers. Statistical analysis was performed in Stata software (Stata Intercooled, version 10; Stata Corporation, College Station, Texas, USA). These strategies of model building have been described previously (Dohoo, et al., 2003).

Results

In total, animal owners from 955 households nested within 37 hamlets of the 5 communes were interviewed about the PHFD outbreak that occurred during 2008. Three hundred and nineteen households (33.4%) were classified as cases of PHFD. These cases appeared throughout the year of 2008, with the highest number of occurrences in June (Figure 3.1). This period was also identified as a temporal cluster with SatScan (P <0.001). Figure 3.2 shows the ratio of kernels of cases to population at risk, which can be interpreted as a risk map. The smoothed estimates of these ratios had a minimum of 0 and a maximum of 0.76, with a mean of 0.33. In this study area, risk higher than 40% occurred in some visible zones in the south.

The Cuzick Edward's test showed no significant spatial clustering of cases, either on the basis of a summary test or after examining each individual level of neighborhoods (P >0.33). A similar result was obtained using the D-function, which suggested no clustering within the distance evaluated. In addition, the most likely spatial cluster, as estimated by the spatial scan test, was not statistically significant (P = 0.12). In contrast, spatial relative risk mapping pointed out a major significant spatial cluster (P < 0.05) in the center of the map (Figure 3.2) and a few minor areas near the southern boundaries of the study area. Interestingly, the non-significant primary cluster detected by the spatial scan test intersected with the major significant cluster detected by the spatial relative risk map, although the extent of the area detected by the latter method was larger.

Knox's test, identifying spatio-temporal clustering, showed a significant space-time interaction in some space-time cut-point combinations. Households within 50 m of each other were associated in term of the occurrence of PHFD during less than 14 days (P < 0.0055, Bonferroni's adjustment for nine comparisons). However, when the cut-point for neighbor identification increased to 200 m, the P values were non-significant (P > 0.0055) for any time cut-point component mentioned. All combinations of time and space of 500 m were significant (P <0.0055). In general, it may be inferred from the results of Knox's test that, at least, there was a significant interaction between time and space in disease occurrence within less than 500 m and 30 days. In contrast, the space-time K-function showed no significant clustering because the D_o plot (Figure 3.3) did not clearly suggest an interaction between time and space over the spatial and temporal proximity evaluated by Monte Carlo simulation (P = 0.22). However, there was a small apparent interaction at approximately 10 m to 20 m in 30 days. Moreover, the result of the space-

time permutation scan test showed no significant clusters ($P = 0.359$). Collectively, these analyses suggested that disease occurrence showed no strong evidence of clustering in space and time.

Variables presented in Table 3.1 includes 14 categorical variables and two continuous variables. These continuous variables, including numbers of sows and finishing pigs in each household, were transformed using a base two logarithm. However, these small scale households at some times had either no sows or no finishing pigs, making the transformed value infinite. Thus, 0.25 was added to these 0 values before transformation (Lawless, 1989). For univariable analysis, the association between each variable and disease status are reported in Table 3.2 in the scale of log odds. Evaluation of contextual variables were also shown in this table. From this, seven variables were significantly associated with logit of PHFD ($P < 0.05$): log sow, log finishing pig, breeding method, using water green crop, having ducks, classical swine fever (CSF) vaccine, and log of means of finishing pigs in hamlets. The final multivariable model is shown in Table 3.3. Variables associated with the PHFD were log number of sows, log number of finishing pigs, receiving pigs before health problems were reported, and interaction between having ducks in a household and using water green crop as a feed for pigs. Table 3.4 shows the odd ratios of interaction combinations between using water green crop and having ducks.

Discussion

The final logistic regression model identified three demographic variables expected to be risk factors (receiving pigs, number of sows, and number of finishing

pigs), and two other risk factors (ducks and water green crop) with their interaction, which had not been previously described, to our knowledge. First, among the expected risk factors, receiving pigs from an external source was identified as a significant variable in the final model. This practice might include either nursery pigs or sows in herds of all sizes. Estimates from the model suggest that introduction of new pigs into a farm increases risk of PHFD 1.7 times. Identification of this variable served to remind us that direct contact between farms due to animal movement was an important means of pathogen transmission and was an expected finding. Many studies have reported introduction of pigs to farms as a major risk factor for numerous diseases, including PRRS (Mortensen, et al., 2002; Mousing, et al., 1997), CSF, and others (Terpstra, 1987). Scientific publications on the pattern of movement of pigs in this or a similar region are not currently available. However, our experience was that producers in this area purchased animals from the local market, from other provinces, or from more distant places far from the province, without knowledge of biosecurity. This might explain the lack of spatial disease clustering or spatial clusters. Thus, studying animal movement is likely to be important for understanding disease spread in this area, and might help veterinary services to design appropriate control measures, such as education in biosecurity and certifying disease-free sources of breeding pigs for farmers.

Farm size factors in the model, including numbers of sows and finishing pigs, might act as confounders for other factors because farm size itself was a risk factor (Mortensen, et al., 2002; Wu, et al., 2008) and was associated with other risk factors concerning production management. For example, large farms do not often raise ducks or feed water green crop to pigs, but they might have received more pigs. In addition, the

possibility of detecting a case by clinical signs is greater on large farms than on small farms, i.e., herd sensitivity to classify a case might be higher for large farms than for small farms. Therefore, it was our decision to keep farm size in the model, regardless of its significance. In fact, farm-size variables were always significant in the model-building process.

The confounding effect of farm size can be seen in the case of CSF vaccine. Classical swine fever virus has been confirmed as a pathogen present in outbreaks of PHFD in China (McOrist and Done, 2007). Typical CSF vaccines are modified live vaccines, and it is unknown to the authors how the vaccine strain and other pathogens interact. Thus, CSF vaccine might be a suspected factor for our study. In fact, CSF vaccine was significant as a risk factor (OR=1.73) in univariable analysis. However, it became insignificant when log numbers of sows and finishing pigs were added in the model, probably because CSF vaccine is strongly associated with farm size. To prove this association, logistic regression was used with CSF vaccine as an outcome and log number of sows in the household as a predictor. We found a strong correlation between farm size and CSF vaccine (OR=1.57, $P < 0.001$). Large-scale farms were aware of the importance of this vaccine, and most farms having sows used CSF vaccine on the recommendation of government veterinary service: 877 of 955 farms (91.8%) vaccinated for CSF. When farm-size variables and CSF vaccine were included in the model, CSF vaccine became insignificant, which suggests that CSF vaccine was not a risk factor for PHFD. However, this should be interpreted with caution, because we did not have information on the efficacy or possible side-effects of the specific vaccine used in this area. Other vaccines, including FMD and PRRS, were not significant during univariable analysis. Foot-and-

mouth disease vaccine had been used before, but was not compulsory at the time of the outbreak, as there had been no major FMD outbreaks recently. Some commercial PRRS vaccines were used, but their efficacies in this area were not evaluated.

The unexpected significant variables in the final model were using water green crop for pig feed, having ducks on the farm (with or without direct contact with pigs), and their interaction. When water green crop is used to feed pigs, local experts suggest that more than 90% of farms use water spinach (*Ipomoea aquatica*), also known as water morning glory, water convolvulus, or "rau muong" in Vietnamese, to feed pigs and ducks. Because this area is located in the Mekong delta, water is always adequate for the crop. Thus, this crop is commonly planted throughout the year. After harvesting, it is cut into small pieces and mixed with commercial pig feed. The interaction effect between water green crop and keeping ducks can be interpreted as follows: when there were no ducks on farms, the risk for the disease on farms that did or did not use water green crop did not differ, and when water green crop was not used, raising ducks did not increase the risk of PHFD. Thus, there is no effect on risk for PHFD from raising ducks or feeding water green crop, independent of each other. However, when ducks were present, the risk for PHFD on farms using water green crop was higher than on farms that did not use water green crop (OR=1.97). The difference in disease risk became even more pronounced when ducks had direct contact with pigs. In these farms, using water green crop increased the odds of having PHFD 2.95 times compared to not using water green crop. Swine producers in this region might buy water green crop from external sources or harvest crops from their own fields to feed their pigs. If ducks were raised, producers fed the same water green crop to both pigs and ducks. Duck breeds in the study area vary

between farms, and those raised for meat or eggs may be Pekin ducks, Khaki Campbell, a new line called CV2000 imported from the UK, or cross breeds. Ducks are often raised all year round in a fenced area to take advantage of leftover pig feed. In some households (39/955 households), farmers raised ducks in the same barn with pigs, and ducks could easily reach pig pens and access the food, i.e., they had direct contact with the pigs. This direct contact might explain the significant increase in risk. Attributing this interaction to PRRSV transmission is controversial. There is some evidence in the current scientific literature implicating PRRSV in this interaction. First, PRRSV survives well in water and can be mechanically transported between farms on fomites (Pirtle and Beran, 1996; Dee, et al., 2003). In addition, PRRSV can replicate in mallard ducks (Zimmerman, et al., 1997). On the basis of this evidence, the interaction between water green crop and the presence of ducks on pig farms is logical and corresponds well with PRRS epidemiology. Water green crop may mechanically transmit PRRSV from a water reservoir into ducks, where it further replicates and infects pigs. However, several other reports do not support this hypothesis. For example, a previous study reported that PRRS was unlikely to be transmitted via contaminated lagoon effluent (Dee, et al., 2005), and infectivity of PRRSV for ducks is still controversial (Zimmerman, et al., 1997; Trincado, et al., 2004). Thus, the interaction between water green crop and ducks on farms needs to be interpreted cautiously until further information about some of the key parameters is obtained.

An alternative explanation for this interaction is that other currently unidentified pathogens from ducks or water green crop might contribute to PHFD occurrence. For example, one or more other pathogens unable to efficiently cause a disease in pigs might

be introduced by water green crops. Crops might serve as fomites introducing the unidentified pathogens from the environment, particularly when there is no appropriate way to handle the effluence from farms affected by PHFD. Once introduced via green crops, these pathogens would need to be amplified in ducks and then further transmitted to pigs. These pathogens might be directly or indirectly transmissible or even vector-borne. Therefore, ducks might act as an intermediate host for disease transmission. Some diseases that can be transmitted from ducks and cause disease in pigs include avian influenza (Kida, et al., 1994) and Japanese encephalitis (Pant, 2006). However, avian influenza typically does not cause high mortality in pigs as was recorded in this study and thus cannot explain this outbreak.

A third possibility is that either variable included in the interaction could be a proxy factor for some other management procedure that was not captured in this study. Finally, this interaction might have been a chance finding. A model containing water green crop and ducks without their interaction would suggest a different epidemiology and would expand the list of potential infectious causes. However, we found that, in addition to the significance of the coefficients of the interaction, the lower Akaike's information criterion (AIC) of the model with the interaction compared with the model without the interaction (1146 versus 1149) and the significance of likelihood ratio test ($P = 0.04$) suggested that the interaction terms should be kept in the model, moreover, this interaction remained significant after most influential points were omitted from the data. The external validity of this finding is not certain because the involvement of water green crops and ducks might have been only one mechanisms explaining how pathogens were

able to spread in this region at that time. However, this interaction does warrant further investigation.

The Cuzick Edward's test and the D-function were unable to identify any evidence of spatial clustering. However, for detection of disease clusters, the findings were not consistent among methods, i.e., the relative risk map identified some significant clusters while the scan test did not. The scan test is commonly used in veterinary research, but use of the spatial relative risk method has not been reported, possibly because of the problem in choosing the appropriate bandwidth and the difficulty with edge correction. Ignoring minor clusters near the border of the study area in our relative risk map, the significant cluster in the center of the map overlapped with the non-significant primary cluster detected by the scan test, suggesting that spatial relative risk might be a sensitive method for cluster detection in this situation. Further research is needed to clarify this. The possible spatial cluster might be explained by environmental factors or common production factors. In terms of environment, we used several spatial variables such as distance to roads, distance to rivers, and distance to the nearest neighbor in the preliminary risk factor analysis, but no significant associations could be identified. However, the potential cluster area has the highest density of pigs in the study area, with a great deal of pig movement. This could possibly explain the existence of such a cluster , because, according to local farmers, households in the same area usually buy nursery pigs or replacement sows from the same sources to share their shipping fee.

Similarly, space-time clustering was inconclusive. This inconsistency in results could be in part due to the different principles of the tests. Knox's test depends greatly on choice of thresholds, while the space-time K-function as used in this study was

influenced by the sequential steps of distance and time and their total extent. Knox's test is significant within 50 meters over 7 days and 50 meters over 14 days, while the Space-time K-function increased beyond expectations at a distance within 10 meters and during a period of 20-30 days from the start of the randomly selected case. However, the distance of 10 meters seems to be inappropriate for the 2 nearest neighbor households (mean, 50 meters; 25^{th} and 75^{th} percentiles, 23 and 92 meters, respectively). Overall, we believe that spatial and space-time clustering were not important contributors to the outbreak in the study area for one or more of the following reasons. (i) The nature of the study population, that consisted mostly of small households: perhaps when disease occurred in such households, not enough virus was produced to allow airborne spread to occur. (ii) The nature of the pathogen, i.e., PRRSV: it is possible that the virus is not easily aerosolized. One study agreed with this when investigators failed to prove airborne transmission of PRRSV between farms short distances apart, i.e., 1 meter (Otake, et al., 2002). In contrast, others studies reported airborne transmission of PRRSV (Dee, et al., 2006; Mortensen, et al., 2002). This gap can be explained by differences in airborne transmission of different PRRSV strains (Torremorell, et al., 1997; Otake, et al., 2010). To our knowledge, no study reported airborne transmission of specific PRRSV strains in the study area. (iii) Environmental factors: the tropical climate in this area might limit survival and infectivity of PRRSV. Average temperature from April to July is approximately 28^{o}C and relative humidity (RH) is approximately 83%-87%. This condition might reduce survival and infectivity of PRRSV. Particularly, at 5^{o}C and 10% RH, the half-life of aerosolized infectious PRRSV was approximately 215 minutes, while at 25^{o}C and 80% RH, the half-life was only 22.8 minutes (Hermann, et al., 2007). (iv)

Other mechanisms of disease transmission: direct movement of pigs as identified in the risk-factor analysis, indirect transmission through potentially infected fomites, or human movement can cause transmission without a local pattern. In addition, the reader should be reminded that the small spatial scale of this study might make it difficult to infer the general pattern of disease spread at a larger scale or in other regions.

Although evidence for local spread was weak, the temporal cluster was significant with a high peak of reported cases in June. The fact that this spike was unusual might be questioned on information bias. Most households were small scale producers, so they could easily recall production practices (e.g., buying a new pig or selling pigs). These farmers often use the lunar calendar, which marks many traditional events, most commonly the full moon every 3 months, to recall production events during the year. The interviewers had been trained in converting the lunar calendar to the conventional calendar for use in the study. In addition, as the disease was severe and directly related to loss of income, it would make a strong impression on the producers and would be easy to recall.

It is possible that the temporal peak in June was associated either with seasonal changes in temperature, humidity, and precipitation or with temporally forced pig movement. In this area, there are two seasons: the rainy season (May – Nov) and the dry season (Dec – April). The start of the rainy season is often characterized by an increase in morbidity. Environmental factors (high humidity and temperature) during the transition months (May or June) might limit airborne transmission of the PRRSV. However, during the same period, the weather change from the dry to the rainy season can make pigs more susceptible to any diseases introduced or present on the farm. Alternatively, temporally

forced pig movement could partly explain the existence of the temporal cluster. However, reasons for such movement are not entirely known. Typically, more frequent movement of nursery pigs was expected in September to prepare for the Tet festival in late January when many pigs are sold at premium prices. Thus, seasonal buying of nursery pigs or indirect transmission of disease through livestock dealers cannot explain the observed pattern. We do not have evidence of a seasonal pattern of farrowing in this area. However, to produce more nursery pigs for the high demand in September, sows would ideally farrow in late June. Thus, during May and June, if even a moderate seasonality in breeding existed in the study area, there would be more pregnant sows susceptible to the disease as defined in the study than in other months. This would warrant further investigation. However, the most logical explanation for this temporal cluster could be existing outbreaks in the neighboring provinces that started in April of 2008. Farmers from these provinces could have sold more nursery pigs because they might have been concerned about implementation of control strategies, including enforced euthanasia of affected pigs and movement restriction. This could lower the price of nursery pigs, leading to a higher number of pig movements to the study area and transmission of the causative agent.

The household prevalence (33.4%) and high sow mortality (9.4%) suggest PHFD was a serious problem in the area, even though it was reported that severity was low compared to other provinces. According to an earlier study, seroprevalence of exposure to PRRSV was approximately 6% to 7% in sows in the Mekong delta (Kamakawa, et al., 2006). This might extrapolate to our study area, suggesting that the outbreak of PHFD might have been underestimated, and that the provincial veterinary service had given it

inadequate consideration. For example, for the short-term, further investigations into disease etiology and discussion of feasible recommendations for animal movement control and biosecurity procedures could be included. As a long-term goal, if this situation continues, community intervention trials with feasible strategies to eliminate pathogens or to reduce the disease impact should be considered.

This study had two major limitations. The case definition was based only on clinical signs. A prospective investigation based on diagnostic assays was not feasible because of prohibitive costs and logistics of such a study. We believe, however, that our case definition had a high specificity for the following reasons: (i) producers were asked to recall clinical signs from a list of suggested signs indicative of this disease, (ii) producers were asked about the same list of clinical signs in two different ways, depending on their initial answer on the unusual clinical disease, (iii) series interpretation of severe clinical signs was used to define a case of PHFD, (iv) the referred time was the outbreak period in the south of the country; and (v) local veterinarian support helped to ensure specificity during the survey. Despite that, we are aware that this definition could at best be equated with a probable case of PHFD, or even a possible case of PHFD. The most likely PHFD misclassification in our study was non-differential, therefore the point estimates for at least some factors are likely underestimated.

The second limitation is related to the scale of the project. Logistic constraints prevented us from investigating larger areas. This limits extrapolation of results from this area to others. However, methods used for investigation of spatial and space-time clustering in this study might be sensitive to large-scale trends, and authors from other areas may opt to limit the extent of the geographical area used for such investigations

(Picado, et al., 2007). Thus, the size of the area was acceptable from this perspective. Most importantly, this study and the associated data-collection instrument were very detailed and could be further refined and used by government veterinary services in investigation of PHFD cases.

From this study of risk factors for PHFD in an area in Vietnam, we conclude that movement of pigs, i.e., between farms, might be the primary means of spreading disease. Evidence of spatial clustering and space-time clustering was weak. In addition, we found the interaction between having ducks with or without direct contact with pigs and feeding pigs with water green crop was a risk factor for PHFD. Thus it can be inferred that a pathogen originating from a water source, and which further replicates in ducks, may have contributed to the occurrence of PHFD in this area of Vietnam.

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Table 3.1.

Variable names and their definitions used for risk assessment to identify cases of PHFD at household level

Variables	Description	No. of households
Breeding method	No breeding*	80
	Natural insemination only	226
	Artificial insemination only	504
	Both natural and artificial insemination	144
By-products	Not used*	646
	Recycling by-products from own household	23
	Bought by-products from outside	286
Chicken	Farm had no chickens*	180
	Farm had chickens without direct contact with pigs	141
	Farm had chickens with direct contact with pigs	634
Cow	Farm had no cows*	782
	Farm had cows without direct contact with pigs	114
	Farm had cows with direct contact with pigs	59
CSF vaccine	No classical swine fever vaccine*	78
	Classical swine fever vaccine as recommended	877
Duck	Farm had no ducks*	553
	Farm had ducks without direct contact with pigs	315
	Farm had ducks with direct contact with pigs	87
Feed deliver	Feed delivered by farm owner*	677
	Feed delivered by feed dealers	267
FMD vaccine	No foot and mouth disease vaccine *	676
	Foot and mouth disease vaccine as recommended	279
Log finishing pig	Logarithm number of finishing pigs in household	
Log sow	Logarithm number of sows in household	
Main water	From drilled well*	599
	From open wells (<10m depth)	317
	Surface water only	33
PRRS vaccine	No PRRS vaccine*	771
	PRRS vaccine as recommended	184
Receiving pigs	No pigs received from other farms*	784
	Received pigs from other farms before unusual disease	171

Table 3.1 (continued)

Receiving other animals	No other animals received *	845
	Received other animals before unusual disease	110
Waste treatment	No waste treatment*	912
	Treatment by bio-gas and composting	43
Water green crop	Water green crop not used as additional feed for pigs*	549
	Water green crop used as additional feed for pigs	406

* used as base-line in risk factor analysis

Table 3.2.

Factors associated with being a case of PHFD at household level based on univariable analysis at log odds scale (in the order of *P*-value from lowest to highest)

	Individual level				Contextual level	
Variables	**Estimate**	**95% CI**		**P-value**	**Estimate**	**P-value**
Log sows	0.38	0.27	0.49	<0.01	0.10	0.62
Log finishing pigs	0.06	0.01	0.10	0.01	0.17	0.02
Breeding method				0.01		
Natural	0.72	0.09	1.35			
Artificial	0.86	0.27	1.45			
Both	1.03	0.38	1.68			
Water green crop	0.33	0.06	0.60	0.02		
Ducks				0.04		
Having ducks	0.06	-0.23	0.36			
Ducks contact pigs	0.61	0.15	1.07			
CSF vaccine	0.55	0.01	1.10	0.04	-1.06	0.48
Feed delivery	0.23	-0.07	0.53	0.13	-0.11	0.87
Waste treatment	0.48	-0.14	1.10	0.13	0.94	0.62
Receiving other animals	0.28	-0.13	0.69	0.18	0.23	0.87
Receiving pigs	0.25	-0.10	0.59	0.16	0.20	0.21
PRRS vaccine	0.14	-0.20	0.47	0.43	0.08	0.92
By-products				0.45		
Self produce	-0.61	-1.62	0.39			
From outside	-0.03	-0.33	0.26			
Chickens				0.47		
Having chickens	-0.27	-0.75	0.20			
Chickens contact pigs	-0.05	-0.40	0.30			
Main water				0.57		
Open well	0.10	-0.19	0.39			
Surface water	-0.28	-1.06	0.51			
Cows				0.65		
Having cows	-0.11	-0.53	0.31			
Cows contact pigs	-0.24	-0.82	0.34			
FMD vaccine	-0.03	-0.32	0.27	0.86	-0.01	0.98

Baseline level can be referred in the Table 3.1

Table 3.3.

Factors associated with being a case of PHFD at household level in final random hamlet multivariable logistic regression (in log odd scale)

Variables	**Estimate**	**95% CI**		***P*-value**
Fixed effect				
Intercept	-1.50	-1.85	-1.16	<0.001
Log sows	0.39	0.28	0.51	<0.001
Log finishing pigs	0.05	0.01	0.09	0.047
Water green crop (WGC) * ducks				0.002
WGC	0.04	-0.34	0.43	
Having ducks	-0.26	-0.70	0.18	
Ducks contact pigs	0.18	-0.49	0.86	
WGC & having ducks	0.63	0.01	1.26	
WGC & ducks contact pigs	1.04	0.04	2.03	
Receiving pigs	0.53	0.15	0.91	<0.001
Random effect				
Variance of intercept	0.07	0.02	0.32	0.020
AIC=1146 ; N=955				

Table 3.4.

