Investigation of the spread, control and surveillance of porcine reproductive and respiratory syndrome (PRRS) using epidemiological approaches

by

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ABSTRACT

INVESTIGATION OF THE SPREAD, CONTROL AND SURVEILLANCE OF PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME (PRRS) USING EPIDEMIOLOGICAL APPROACHES

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This thesis aims to investigate the use of numerous epidemiological tools to improve our understanding of porcine reproductive and respiratory syndrome (PRRS) transmission, control and surveillance at the herd and regional levels. These tools include spatial analysis, molecular epidemiology, network analysis, and infectious disease modelling. The main source of data for the thesis chapters was the Ontario PRRS area regional control and elimination projects.

There are a few important conclusions from this thesis. First, spatial dependence in the patterns of PRRS positivity were not detected for three regions in Ontario, even though defined spatial clusters could be found. Secondly, an investigation of the occurrence of PRRS revealed that the importance of area spread and truck network connections were dependent on the virus genotype. Thirdly, description of networks showed that the Ontario swine industry is highly connected through multiple service providers, which can represent a challenge for outbreak investigations and disease surveillance and control. Lastly, the development of two types of mathematical models (hybrid and agent-based) allowed for the evaluation of herd- and regional-level control and surveillance strategies for PRRS. One of the take home messages from the herd-level
model was that major PRRS outbreaks could occur in breeding herds long after the initial virus introduction, even in cases where the herd is naïve. Furthermore, neither vaccination of gilts with a modified-live PRRS virus vaccine nor their exposure through live-virus inoculation guaranteed that the introduced virus would not circulate in the piglet population in cases of novel viral introductions. The regional-level model suggested that contemporary approaches to implement risk-based surveillance based on site demographic characteristics (e.g. production type) do not appear to necessarily improve surveillance system sensitivity; which suggest novel strategies need to be explored to assure rapid detection of emerging PRRS virus strains.

Conclusions from this thesis support the use of databases obtained from industry-initiated regional control projects to inform models and characterize risk, and development of epidemiological tools should continue in the future for the benefit of the swine industry as a whole.
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Many thanks to the Department of Population Medicine staff, professors, colleagues and friends for amazing discussions from ‘critical appraisal of the literature’ to ‘what is the world’s best dessert’. Thank you to the Pig Palace family for improving my euchre skills constantly, for caring, and for doing a great job on making me feel less away from home. You have made it easier, you have made it fun, you have made every single day count.

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STATEMENT OF WORK

The Ontario area regional control and elimination (ARC&E) projects was a program conducted independently from this thesis by the Ontario Swine Health Advisory Board (OSHAB). Jane Carpenter was the program coordinator responsible for declaring disease status individually for swine sites participating in the project as well as guiding data entry, which was performed by trained personnel including Andréia Arruda. Diagnostic data for the project were obtained from diverse laboratories across Ontario, but all ORF5 gene sequencing data utilized in Chapter 3 were obtained from the Animal Health Laboratory (University of Guelph, Canada). Karen Hand was responsible for OSHAB database management and extraction of all data used by the author for analyses. The initial proposal for this study was prepared by Zvonimir Poljak.

All data cleaning, merging, assessment, and statistical analyses were performed by Andréia Arruda, with help from Zvonimir Poljak and William Sears. Mathematical models were created in Anylogic© by Andréia Arruda, with help from Amy Greer (Chapter 5), Dylan Knowles (Chapter 6), Allen McLean (Chapter 6), Winchell Qian (Chapter 6), and the Anylogic© support team (Chapters 5 and 6). Parameters estimated for use in such models were gathered during meetings with Andréia Arruda, Robert Friendship, Zvonimir Poljak and Jane Carpenter. All chapters were written entirely by Andréia Arruda, reviewed and edited by all committee members including Zvonimir Poljak, Jane Carpenter, Karen Hand and Robert Friendship. Further edits were provided by Montse Torremorrell, Terri O’Sullivan and Olaf Berke after the PhD oral examination.
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CHAPTER 1: Literature Review of Porcine Reproductive and Respiratory Syndrome and Research Objectives

1.1. Prevalence and Economic Impact

Porcine reproductive and respiratory syndrome (PRRS) was first recognized in the late 1980s in North America and Europe. At that time, the causative agent was unknown, and terms such as “mystery swine disease” and “blue-ear pig disease” were used to refer to this syndrome that was characterized by reproductive failure in sows and respiratory disease in pigs of all ages. In 1991 and 1992, the causative agent was identified as a small single-stranded enveloped RNA virus in Europe (Wensvoort et al., 1991) and North America (Collins et al., 1992), and it was discovered that these so-called PRRS viruses (PRRSV) had properties similar to viruses in the family Arteriviridae, genus Arterivirus.

Porcine reproductive and respiratory syndrome has been diagnosed worldwide and is known to be endemic in most pig producing countries including the United States (US), Canada, China, Germany, France, Poland, Japan, Korea, and Thailand with a few exceptions that include New Zealand, Australia, Sweden and Switzerland (OIE, 2008). In Ontario, analysis of sera collected in the late 1970’s showed the presence of PRRSV antibody response as early as 1979 (Carman et al., 1995). Its prevalence and incidence vary among countries; and in the United States a study conducted in 2006 by the National Animal Health Monitoring System (NAHMS) reported that out of 173 sites (5,793 tested samples), 49.8% of unvaccinated grower/finisher pigs were seropositive for PRRS and 71.1% of unvaccinated sites had at least one enzyme-linked immunosorbent assay (ELISA) positive sample out of 35 (APHIS, 2009). An on-going national PRRSV incidence project that currently includes more than 1.2 million sows across 15 states...
estimated that each year 29-38% of sow herds report a new PRRS infection (Tousignant et al., 2013, unpublished data). In Canada, 40 to 80% of herds in eastern and central Canada were seropositive in 1995. Ten years after these estimates were published, an increase in the incidence and severity of the disease was reported in Ontario by field veterinarians and confirmed by a diagnostic laboratory (Young et al., 2010), and it was later estimated that the prevalence of PRRS in swine herds was approximately 55% (Carpenter et al., 2010, unpublished data). A cross-sectional study conducted between 2006 and 2008 reported a PRRS apparent prevalence of 74% for sow herds located in a moderate pig-dense area in Quebec (Lambert et al., 2012). In the provinces of Quebec and Manitoba, a study conducted in slaughterhouses found that 74% of serum samples were seropositive for PRRSV, and 4 and 2% of serum and meat samples, respectively, were positive for PRRSV by PCR (Magar and Larochelle, 2004).

Economic impacts of PRRS include; direct costs (decreased production of weaned pigs, reduced growth and average daily gain, increased mortality and poorer feed conversion) and indirect costs (animal health effects such as prevention and treatment of accompanying infections, increased labor and veterinary services), and both should be considered when estimating the cost of this infectious disease. It has been recently estimated that the cost of PRRS for the United States swine industry is approximately $664 million per year or $115 per female per year for every sow in the US breeding inventory, and herd level productivity losses were estimated to result in 9.93 million fewer pigs marketed per year (Holtkamp et al., 2013). Forty-five percent of this cost was attributed to losses in the breeding herd, compared to 12% estimated in a US study conducted in 2005 (Neumann et al., 2005). In addition, an extra $448 million annually was estimated for additional costs including animal-health, biosecurity and other outbreak-related costs (Holtkamp et al., 2013). In Europe, Nieuwenhuis et al. (2012) estimated that a PRRS
outbreak in a sow herd in The Netherlands costs €75 per sow in a regular commercial production herd considering an 18-week outbreak, and in Canada the disease is estimated to cost approximately $130 million per year to the swine industry (Mussell et al., 2011).