Odd ratios of interaction combinations between using water green crop and having ducks

Group	Risk factor	Baseline	OR	95% CI		*P*-value
No water green crop						
	Having ducks	No ducks	0.77	0.50	1.19	0.246
	Having ducks contact pigs	Having ducks	1.56	0.75	3.22	0.233
	Having ducks contact pigs	No ducks	1.20	0.61	2.36	0.530
Use water green crop						
	Have ducks	No ducks	1.45	0.93	2.28	0.105
	Having ducks contact	Having ducks	2.31	1.09	4.96	0.028
	Having ducks contact	No ducks	3.38	1.62	7.06	0.001
No ducks						
	Crop	No crop	1.04	0.71	1.54	0.821
Having ducks						
	Crop	No crop	1.97	1.19	3.25	0.008
Having ducks contact pigs						
	Crop	No crop	2.95	1.17	7.39	0.021

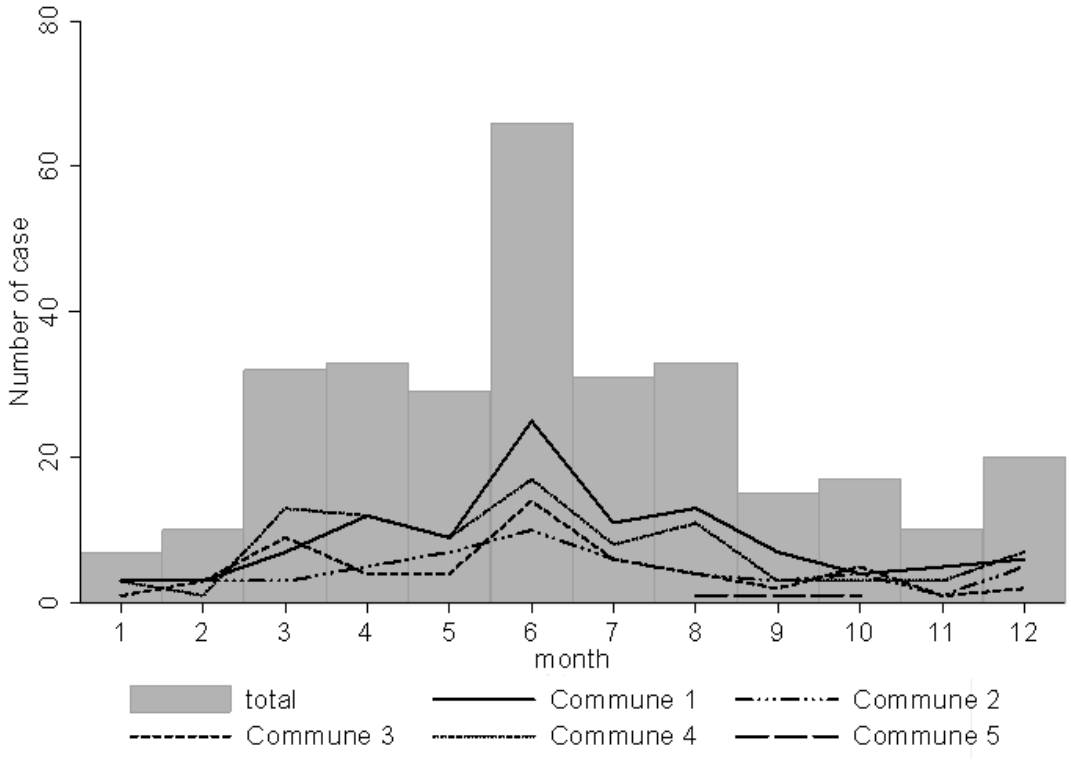

Figure 3.1.

Distribution of cases of PHFD during the year of 2008 in 5 communes and in total of the study area with the high number of case (peak) in June, 2008

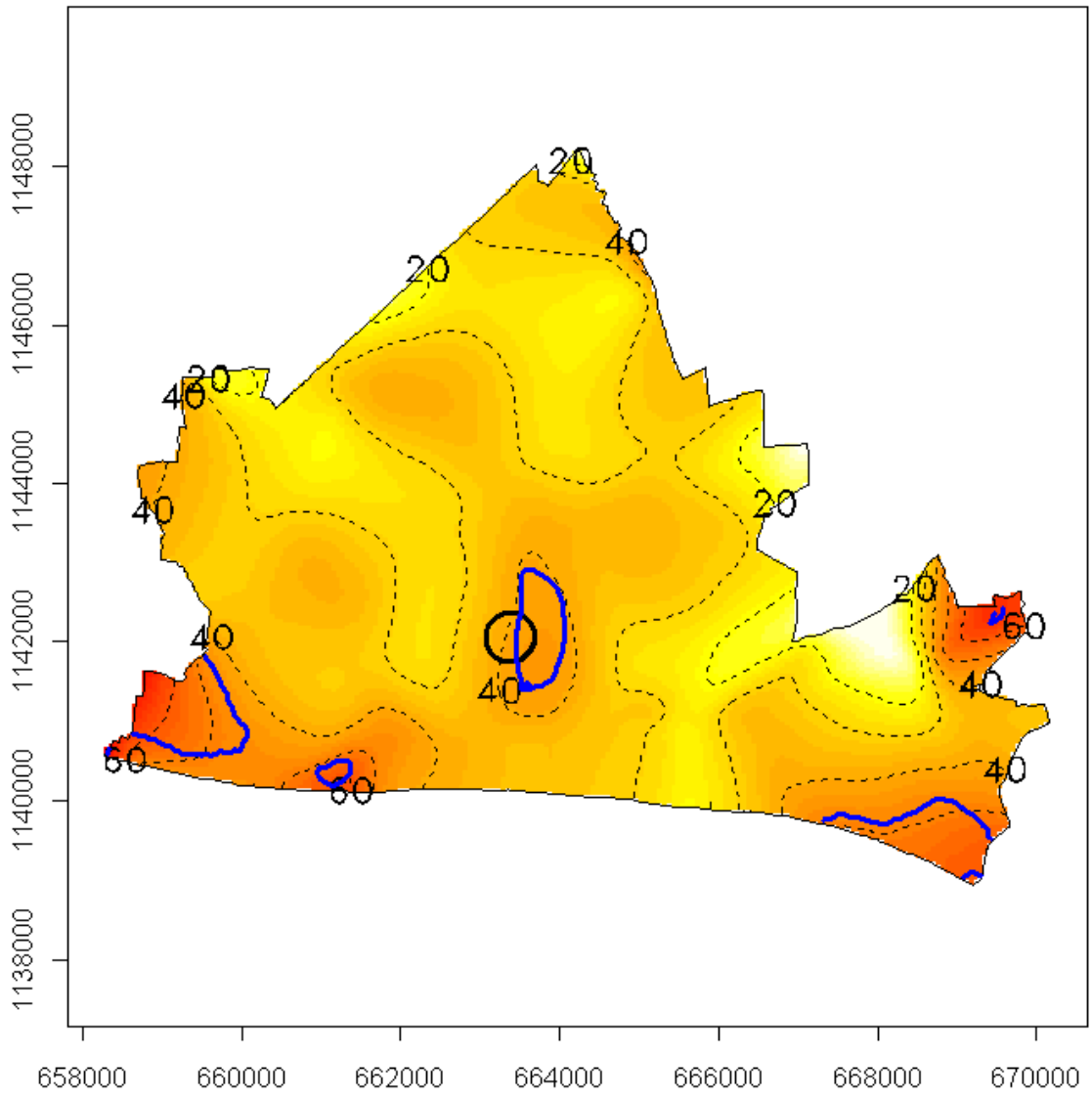

Figure 3.2.

Kernel smoothing prevalence map of PHFD in percentage and potential clusters (the blue line areas representing significant clusters detected by Kernel estimation of spatial relative risk; the black circle in the middle of the map is the primary potential cluster from spatial scan test)

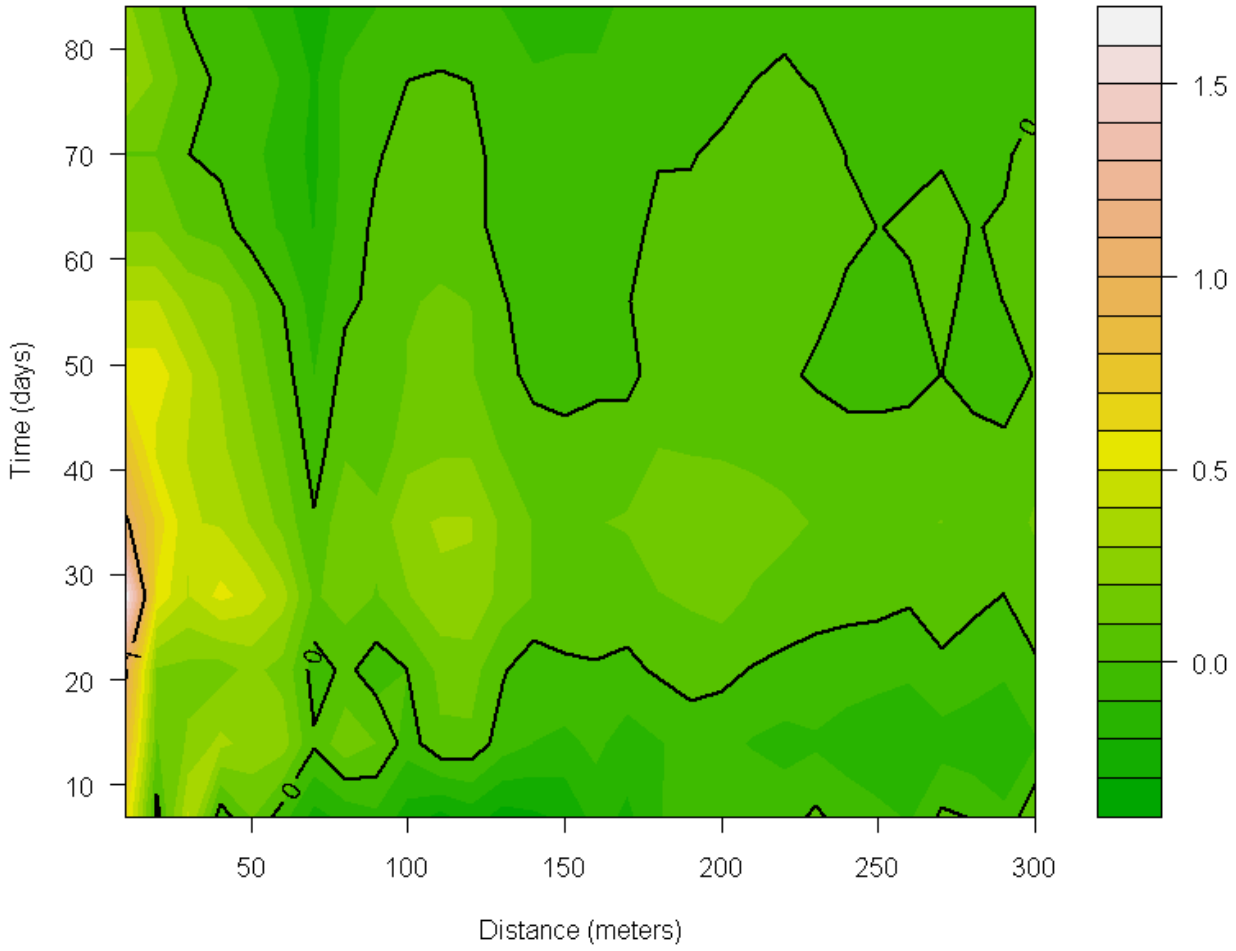

Figure 3.3.

Space-time K-function to explore time and space clustering of PHFD in the area during 2008

Chapter 4

Mathematical Modeling of Porcine Reproductive and Respiratory Syndrome Virus Infection in a Pig herd

Written in the style of "Preventive Veterinary Medicine"

Abstract

This study analyzed observational data using statistical models to extract parameters of importance for building a dynamic model of porcine reproductive and respiratory syndrome (PRRS) virus transmission within a herd. These parameters were then used to build mathematical models of PRRSV (PRRSV) spread and control in sows and nursery pigs. The observational data included results of a PRRS enzyme-linked immunosorbent assay (ELISA) on blood samples from 795 newborn piglets sampled longitudinally from seven swine herds in Ontario. A random effect linear model was used to estimate the decay of maternal immunity, and random effect Poisson regression was used to estimate a transmission parameter. Based on the parameters estimated from the

data and additionally obtained from the literature, the production-stage structured susceptible-infectious-resistant (S-I-R) deterministic mathematical model for sows and the age-structured maternally immune-susceptible-infectious-resistant (M-S-I-R) model for nursery pigs were built to include herd demographics and dynamics of PRRSV infection in a 1000-sow herd. The herd was assumed to be completely susceptible and was seeded with one infectious sow. It was further assumed that transmission from piglets to sows does not occur, and that transmission between different batches of growing pigs does not occur. Control strategies included gilt acclimatization, herd closure, and mass immunization in different combinations. Under the assumptions used in the mathematical model, herd closure for at least 40 weeks can eliminate the virus when duration of sow immunity is long (approximately 80 weeks) but not when it is short (approximately 36 weeks). Mass immunization with 100% efficacy applied simultaneously to all sows can eliminate the infection if a herd is closed for at least 5 weeks after immunization. If the efficacy of mass immunization is below 100%, a longer period of herd closure should be planned to control the disease. A mathematical model is a useful tool to guide control strategies. This study showed the possibility of PRRSV elimination in a herd, in general agreement with the results of field observations. A transmission parameter, which was relatively simple to obtain from diagnostic data, was critical in building the model that described PRRSV dynamics. Duration of sow immunity was another parameter critical for output of the examined control strategies. Using diagnostic data, particularly from sow herds in the early stages of infection, would allow building of stochastic models and further progress in this field.

Introduction

Porcine reproductive and respiratory syndrome (PRRS) was first reported in the U.S in the late 1980s (Keffaber, 1989) and then rapidly spread throughout the world, affecting almost all countries (Zimmerman et al., 2006). It is caused by PRRSV (PRRSV) which was first isolated in the Netherlands in 1991 (Wensvoort et al., 1991). The disease is characterized by a variety of reproductive problems in sows and respiratory disease in pigs of all age groups (Zimmerman et al., 2006). For a long period of time, PRRS has been considered primarily a production-limiting disease, and as such is of primary interest for individual producers and for their herd veterinarians.

Several factors have contributed to a change in attitude toward this disease in recent times. On the negative side, there has been the emergence of porcine high fever disease that was associated with highly pathogenic PRRSV in Asia (McOrist and Done, 2007). This disease was characterized by its fast spread and high mortality in breeding-age animals. Although to a much lesser extent, a similar trend has been observed in parts of the United States with emergence of PRRS virus (PRRSV) restricted fragment length polymorphisms (RFLP; genotype) 1-8-4, and parts of Canada with PRRSV genotype 1-?-4 (Rosendal et al., 2010). These genotypes were associated with outbreaks characterized by more severe clinical signs. In 2005, the annual cost of PRRS to the US swine industry was estimated at $560 million (Neumann et al., 2005). More recently, the estimate has been updated to an annual cost of $641 million (Pork Checkoff Study, August 17, 2011). In Canada, the cost was estimated by the George Morris Centre at $130 million per year (Mussell, 2010). On the positive side, there have been some success stories as well. The Chilean commercial swine industry reported elimination of

PRRSV although a formal freedom from disease test has not been published in the scientific literature (Osorio, 2010). The Swedish veterinary authority identified incursion of an outbreak in Sweden, and successfully contained the outbreak and eliminated the infection using demographic measures, including zoning, movement control, “stamping out”, cleaning and disinfection, and a vacancy period of 3 weeks before the herds were repopulated (Carlsson et al., 2009). Finally, a project led by the University of Minnesota reported PRRS elimination from a zone in Minnesota (Corzo et al., 2010). In addition the development of new surveillance and infection control technologies and approaches at the industry level, provide some rationale to suggest that better control of this disease in areas with different potential for internal circulation and external introduction is possible.

The first step toward regional elimination is to have in place methods, strategies, and approaches for disease elimination at the herd level. In addition to field observational studies, clinical trials, and program evaluation, mathematical modeling may be a good tool to help understand the dynamics of infectious diseases in a population. They might also help to evaluate the effects of infection control strategies under simulation conditions without excessive cost. This tool has been considered important in veterinary medicine (de Jong, 1995). For PRRS, mathematical models have been used to explain the dynamics of infection or vaccine strategy (Nodelijk et al., 2003; Evans et al., 2010). All of these models are performed under European conditions using a farrow-to-finish herd. The epidemiological situation in North American (NA) swine-production regions is different with respect to the type of PRRSV and herd demographics. For the most part, the NA type of virus is present, and in some regions highly pathogenic strains circulate (Otake et al., 2010; Rosendal et al., 2010). The three-site production system is a common

organizational structure. This system is characterized by farrow-to-wean herds, good age separation, and generally a large sow herd size. Finally, herd veterinarians in some regions are commonly included in successful disease elimination efforts at the herd level. As a part of a larger project, this study aims to address two objectives. The first is to estimate parameters important for understanding PRRSV infection dynamics in Ontario herds using observational data. The second objective is to gain qualitative understanding of PRRSV dynamics when common infection control practices are applied alone or in combination. The latter objective was addressed by building simple deterministic mathematical models.

Materials and Methods

This study included two major parts. First, observational data were analyzed using statistical models to extract parameters of importance for building a dynamic model of PRRSV transmission within a herd. In the second phase, a deterministic mathematical model of PRRSV transmission was built in order to describe PRRS control strategies commonly considered under North American conditions.

Observational data

Sampling and diagnostic tests

A longitudinal study included data collected from seven Ontario commercial swine herds during 2003. The veterinarians for these herds reported a history of clinical problems in the nursery barns due to PRRSV. All herds included a minimum of 150 and a maximum of 1000 sows. The study population consisted of 4 farrow-to-wean and 3

farrow-to-finish operations which had not been vaccinated for PRRS. At the first farm visit, litters with piglets that were 2-6 days old and had not been cross-fostered were included in the study. The number of litters included per farm was 20-21. Approximately half of each study litter was randomly selected for inclusion in the study, with poor-doing and weak pigs excluded. Additional information collected at the baseline included sow's parity and piglet's weight and gender. In addition, blood samples were collected from all study sows and all selected piglets. After this first visit, the study farms were visited on alternate weeks (every 10 to 14 days) until the end of the nursery stage to collect blood samples from all study piglets.

Blood samples were submitted to the Animal Health Laboratory (University of Guelph, Guelph, Ontario, Canada) to test for exposure to PRRSV using a commercial PRRS ELISA antibody test kit (HerdChek; IDEXX Laboratories, Inc., Westbrook, Maine) with results reported as sample-to-positive (S/P) ratios. An S/P ratio of 0.4 was set as the cut-point between positive ($S/P \geq 0.4$) and negative ($S/P < 0.4$) results.

Statistical analysis of maternal immunity decay

Only data from piglets that were PRRS-positive by ELISA at the first visit were used for this analysis. The S/P values of these pigs were included in the analysis using two criteria. If the piglets had values of < 0.4 at a point of time then itself and all measurements before this point were included. If all S/P values of a piglet were ≥ 0.4, then all measurements until a minimum S/P ratio was reached were included. This sampling schedule resulted in multiple repeated measurements for each pig. The selected S/P values were log-transformed and used as the outcome. A linear mixed model was

used to evaluate the decay of maternal antibodies in individual pigs over time. The log-transformed S/P values of each included sow were offered to analysis as explanatory variables. Other variables offered as fixed effects were sow's parity, piglet's gender, and time-variant linear and quadratic effect of piglet's age. Random effects included in the model were effects of litter and of individual pigs on intercept, and random effect of linear effect of age at the pig-level (i.e., random slope). The final model was selected on the basis of statistical criteria (Akaike information criterion; AIC) and was back-transformed to the S/P scale and used to predict the age when the S/P value dropped below 0.4. The analysis was performed in Stata software (Stata Intercooled, version 10; Stata Corporation, College Station, Texas, USA).

Statistical analysis of PRRSV spread

The data from all study piglets were used in this analysis. Originally recorded S/P ratios for each animal at each sampling time were first dichotomized into positive ($S/P \geq 0.4$) and negative ($S/P < 0.4$) test results. The dichotomous data were then expanded to the daily data for each animal. The minimum age for each pig was the first day of testing, and the maximum age was the date of the last reported test.

The expansion process is based on assumptions of two considerations: the age of seroconversion and the infection status of the pig. The age of seroconversion was then determined for each piglet in one of the following ways. (*i*) If a pig was farrowed with a negative ELISA result, seroconversion occurred when the pig was detected as seropositive for the first time; (*ii*) if a pig was farrowed with a positive ELISA result and then became ELISA-negative, the age at which the pig became ELISA-positive again was

the age of seroconversion; and (*iii*) if a pig was farrowed ELISA-positive and continued to be seropositive throughout the study period, the age when a minimum S/P ratio was recorded was considered to be the age when maternal antibodies were the lowest, and the age of the first subsequent test with a higher S/P ratio was assumed to be the age of seroconversion. Then, it was assumed that detectable levels of antibodies would be observed on average 10 days after exposure to PRRSV. Thus, the age of infection for each pig (case) was calculated as the age of seroconversion minus 10 days. As we knew that antibody detectable by ELISA appears approximately 7 to 10 days after infection (Yoon et al., 1995; Loemba et al., 1996), and pigs were sampled at consecutive 10-14 day intervals, 10 days might be a reasonable estimate of the day of infection.

The infection status of pigs for each day was based on the following assumptions: (*i*) if a pig was farrowed with a negative ELISA result, the status of that pig prior to the age of infection was "susceptible" (*S*); (*ii*) if a pig farrowed ELISA-positive, became ELISA-negative, and then seroconverted, it was assumed to be maternally immune (*M*) until the day that it was detected as negative for the first time, and after that was assumed to be *S* until the day infection occurred; (*iii*) if a pig was farrowed ELISA-positive and continued to be seropositive throughout the study period, it was considered to be *M* before the day of infection; and (*iv*) a pig that became infected immediately became infectious (I) for the next 56 days (8 weeks) (Terpstra et al., 1992; Nodelijk et al., 2000), after which it became resistant (*R*) until the end of the study period. Pigs that did not seroconvert were assumed to be *S* throughout the observation period.