1.2. Virus Characterization

Porcine reproductive and respiratory syndrome virus is a 15kb enveloped, single-stranded, non-segmented, positive-sense RNA virus that belongs to the order Nidovirales, family Arteriviridae, genus Arterivirus. Other viruses from this family include the equine arteritis virus, the lactate dehydrogenase-elevating virus and the simian hemorrhagic fever virus (Rowland, 2007). There are two main variants of PRRS virus: the Lelystad or Type 1, which refers to the European virus, and the VR-2332 or Type 2, which refers to the North American strain, but both strains are present worldwide (Lunney et al., 2010). It has been suggested that PRRSV is a mutant from the mouse lactate dehydrogenase-elevating virus and that wild boars were an intermediate host, spreading the virus from central Europe to North Carolina in 1912 through importation of animals (Plagemann, 2003). The viruses then evolved independently for 70 years, and selection for mutants with better growth potential in the different wild populations on the two continents might explain the virus diversification (Plagemann, 2003). The genetic difference between the two viruses is about 40% for the whole genome sequence (Murtaugh et al., 2010).

The RNA genome encodes nine open-reading frames (ORFs). Approximately 80% of the virus genome is composed of the open reading frames 1a and 1b, which encodes non-structural proteins necessary for viral replication (Meulenberg, 2000). The remaining genome is composed of the ORFs 2a, 2b and 3-7, which encode 7 structural proteins. The minor envelope proteins GP2a, GP3 and GP4 are encoded by ORFs 2, 3 and 4, respectively. They are not required for
particle assembly but interact with each other, and are essential for infectivity (Wissink et al., 2005). The 2b protein is also encoded by ORF2 and is an integral but minor structural component of the virion (Wu et al., 2001). The major structural proteins GP5, Matrix and Nucleocapsid are encoded by ORFs 5, 6 and 7, respectively, and they are required for particle formation and infectivity (Wissink et al., 2005).

The PRRS viruses are highly heterogeneous, and this genetic variability of PRRS viruses probably results from more than one factor. The simplest explanation would be the infidelity of the RNA polymerase but recombination events also play an important role (Murtaugh et al., 2010), causing insertions and deletions. The ORF 5 gene has shown to be the most diverse PRRS gene, prone to mutational changes during replication, which raises concerns regarding antigenic shift and immunity since this gene encodes the major envelope protein GP5 (Chang et al., 2009). The variability in GP5 might explain some of the failure in developing an efficacious vaccine (Lunney et al., 2010). The ORF5 is often used for phylogenetic analysis because of its high variability (Shi et al., 2010). According to Murtaugh et al. (2010), a major limitation in the understanding of PRRS is the difficulty in connecting genetic relationships to functionally relevant phenotypical characteristics, especially those related to immunology and virulence.

1.3. Pathogenesis and Clinical Signs

Swine are the only known hosts for PRRSV (Murtaugh et al., 2010) and are susceptible by several routes of exposure including parenteral, intranasal, intramuscular, oral, intrauterine and vaginal (Zimmerman et al., 2012). Following exposure, the virus replicates in local permissive macrophages and spread to lymphoid organs, lungs and less commonly to other tissues, causing an initial viremia within 12 to 24 hours after inoculation (depending on the virulence of the
strain) that peaks by days 7 to 14 post-infection (Zimmerman et al., 2012). Porcine reproductive and respiratory syndrome virus has a restricted cell tropism for cells of the monocytic lineage, more specifically, fully differentiated macrophages that have the receptors sialoadhesin and CD163 on their surface; such as pulmonary alveolar macrophages, pulmonary intravascular macrophages and macrophages in lymphoid tissues. Therefore lungs and lymph nodes are the organs with higher titers of PRRSV antigen and nucleic acid (Welch and Calvert, 2010; Zimmerman et al., 2012).

It is known that viremia can be detected up to 28 days post-infection or 48 days post-infection in the case of congenitally infected pigs, but persistent virus is continuously produced in lymphoid tissues by a low level of virus replication (Allende et al., 2000, Zimmerman et al., 2012). If the animal is pregnant, PRRSV is able to cross the placenta around 90 days of gestation and infect fetuses (Lunney et al., 2010). Viral RNA can be detected in serum and tonsils of pigs infected under experimental conditions for up to 251 days (Wills et al., 2003) and up to 130 days in tonsil and lymphoid organs of piglets infected in-utero (Rowland, 2010). In congenitally infected pigs, virus can be isolated from many tissues, but the thymus is the organ with the largest quantity of virus, followed by spleen and lymph node, indicating that the thymus is likely the site of virus replication (Rowland, 2010).

The lesions caused by PRRSV replication are known to be caused by the death of infected and proximal non-infected cells, secretion of inflammatory cytokines, B-cell activation and reduction of the animal’s overall immune defense due to the killing of macrophages (Zimmerman et al., 2012). Death of infected cells can be induced by necrosis and apoptosis (Lee and Kleiboeker, 2007), but the cause of indirect apoptosis in non-infected cells remains unknown (Zimmerman et al., 2012). Inflammatory cytokines have beneficial effects on the host since they stimulate innate
immune responses, but detrimental effects can also happen. For example, the cytokines TNF-α, IL-1 and IL-6 are involved in causing increased vascular permeability resulting in pulmonary edema and bronchial constriction, as well as induction of systemic effects such as fever, anorexia and lethargy (Gomez-Laguna et al., 2010, Zimmerman et al., 2012). In addition, the induction of polyclonal B-cells can lead to circulation and deposition of immune complexes, resulting in cellular damage (Lemke et al., 2004).

The severity of clinical signs of PRRS is highly variable and can be affected by factors such as herd management practices, presence of other concurrent diseases, specific PRRSV strains, PRRS history on the specific site, level of immunity of the animals, age, and a variety of other factors. A PRRS outbreak or epidemic occurs when PRRSV is introduced into a naïve population of animals, or when a relatively unrelated genetic variant is introduced into a herd that has been previously exposed. In populations that have a certain level of immunity due to previous exposure, PRRS can be endemic and clinical disease will only be seen in susceptible sub-populations such as sick animals and animals where maternal immunity is low (Zimmerman et al., 2012).

Infection of a herd with PRRSV can be divided into two phases: the acute phase, which lasts about two weeks or more, and is characterized by the disease being spread throughout the site and causing viremia, and the second phase, which can last for one to four months and is characterized by long-term reproductive and respiratory syndromes (Zimmerman et al., 2012). The reproductive aspect of the disease may include clinical signs such as late-term abortions, premature parturition (5-7 days early), increased numbers of weak born, stillbirths (50-70%) and mummified piglets, as well as increased return-to-estrus intervals, increased numbers of sows with irregular returns to estrus; and an increase in sow mortality in some cases (Wensvoort et al.,
1991; Goyal, 1993). Boars may show respiratory clinical signs, lethargy, anorexia, lack of libido and reduction in sperm quality (Prieto et al., 1996). In transplacental infections, surviving neonates can exhibit immune system lesions that may enhance piglet susceptibility to other microbial infections such as *Streptococcus suis*, and respiratory pathogens (Feng et al., 2001). The respiratory form of the disease has been reported in pigs of all ages even though it is more pronounced in younger pigs, and can be characterized by an influenza-like illness, including depression, lethargy, inappetence, growth retardation and potential high mortality especially when there are increased concurrent bacterial infections (Goyal, 1993). In nursery pigs, clinical signs are more pronounced, with a respiratory disorder called “thumping” caused by interstitial pneumonia, and mortality can reach 60% (Goyal, 1993). In grow-finish pigs, clinical signs are usually milder than in younger aged pigs and may include; anorexia, increased respiration rate, hyper-excitability, chronic pneumonia, dermatitis and uneven growth rate (Keffaber, 1989). Similar gross lesions are consistently described from 4 to 28 days post-infection for pigs of all ages and include interstitial pneumonia and enlarged lymph nodes (2 to 10 times the normal size) (Zimmerman et al., 2012). Infected litters will have variable numbers of normal pigs, weak pigs, fresh and autolytic stillborn pigs, partially mummified and completely mummified fetuses (Zimmerman et al., 2012).