After infection status had been defined, daily periods were aggregated to weekly periods, and the status of a pig (*M*-*S*-*I*-*R*) in any week was the status maintained for the

longest time in that week. Additionally, new-case status (C) was assigned the week when a pig was infected. Finally, the number of pigs in each infectious state (*M*-*S*-*I*-*R*) and the number of new cases for each week was generated for each herd. This represented the dataset offered for the Poisson regression to estimate the transmission parameter. This approach has been described by Vynnycky and White (2010), and has also been performed to estimate the transmission parameter in other studies of within-herd spread (Schouten et al., 2009). Specifically, the number of *C*s in each interval can be explained by a mathematical equation where *S* is the number of susceptible pigs at the start of each interval, *I* is the number of infectious pigs, *N* is the population size ($N = M+S+I+R$) at time t, and β is the transmission parameter, which is assumed to be constant.

$$C \sim (\beta \times S \times I)/N \times \Delta t$$

Converting to a logarithm in this equation we have

$$\text{Log } C \sim \log(\beta) + \log((S \times I/N) \times \Delta t)$$

Poisson regression including only the intercept and log(S×I/N) as the offset was used to estimate the transmission parameter for each farm. In addition, Poisson regression with farm identification as a random effect was used to estimate the mean of the transmission parameter. Random effects in this model were based on a gamma distribution. The exponential function of the intercept from each model was used to estimate the corresponding β. Then these β and their 95% confidence intervals were used to calculate the basic reproductive ratios R_o as β/α (Schouten et al., 2009; Vynnycky and White, 2010), where α is the recovery rate defined as the inversion of the length of the

infectious period (8 weeks). All analyses were performed in Stata software (Stata Intercooled, version 10; Stata Corporation, College Station, Texas, USA).

Deterministic mathematical models of PRRSV transmission

Baseline model

A production-stage structured susceptible-infectious-resistant (*S-I-R*) deterministic frequency-dependent mathematical model was developed for the sow herd. The model incorporated production movement and dynamics of PRRSV infection in a 1000-sow population, consisting of three production stages (breeding [^{b}], gestation [^{p}], and farrowing [^{f}]). The sow flow in the gestation stage was modeled according to Equation 1 and Figure 4.1. Parameters used to model the flow (Table 4.1) were all used on a weekly time scale. Notably, the transmission coefficient (τ) was obtained from a previous study that reported an R_0 of ~3 (Nodelijk et al., 2000), and the infectious period was assumed to be 4 weeks (Batista et al., 2002). In addition, the duration of immunity was assumed to be on average 36 weeks (Yoon et al., 1995; Evans et al., 2010) or 80 weeks, (Lager et al., 1997). Sows in *S* and *R* compartments of the gestation stage were assumed to be transferred to the farrowing stage at a rate ρ while sows in *I* compartment were transferred at a rate ρ'.

Equation 1 (sows during gestation stage)

$$\frac{dS^p}{dt} = -\tau \mathrm{S^p}\left(\frac{\mathrm{I^p}}{\mathrm{N^p}}\right) - \delta \mathrm{S^p} - \rho \mathrm{S^p} - \sigma \mathrm{S^p} + \gamma \mathrm{R^p} + \pi \mathrm{S}^b$$

$$\frac{dI^p}{dt} = \tau \mathrm{S^p}\left(\frac{\mathrm{I^p}}{\mathrm{N^p}}\right) - \delta \mathrm{I^p} - \acute{\rho} \mathrm{I^p} - \acute{\sigma} \mathrm{I^p} - \vartheta \mathrm{I^p} + \pi \mathrm{I^b}$$

$$\frac{dR^p}{dt} = \vartheta \mathrm{I^p} - \delta \mathrm{R^p} - \rho \mathrm{R^p} - \sigma \mathrm{R^p} - \gamma \mathrm{R^p} + \pi \mathrm{R^b}$$

In the farrowing stage, sow flow was modeled in a similar manner with a few modifications (Equation 2). Notably, sows from each compartment in the farrowing stage was assumed to be returning to the breeding production stage at a rate of ζ which was calculated from weaning age. In addition, the number of sows in the farrowing stage influenced the average number of piglets born alive per week through parameter η (for farrowing sows from S^f or R^f compartments) or η' (for farrowing sows from I^f compartment).

Equation 2 (sows during the farrowing stage)

$$\frac{dS^f}{dt} = -\tau \mathrm{S}^{\mathrm{f}}\left(\frac{\mathrm{I}^{\mathrm{f}}}{\mathrm{N}^{\mathrm{f}}}\right) - \delta \mathrm{S}^{\mathrm{f}} - \zeta \mathrm{S}^{\mathrm{f}} + \rho \mathrm{S}^{\mathrm{p}} + \gamma \mathrm{R}^{\mathrm{f}}$$

$$\frac{dI^f}{dt} = \tau \mathrm{S}^{\mathrm{f}}\left(\frac{\mathrm{I}^{\mathrm{f}}}{\mathrm{N}^{\mathrm{f}}}\right) - \delta \mathrm{I}^{\mathrm{f}} - \vartheta \mathrm{I}^{\mathrm{f}} - \zeta \mathrm{I}^{\mathrm{f}} + \acute{\rho} \mathrm{I}^{\mathrm{p}}$$

$$\frac{dR^f}{dt} = \vartheta \mathrm{I}^{\mathrm{f}} - \delta \mathrm{R}^{\mathrm{f}} - \gamma \mathrm{R}^{\mathrm{f}} - \zeta \mathrm{R}^{\mathrm{f}} + \rho \mathrm{R}^{\mathrm{p}}$$

In the breeding stage, sow flow was modeled according to Equation 3. Notably, sows were culled from all compartments of this production stage according to the rate ω. In addition, in the baseline model, gilt replacements were introduced into the S^b compartment to offset the number of sows that were culled from each breeding stage compartment, as well as mortalities from each production stage compartment.

Equation 3 (sows during the breeding stage)

$$\frac{dS^b}{dt} = -\tau \mathrm{S}^{\mathrm{b}}\left(\frac{\mathrm{I}^{\mathrm{b}}}{\mathrm{N}^{\mathrm{b}}}\right) - \delta \mathrm{S}^{\mathrm{b}} + \zeta \mathrm{S}^{\mathrm{f}} - \omega \mathrm{S}^{\mathrm{b}} + \sigma \mathrm{S}^{\mathrm{p}} + \gamma \mathrm{R}^{\mathrm{p}} - \pi \mathrm{S}^{b} + [\delta(N^{\mathrm{p}} + \mathrm{N}^{\mathrm{f}} + \mathrm{N}^{\mathrm{b}}) + \omega \mathrm{N}^{\mathrm{b}}]$$

$$\frac{dI^b}{dt} = \tau \mathrm{S}^{\mathrm{b}}\left(\frac{\mathrm{I}^{\mathrm{b}}}{\mathrm{N}^{\mathrm{b}}}\right) - \delta \mathrm{I}^{\mathrm{b}} + \zeta \mathrm{I}^{\mathrm{f}} - \omega \mathrm{I}^{\mathrm{b}} + \acute{\sigma} \mathrm{I}^{\mathrm{p}} - \vartheta \mathrm{I}^{\mathrm{b}} - \pi \mathrm{I}^{\mathrm{b}}$$

$$\frac{dR^b}{dt} = \vartheta \mathrm{I}^{\mathrm{b}} - \delta \mathrm{R}^{\mathrm{b}} + \zeta \mathrm{R}^{\mathrm{f}} - \omega \mathrm{R}^{\mathrm{b}} + \sigma \mathrm{R}^{\mathrm{p}} - \gamma \mathrm{R}^{\mathrm{p}} - \pi \mathrm{R}^{\mathrm{b}}$$

For nursing [^{n}] and nursery [^{g}] periods, the age-structured maternally immune-susceptible-infectious-resistant (*M-S-I-R*) deterministic frequency-dependent mathematical model was developed. This model was linked to the sow *SIR* model and was used to model the dynamics of PRRSV infection in growing pigs. Newborn piglets for each week will be kept in the same facility and moved through the nursery period together. This is consistent with the assumption that facilities were managed all-in, all-out and weekly management is applied.

Equation 4 (nursing piglets from week 1 to week 3)

$$\left\{\frac{dS^n}{dt} = \boxed{\eta S^f} + \psi M^n - \beta \mathrm{S}^{\mathrm{n}}\left(\frac{\mathrm{I}^{\mathrm{n}}}{\mathrm{N}^{\mathrm{n}}}\right) - \varepsilon \mathrm{S}^{\mathrm{n}} - \mathrm{S}^{\mathrm{n}}\right\}_{i:1\to 3}$$; for week 2-3, $\boxed{\eta S^f}$ *is* changed into $\boxed{\mathrm{S}^{\mathrm{n}}{}_{i-1}}$

$$\left\{\frac{dI^n}{dt} = \boxed{\acute{\eta} S^f} + \beta \mathrm{S}^{\mathrm{n}}\left(\frac{\mathrm{I}^{\mathrm{n}}}{\mathrm{N}^{\mathrm{n}}}\right) - \acute{\varepsilon} \mathrm{I}^{\mathrm{n}} - \alpha \mathrm{I}^{\mathrm{n}} - \mathrm{I}^{\mathrm{n}}\right\}_{i:1\to 3}$$; for week 2-3, $\boxed{\acute{\eta} S^f}$ is changed into $\boxed{\mathrm{I}^{\mathrm{n}}{}_{i-1}}$

$$\left\{\frac{dR^n}{dt} = \boxed{0} + \alpha \mathrm{I}^{\mathrm{n}} - \varepsilon \mathrm{R}^{\mathrm{n}} - \mathrm{R}^{\mathrm{n}}\right\}_{i:1\to 3}$$; for week 2-3, $\boxed{0}$ is changed into $\boxed{\mathrm{R}^{\mathrm{n}}{}_{i-1}}$

$$\left\{\frac{dM^n}{dt} = \boxed{\eta R^f} - \psi M^n - \varepsilon M^n - M^n\right\}_{i:1\to 3}$$; for week 2-3, $\boxed{\eta R^f}$ is changed into $\boxed{\mathrm{M}^{\mathrm{n}}{}_{i-1}}$

Equation 5 (nursery pigs from week 4 to week 10)

$$\left\{\frac{dS^g}{dt} = \boxed{S^n_{i=3}} + \psi M^g - \beta \mathrm{S}^{\mathrm{g}}\left(\frac{\mathrm{I}^{\mathrm{g}}}{\mathrm{N}^{\mathrm{g}}}\right) - \chi \mathrm{S}^{\mathrm{n}} - \mathrm{S}^{\mathrm{g}}\right\}_{i:4\to 10}$$; for week 5-10, $\boxed{S^n_{i=3}}$ *is* changed into $\boxed{\mathrm{S}^{\mathrm{g}}{}_{i-1}}$

$$\left\{\frac{dI^g}{dt} = \boxed{I^n_{i=3}} + \beta \mathrm{S}^{\mathrm{g}}\left(\frac{\mathrm{I}^{\mathrm{g}}}{\mathrm{N}^{\mathrm{g}}}\right) - \acute{\chi} \mathrm{I}^{\mathrm{g}} - \alpha \mathrm{I}^{\mathrm{g}} - \mathrm{I}^{\mathrm{g}}\right\}_{i:4\to 10}$$; for week 5-10, $\boxed{I^n_{i=3}}$ is changed into $\boxed{\mathrm{I}^{\mathrm{g}}{}_{i-1}}$

$$\left\{\frac{dR^g}{dt} = \boxed{R^n_{i=3}} + \alpha \mathrm{I}^{\mathrm{g}} - \varepsilon \mathrm{R}^{\mathrm{g}} - \mathrm{R}^{\mathrm{g}}\right\}_{i:4\to 10}$$; for week 5-10, $\boxed{R^n_{i=3}}$ is changed into $\boxed{\mathrm{R}^{\mathrm{g}}{}_{i-1}}$

$$\left\{\frac{dM^g}{dt} = \boxed{M^n_{i=3}} - \psi M^g - \chi M^n - M^g\right\}_{i:4\to 10}$$; for week 5-10, $\boxed{M^n_{i=3}}$ is changed into $\boxed{\mathrm{M}^{\mathrm{g}}{}_{i-1}}$

This mathematic model based on differential equations was run in a 1000-sow population. At time t=0, it was assumed that a herd was fully susceptible (999 susceptible sows) with the exception of one infected sow (or gilt) in the breeding stage. The following assumptions were made. All replacement gilts were assumed to be from a

PRRS-free herd. Susceptible pigs become infectious at a transmission rate (τ and β for sows and piglets, respectively) which is calculated from the corresponding reproductive number (R_o) (R_s and R_{ng} for sows and piglets, respectively) and a recovery rate (υ and α for sows and piglets, respectively) defining the time for an infectious pig to become resistant. We assume that R_o in nursing or nursery pigs is higher than in sows, while recovery rate in sows is larger. Transmission can occur among sows in all production stages. Piglets born in the same week are kept in the same group until the end of the nursery stage and can be infected by the piglets in the same weekly batch. This would be equivalent to perfect all-in, all-out management by weekly batch, where there is no infection between different weekly cohorts in the nursing or nursery stage, nor is there transmission between the nursing or nursery pigs and sows with the assumption of no cross-fostering. Only resistant sows can become susceptible again at a losing-immunity rate (γ) calculated from duration of immunity. Because of the differences in the literature, duration of immunity was evaluated at two different levels: short and long duration corresponding to 36 weeks and 80 weeks, respectively (Yoon et al., 1995; Evans et al., 2010; Lager et al., 1997). For piglets with maternal immunity, the rate of decay of maternal immunity (ψ) was calculated from the duration of maternal immunity which was estimated from the observed data. Figure 4.1 shows the model of movement and dynamics of PRRS infection in sows and nursing or nursery pigs. All production parameters and infectious parameters are displayed in Table 4.1.

Herd-level control strategies

After developing this baseline scenario, three broad control measures commonly applied under North-American commercial conditions were incorporated. From the

description of the strategies below, it will follow that these strategies, in the broadest sense, are a combination of different approaches to movement control and immunity management. For convenience, we selected the time to apply the control measures to 10 weeks after introduction of the first infectious animal into a sow population. The impact of control strategies was evaluated on the basis of expected prevalence of infectious sows in the sow herd, including a special case when infection was eliminated; as well as on the expected prevalence of infectious nursery pigs at 10 weeks of age, including a special case when infection was eliminated.

Scenario 1- Gilt acclimatization: In the context of PRRS control, the process of gilt acclimatization consists of exposure of susceptible gilts to the PRRSV circulating in the recipient sow herd (resident virus). The process of exposure could be managed through exposure of gilts to viremic nursery pigs (Vashisht et al., 2008; Corzo et al., 2010) or alternatively, through injection of the live resident virus (Batista et al., 2002; Corzo et al., 2010). For the purpose of this paper, we will also consider vaccination of gilts before introduction into a herd as a process of acclimatization, which is consistent with Corzo et al. (2010), although not with Dee et al. (1995). Ideally, this process would be performed in an isolated facility and it would allow sufficient time for complete cessation of virus shedding and development of complete immunity in all animals. In the model for this scenario, we assumed two situations: first, successful gilt acclimatization in which all replacement gilts are introduced in the resistant compartment (Scenario 1a). Thus, Equation 3 was modified into Equation 6 starting at time t=10 weeks.

Equation 6 (sows during the breeding stage in Scenario 1a)

$$\frac{dS^b}{dt} = -\tau \mathrm{S^b}\left(\frac{\mathrm{I^b}}{\mathrm{N^b}}\right) - \delta \mathrm{S^b} + \zeta \mathrm{S^f} - \omega \mathrm{S^b} + \sigma \mathrm{S^p} + \gamma \mathrm{R^p} - \pi S^b$$

$$\frac{dR^b}{dt} = \vartheta \mathrm{I^b} - \delta \mathrm{R^b} + \zeta \mathrm{R^f} - \omega \mathrm{R^b} + \sigma \mathrm{R^p} - \gamma \mathrm{R^p} - \pi \mathrm{R^b} + [\delta(N^{\mathrm{p}} + \mathrm{N^f} + \mathrm{N^b}) + \omega \mathrm{N^b}]$$

This scenario would be consistent with a situation when duration of acclimatization is sufficient and gilts enter into a herd with completely developed immunity. In the second situation, unsuccessful acclimatization, 50% of acclimatized replacement gilts will be introduced to a resistant compartment and the other 50% will be introduced to the infectious compartment (Scenario 1b). Thus, Equation 3 was modified into Equation 7 starting at time t = 10 weeks.

Equation 7 (sows during breeding stage in Scenario 1b)

$$\frac{dS^b}{dt} = -\tau \mathrm{S^b}\left(\frac{\mathrm{I^b}}{\mathrm{N^b}}\right) - \delta \mathrm{S^b} + \zeta \mathrm{S^f} - \omega \mathrm{S^b} + \sigma \mathrm{S^p} + \gamma \mathrm{R^p} - \pi S^b$$

$$\frac{dI^b}{dt} = \tau \mathrm{S^b}\left(\frac{\mathrm{I^b}}{\mathrm{N^b}}\right) - \delta \mathrm{I^b} + \zeta \mathrm{I^f} - \omega \mathrm{I^b} + \acute{\sigma} \mathrm{I^p} - \vartheta \mathrm{I^b} - \pi \mathrm{I^b} + 0.5[\delta(N^{\mathrm{p}} + \mathrm{N^f} + \mathrm{N^b}) + \omega \mathrm{N^b}]$$

$$\frac{dR^b}{dt} = \vartheta \mathrm{I^b} - \delta \mathrm{R^b} + \zeta \mathrm{R^f} - \omega \mathrm{R^b} + \sigma \mathrm{R^p} - \gamma \mathrm{R^p} - \pi \mathrm{R^b} + 0.5[\delta(N^{\mathrm{p}} + \mathrm{N^f} + \mathrm{N^b}) + \omega \mathrm{N^b}]$$

Scenario 2- Herd closure. This strategy has been described as a method of PRRS elimination (Torremorell et al., 2003; Zimmerman et al., 2006; Schaefer and Morrison, 2007) which consists of closing a sow herd to replacement animals for a period of time (e.g., 6 months) while breeding of remaining animals continues. For the purposes of this study, we assumed that the herd will stop introduction of gilts for a certain length of time (i.e., 30 weeks or more). After this, the herd will be opened and all replacements gilts will be either susceptible (100% susceptible), or successfully acclimated (100% resistant) or unsuccessfully acclimatized (50% resistant and 50% infectious). Thus, during herd

closure, Equation 3 will be further modified into Equation 8 to remove the inflow due to replacement animals. When herd closure ends, we assume that a number of gilts (to replace all sow mortalities and sows culled during closure) were first introduced, followed by weekly gilt introductions as described in Equations 3, 6, and 7 for all susceptible, successfully acclimated, and unsuccessfully acclimatized gilts, respectively.

Equation 8 (sows during breeding stage and herd closure)

$$\frac{dS^b}{dt} = -\tau \mathrm{S^b}\left(\frac{\mathrm{I^b}}{\mathrm{N^b}}\right) - \delta \mathrm{S^b} + \zeta \mathrm{S^f} - \omega \mathrm{S^b} + \sigma \mathrm{S^p} + \gamma \mathrm{R^p} - \pi S^b$$

Scenario 3- Mass immunization. For the purposes of this study, mass immunization was considered to be a one-time application of live virus either in the form of a live modified vaccine virus (Dee and Philips, 1998; Gillespie and Carroll, 2003) or the resident live virus (Batista et al., 2002; Opriessnig et al., 2007). First we assumed that this mass immunization has 100% efficacy (Scenario 3a). However, since sows were immunized with live virus, we had to make some additional assumptions. At the time of immunization (week 10), we assumed that the herd was closed, all sows from susceptible compartments of all production stages were moved to the infectious compartments while all sows from infectious compartments of all production stages remained in the infectious compartments and all sows in the resistant compartments remained in the resistant compartments. During 4 weeks (week 11 to week 14), sows in the infectious and resistant compartments did not change their status. The equations 1, 2, and 3 were modified to equations 9, 10, and 11. After week 14, we assumed that all sows from the infectious compartments moved to the resistant compartments. All sows in the resistant compartments from this point start the typical flow from resistant to susceptible with the rate γ. All sow flows become similar to Scenario 2 (herd closure). The herd was closed at

time 10, when all sows were immunized, and remained closed for a minimum of five weeks after mass immunization. At the end of the period of closure, the herd is assumed to be re-opened, with all gilts coming from negative herds, or from successful or unsuccessful acclimatization according to Equations 1,2, 3 or 6 or 7, respectively.

Equation 9 (sows during the gestation stage – Scenario 3a – week 10-14)

$$\frac{dS^p}{dt} = 0$$
$$\frac{dI^p}{dt} = -\delta \mathrm{I}^{\mathrm{p}} - \rho \mathrm{I}^{\mathrm{p}} - \acute{\sigma} \mathrm{I}^{\mathrm{p}} + \pi \mathrm{I}^{\mathrm{b}}$$
$$\frac{dR^p}{dt} = -\delta \mathrm{R}^{\mathrm{p}} - \rho \mathrm{R}^{\mathrm{p}} - \sigma \mathrm{R}^{\mathrm{p}} + \pi \mathrm{R}^{\mathrm{b}}$$

Equation 10 (sows during the farrowing stage– Scenario 3a – week 10-14)

$$\frac{dS^f}{dt} = 0$$
$$\frac{dI^f}{dt} = -\delta \mathrm{I}^{\mathrm{f}} - \zeta \mathrm{I}^{\mathrm{f}} + \rho \mathrm{I}^{\mathrm{p}}$$
$$\frac{dR^f}{dt} = -\delta \mathrm{R}^{\mathrm{f}} - \zeta \mathrm{R}^{\mathrm{f}} + \rho \mathrm{R}^{\mathrm{p}}$$

Equation 11 (sows during the breeding stage– Scenario 3a – week 10-14)

$$\frac{dS^b}{dt} = 0$$
$$\frac{dI^b}{dt} = -\delta \mathrm{I}^{\mathrm{b}} + \zeta \mathrm{I}^{\mathrm{f}} - \omega \mathrm{I}^{\mathrm{b}} + \acute{\sigma} \mathrm{I}^{\mathrm{p}} - \pi \mathrm{I}^{\mathrm{b}}$$
$$\frac{dR^b}{dt} = -\delta \mathrm{R}^{\mathrm{b}} + \zeta \mathrm{R}^{\mathrm{f}} - \omega \mathrm{R}^{\mathrm{b}} + \sigma \mathrm{R}^{\mathrm{p}} - \pi \mathrm{R}^{\mathrm{b}}$$

In addition, immunization with less than 100% efficacy (Scenario 3b) is considered. We assumed different immunization efficacies (IE), i.e., 60%, 70%, 80%, and 90%. In this scenario, after immunization of all sows, the percentage of susceptible sows (equivalent to IE) become infected and infectious identical to Scenario 3a, while the others (equivalent to 1-IE) remain susceptible and continue to transition according to Equations

1, 2, 8. The herds are also closed for an identical number of weeks as in Scenario 3a. To make a scenario of elimination possible in this deterministic mathematical model, we used the actual number of animals as our herd size (instead of proportions) in all scenarios, further conditioning the transition rate from susceptible to infectious to be 0 if the number of infectious animals was < 1.

The effect of elimination of PRRS is considered by comparing the prevalence and number of infectious sows and nursery pigs at 10 weeks of age between scenarios and within scenarios with different values of parameters of interest. The modeling process was performed in Anylogic 6.5 (XJ Technologies, St. Petersburg, Russian Federation) and data extracted from the modeling scenarios will be plotted using Stata software (Stata Intercooled, version 10; Stata Corporation, College Station, Texas, USA) to allow visual interpretation.

Results

Observational data

Included in the study were 795 newborn piglets from 146 sows. The observed prevalence of ELISA-positive sows ranged from a minimum of 28.2% to a maximum of 50.5%. The mean S/P ratio for the positive sows was 0.82 (95% CI, 0.75-0.90). Among variables used to estimate antibody level in piglets, only the transformed S/P ratio in sows and age of piglets were statistically significant in the final linear model. On the basis of this statistical model and the 95% confidence interval of the mean sow S/P ratio, maternal immunity dropped below 0.4 at an estimated 19 days of age (95% CI:16 to 22 days) for pigs from sows with the lower mean S/P ratio (S/P = 0.75). For pigs from sows

with the higher mean S/P ratio (S/P = 0.90), the duration of maternal immunity was expected to be 21 days (95% CI, 19-24). Figure 4.2 shows that the S/P values for piglets changed with age and sow S/P ratio. From these field study results, the maternal immunity duration was defined as 3 weeks for use in the mathematic model in suckling-nursery pigs.

The number of piglets in each compartment (M, S, I, R) and the number of cases at each week of age in the populations of seven farms are shown in Table 4.2. The result of R_o and the 95% confidence interval calculated from the Poisson regression for each farm and from the Poisson random effect for all seven farms are shown in Table 4.3. The value of R_o is variable in different farms with an average R_o of 9.76 (95% CI, 7.26-13.13). From these field study results, the R_o was defined as 9.76 for use in the mathematic model in suckling-nursery pigs.

Mathematical modeling

The baseline scenario model explained the dynamics of PRRS infection in sows and nursery pigs when the first infectious gilt was introduced into the naïve herd (Figure 4.3). The number of infectious sows increase to peak at week 16 with a prevalence of 30.1%. After this peak, the number of infectious sows diminish rapidly and then reach a steady state from week 100 until week 200, when the simulation ended. During the steady-state period, the proportions of susceptible, infectious, and resistant sows were approximately 35.5%, 7.9%, and 56.6% respectively. Similarly, the prevalence of infectious 10-week old nursery pigs increases, reaches a peak (67.9%) at week 15, and remains stable (60.7%) from week 100 until week 200. However, the steady-state

prevalence of infectious and resistant animals differs between sows and 10-week old nursery pigs. The number of resistant sows is higher than that of infectious sows, while in 10-week old nursery pigs, the number of infectious pigs is higher than the number of resistant pigs. When the longer duration of sow immunity was assumed, prevalence of infection in sows during the steady-state period was lower (4.7%), while the prevalence of infection in 10-week-old nursery pigs was slightly higher (64.8%) than in the model that assumed a shorter duration of sow immunity. Table 4.4 shows the prevalence of infection in sows and 10-week-old nursery pigs for each scenario during the steady-state period, e.g., 200 weeks after the first animal is infected.

Scenario 1 model results for the short duration of sow immunity shows that implementation of successful gilt acclimatization reduces the prevalence of infectious sows during the steady state period to 5.5%. Similarly, unsuccessful acclimatization reduces the prevalence of infectious sows during the steady-state period to 7.4%. In nursery pigs, successful and unsuccessful gilt acclimatization result in a slight increase in the prevalence of infection in 10-week-old pigs during the steady-state period to approximately 63.4% and 62.2%, respectively. In this same scenario, for the longer duration of sow immunity, a decrease in the prevalence of infection in sows relative to the baseline scenario was observed, as well as an increase in the prevalence of infection in nursery pigs. In general, the patterns of prevalence of infection in sows and 10-week-old nursery pigs under the assumptions of both acclimatization strategies are similar to those of the baseline scenario. In this model, neither acclimatization strategy alone could eliminate the virus.

Under the assumption of short duration of sow immunity, when herd closure for 30 to 50 weeks was followed by opening the herd and introducing only susceptible replacement gilts, or successful, or unsuccessful gilt acclimatization, PRRSV either in sows or in nursery pigs could not be eliminated (Figure 4.4). Prevalence during the steady-state period was similar to the prevalence during baseline model, scenario 1a, or 1b (Table 4.4) depending on the assumptions used about gilt replacement after herd closure. With successfully acclimatized or susceptible replacement gilts, elimination of PRRSV was possible when a longer duration of sow immunity was assumed, and when closure time was > 31 weeks (Figure 4.4; Table 4.4). Unsuccessful acclimatization with the assumption of a long duration of sow immunity could not eliminate the virus even when the herd was closed for 50 weeks.

With the assumption of short duration of sow immunity and mass immunization with 100% immunization efficacy (IE) at week 10, PRRSV can be successfully eliminated from the herd if the herd is closed > 5 weeks after immunization and that is followed by introducing only susceptible gilts or by introducing successfully acclimatized gilts (Figure 4.5). When the period of herd closure after immunization is followed by unsuccessful acclimatization, the virus cannot be eliminated and prevalence of infection persists during the steady-state period similar to scenario 1b. With assumption of a longer duration of sow immunity, successful elimination can be achieved when the herd is closed for only 5 weeks, followed by introduction of only susceptible gilts or with successful gilt acclimatization. Unsuccessful acclimatization cannot eliminate the virus even with a closure time of 50 weeks. Thus, for this situation, instead of considering complete elimination, we considered a critical time when the prevalence of infection in

sows might become very low e.g., 0.1% to 0.2%, which may occur when herd closure is > 6 weeks.

Under the assumption of mass immunization with IE < 100% and a short duration of sow immunity, in general, cannot completely eliminate the virus. With IE of 90%, herd closure for > 15 weeks and introduction of only susceptible replacement gilts, the critical time for a low prevalence of infection may occur at weeks 40 to 50. With a 90% IE, herd closure for > 6 weeks and successful acclimatization, the critical time for a low prevalence of infection may occur at weeks 30 to 60. However, with this IE and herd closure followed by unsuccessful acclimatization, a low prevalence of infection does not occur no matter how long the duration of herd closure. When mass immunization has an IE < 90%, the virus cannot be eliminated and a low prevalence of herd infection is never achieved.

Under the assumption of an IE of < 100% and a long duration of sow immunity, elimination of the virus can be attained with immunization with an IE of > 60% and herd closure for a minimum of 6 weeks followed by successful gilt acclimatization. When herd closure is followed by introduction of susceptible gilts, this strategy could still successfully eliminate the virus, but this depends on a combination of duration of herd closure and IE. For instance, the duration of herd closure in this model needs to be > 34 weeks, > 26 weeks, > 17 weeks, and > 5 weeks for the IEs of 60%, 70%, 80%, and 90%, respectively. The virus cannot be eliminated if this strategy of herd closure and mass immunization is followed by unsuccessful gilt acclimatization, regardless of duration of herd closure and IE.

Discussion

The duration of maternal immunity in piglets in this study (3 weeks) agrees with that reported in studies by Zimmerman (2006), but not with the results of other reports. For example, the time for maternal antibody decay has been reported in the range of 4 to 10 weeks of age (Houben et al., 1995; Liu et al., 2008; Nodelijk et al., 1997). This long period of maternal immunity (up to 10 weeks of age) seem unlikely as, in our study, many piglets became infected at 4 weeks of age or even earlier, suggesting that maternal immunity was already waning. In addition, in the model developed in this study, decay of maternal immunity, reported as an S/P ratio, depended on the sow's level of immunity (also reported as an S/P ratio). Thus, rates of maternal antibody decay might vary due to a different status of PRRSV infection on these farms, for example, time since exposure, level of sows' immunity, strains of virus, serological test used, exposure route, and dosage (Johnson et al., 2004). The large number of samples from seven different farms in this study may have contributed to the validity of the results. In fact, in our observational study, we found that the majority of infectious cases occurred when the piglets lost their maternal immunity beginning at 4 weeks of age. The short duration of maternal immunity implies that PRRS control should not rely much on the protective capacity of maternal immunity. However, under the assumptions of this study, only a very small number of pigs were infected before three weeks of age. This information may be considered in the context of the serological status of included herds, since a maximum of 50.5% of sows had detectable antibodies. It would be interesting to explore whether a high level of maternal antibodies in the population of piglets in a single weekly batch (e.g., produced by recent exposure of the sows to live virus) could protect individual piglets from

circulation of PRRSV directly, or the entire population of piglets through an indirect effect (i.e., herd immunity). We did not pursue this investigation as our data were not sufficient for such purposes.

The average R_o in suckling nursery pigs estimated in this study (9.76) was considerably higher than that previously reported in sows. Nodelijk (2001) estimated that R_o in vaccinated nursery pigs was > 4.9 (Nodelijk et al., 2001). Since PRRS vaccine was believed to reduce transmission between piglets, the actual R_o in an unvaccinated population should be higher than 4.9. Another study performed by Mondaca-Fernandez (2007) estimated a low value of R_o with 95% confidence interval 0.136 to 3.218, and there was no difference between unvaccinated and vaccinated groups (Mondaca-Fernandez et al., 2007). Few previous studies estimated R_o in nursery pigs. Values of R_o differ between populations and from this perspective it is not surprising that study results differ. This was clearly depicted even from the reported values of R_o in this study, which varied considerably among different herds, with a minimum value of ~3.6 and a maximum of ~14.7. Several factors may contribute to high R_o values in nursery pigs. In our study, we assumed that piglets and sows were infectious for 8 and 4 weeks, respectively (Terpstra et al., 1992; Batista et al., 2002; Wills et al., 2003). This assumption was based on the finding that PRRSV infection is age dependent, with viremia persisting for a longer period in younger pigs than in finishers or sows (Klinge et al., 2009). The housing system may also explain that R_o in nursery piglets is expected to be larger than in sows (e.g., management of the nursery provides a great opportunity for transmission between pigs) (Nodelijk et al., 2003). In addition, the pathogenicity of PRRSV has been reported to vary with genotype (Meng, 2000). The more pathogenic

MN-184 virus was shown to be more infectious by aerosol than the VR-2332 genotype under experimental conditions (Cutler et al., 2011). The study of Otake et al (2010) showed that infectious PRRSV genotype 184, but not the other PRRSV genotypes, could be found 9.1 km from the from the source barn. It is thus possible that some PRRSV persist in higher quantities in air, and at the same time are also more infectious. This situation alone, even without considering other factors, might explain the high variability in R_o found among different farms in this study. The observational study was conducted before emergence of genotypes that were considered highly virulent. This emphasizes the need for more updated information using serological and molecular studies, particularly when combined with regular diagnostics.

The high value of R_o in this study can explain the very high prevalence of PRRSV infection in nursery pigs. In our data, the prevalence of infection of 68% (421/619 at 10 weeks of age) was in concordance with studies that reported a 72% prevalence of infection (Sasaki et al., 2010) in this age group. Thus, 9- to 16-week- old pigs are the preferred age group to detect PRRSV in a herd (Duinhof et al., 2011).

In this study, duration of immunity in the sow population was of utmost importance in control and elimination of PRRSV infection. However, there is little agreement about this duration among studies. For this reason, we defined duration of immunity as short (36 weeks) and long (at least 80 weeks), as reported in previous studies (Zimmerman et al., 2006; Yoon et al., 1995; Evans et al., 2010; Lager et al., 1997). Models based on a short duration of immunity will be discussed when the results obtained under different duration of immunity do not differ. When the results differ,

results from both models will be discussed. In general, the assumption of a short duration of immunity posed a greater challenge to the control strategy in this study.

From the baseline model, when the virus was introduced into the herd, the peak of infection was 16 weeks later, with a prevalence of infection in sows up to 30.1%, and at about the same week, infection peaked in 10-week-old nursery pigs. It would be logical to expect that peak of prevalence would occur in the 10-week nursery pigs later than in the sows. However, with the very high value of R_o in nursery pigs, the disease spreads very fast and reaches the peak of infection at the same time as in the sows. Thus, in practice, when a PRRS outbreak is recognized, clinical disease in one or all production stages can be observed (Zimmerman et al., 2006). The baseline model describes the herd with endemic PRRS infection. The proportions of resistant sows and nursery pigs in the steady state in the baseline model are very similar to the seroprevalence in our observed data and in endemic herds as reported by other investigators (Bautista et al., 1993; Anonymous 2009). In the model, for both short and long duration of sow immunity, the prevalence of infection at the endemic level is low in sows compared to that in 10-week-old nursery pigs. It should also be noted that in the steady state, the prevalence of immune sows was high and prevalence of infectious was low. In contrast, the prevalence of infectious 10-weeks old nursery pigs was high and prevalence of immune nursery pigs was low.

The prevalence of immune animals does not necessarily correspond to seroprevalence. In the modeling approach developed in this paper, animals could belong only to mutually exclusive infectious groups, thus the proportion of that population that is immune cannot be infectious. This does not necessarily correspond to results of

virological and serological tests. Antibodies, as measured by ELISA, may be present in the part of the population that is still PCR-positive for virus and presumably infectious. This was shown in the results of chapter 5, when 48% of 117 finisher pigs were ELISA-positive, but 16% and 34% of these ELISA-positive pigs were also serum and tissue PCR- positive, respectively. Thus, seroprevalence, as measured by ELISA, can overestimate the prevalence of immune animals. In contrast, infection may be present even when antibodies are not detectable by ELISA. Batista (2004) found that pigs with negative or low positive ELISA S/P ratios may still harbor infectious PRRSV.

According to the model, acclimatization is considered a control strategy (Dee et al., 1995) in which the incoming gilts can be exposed to the virus by many means such as inoculation, direct contact, or feed back of tissue. A previous study showed that acclimatization through contact with infectious animals can infect naive gilts (Vashisht et al., 2008). Similarly, delivery of live virus to susceptible and to seropositive gilts through injection results in viremic gilts (Batista et al., 2002). However, the time to clear the virus for these acclimatized gilts is unclear. In reality, this time is very variable between farms and is often set at a standard value, e.g., 30 days. Vaccination is considered part of acclimatization by some authors (Corzo et al., 2010), but not by others (Dee et al., 1995). For these reasons, we used two types of acclimatization: successful and unsuccessful acclimatization. For both types of acclimatization, the prevalence of infection in sows was considerably lower during the steady-state period than in the baseline model; in contrast, the prevalence of infection in 10-week-old nursery pigs was higher during the steady-state period than in the baseline model. This explains that the low level of infected

sows will produce more susceptible piglets which become infected during the suckling and nursery stages.

Acclimatization alone was not successful in eliminating PRRSV infection from a sow herd, although success using acclimatization alone has been reported (Fano et al., 2005). The type of modeling approach could be one reason for that finding. Our assumption was that successfully acclimatized gilts would enter a herd and mix homogeneously with sows already existing in that herd. Immunity decay would then be equally distributed among breeding animals of all ages. Presumably, animals with more recent exposure to the virus would lose their immunity later, and this was a limitation that was not addressed in the study. An age-structured model would be better to address this issue, or even an individual-based model. In addition, stochastic models would be much better suited to address the issue of variability inherent to small populations. However, gilt acclimatization alone has not been recently listed as a PRRS elimination strategy (Corzo et al., 2010), but as a control strategy and an integral part of other control strategies. Its importance is further emphasized by the finding that no other PRRS control strategy was successful in elimination of virus if acclimatization was not successful. This seems intuitive. However, a recent study in Quebec performed by Lambert (2011) from the University of Montreal (personal communication) found that many herds did not allow sufficient time between cessation of exposure and introduction of gilts. This practice would result in a scenario that is similar to our unsuccessful acclimatization. In the Quebec study, it was also found that only a limited number of herds performed diagnostic monitoring to measure the success of exposure. This lack of diagnostic

monitoring might also result in unsuccessful acclimatization, even when the period of acclimatization appears to be adequate.

Herd closure under the assumption of a short duration of sow immunity could not eliminate PRRSV infection even when the closure time was > 60 weeks. Under field conditions, successful elimination of infection in sow farms of different sizes was reported after a herd closure period of 6 months (Schaefer and Morrison, 2007), although unsuccessful elimination has also been reported after 11 months of herd closure (Sandri et al., 2010). Torremorell et al (2003) concluded that it is difficult to eliminate PRRS in large-scale farms unless other factors are applied, for example, good biosecurity programs (Torremorell et al., 2003). In our study, the virus was successfully eliminated under the assumption of long duration of immunity. Thus duration of immunity is an important factor in the success of the herd closure strategy. More studies on duration of immunity should be conducted. Stochastic models would be much more suited to address this question for dynamic models.

Mass immunization over a short period of time can be performed for all sows in a herd by one of the following strategies: vaccination with modified live virus (Dee and Philips, 1998; Gillespie and Carroll, 2003), serum inoculation (Batista et al., 2002; Opriessnig et al., 2007), or tissue feedback (McCaw et al., 2003). The strategy that was assumed here was one-time inoculation of the entire herd, although herd demographics and the type of immunization under field conditions may necessitate a one-time application to each animal over a period of time (i.e., so that sows are immunized after weaning rather than at > 90 days of gestation. We assumed that immunization with a PRRSV that (i) is genetically homologous to the virus circulating in the herd, (ii) contains

an optimal quantity of immunogenic virus, and (iii) is applied properly to animals that are able to mount an immune response, will result in an efficacy of 100% in preventing infection. Under these three assumptions, elimination could be achieved after 5 weeks of herd closure. Five weeks is also the time after which all infectious sows were assumed to transition from the infectious compartment to the resistant compartment.

However, in reality, we cannot be certain that immunization will be 100% efficacious. Under the assumption of < 100% immunization efficacy was assumed, the results were different. We assumed that unsuccessful immunization will result in a small number of sows that remain fully susceptible to PRRS infection after immunization. Some of these sows will become infected while successfully immunized sows are shedding the virus. Because of the small values of R_o assumed in this study, these susceptible sows will become infected slowly, maintaining a small proportion of infectious sows in the herd. Thus a longer period of herd closure is needed after immunization in case susceptible gilts are introduced into the herd. Under the assumption of short duration of sow immunity, the number of susceptible sows increases before the end of herd closure, and the virus cannot be eliminated from the population. However, when long duration of sow immunity is assumed, PRRS elimination is possible even when IE < 100%.

Under the assumption of short duration of sow immunity with IE < 100% the virus cannot be eliminated, but at some point, referred to as the critical time, the prevalence of infection in the sow population becomes very low (0.1% to 0.2%). For sow populations of > 1000, this prevalence is equivalent to one or more infected sows. Thus, incoming susceptible gilts, or sows that lose their immunity and become susceptible,

would continue to maintain infection in the herd, and the virus cannot be eliminated from the herd. However, when the sow population is <1000 sows, the number of infectious sows calculated by model will be < 1, and transmission cannot occur, according to the model specification. In this case, successful elimination is achieved. This circumstance reveals a limitation of deterministic mathematic modeling, when an unrealistic persistence of infection occurs, making elimination impossible (Maude et al., 2010). From this it may be inferred that it is easier to control the disease in a small size herd than in a large herd. Additionally, studies with PRRS vaccines have shown variable results and vaccine efficacy has not always been tested.

Interestingly, in one example of unsuccessful immunization in the model, less than perfect immunization (90% IE) was apparently able to eliminate PRRSV infection from nursery pigs, but this was followed by a secondary outbreak of large magnitude in the nursery, and a slowly developing secondary outbreak in sows which peaked at a later time than the nursery outbreak. This is important because it mimics the scenario of successful elimination (i.e., nursery pigs appear PRRS-negative), and occurs because the virus continues to circulate in gestation areas but not in the farrowing area until the number of infectious animals becomes substantial.

A mathematical model is a useful tool, having been much used to simulate processes relating to medical issues and should be applied more in disease control in animal health. This tool can act as decision support to choose the strategy for a herd, or for policy makers with a broader point of view. However, with this deterministic model there are some limitations: (i) elimination of infection in a deterministic SIR mathematical model is typically not possible, so we defined the transition from

susceptible to infectious as 0, if the population of infectious animals was < 1, (ii) stochasticity is an important element in small populations and was not considered here, and (iii) immunization under North American conditions primarily uses live virus (modified or field virus), forcing us to make some simplifying assumptions about immunization efficacy. In a herd that is experiencing a PRRS outbreak, all animals are immunized, including those that are serologically positive, which this may result in prolonged infectiousness. We also assumed a duration of infectiousness of 4 weeks in sows, based on information that duration of viremia is age dependent (Klinge et al., 2009). However, prolonged infectiousness of sows during nose-to-nose contact, as assessed by contact with susceptible animals has also been reported (Bierk et al., 2001). The R_o in this study originated from a previous report (Nodelijk et al., 2000). With the extensive testing occurring in previously PRRS-naïve herds that experience outbreaks of PRRS, there should be an abundance of data available for estimation of parameters of importance for dynamic disease modeling. In the future, models should incorporate additional control strategies and stochastic models in order to evaluate their ability to eliminate the virus from sow and growing pig populations. Despite these limitations, modeling helps to explain some commonly used PRRS control strategies in a quantitative way, and factors that determine the failure or success of these strategies.

In conclusion, maternal immunity to protect piglets from the spread of PRRS is short, and transmission of PPRS virus is very rapid in suckling and nursery pigs (R_o = 9.76). To reduce the disease in sows, acclimatization or herd closure can be applied. Immunization promises the possibility of eliminating the virus both from the sow population and 10-week-old nursery pigs. However, duration of immunity and

infectiousness are critical parameters that should be investigated in future studies to assist the model to produce a more accurate evaluation.

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Table 4.1.

Production and infectious parameters used for mathematic modeling in sows and nursing-nursery pigs

N[§]	Definition	Value	Unit	Source
a	Infectious duration in sows	4	weeks	(Batista et al., 2002)
d	Active immunity duration in sows (short and long time)	36	weeks	(Yoon et al., 1995; Evans et al., 2010)
		80	weeks	(Lager et al., 1997)
m	Maternal immunity duration	3	weeks	*Our study*
N	Number of animal in each stage at one point of time			
R_s	R_o in sows	3		(Nodelijk et al., 2000)
R_{ng}	R_o in nursing-nursery pigs	9.76		*Our study*
s	Infectious duration in nursing-nursery pigs	8	weeks	(Terpstra et al., 1992; Nodelijk et al., 2000)
w	Weaning	3	weeks	(Almond et al., 2006)
α	Recovery rate in nursing-nursery pigs	0.125	$Week^{-1}$	*Calculated from "s"*
β	Transmission rate in nursing-nursery pigs	1.22	$Pig^{-1}\ week^{-1}$	*Calculated from R_p and "s"*
γ	Losing-immunity rate in sows (short and long time)	0.027 0.0125	$week^{-1}$	*Calculated from "d"*
δ	Death rate in sow	5%	$Year^{-1}$	(Almond et al., 2006)
ε	Mortality in nursing pigs	11.8%	3 weeks	(Cutler et al., 2006)
ε'	Mortality in infected nursing pigs	17.5%	3 weeks	(Hurd et al., 2001)
ζ	Farrowing to weaning rate	0.33	$Week^{-1}$	*Calculated from "w"*
η	Alive newborn piglets	10.5	$Litter^{-1}$	(Almond et al., 2006)
η'	Alive newborn piglets from infectious sows	6	$Litter^{-1}$	(Pejsak and Markowska-Daniel, 1997; Evans et al., 2010)
υ	Recovery rate in sows	0.25	$week^{-1}$	*Calculated from "a"*
π	Breeding rate	90%	3 weeks	(Almond et al., 2006)
ρ	Normal farrowing rate	90%	16 weeks	(Almond et al., 2006)
ρ'	Infectious farrowing rate	69.8%	16 weeks	(Hurd et al., 2001)
σ	Normal abortion rate	10%	16 weeks	*Calculated from ρ*
σ'	Infectious abortion rate	30.2%	16 weeks	*Calculated from ρ'*
τ	Transmission rate in sow	0.75	$sow^{-1}week^{-1}$	*Calculated from R_s and "a"*
χ	Mortality in nursery pigs	6.8%	7 weeks	(de Grau et al., 2005)
χ'	Mortality in infected nursery pigs	12%	7 weeks	(Stevenson et al., 1993)
ψ	Losing-maternal immunity rate	0.33	$Week^{-1}$	*Calculated from "m"*
ω	Culling rate	30%	$Year^{-1}$	(Almond et al., 2006)

N[§] Notation

Table 4.2.