### 1.4. Epidemiology

Once PRRSV is introduced into a population of susceptible animals, infection will occur and infected animals will begin to shed the virus via saliva, nasal secretions, urine, semen, mammary secretions and feces (Wills et al., 1997; Rossow et al., 1994). Duration of shedding of PRRSV is dependent on type of sample and strain of the virus and is yet not precisely known. It has been
reported that the virus can be shed in saliva from day-1 post-infection up to 42 days (Wills et al., 1997), in nasal secretions for up to 21 days (Rossow et al., 1994) and from oropharyngeal samples up to 84 days, possibly being associated with tonsil samples. The virus can also be isolated sporadically from urine and feces during the first two weeks of infection and up to 28 days post-infection (Wills et al, 1997; Rossow et al., 1994). In boars, it has been reported that PRRSV can be shed in semen from 3 days post-infection up to 92 days (Christopher-Hennings et al., 1995); and in mammary secretions, the virus can be isolated up to 9 days post-farrowing (Wagstrom et al., 2001).

All animals infected with PRRSV are commonly reported to be viremic by day-14 post-infection, up to day-21 (Wills et al, 1997). The virus can persist and replicate at low levels within individual animals, allowing viral RNA isolation from serum and tonsils up to 251 days under experimental conditions (Wills et al., 2003), which may explain why it can be shed in oropharyngeal samples for long periods of time. However, shedding and persistence of infection might be dependent on the age of the host, as it has been reported that infected breeding-age gilts showed short duration of persistence and shedding (less than 90 days according to Batista et al., 2002). Seroconversion occurs by 14 days post-infection, but can occur as early as day-7, being at times concurrent with viremia (Rossow et al, 1994; Wills et al., 1997). This indicates that primary antibody response is not protective against infection with PRRSV, but neutralizing antibodies are known to appear after approximately 4 weeks of infection, and clear viremia (Lopez and Osorio, 2004). Cellular immune response is active later after infection and is responsible for eliminating the virus from infected cells (Lopez and Osorio, 2004).

As previously mentioned, there are several routes of exposure for PRRSV, and transmission of the virus can occur directly, indirectly or in-utero. Any type of direct contact between animals
such as nose-to-nose contact, fights, tail biting, ear biting, suckling, etc., exposes pigs to the virus, therefore putting them at risk for being infected. Transmission of PRRSV to naïve pigs from ingesting contaminated meat from infected pigs yielded contradictory results (Magar and Larochelle, 2004; Molina et al., 2009). Indirect routes are also known to be very important in PRRSV transmission. Activities such as ear notching, tail docking, teeth clipping, castration and medicine administration can spread infection from infected to susceptible animals if the handler or equipment are contaminated with PRRSV. Other indirect transmission pathways that have been previous explored include; contact with contaminated coveralls, boots, gloves and overall clothing, as well as insect vectors and artificial insemination with contaminated semen. Finally, vertical transmission is an essential way for PRRSV transmission and maintenance in a herd. It has been shown that even low doses of a mildly virulent strain administered intramuscularly to naïve gilts at 90 days of gestation can cause clinical signs in gilts and be effectively transmitted to the litter (Cano et al., 2009). In this study, a high percentage of the piglets in a litter were shown to be PRRSV PCR-positive at birth (55-100%), with all piglets being positive by 4 days of age, and most of the piglets (89%) were still viremic by 17 days of age. Another study showed that a fetus from PRRSV- challenged pregnant sows can generate an antibody response to PRRSV in-utero (Rowland, 2010).

It is known that many risk factors at the virus, host, herd and regional levels affect PRRSV transmission and persistence, and some of these will be explored in the following sections.