Number of piglets in each compartments (Maternally immune, Susceptible, Infectious, and Resistant) from 1-10 weeks

Age (week)	N	Maternal immunity	Susceptible	Infectious	Resistant	Case
1	795	445	350	0	0	1
2	795	439	355	1	0	4
3	790	370	416	4	0	0
4	695	227	448	20	0	22
5	670	141	498	31	0	14
6	667	81	515	71	0	62
7	665	49	441	175	0	121
8	667	16	348	303	0	157
9	660	9	243	408	0	55
10	619	5	192	421	1	47

Table 4.3.

Basic reproductive number (R_o) of nursing-nursery pigs in each farm and average value adjusted for the farm effect

Farm	R_o (transformed from coeficient of Poisson regression model)		
	Mean	95% confidence interval	
1	14.68	11.32	19.03
2	10.41	7.82	13.85
3	8.12	6.30	10.45
4	14.49	12.51	16.78
5	3.65	2.23	5.95
6	10.70	7.17	15.96
7	10.42	8.32	13.05
All farms	9.76	7.26	13.13

Table 4.4.

Results of modeling PRRSV control strategies with prevalence of infection in sows and in10-week old nursery pigs at the steady level (i.e., 200 weeks after the first infection)

	Scenario	Immunity in sow	Duration (weeks)	Followed by	Prevalence at stable level	
					Sows	10-week pigs
0	Baseline					
		short			0.079	0.607
		long			0.047	0.648
1	Acclimatization					
[a]	successfully	short			0.055	0.634
[b]	unsuccessfully	short			0.074	0.622
[a]	successfully	long			0.017	0.680
[b]	unsuccessfully	long			0.042	0.662
2	Herd closure					
		short	>30	Open[1]	0.054	0.634
		short	>30	Successful[2]	0.055	0.634
		short	>30	Unsuccessful[3]	0.074	0.622
		long	>31	Open[1]	0	0
		long	>31	Successful[2]	0	0
		long	30-50	Unsuccessful[3]	0.042	0.662
3	Immunization					
	100% efficacy	short	>5[*]	Open[1]	0	0
	100% efficacy	short	>5[*]	Successful[2]	0	0
	100% efficacy	short	>6[*]	Unsuccessful[3]	0.074	0.622
	100% efficacy	long	>4[*]	Open[1]	0	0
	100% efficacy	long	>4[d]	Successful[2]	0	0
	100% efficacy	long	>6[*]	Unsuccessful[3]	0.042[§]	0.662
	60%-90% efficacy	short	>15[*]	Open[1]	0.079[§]	0.607
	60%-90% efficacy	short	>6[*]	Successful[2]	0.079[§]	0.607
	60%-90% efficacy	short	>30[*]	Unsuccessful[3]	0.074	0.622
	60%-90% efficacy	long	>5[*]	Open[1]	0	0
	60%-90% efficacy	long	>5[*]	Successful[2]	0	0
	60%-90% efficacy	long	>25[*]	Unsuccessful[3]	0.042	0.662

[1]Open to all susceptible gilts
[2]Successful acclimatization
[3]Unsuccessfully acclimatization
[*] Duration of herd closure after immunization
[§] At the end of herd closure, the prevalence drop to 0.2%-0.3% in sow (the critical time), applicable for 90% efficacy only

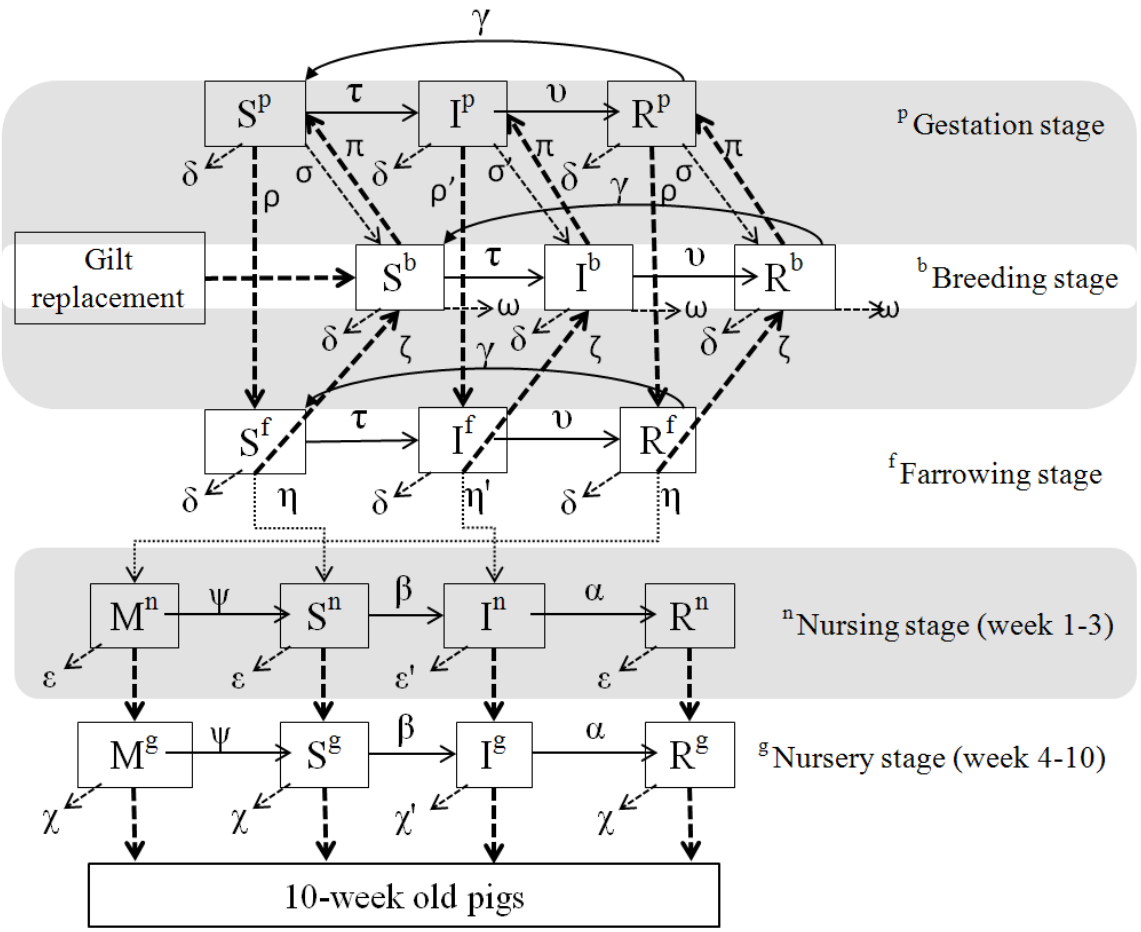

Figure 4.1.

The production stage-structured susceptible-infectious-resistant (S-I-R) mathematical model for sow and the age-structured maternally immune-susceptible-infectious-resistant (M-S-I-R) mathematical model for nursing-nursery piglet with the Greek letters representing the rates of movement (detailed values of Greek letters in Table 4.1)

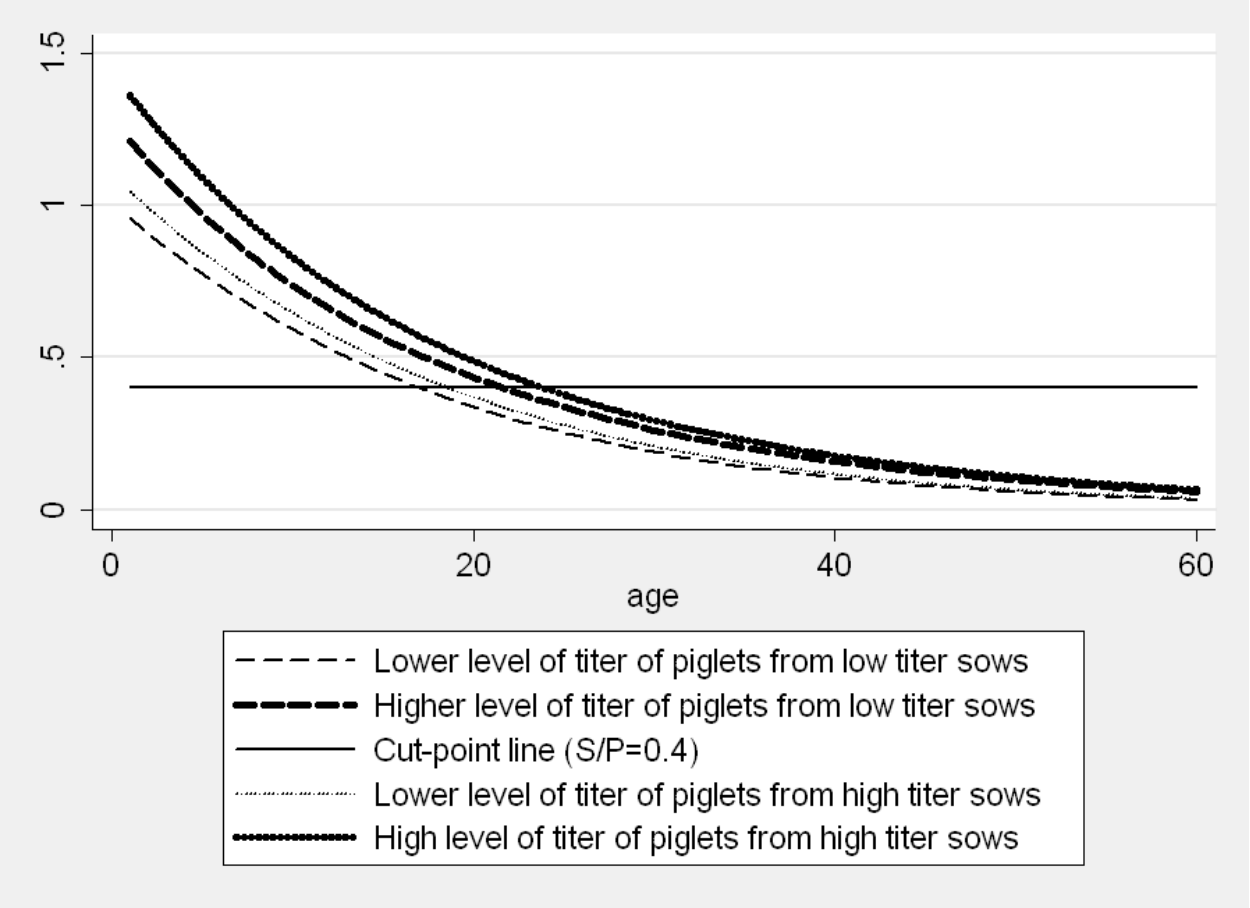

Figure 4.2.

Development of maternal immunity (S/P value) by age of piglets farrowed in litters from sows with different level of immunity and cut-point of ≥0.4 to define a positive test on ELISA

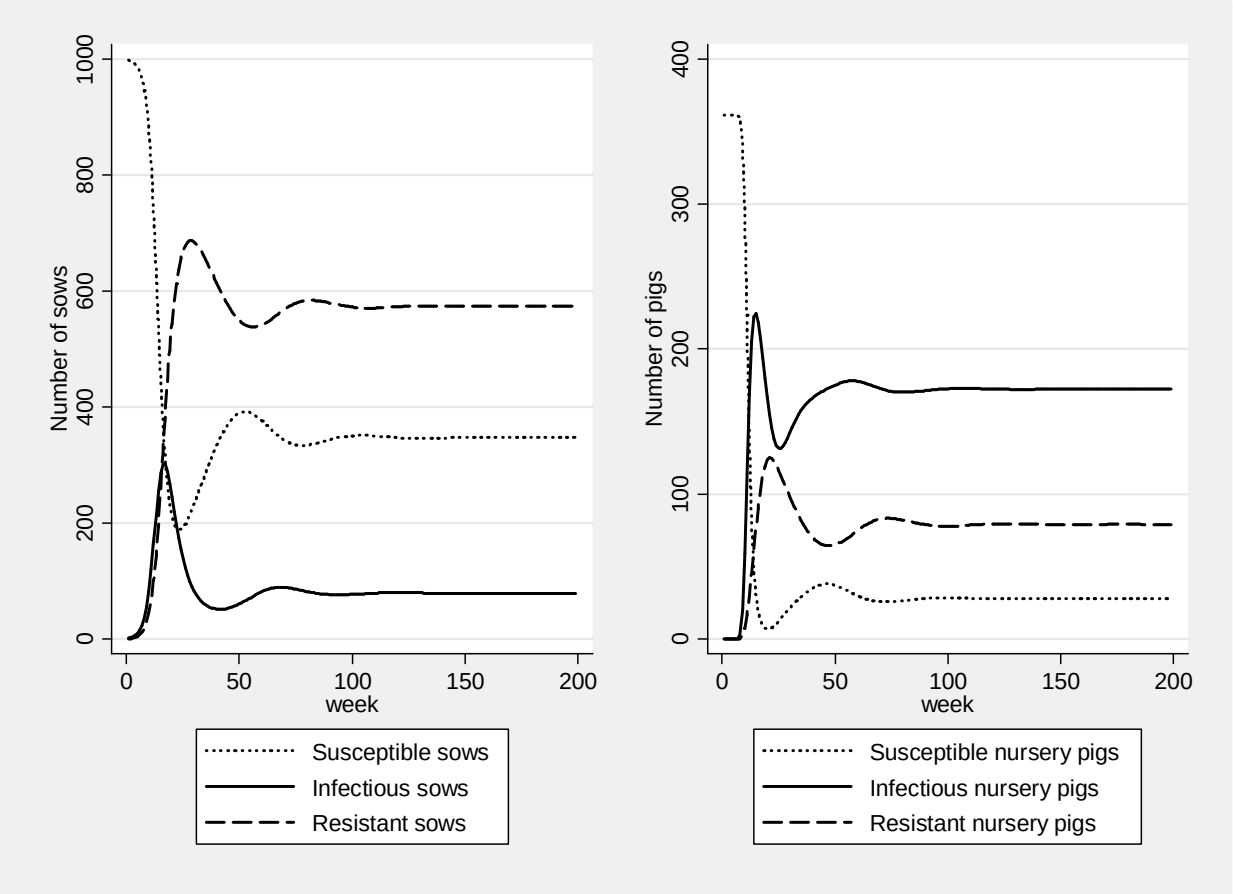

Figure 4.3.

Dynamics of PRRSV infection in sows and 10-week old nursery pigs after introduction of one infectious animal into a completely susceptible herd with assumption of short duration of sow immunity

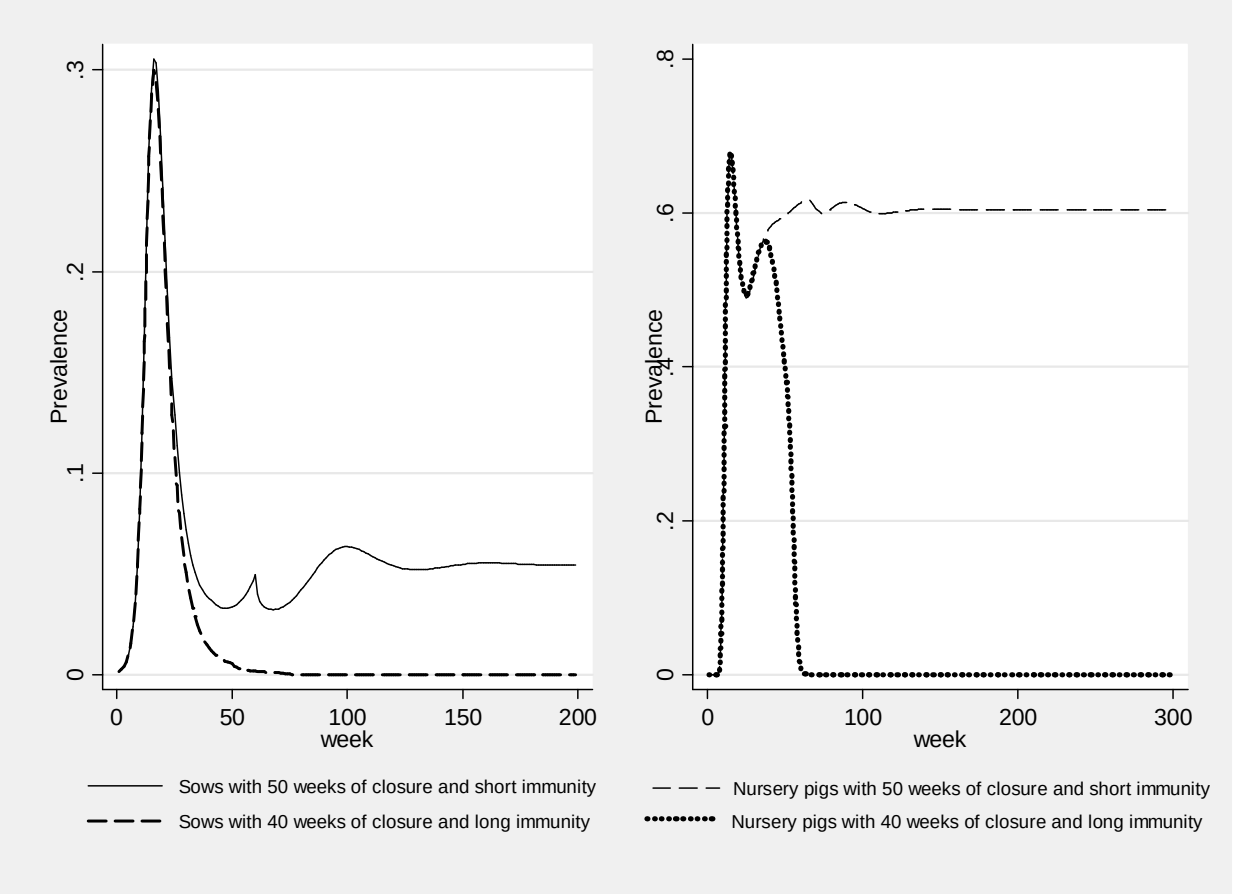

Figure 4.4.

Prevalence of infection in sows and in10-week old nursery pigs in a 1000-sowherd where herd closure started at 10 weeks after first infection and lasted for 40 weeks and 50 weeks, followed by introduction of susceptible gilts, and under the assumption of long and short duration of sow immunity

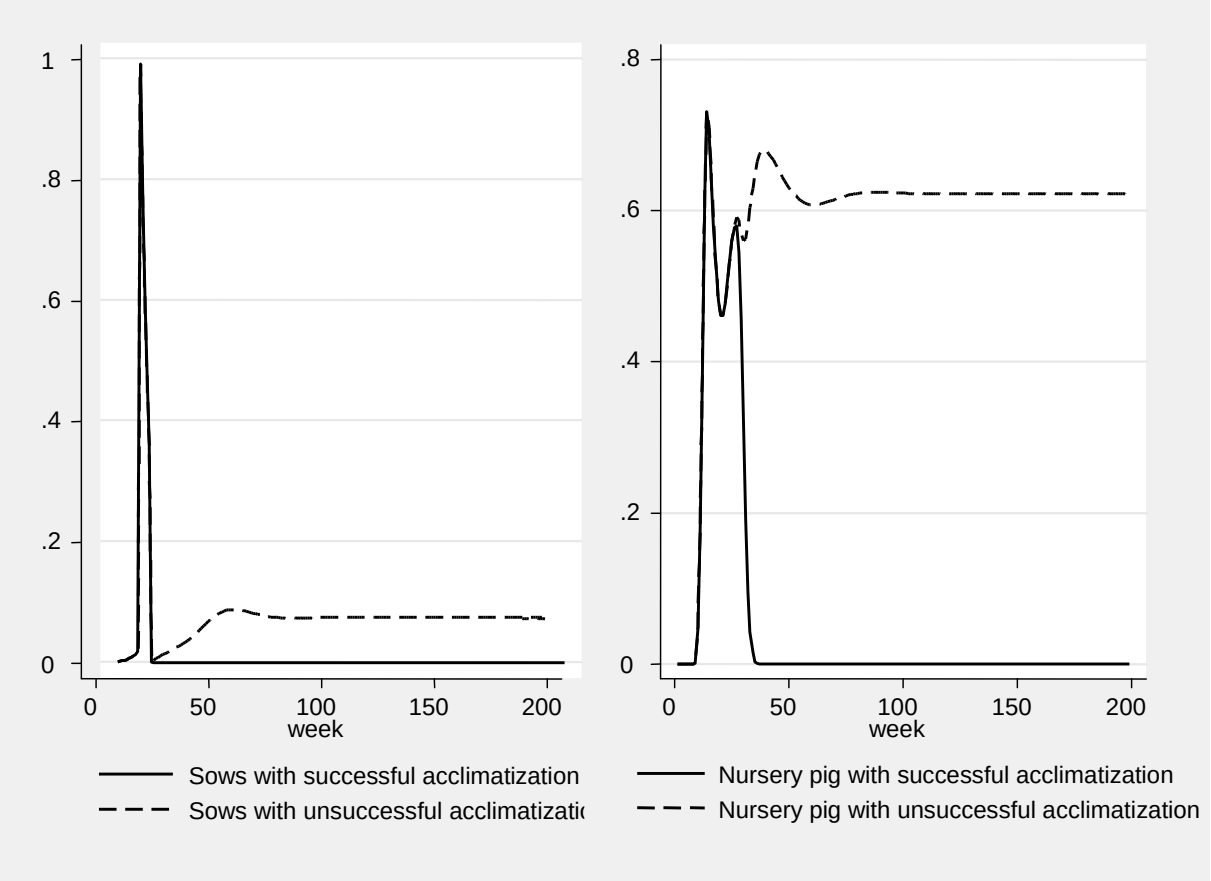

Figure 4.5.

Prevalence of PRRSV infection in sows and 10-week old nursery pigs in a 1000-sow herd immunized with a product with 100% immunization efficacy at 10 weeks after the first infection, with concurrent application of herd closure that lasted for affitional 6 weeks and was followed by introduction of successfully or unsuccessfully acclimatized replacement gilts, and under assumption of short duration of sow immunity

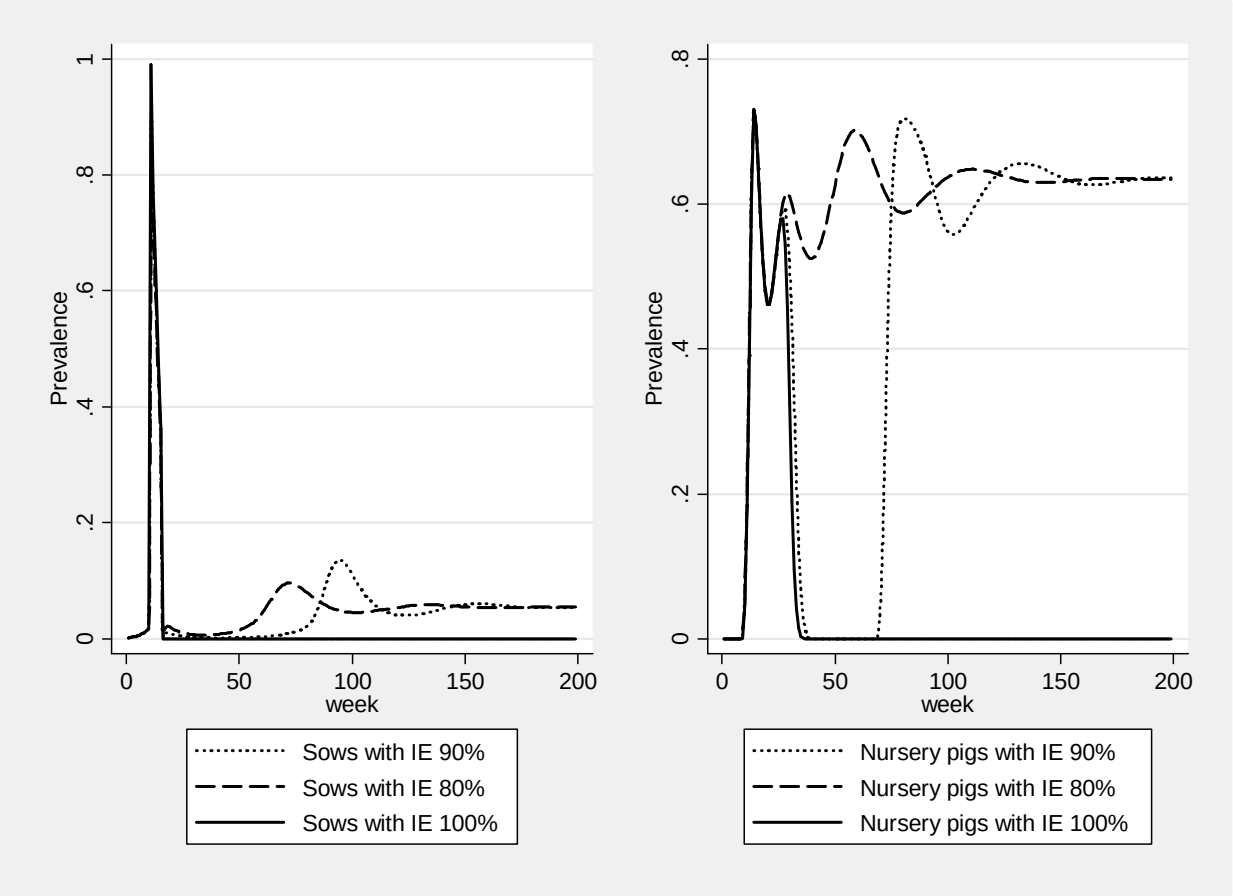

Figure 4.6.

Prevalence of PRRSV infection in sows and 10-week old nursery pigs in a 1000-sowherd immunized with a product with different immunization efficacy (IE) at 10 weeks after the first infection, with concurrent application of herd closure that lasted for additional 5 weeks and was followed by introduction of succsessfully acclimatized gilts, and under assumption of short duration of sow immunity

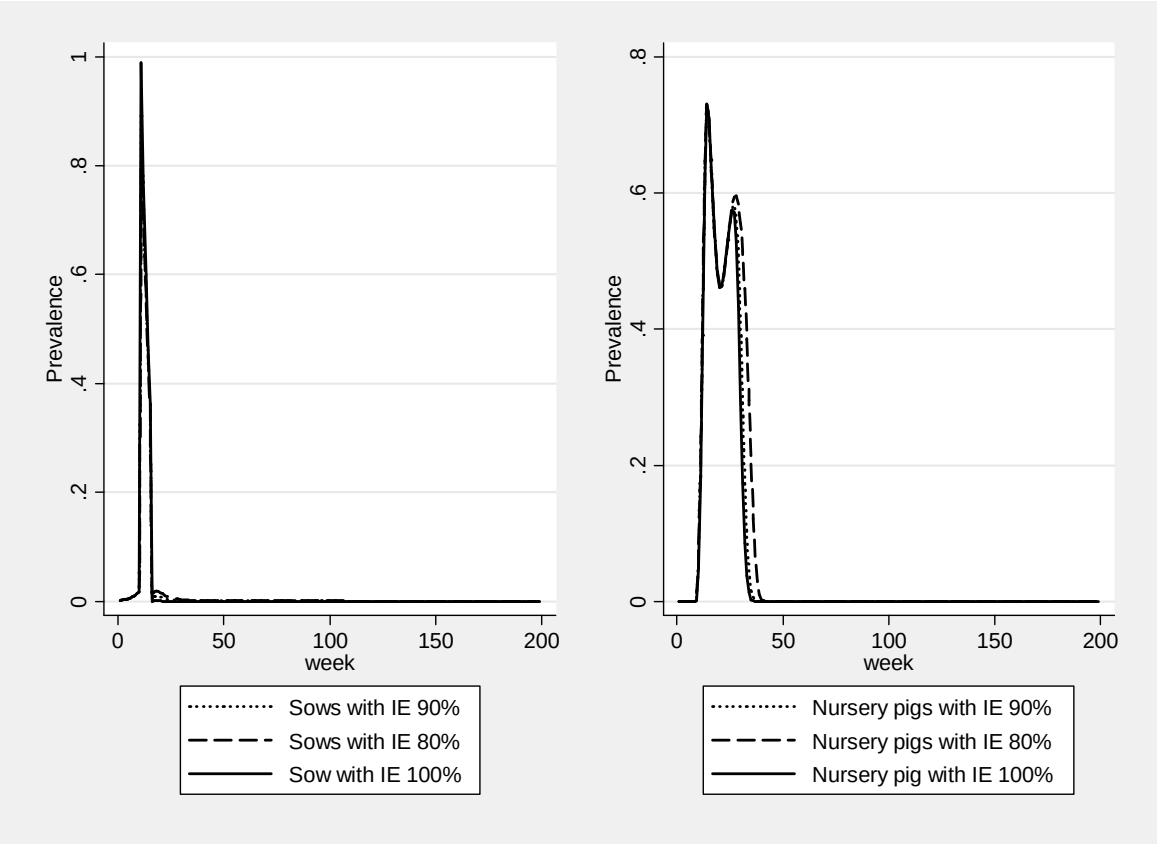

Figure 4.7.

Prevalence of PRRSV infection in sows and 10-week old nursery pigs in a 1000-sowherd immunized with a product with different immunization efficacy (IE) at 10 weeks after the first infection, with concurrent application of herd closure that lasted for additional 5 weeks and was followed by introduction of succsessfully acclimatized gilts, and under assumption of long duration of sow immunity

Chapter 5

Contributions to surveillance of porcine reproductive and respiratory syndrome virus

Written in the style of "Journal of Swine Health Production"

Abstract

Objective**:* to determine the diagnostic sensitivity and specificity of commonly used tests for PRRSV and to identify prognostic factors that determine positivity to PRRSV in a group of finisher pigs with lower health status**.** ***Methods: From 25 finisher barns and 4 nursery barns, 117 pigs at an age close to market weight and 15 nursery pigs with poor health and selected for euthanasia were sampled. PRRSV antibody was tested from serum using an ELISA test. Serum samples and pooled tissue samples (lung, lymph nodes and tonsils) were also tested for RNA of PRRSV using real-time PCR tests. Bayesian analysis was used to estimate sensitivity and specificity of serum PCR and tissue PCR. Logistic regression based on generalized estimating equation (GEE) was

used to estimate the relationship between clinical signs considered prognostic factors and the presence of virus in tissue. ***Results:*** For all herds, 62%, 24%, and 41% were positive by ELISA, serum PCR, and tissue PCR, respectively. Sensitivities of the serum PCR and tissue PCR tests were estimated to be 85.8% and 94.8%, and specificities were 98.0% and 90.2%, respectively. The likelihood of detecting the virus in tissue was higher in pigs with dyspnea or rough hair coat, but lower in lame pigs. ***Implications:*** To increase the sensitivity of detecting PRRSV in a finisher barn, pigs with dyspnea or rough hair coat, rather than pigs with lameness should be selected. Lung and tonsil samples should be used for PRRSV PCR rather than sera. Diagnostic accuracy of PCR tests when applied in these populations might have limitations that should be considered in the design of monitoring programs.

Introduction

Since its emergence in the late 1980s, porcine reproductive and respiratory syndrome (PRRS) has become a challenging disease to control. It is caused by PRRS virus (PRRSV), an RNA virus classified in the family *Arteriviridae*.[1] The virus is characterized by a long duration of infectiousness, which is age-, and likely genotype-, related.[2-5] Different segments of the RNA genome are subject to mutation and recombination, which further complicates the duration of infectiousness and immunity, including the yet to be elucidated question of cross-protection.[6] At the herd level, infection can be transmitted in a variety of ways, including movement through infected animals or contaminated semen, indirectly through contaminated vehicles, equipment, and supplies, vectors such as insects and people, and finally, through aerosols.[1] The

prevalence of wild-type virus is not well documented but is likely high. Thus, in typical situations under North American husbandry conditions, infection is easy to contract and difficult to control or eliminate from individual herds.

Different types of commercial vaccines exist and have been used in an attempt to control clinical disease on farms, with varying reported efficacy.[7, 8] Vaccines have also been used to control infection when used in combination with other demographic measures. The latter approach requires well developed and structured programs. Elimination strategies at the herd level have been developed, typically requiring a combination of immunization, demographic measures, strategic movement, and movement control, as well as thorough cleaning and disinfection. Prevention measures rely on strict biosecurity, including filtration of incoming air in pig-dense areas.[9] It is thus not surprising that the cost of disease, through actual losses and expenditures to control or prevent, is high for individual herds. At the industry level, it has been estimated that annual costs vary from $560 million in the United States [10] to $130 million in Canada.[11] Starting in 2004, attitudes of producers and others involved in the industry have changed. First, although the virus has always been considered capable of causing severe clinical signs, according to field reports, the PRRSV genotypes in some North American regions became more virulent. The most drastic example was the emergence of porcine high fever disease in Asia.[12] Second, starting with successful elimination in Stevens county, Minnesota,[9] a number of different ongoing PRRS elimination projects have been initiated in the United States, Mexico, and Canada.[13]

Researchers from other fields recently discussed the lessons learned in elimination of three viral diseases from human population to design elimination programs for

malaria.[14] Surveillance was one of the nine most important lessons, but at least four other lessons may also be linked with surveillance. For accurate surveillance, a fundamental requirement is to have estimates of diagnostic sensitivity and specificity for commonly used tests. This could substantially contribute to the design of output-based surveillance standards where a desired output is required (eg., the sufficient probability of detecting an outcome), regardless of the test used, instead of input-based approach where activities are prescribed with expectation that they will provide the desired output [15]. Development of the output-based standards could be particularly attractive to PRRSV control and herd elimination, as clinicians may select sample type, test subjects, and test used. Unfortunately, diagnostic accuracy is not well documented for PRRSV diagnostic tests. Another important factor in design of the surveillance system is the expected minimum within-herd prevalence. If this prevalence is high, then sample size can be reduced when we are interested only in a herd-level test [16]. It would thus be useful to understand which factors increase the risk of being positive for exposure to PRRSV or its presence. Growing pigs could be sampled for PRRSV in the finisher barn to assess herd status for PRRS infection for the following reasons: first, their status might reveal the effectiveness of both the control measure and elimination of the virus in the previous production phase, and secondly some pigs may be available for euthanasia and tissue sampling because of acute or chronic disease with poor prognosis, body weight below the acceptable minimum market weight, or animal welfare reasons.

Thus, the objectives of this project were to determine the sensitivity and specificity of commonly used tests for PRRSV and to identify prognostic factors that determine positivity to PRRSV in a group of finisher pigs with lower health status. These

objectives were addressed within a study that aimed to investigate monitoring of respiratory pathogens in a broader sense.

Materials and methods

This study was approved by the Animal Care Committee and the Ethical Committee of the University of Guelph.

Study area and population

The study population consisted of pigs from finisher barns in Ontario, Canada, and herds at an age close to market weight, between April 2010 and July 2011. These barns had good production records and availability of at least two animals for euthanasia during the herd visit. Originally, animals to be sampled were selected from the last shipment of pigs to go to market from a barn (the close-out group). However, subsequently this criterion was relaxed to include the last period of the finisher phase in order to introduce higher variability in clinical signs and to encourage participation of herds in the project. In addition, a small number of nursery barns were included in the study at the time when nursery pigs were scheduled to be moved to finisher barns. Herds were included in the study by contacting veterinarians specializing in swine production who could provide contacts with managers overseeing large numbers of finisher herds. Timely access to the source herds was provided either when the last group of pigs was ready to be sent to market or immediately after an outbreak of respiratory or systemic disease was reported.

During the herd visit, two to six pigs were selected for euthanasia and diagnostic follow-up. The inclusion criteria for euthanasia were, in descending order of importance,

clinical signs consistent with a respiratory or systemic disease, preferably in early onset and before application of parenteral treatment; weight loss; and other conditions that warranted euthanasia.

Clinical observations

Selected pigs were observed for clinical signs both at rest and after movement, then were palpated when possible. Clinical signs included systemic (eg, fever), respiratory (eg, dyspnea, coughing and sneezing), neurological (eg, depression, signs consistent with middle ear infection, ataxia), locomotor, and integumentary signs (eg, scratches, rough hair coat, abscesses, hematoma).

For each selected pig, a standardized form was used containing 18 questions pertaining to clinical signs described by either nominal or binary variables. The list of clinical variables is shown in Table 5.1, including 14 binary variables (yes, no); three ordinal variables (dyspnea, weight loss, and distended abdomen) described by degree of clinical signs (normal, moderate, severe); and one nominal variable, neurological signs (neuro-sign), describing the signs observed.

Sampling and diagnostic testing

Blood samples were collected using jugular venipuncture. Pigs were then first anesthetised and then euthanized using intravenous administration of an overdose of anaesthetic. After euthanasia, animals were necropsied and gross lesions were described in a standardized manner. Diagnostic material was selected primarily from lungs, tonsils, and lymph nodes. Specifically, all six lobes of the lungs were sampled and pooled with a

section of tonsil and a section of inguinal lymph node. These pooled tissues are referred to as the tissue sample. All blood and tissue samples were transported at 4°C and submitted to the Animal Health Laboratory (AHL) of the University of Guelph, Guelph, Ontario, Canada, within a few hours of collection. At the laboratory, sera were separated by centrifugation and were tested for PRRSV antibody using the enzyme-linked immunosorbent assay (ELISA X3 Ab Test; IDEXX Laboratories, Inc, Westbrook, Maine). Sera and the pooled tissue samples were tested for RNA of PRRSV using real-time polymerase chain reaction (PCR) tests, according to the standard procedures of the AHL.

Statistical analysis

Data on each diagnostic test were received from the AHL. For the ELISA, samples with sample-to-positive (S:P) ratios ≥ 0.4 were considered positive, according to the manufacturer's instructions. For quantitative real-time PCR, detection of viral copies > 0 was considered positive. Based on the binary outcome for each test or combination of tests, the prevalence of pigs with PRRSV antibody or virus infection was calculated. The binary outcomes for all tests were cross-tabulated between any two tests to evaluate their agreement using the kappa statistic.[17] A value of kappa between 0.4 and 0.5 indicates moderate agreement, while values > 0.6 confirm good agreement. Diagnostic sensitivity and specificity of serum PCR were first estimated under the assumption that tissue PCR is the gold standard. In addition, under the assumption of no gold standard, diagnostic sensitivity and specificity of serum PCR and tissue PCR were calculated using Bayesian analysis for two dependent tests in one population.[18]

In Bayesian analysis, all parameters are expressed as random variables. Prior distributions for the test properties (sensitivity and specificity) and the prevalence in the population of interest must be specified. Because little published data is available, the prior distribution for the diagnostic sensitivity and specificity of the two tests were based on the range between 0.80 to 0.99,[19] in which the modes were assumed to be 0.98, and with the 95% certainty that the diagnostic sensitivity and diagnostic specificity were higher than 0.80 and 0.9, respectively. The prior distribution for the prevalence of infection in the population was based on the range between 0 to 0.3,[20] with the assumption that the mode was 0.2, and with the 95% certainty that the prevalence was less than 0.3. These assumptions were then transformed to the parameters (a,b) of the beta distributions using BetaBuster 1.0 (University of California, Davis, California). Under the assumed prior information and the observed data, a Bayesian analysis through Markov chain Monte Carlo (MCMC) sampling was performed using WinBUGS version 1.4 (Imperial College & Medical Research Council, London, UK) with 50,000 iterations to estimate the posterior distribution of the sensitivity and specificity of the serum and tissue PCR tests. Median and 2.5 and 97.5 percentiles of the posterior distribution were used to characterize the posterior distributions of sensitivity and specificity for two tests. Because of the relation of tissue sample and serum sample from each sampled pigs, the assumption of two dependent tests for one population was applied. The WinBUGS code for Bayesian analysis are shown in Appendix 2.

Finally, to determine prognostic factors, only finisher pigs were used. This was conducted in two steps. First, univariable logistic regression was performed with each clinical sign as a prognostic factor and the result of each test as the outcome. The

likelihood ratio test or Fisher's test calculated by the exact method was used for this purpose. Then all factors significant at $P < .20$ were offered to a backward elimination procedure in order to identify a subset of factors associated with the outcome of interest. The cut-point of $P < .10$ was considered significant. The model identified by this procedure was refitted using generalized estimating equations with independent correlation structure to acknowledge the sampling design. All statistical analysis (with the exception of Bayesian analysis) was performed using Stata software (Stata Intercooled, version 10; Stata Corporation, College Station, Texas).

Results

A total of 132 pigs (117 finisher pigs and 15 nursery pigs) from 29 herds (25 finisher barns and four nursery barns) were included in this study. Most herds (n = 23) were located on finisher-only premises, two finisher herds were a part of farrow-to-finish premises, and four nursery herds were from nursery-only premises. Number of animals sampled per herd varied between a minimum of two and the maximum of seven. Two animals were sampled in three herds, three animals were sampled in five herds, four animals were sampled in six herds, five animals were sampled in four herds, six animals were sampled in 10 herds, and seven animals were sampled in one herd. In three of the four nursery herds, three animals were sampled, and six animals were sampled in the fourth herd.

At the herd level, 62% of all herds and 64% of finisher herds were positive by ELISA (herds with at least one ELISA-positive pig) (Table 5.2). Smaller numbers of herds were positive for PRRSV by PCR than by ELISA. More finisher herds were

PRRSV-positive by tissue PCR than by serum PCR. Four finisher herds were positive by both tissue and serum PCR tests and 14 were negative by both tests. Six herds were declared negative by serum PCR but positive by tissue PCR, and one herd was positive by serum PCR but negative by tissue PCR. Overall, 68% of finisher herds were positive by at least one of the three tests used. Five herds were positive by both ELISA and serum PCR, and nine herds were negative by both ELISA and serum PCR. Eleven herds were positive by ELISA but negative by serum PCR; however, no herds tested negative for ELISA and positive for serum PCR. Nine herds tested positive by both ELISA and tissue PCR, and eight herds tested negative by both ELISA and tissue PCR. Seven herds were positive by ELISA but negative by tissue PCR, and one herd tested negative by ELISA and positive by tissue PCR.

ELISA results showed that the distribution of within-herd prevalence of all finisher herds followed a U-shaped pattern, with 40% of herds having a prevalence between 80% and 100%, and 36% of herds having a prevalence of 0% (Figure 5.1). In ELISA-positive finisher herds, mean and median prevalences were 79.7% and 83.3%, respectively (interquartile range [IQR], 29.2%) with a minimum of 33.3% and maximum of 100%. Serum PCR results showed that within-herd prevalence had a uniform distribution (Figure 5.2) with 80% of finisher herds serum PCR-negative. In finisher herds positive by serum PCR, mean and median prevalences were 40.1% and 50.0%, respectively (IQR, 30.0%) with a minimum of 16.7% and maximum of 66.7%. Tissue PCR results showed that within-herd prevalence had a uniform distribution (Figure 5.3), with 60% of finisher herd tissue PCR-negative. In finisher herds positive by tissue PCR, mean and median prevalences were 47.6% and 29.2%, respectively (IQR, 83.3%), with a

minimum of 16.7% and maximum of 100.0%. In finisher herds in which both PCR tests were positive (at least one pig tested positive by either serum or tissue PCR), within-herd prevalence also had a uniform distribution (Figure 5.4), with 56% of finisher herds both tissue and serum PCR-negative. In finisher herds defined as positive if at least one pig was positive by either serum or tissue PCR, mean and median prevalences were 45.1% and 25.0%, respectively (IQR, 83.3%), with a minimum of 16.7% and maximum of 100.0%.

Cross tabulations of diagnostic results obtained from the three assays at the individual-pig level in the entire study population and in finisher pigs only are listed in Table 5.3. The kappa statistic showed that agreement between ELISA and each of the PCR assays was low, while the agreement between serum PCR and tissue PCR assays was good. Under the assumption that the tissue-based PCR correctly classified the infectious status of individual animals, for all pigs, the diagnostic sensitivity of serum PCR was 54.3% (95% CI, 36.6% -71.2%) and specificity was 99% (95% CI, 94.4% - 100.0%). Under the same assumption, in finisher pigs only, the sensitivity of serum PCR was 40.7% (95% CI, 22.4%- 61.2%) and specificity was 98.9% (95% CI, 94% - 100.0%). The result of the Bayesian analysis to evaluate two dependent tests (serum PCR and tissue PCR) in one population without a gold standard is shown in Table 5.4. Sensitivities of the serum PCR and tissue PCR tests were 85.8% (2.5 to 97.5 percentile, 64.1%-99.1%) and 94.8% (2.5 to 97.5 percentile, 84.4%-99.7%), and specificities were 98.0% (2.5 to 97.5 percentile, 94.7%-99.8%) and 90.2% (2.5 to 97.5 percentile, 83.5%-97.0%), respectively.

Prognostic factors identified in the univariable analysis using ordinary logistic regression are listed in Table 5.5. The lowest number of factors were associated with the ELISA test results, and the highest number of factors were associated with tissue PCR results. For multivariable models, no factors remained positive for ELISA at $P < 0.1$. For serum PCR, the only factor that remained in the model (generalized estimating equations or ordinary logistic regression) was anemia, therefore no further results were reported. Table 5.6 lists the prognostic factors for detection of the virus in tissue in the final multivariable logistic regression model that accounted for membership in the herd. Briefly, the likelihood of detection was higher in pigs with moderate and particularly with severe dyspnea, and with rough hair coat. The likelihood was lower in pigs that were lame.

Discussion

Our results showed that the ELISA prevalence was higher than the serum PCR prevalence in the positive herds. The U-shaped distribution of ELISA prevalence indicates some herds may be free of PRRS, but when PRRS occurs in a herd, prevalence of exposure in this group of pigs will be high. In fact, we know that the transmission rate of PRRSV is high and the value of its basic reproductive number (R_0) is high, [21] resulting in a large number of pigs becoming infected early in life (ie, during the nursery period). Thus, most pigs have anti-PRRSV antibody at the end of the finishing phase. During the finishing period, infected pigs usually clear the virus from blood,[22] while some continue to shed the virus up to 22 weeks after infection.[4] Thus, in this study, serum PCR-prevalence was lower because the source populations were pigs in the late finisher phase.

Similarly, tissue PCR prevalence was also lower than ELISA prevalence late in the finisher phase, but within-herd variability was wider for tissue PCR prevalence than for serum PCR prevalence. Our results are similar to those reported in a USDA study in which within-herd seroprevalence of PRRS was high (40% to 60%) and infectious prevalence (serum PCR) was 5% to 35% in positive herds.[23] The wide range of these prevalences might be due to the different stages of infection in each herd or the difference in virus strains and their duration of circulation in the herds.

Estimates of diagnostic sensitivity and specificity of a test at the animal level are frequently uncertain in practice because they are typically based on experimental studies in which samples are obtained at known times after challenge with a pathogen of interest [19, 24]. Thus their results seem to be overly optimistic estimates of test accuracy. In our study, using the field observational data, the sensitivity and specificity of two PCR tests (serum and tissue) were calculated on the basis of the assumption of one population and two dependent tests. These tests are dependent because the principle of the tests (PCR) is the same and sample types (blood and tissue) are related. Without any gold standard test, as in this situation, Bayesian analysis would be the appropriate method for estimating sensitivity and specificity. This analysis has been used for evaluation of diagnostic test accuracy and is well documented in medicine.[25] The data came from two phases of pig production (nursery and finisher) and from 29 herds. In order to simplify the situation, we assumed all data came from one population of growing pigs. A prior prevalence for this population was calculated proportionally from that reported in the literature. [20, 26]

Our results showed that the sensitivity of serum PCR is lower than that of tissue PCR. That is clearly explained by the duration of viremia in PRRS-infected pigs. In one

study, after infection with the virus, all pigs were viremic by 7 days.[27] In a different study, viremia was resolved by 56 days, [28] while it has been reported that the virus can persist in tissues for much longer.[22] Thus there is a greater possibility of detecting virus in tissue than in serum. In addition, depending on the stage of infection, the possibility of detection of virus in serum may be different. For instance, Rovira et al (2007) found that the sensitivity of serum PCR was lower during the early stage of infection.[29] From that perspective it is logical that a serum-based test will have lower sensitivity but it will also likely continue to be used in practice because venipuncture is much more convenient than the tissue sample, and sometimes the only possible sample type. However, what perhaps is more important, is the actual estimate of diagnostic sensitivity in this particular population so that the estimate could be used for sample size estimation or for the design of risk-based surveillance.

However, the specificity of tissue PCR is lower than that of serum PCR. It can be inferred that more false-positives may be detected by tissue PCR than by serum PCR. The reason for this might be related to the definition of true prevalence. The principle of PCR is detection of the RNA of virus without discrimination between live and dead virus. In some pigs, PRRSV is cleared from the blood (viremic duration is over) and by then the virus has also been inactivated in tissue, but virus RNA may still exist and is localized in the tissue, so that it is detectable by the PCR test, but virus cannot be isolated.[22] In addition, the possibility of false-positives has been reported as an important weakness of PRRS PCR testing.[30] False-positives have previously been reported to be caused by contamination or amplification of nonspecific products due to suboptimal assay stringency conditions.[31] Tissue samples were comprised of several tissues including

lungs, lymph nodes and tonsils. It certainly takes more steps to collect these tissues and under field conditions is subject to cross-contamination to a greater degree than collection of blood through venipuncture which is essentially aseptic. This could be a point where cross-contamination could occur. Once tissue samples are submitted to the laboratory, at least under conditions of this study, possibility of cross-contamination is drastically reduced because the tissues are extracted by a robot with a decontaminating UV light.

In addition, it is also possible that tissue samples might contain many other micro-organisms (particularly tonsils) considered either as normal flora or pathogenic agents and a large amount of cell DNA. These factors may result in a false positive PCR test due to the primer specificity of less than 100%. However, the test used in this study was the Tetracore Next Generation PRRSV real time PCR and it includes 19 primers, with 6 sequence specific probes, so problems with primer sensitivity (19) and specificity (6) is likely minimized. It also needs to be pointed out that samples collected in this study were coming from animals that were infected by multiple pathogens; some of them were chronically diseased; and under such conditions the lower specificity of any test is not completely unexpected.

When interpreting the sensitivity and specificity of a PCR test, the reader should also keep in mind that the source populations of pigs were not at the peak of an outbreak of respiratory disease, but may also have been affected by other conditions. Given the nature of the study population, it would be expected that many of these pigs would be near the upper end of their positivity for PCR. Thus, these were not estimates of sensitivity and specificity for a population at the peak of an outbreak of disease. One might expect that test accuracy for this population at the peak of an outbreak to be higher.

The sensitivity of serum PCR is also found to be low when tissue-based PCR is assumed to be the perfect test. The explanation for this is similar to the above where the sensitivity of serum PCR was lower than that of tissue PCR. This reminds us that although blood is a convenient sample, there are situations when it is not sufficiently sensitive to identify a positive pig in an infected herd.

The values of sensitivity and specificity at animal level can be applied to estimate the sensitivity and specificity of the test at herd level and set up an appropriate procedure to examine PRRSV herd status. Herd-level sensitivity and specificity depend on the sensitivity and specificity at animal level and on other factors including the number and type of animals sampled, and within-herd prevalence.[32] The test to be used and the number of animals sampled depend on the purpose of herd testing. For risk-based surveillance, higher sensitivity is preferred, thus tissue samples might be appropriate.[33] However, lung tissue cannot be collected from live animals. Thus the appropriate procedure should be selected to test the status of PRRSV infection of a herd. For example, tissue can be collected at the slaughterhouse or from euthanized pigs. This raises the question of which pigs should be euthanized for tissue sampling to optimize the sensitivity of the test for PRRSV. Prognosis factor analysis could help answer this question.

In this study, prognosis factor analysis recommended that pigs with dyspnea and rough hair coat and with no lameness should be sampled. There is no doubt that dyspnea relates to PRRS as the name of the disease suggests. Rough hair coat is a general sign indicating body condition of many diseases, including PRRS.[34] Perhaps in these pigs, ability to clear the PRRSV is low, and the disease becomes chronic and persists for a

longer time. Such pigs have been used as a source of virus to acclimatize gilts for PRRS control.[35] Lameness is not known to be associated with the disease.[1] However, in practice, these pigs are easily restrained for herd testing. Our results suggest that these pigs should be excluded from testing for PRRSV.

This study had a limited number of selected pigs from farms. Thus the within-herd prevalence might be over-estimated. However the herd classification might not be influenced by this targeted sampling and this factor is appropriate for risk-based surveillance. Clinical signs were assessed only at single farm visits, thus the evaluation of them might be considered subject to bias. But all sampled pigs were in abnormal condition and planned to be euthanized, the clinical sign would be recognized easily. In general, the results of this study could provide a rationale for making diagnostic decisions to swine veterinarians at the level of an individual herd, and could also be used to design a robust system which will enhance population-level surveillance. Diagnostic accuracy of PCR was lower than originally anticipated and this could be due to the nature of the source population. More results of test accuracy estimation should be made routinely available, and this would allow more accurate design of PRRSV monitoring systems.

Implications

- The PCR test using tissue samples has higher sensitivity to detect PRRSV than that using serum samples.
- When using tissues for monitoring purposes, issues due to lower specificity may arise if it is applied in a target population that is similar to our study population.

- To increase likelihood of detecting PRRSV in a finisher barn in pigs near marketing, finisher pigs with clinical signs including dyspnea and rough hair coat rather than pigs with lameness should be selected.

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Table 5.1.

Description of clinical signs and their categories in the standardized form used on farms to evaluate clinical signs of selected pigs

Clinical signs	Description	Level
Abscess	A pig has an abscess/hematom/tumor	No*/ Yes
Anemia	A pig is noted with signs of anemia	No*/ Yes
Cough	A pig is noted coughing	No*/ Yes
Depression	A pig is visibly depressed	No*/ Yes
Diarrhea	Diarrhea is visible in a pig	No*/ Yes
Eye-discharge	A pig is noted with ocular discharge	No*/ Yes
Fever	A pig has signs of fever	No*/ Yes
Hairy coat	A pig has rough hair coat	No*/ Yes
Head-tilt	Tilting head is suspected middle ear infection	No*/ Yes
Lameness	A pig is lame on one or more legs	No*/ Yes
Nose-discharge	A pig is noted with nasal discharge	No*/ Yes
Open wound	A pig has an open wound	No*/ Yes
Skin-scratch	A pig has scratches on skin	No*/ Yes
Sneeze	A pig is noted sneezing	No*/ Yes
Distended abdomen	A pig with abnormal abdomen size might be due to rectal stricture	No signs* Suspected Pronounced
Dyspnea	Dyspnea is visible in a pig	No signs* Moderate (some after moving) Severe (some in resting)
Weight loss	Wasting status presents	Normal* Moderate Severe
Neurological sign	A pig has acute neurological signs	Normal* Ataxia Head pressing

* this category will be defined as reference groups in prognostic factors analysis

Table 5.2.

Proportion of herds and pigs test positive to PRRSV for 29 pig farms in Ontario between 2010 and 2011

Production class	ELISA	Serum PCR	Tissue PCR	Any PCR	Any test
Nursery herds (n=4)	50%	50%	50%	50%	50%
Finisher herds (n=25)	64%	20%	40%	44%	68%
Overall (n=29)	62%	24%	41%	45%	66%
Nursery pigs (n=15)	40%	53%	53%	53%	53%
Finisher pigs (n=117)	48%	10%	23%	24%	55%
Overall (n=132)	47%	15%	27%	27%	55%

Table 5.3.

Cross tabulations of result of three tests (ELISA, serum PCR, and tissue PCR) in all study pigs and in the finisher pigs only with the agreement for each of the two tests represented by kappa values

		Result (test one / test two)				Kappa (SE*)
Test one	Test two	(+) / (+)	(+) / (-)	(-) / (+)	(-) / (-)	
All pigs						
ELISA	Serum PCR	15	47	5	65	0.18 (0.065)
ELISA	Tissue PCR	25	37	10	60	0.27 (0.079)
Tissue PCR	Serum PCR	19	16	1	96	0.62 (0.082)
Finisher pigs only						
ELISA	Serum PCR	9	47	3	58	0.12 (0.058)
ELISA	Tissue PCR	19	37	8	53	0.21 (0.080)
Tissue PCR	Serum PCR	11	16	1	89	0.49 (0.083)

*Standard error of the mean

Table 5.4.

Estimation of sensitivity and specificity of serum PCR and tissue PCR tests using Bayesian analysis for two dependent tests in one population without gold standard

Test		Mean	Percentile		
			2.5	Median	97.5
Serum PCR	Sensitivity	0.858	0.641	0.874	0.991
	Specificity	0.980	0.947	0.983	0.998
Tissue PCR	Sensitivity	0.948	0.844	0.957	0.997
	Specificity	0.902	0.835	0.901	0.970

Table 5.5.

Univariable associations between the results of each individual test and prognostic factors with P ≤.20 in finisher pigs

		ELISA					Serum PCR					Tissue PCR				
Variable		OR	95% CI		P	P*	OR	95% CI		P	P*	OR	95% CI		P	P*
Abscess	Yes											0.3	0.1	0.9	0.038	0.020
Anemia	Yes						6.3	1.8	21.9	0.004	0.004§	4.3	1.7	11.1	0.003	0.003
Fever	Yes						4.0	0.7	23.1	0.126	0.162					
Hairy coat	Yes											2.7	1.1	6.8	0.035	0.037
Lameness	Yes	0.6	0.3	1.2	0.129		0.3	0.1	1.3	0.100	0.066	0.3	0.1	0.8	0.013	0.007
Dyspnea	Mod.						2.4	0.6	9.5	0.226	0.122	2.8	1.0	7.8	0.048	0.002
	Sev.						5.3	1.1	26.6	0.041		10.3	2.6	41.3	0.001	
Weight loss	Mod.						5.7	0.9	-	0.076	0.064	8.6	1.1	68.7	0.042	0.024
	Sev.						2.0	0.1	-	0.612		8.4	1.0	72.9	0.054	
Distended abdomen	Suspected.	0.4	0.0	3.8	0.419	0.067						0.6	4.7	-	-	0.164
	Pronounced	7.0	0.8	60.0	0.077							0.3	2.2	-	0.000	

* The overall P-value from likelihood-ratio based, or alternatively estimated by exact method if the likelihood ratio solution was not available
§ The only variable is significant in multivariable analysis
CI= Confidence interval

Table 5.6.

The final multivariable model of prognostic factors for detection of PCR positive results based on pooled tissue samples

	Tissue PCR			
Variable	OR	P	95% confidence interval	
Dyspnea (moderate in resting)	3.2	0.034	1.1	9.6
Dyspnea (severe in resting)	8.8	0.003	2.1	37.2
Lameness (Yes)	0.3	0.03	0.1	0.9
Rough hair coat(Yes)	2.8	0.054	1.0	7.8

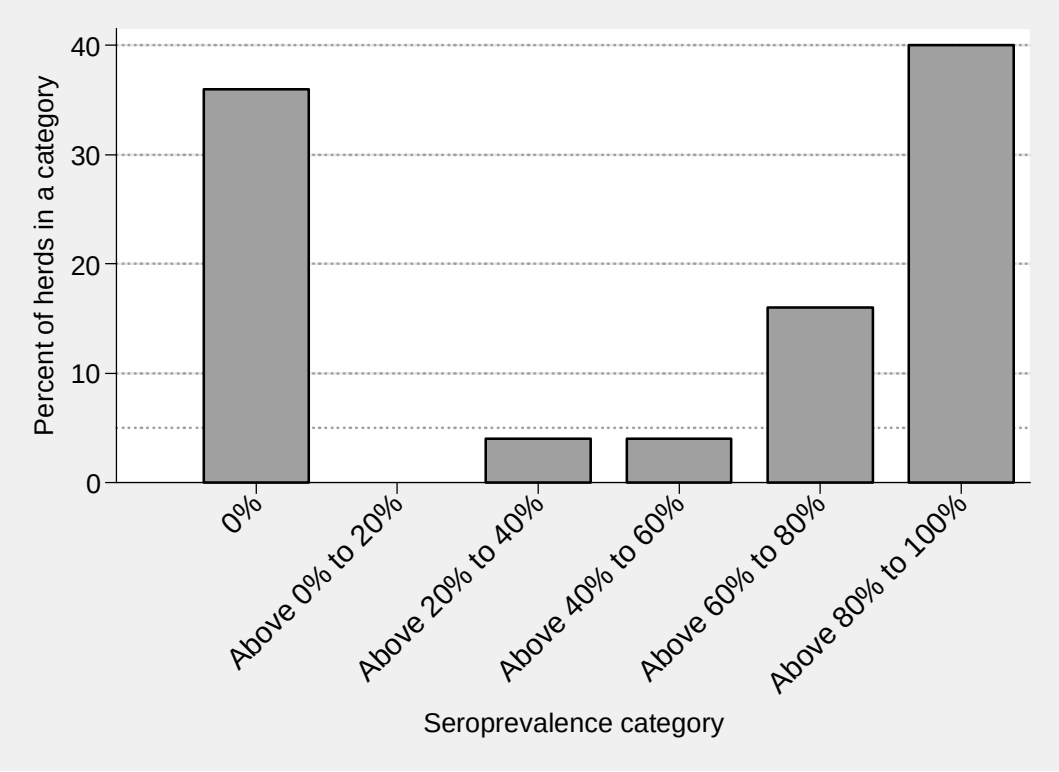

Figure 5.1.

Distribution of within-herd prevalence of exposure to PRRSV based on ELISA test

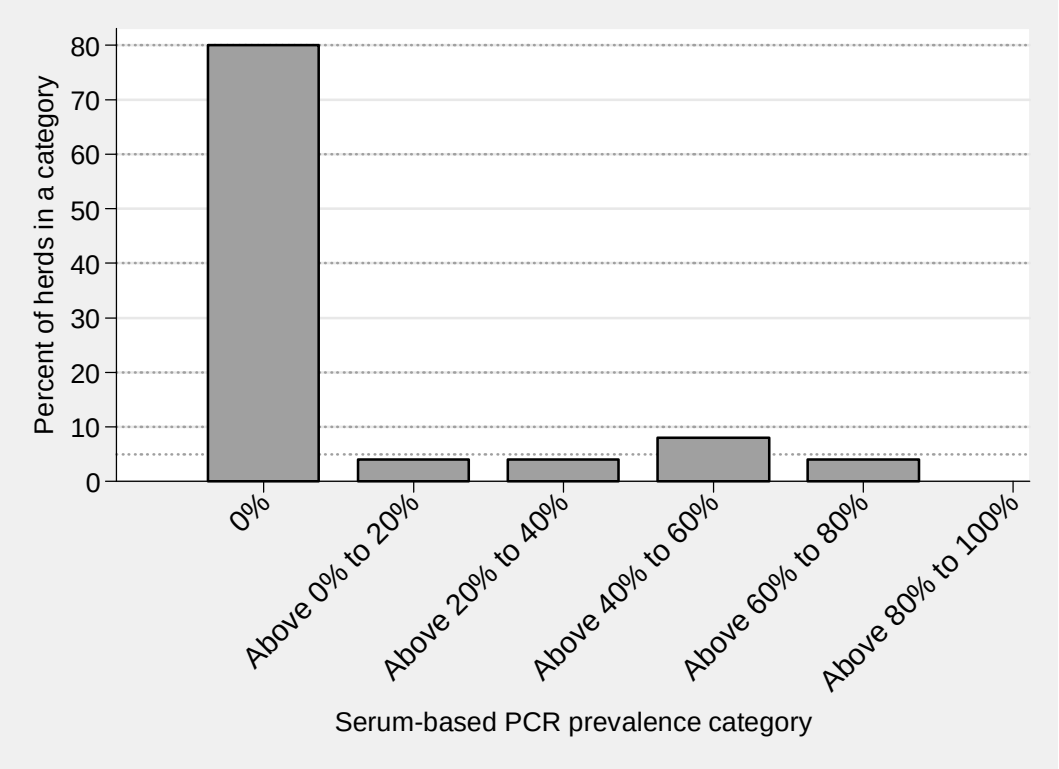

Figure 5.2.

Distribution of within-herd prevalence of PRRSV infection based on serum PCR test

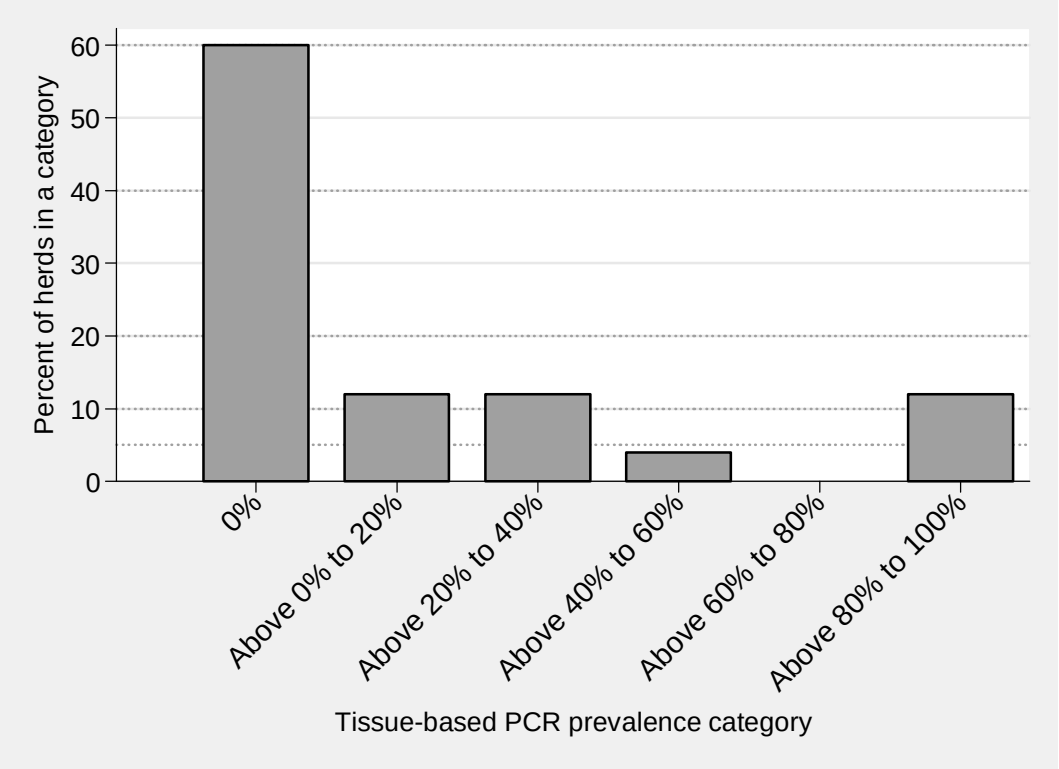

Figure 5.3.

Distribution of within-herd prevalence of PRRSV infection based on tissue PCR test

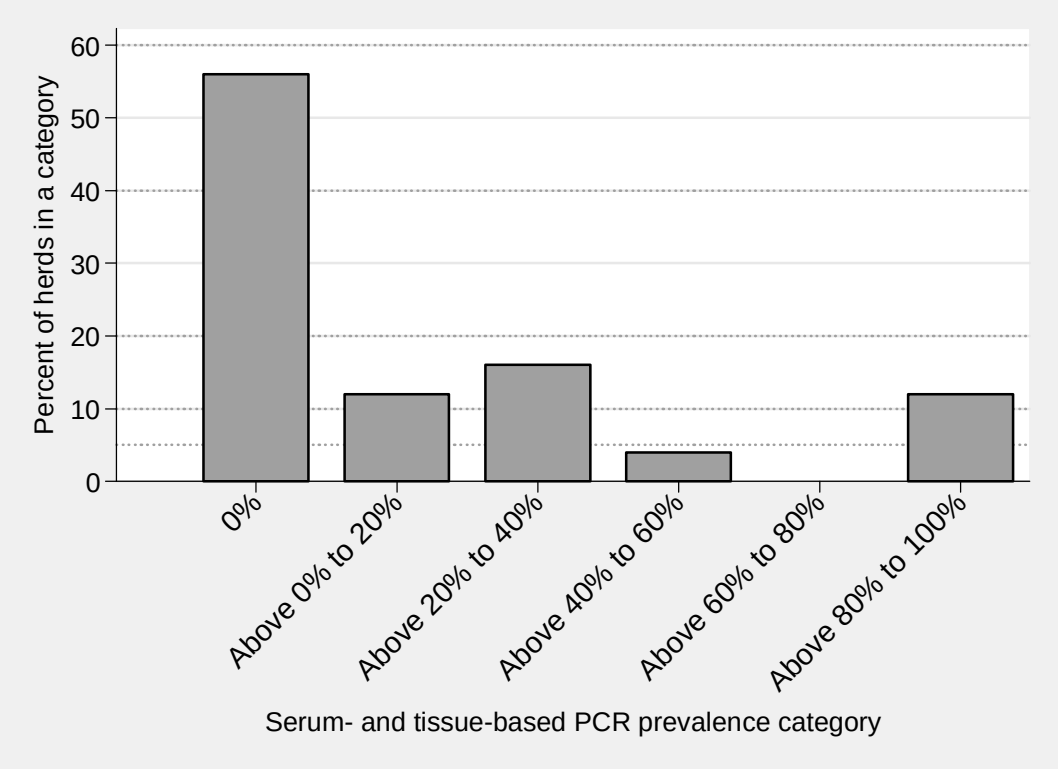

Figure 5.4.

Distribution of within-herd prevalence of PRRSV infection based on either serum or tissue PCR test

Chapter 6

Summary conclusions and recommendations

Porcine reproductive and respiratory syndrome (PRRS) was first recognized in late 1980s in North America, and subsequently in Europe and in other swine-producing regions of the world. The causative agent, PRRS virus (PRRSV) is an RNA virus classified into family *Arteriviridae*, genus *Arterivirus* and could further be subdivided into two major genotypes (European and North American). The pathogen is endemic in most swine producing regions of the world, except Australia, New Zealand, Finland, Norway, Sweden, and Switzerland. Because of the high cost of PRRSV circulation in at least some regions, PRRS has been considered primarily as a production disease of primary interest to producers and veterinarians.

In many swine producing regions, the approach to control of endemically circulating PRRSV has started to change in recent years.The change is due to the following issues. One of the important issues was a report of successful elimination of PRRSV from the commercial swine industry in Chile and the successful regional elimination of the PRRSV from Stevens county in Minnesota (USA). This prompted the initiation of several regional disease and elimination projects, the objective of which is the successful elimination of PRRSV from individual herds. General protocols for PRRSV elimination are described and are commonly applied by veterinary practitioners in North America. However, mathematical modeling of PRRSV elimination that would further contribute to the understanding of PRRSV spread and control has not been attempted earlier. An important component of any approach to disease control and elimination is its surveillance. Critical components of a surveillance system for any disease, including PRRS, are estimates of test accuracy (sensitivity and specificity) and prognostic factors that could be used for identification of subsets of population that could be targeted for more efficient surveillance. This topic has not been studied in great detail with regard to PRRS.

Another issue is represented by the attempted control measures in different regions of south-east Asia in order to control the spread of porcine high fever disease (PHFD) in 2006 and later, which was associated with PRRSV. Despite the efforts undertaken in the control of PHFD, the outbreak spread across the large region affecting several countries and had a large impact on the mortality of pigs and the well-being of farming communities. Epidemiology of PHFD was poorly understood and has not been studied in great detail.

The work reported in this thesis has contributed to the understanding of the factors involved in the emergence of probable cases of PHFD in a region of Vietnam, to the better understanding of how certain intervention strategies commonly applied in the control of PRRSV infection are influencing the level of PRRSV infection and elimination from individual herds, and to enhance the effectiveness of risk-based surveillance programs.

In our investigation of an outbreak of PHFD in a small area of Vietnam, we found that 33.4% of households in the area were affected by PHFD and the mortality in these case households was 24.3%, 22.8%, and 6.7% in sows, suckling-nursery pigs, and finishing pigs, respectively. Case households exhibited clinical signs consistent with the multifactorial nature of PHFD. Analysis of mortality in a three-level random intercept model suggested that the highest proportion of variability in the mortality was attributed to the household level, and a lower proportion to the hamlet level. Since mortality was an important component of the case definition for PHFD, this was suggestive that factors that have contributed to the emergence of PHFD likely operate at the household or herd level.

This was further confirmed in the spatial analysis of this investigation, where we first investigated the existence of local spread of the disease by specifically looking at existence and characteristics of the spatial and spatial-temporal trends and clustering. Spatial and spatio-temporal clustering of PHFD cases at the household level in this investigation was of very limited scope. This further suggested that spatial spread of disease in this study area was of very limited nature and was in close agreement with the results that were obtained by the three-level random effect model to estimate mortality.

As another part of this investigation, we determined management factors that could contribute to disease occurrence using logistic regression. The introduction of pigs into a farm prior to disease outbreak, as well as the interactive effect of raising ducks in proximity to pigs and the feeding of water green crop to pigs increased the risk for PHFD. Thus, it can be inferred that a pathogen originating from a water source, and which further replicates in ducks, may have contributed to the occurrence of PHFD in this area of Vietnam.

The result of this investigation cannot identify the exact pathogens or their pathogenesis but it can help us to understand the pattern of spread and related factors. This finding can be used to guide further epidemiological studies as well as control and prevention programs. From the disease etiology standpoint, the finding of the involvement of ducks and water green crop in the emergence of PHFD could be used to initiate studies that would look into transmission dynamics of pathogens that could circulate when waterfowl such as ducks and pigs are kept in close proximity. The role of ducks in the epidemiology of PRRSV is still not well elucidated, particularly in this region. In addition, pathogens other than PRRSV could have contributed to clinical disease and studying this interaction warrants further investigation.

For veterinary services in the study and target areas, results of this study indicate that measures aimed at controlling movement of pigs and respecting biosecurity practices are most important in preventing future disease outbreaks. This knowledge could be used in order to help create educational programs targeted to small households with aim to enhance understanding and implementation of basic external biosecurity principles. As a part of this effort, in the long run and under ideal conditions, replacement animals (ie.

gilts for breeding) and nursery animals should be obtained from sources that are free of infection from PRRSV and other pathogens, and ideally are certified for that. Knowledge of internal biosecurity principles as it relates to the existence of multiple species in a farm, and risks associated with such practices should also be transferred to farmers.

For inclusion into this study, we aimed to have a study area that would be approximately 10x10 kilometers in shape. It was anticipated that such an area would allow us to have sufficient spatial extent to accurately study spatial patterns (ie. space-time clustering) over a certain spatial distance. Studying such an area was also feasible from the resources standpoint. However, investigation of such patterns in any future studies would ideally be done in more than one area and should ideally be done in a prospective study that would also include some diagnostic tests.

On the other hand, in regions where knowledge and implementation of biosecurity practices in commercial swine herds is already high (such as pig-dense area of North America), the producer and industry groups are actively involved in PRRSV elimination from individual herds or from entire regions. The basis of these strategies are measures applied to the flow of pigs and their immunity. These approaches have been summarized previously and the objective of this work was to investigate this further in a quantitative way using relatively simple mathematical modeling. The starting point of this analysis was the estimation of the basic reproductive number (R_o) from an observational study recorded previously in Ontario swine herds. The analysis of this observational study from seven herds suggested that the basic reproductive number for PRRSV in suckling and nursery pigs is high on average and in the majority of herds. However, in at least some herds the spread of PRRSV is relatively slow as indicated by a low R_o estimate.

Statistical analysis also suggested that detectable maternal antibodies are present in piglets for a short period of time. Thus, it was concluded that PRRSV spread efficiently in suckling and nursery pigs but with some variability, and that control of the disease cannot be based on the protective ability of the maternal antibodies.

The data that were analyzed to extract the R_o are similar to the dataset that would be obtained during many types of disease investigations in herds undertaken by practicing veterinarians. One recommendation based on this analysis is that such datasets should be made available for the extraction of parameters important for simulation modeling, such as R_o. The availability of an analysis of such datasets is particularly important for sow herds since the only information currently reported for sow herds is coming from the study in the Netherlands in 2000. One of the most convenient ways to obtain such data would be to provide serological and virology data for diagnostic tests at the sow level, performed in a population that was not previously exposed to PRRSV, together with the exact date of confirmed introduction of PRRSV positive animals and the date of testing. In addition to being used to obtain transmission parameters, such data could then be used to relate these values to genetics of virus, genetics of animals, and environmental conditions.

Our approach to modeling has identified that the duration of sow immunity could play a critical role in the control and elimination of PRRSV from herds. Under the assumption of a long duration of sow immunity (i.e. 80 weeks) it is possible to eliminate PRRSV infection from a herd of 1000 sows using herd closure only, if this herd closure lasts for at least 31 weeks. Mass immunization could also eliminate infection if the efficacy of immunization is 100%. The results of mass immunization that had efficacy of

less than 100% were dependent on the duration of sow immunity. If the duration of sow immunity was assumed to be long, the success of immunization was dependent on how long the herd was closed. The lower the efficacy of the immunization, the longer the duration of herd closure was needed for elimination to be achieved. One interesting finding from the examples of unsuccessful immunization, under assumption of short duration of sow immunity (i.e. 36 weeks), was that an immunization efficacy of 90% could result in the apparent elimination of PRRSV infection in nursery pigs, which is then followed by a secondary outbreak of large magnitude in nursery pigs, and a slowly developing secondary outbreak in sows. However, the secondary outbreak in sows does peak later than the outbreak in nursery pigs. Therefore, nursery pigs could be free of PRRSV for an extended period of time, and could then experience outbreak at a higher level than what could be detected in the sow population. This could manifest as the outbreak in a nursery herd first, and one could question whether an outbreak in the nursery is due to the lateral introduction of infection into the nursery herd. Our results show that such an event could occur naturally during mass immunization that has high, but not perfect efficacy. Most herds under practical conditions first introduce large supply of gilts into herds. Herds are then closed for a long period of time, mass immunized and then open to new introductions of replacement animals. Since the immunization efficacy is difficult to evaluate, and since it is very important to have high efficacy in large herds, the recommendation for practice is that together with herd closure and mass immunization, herd managers should perhaps consider delivery of very young gilts that could be properly acclimatized over a period of time and then introduced into a herd once the herd closure ends. Such a strategy would be applicable for herds that are very large

and where producer want to be very confident that the virus will not emerge, and could be followed by the introduction of naïve gilts. This, however, is one area that needs to be considered for future research, both from the perspective of dynamic modeling and field application.

Deterministic mathematical modeling is a useful tool that should be applied more frequently to evaluate disease control strategies in animal health. Based on the experience gained in our study, other types of dynamic models should be considered for such evaluations. For example, stochastic models would allow incorporation of variability in behavior of certain critical parameters and would allow incorporation of the chance elements which is very important for small populations. Similarly, agent-based models would allow explicit modeling of each individual in a herd. Using either of these approaches would help investigators to quantify the impact of some of these control strategies and should be considered in future studies.

Application of risk-based surveillance is becoming increasingly important for the purpose of prudent and efficient allocation of resources. For an application of such surveillance for PRRSV in individual herds we need to understand what are the risk factors, their magnitude as well as diagnostic accuracy of tests commonly applied for diagnostics and monitoring. The results revealed that the sensitivity of tissue PCR is higher than PCR based on serum and the likelihood of detecting the virus in tissue was higher in pigs with dyspnea or rough hairy coat, but lower in lame pigs. This finding can help to increase the sensitivity of risk-based surveillance programs and choose the appropriate number of animal needed to be tested and calculate herd level sensitivity in the future.

Appendices

Appendix 1.

Questionaire for the investigation of PHFD in an area of Vietnam

Section 1: General information

No	Question	Answer	Explanation
	Questionnaire ID		
1	Date when questionnaire was filled		This section is planned to be filled by a person who is interviewing without asking pre-specified questions
2	Person who filled questionnaire		
3	Name of a person interviewed		
4	Age of a person interviewed		
5	Gender of a person interviewed		
6	Address		
	Hamlet		
	Commune		
7	Longitude		
8	Latitude		
9	Additional note about location		
	Image IDs		

Section 2: Outbreak information

No	Question	Answer	Explanation
10	Did you have any pigs on your farm during the year **of 2008**?	No: ☐ Yes: ☐	
11	What was approximate number of pigs of each age category that you had on your property **at that time**.	Sows: Boars Suckling pigs Nursery pigs Growing pigs	
12	Did you see any unusual disease in your pigs **in 2008**	No: ☐ Yes: ☐	If answer to this question is YES, then proceed with question 13. If answer is NO, then proceed with question 20.
13	What was the exact or approximate date when this unusual disease first occurred?	_____/ _____/ _____DD/MM/YYYY	Must be entered
14	How long this disease lasted in your farm?	_____/days/ weeks/months	Must be entered
15	How many pigs died at the time of this unusual disease?	Sows (S) Boars (B) Suckling pigs (SP) Nursery pigs (N) Growing pigs (F)	

16	Which of the following signs did you see in your pigs when they experienced this unusual disease (***Then go to question 22***)	
17	Did you observe any of the following signs of disease in your pigs in the first half of 2008? *(If none is chosen, go to question 21)*	

Clinical signs	**Sow**	**B**	**SP**	**N**	**F**
Abortion					
Stillbirth					
High fever (40-41.5)					
Blue ears					
Bleeding spots on skin					
Redness					
Depression					
Hard breathing					
Eye discharge					
Swelling lip-eye					
Skin disease					
Agalactia					
Lameness					
Weak born piglet					
Off-feed					
Coughing					
Paddling					
Excess wasting					
Bleeding from nose					
Hard stool					
Diarrhoea					
Others					

<table>
<tr><td>18</td><td>What was the exact or approximate date when these signs were observed?</td><td>_____/ _____/ _____DD/MM/YYYY</td></tr>
<tr><td>19</td><td>For how long these signs were observed</td><td>_____/days/weeks/months</td></tr>
<tr><td>20</td><td>How many pigs died at the time when these signs were observed</td><td><table><tr><td>Sows</td><td>Boars</td><td>Suckling pigs</td><td>Nursery pigs</td><td>Finisher pigs</td></tr><tr><td></td><td></td><td></td><td></td><td></td></tr></table></td></tr>
<tr><td>21</td><td>Could you tell us how many pigs died during June of 2008?</td><td>Sows (S)
Boars (B)
Suckling pigs (SP)
Nursery pigs (N)
Growing pigs (F)</td></tr>
</table>

Section 3: Management practices

22	How did you house your pigs at that time?	In closed building with pigs alone. ☐ In open building or pen with pigs alone ☐ In closed building or pen with other animals ☐ In open building or pen with pigs other animals ☐ Free in farm's backyard ☐ Free to go to neighbours ☐ Other, _____ ☒	Check all that apply
23	How many animals of other species did you have on your farm at that time? Could animals of these species mix or had direct nose-to-nose contact with your pigs?	Species / Number / Contact with pigs Cattle Water buffalo Goats Chicken Ducks Geese Other, _____ ____ The time here depends on their previous response. It could be time of disease, unusual signs, or April of 2008. Please check if answer is yes, leave unchecked is answer is no and write if answer is "I don't know"	Provide number of animals that were on that property. If there was no animals, enetr 0. If number if animals is more than 0, check the box under contact with pigs if this animal species was in direct contact with pigs.
24	What kind of water used for your farms	Pipeline water ☐ Open well ☐ Drilled well ☐ Surface water ☐ Other, _____ ☒	

25	How is your waste water from farm treated	Directly to surface water Compost to use as fertilizer Biogas Sold Feed another animal (fish) _____ Other, _____	
26	How did you feed your pigs before that time?	Compound feed Delivery by store Delivery by farm Additional feed Garbage from outside Garbage from home Seed/plant grown on farm Seed/plant from outside Other, _____	Check all that apply
27	Did you buy or receive any pigs prior to this time?	No / Yes / Don't know	
28	Did you buy or receive any other animals prior to this time?	No / Yes / Don't know	
29	Did you have pigs, for example boars, from other farms on your farm prior to this time?	No / Yes / Don't know	
30	Did your pigs visited other farms prior to this time	No / Yes / Don't know	
31	Did you vaccinate your animals prior to this time for the following swine disease	Vaccine / S / B / NP / F Classical Swine Fever PRRS Name,_____ FMD Other, _____	

<table>
<tr><td>32</td><td>How did you breed your animals prior to this time</td><td><table>
<tr><td>Natural breeding</td><td></td></tr>
<tr><td>Boars on farm</td><td></td></tr>
<tr><td>Boars from outside</td><td></td></tr>
<tr><td>Artificial insemination</td><td></td></tr>
<tr><td>Semen on farm</td><td></td></tr>
<tr><td>Semen from outside</td><td></td></tr>
<tr><td>Done by yourself</td><td></td></tr>
<tr><td>Done by specialist</td><td></td></tr>
<tr><td colspan="2">Other, _____</td></tr>
</table></td><td></td></tr>
<tr><td>33</td><td>How frequently do you buy gilts or sows</td><td>☐ Never
☐ _____Month/Year
☐ Other, _____</td><td>Answer to this will either be never, or number of times per appropriate time period</td></tr>
<tr><td>34</td><td>How often do you buy or receive nursery pigs on this farm</td><td colspan="2">☐ Never
☐ Buy all nursery pigs for every production cycle
☐ Buy only a portion of pigs
☐ Produce only own
☐ Other, _____</td></tr>
<tr><td>35</td><td>From how many different farms you purchase sows</td><td>_____
☐ Buy from a dealer</td><td>Provide number of sources on the line.</td></tr>
<tr><td>36</td><td>From how many different farms you purchase nursery pigs</td><td>_____
☐ Buy from a dealer</td><td>Provide number of sources on the line.</td></tr>
<tr><td>37</td><td>When did you last time purchase or receive pigs of any age on this farm?</td><td>_____/_____/_____ (dd/mm/yyyy)</td><td></td></tr>
<tr><td>38</td><td>What production class did you buy? How many pigs did you buy?</td><td><table>
<tr><td>Sows</td><td>Boars</td><td>Suckling pigs</td><td>Nursery pigs</td><td>Finisher pigs</td></tr>
<tr><td></td><td></td><td></td><td></td><td></td></tr>
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<table>
<tr><td>39</td><td>Where did you buy these pigs</td><td>☐ From another small farm
☐ From a large breeding farm
☐ Dealer
☐ Other, _____</td><td>Check all that apply</td></tr>
<tr><td>40</td><td>How close was the source farm of these pigs (in kms)</td><td>_____</td><td>In kms (Road distance)</td></tr>
<tr><td>41</td><td>When did you last time sell pigs of any age from your farm?</td><td>_____/_____/_____ (dd/mm/yyyy)</td><td></td></tr>
<tr><td>42</td><td>What production class did you sell?</td><td><table><tr><td>Sows</td><td>Boars</td><td>Suckling pigs</td><td>Nursery pigs</td><td>Finisher pigs</td></tr><tr><td></td><td></td><td></td><td></td><td></td></tr></table></td><td>number</td></tr>
<tr><td>43</td><td>Where did you sell these pigs</td><td colspan="2">☐ To another farm , _____
☐ To a dealer,_____
☐ Other, _____</td></tr>
<tr><td>45</td><td>How frequently you have visitors to your farm. (general guest)</td><td><table><tr><td>Per day</td><td>Per week</td><td>Per month</td></tr><tr><td></td><td></td><td></td></tr></table></td><td></td></tr>
<tr><td>46</td><td>How frequently you have visitors to your farm that are so close that could touch your pigs (dealers, advisors, ...)</td><td><table><tr><td>Per day</td><td>Per week</td><td>Per month</td></tr><tr><td></td><td></td><td></td></tr></table></td><td></td></tr>
<tr><td>47</td><td>From how far away was the last visit you received</td><td>_____</td><td><u>In kms</u> Road distance</td></tr>
</table>

<table>
<tr><td>48</td><td>How frequently do you have any animals coming to your farm for any reason (e.g., either if you purchased them or if neighbour's animals are on your property)., and from how many different farmers. Please fill one column, depending on how farmer answer the question.</td><td>
<table>
<tr><th>Species</th><th>Day</th><th>Week</th><th>Month</th><th>N sources</th></tr>
<tr><td>Cattle</td><td></td><td></td><td></td><td></td></tr>
<tr><td>Water buffalo</td><td></td><td></td><td></td><td></td></tr>
<tr><td>Goats</td><td></td><td></td><td></td><td></td></tr>
<tr><td>Chicken</td><td></td><td></td><td></td><td></td></tr>
<tr><td>Ducks</td><td></td><td></td><td></td><td></td></tr>
<tr><td>Geese</td><td></td><td></td><td></td><td></td></tr>
<tr><td>Wild birds</td><td></td><td></td><td></td><td></td></tr>
<tr><td>Wild waterfowl</td><td></td><td></td><td></td><td></td></tr>
<tr><td>Wild animals</td><td></td><td></td><td></td><td></td></tr>
<tr><td>Other, _____</td><td></td><td></td><td></td><td></td></tr>
</table>
</td></tr>
<tr><td>49</td><td>Is there anyone in your farm work as veterinarian</td><td>☐ No ☐ Yes Specify:_____</td></tr>
<tr><td>50</td><td>Who will do animal treatment / birth assistance for your farm</td><td>
<table>
<tr><td>Veterinarian</td><td></td></tr>
<tr><td>By yourself</td><td></td></tr>
<tr><td>Other: _____</td><td></td></tr>
</table>
</td></tr>
</table>

Appendix 2.

The WINBUGS code to estimate the sensitivity and specificity of two PCR test

```
model;
{
x[1:4] ~ dmulti(p[1:4], n)
p[1] <- pi*(Setiss*Seblood+covDp) + (1-pi)*((1-Sptiss)*(1-Spblood)+covDn)
p[2] <- pi*(Setiss*(1-Seblood)-covDp) + (1-pi)*((1-Sptiss)*Spblood-covDn)
p[3] <- pi*((1-Setiss)*Seblood-covDp) + (1-pi)*(Sptiss*(1-Spblood)-covDn)
p[4] <- pi*((1-Setiss)*(1-Seblood)+covDp) + (1-pi)*(Sptiss*Spblood+covDn)
ls <- (Setiss-1)*(1-Seblood)
us <- min(Setiss,Seblood) - Setiss*Seblood
lc <- (Sptiss-1)*(1-Spblood)
uc <- min(Sptiss,Spblood) - Sptiss*Spblood
pi ~ dbeta(12.82, 48.28) ## Mode=0.2, 95% sure pi < 0.3
Setiss ~ dbeta(15.7987, 1.302) ## Mode=0.98, 95% sure Setiss > 0.80
Sptiss ~ dbeta(42.11, 1.84) ## Mode=0.98, 95% sure Sptiss > 0.90
Seblood ~ dbeta(15.7987, 1.302) ## Mode=0.98, 95% sure Setiss > 0.80
Spblood ~ dbeta(42.11, 1.84) ## Mode=0.98, 95% sure Sptiss > 0.90
covDn ~ dunif(lc, uc)
covDp ~ dunif(ls, us)
rhoD <- covDp / sqrt(Setiss*(1-Setiss)*Seblood*(1-Seblood))
rhoDc <- covDn / sqrt(Sptiss*(1-Sptiss)*Spblood*(1-Spblood))
}
list(n=132, x=c(19, 16,1,96))
list(pi=0.2, Setiss=0.9, Sptiss=0.98, Seblood=0.98, Spblood=0.98)
```