1.4.1. Risk factors at the virus level

Strain type: Different genetic variants of the virus can cause different types and severity of disease. As an example, a study using samples collected between 2004 and 2007 showed that restriction fragment length polymorphism (RFLP) patterns were associated with specific clinical
signs in different types of swine herds in Ontario, and also that a few RFLP types (1-3-4 and 1-8-4) were associated with epidemics in that province (Rosendal et al., 2010).

**Virus stability in environmental conditions:** PRRSV is quickly inactivated by heat and drying, but is able to survive in extremely cold temperatures and in moist environments. It is known to be stable for months or years at temperatures of -70 and -20°C and in moist conditions (Dee et al., 2002, Zimmerman et al., 2012), and it was estimated that approximately 8 hours of 50°C are needed in order to inactivate PRRSV from manure (Linhares et al., 2012b). It has been reported that case submissions to diagnostic laboratories in the US and Canada increases during periods of cold weather (Dee et al., 2002).

1.4.2. **Risk factors at the host level**

**Age:** Pigs acquire innate resistance to PRRSV infection as they age as indicated by reduced duration of viremia and viral loads in blood in finishing pigs and adults compared to weaned pigs (Murtaugh and Genzow, 2011). The late-gestation sow, even in the face of stronger innate resistance, is a critical population at risk since the virus has a predilection for the reproductive tract (Murtaugh and Genzow, 2011). It is important to note that pigs born to seropositive dams can remain positive on serologic tests until 3 to 16 weeks of age (Yoon, 2003), but when maternal antibodies begin to wane, pigs will be susceptible to infection and this might contribute to the severity of the disease seen in nursery pigs compared to the mild and even subclinical forms seen in finishing pigs.

**Immune status of the animal:** The appearance of PRRSV neutralizing antibodies is slow and irregular, but can provide protection against homologous PRRSV strains and even partial protection against heterologous strains that are somewhat genetically similar to the one they have been previously exposed. Animals that have been previously exposed to PRRSV through natural
exposure or vaccination have shown less severe clinical signs when they encounter a new PRRSV (Cano et al., 2007). Young et al. (2010) investigated vaccination use in sows and reported it was associated with a decrease in weak-born pigs and pre-weaning mortality. It has also been reported that animals vaccinated with a modified-live vaccine (MLV) have reduced shedding in aerosol and oral fluid samples (Linhares et al., 2012a).

1.4.3. **Risk factors at the herd level**

Overall, when introduced to a susceptible population, the infection tends to spread quickly throughout the herd, and may then continue to circulate in the swine population indefinitely. It has been estimated that 80 to 95% of the pigs in a herd experience seroconversion within 2-3 months of initial introduction (Albina, 1997).

**Herd size:** Both the numbers of direct and indirect contacts are increased in larger herds, as well as number of interactions with the outside world such as removal of dead stock and introduction of animals. A study conducted in Quebec showed that having a large pig inventory was positively associated with being PRRS positive (Lambert et al., 2012). Likewise, other studies reported that increased number of sows, nursery and finisher pigs were associated with increased PRRS clinical signs and mortality (Young et al., 2010; Goldberg et al. 2000b).

**Herd flow and biosecurity measures:** Continuous flow systems provide a constant introduction of potentially susceptible animals in a herd, which, combined with the persistence of PRRSV in long-term carrier animals, leads to maintenance of the virus in a population of pigs. It has been reported that all-in all-out (AIAO) management practices were protective against clinical signs associated with the reproductive syndrome, possibly through reduction of transmission rate of PRRSV within herds (Goldberg et al., 2000b). Other biosecurity measures such as use of shower-in facilities, entry protocols, Danish entry system, use of clean and disinfected gloves,
coveralls and boots and control of fomites, besides others, have been highly recommended for infectious disease prevention and control (Pitkin et al., 2009). Lambert et al. (2012) has shown that PRRS infected sites were less likely to have a shower in facility for people entering the barn (Lambert et al., 2012). Dead stock removal might be an important risk factor for PRRS infection as incoming trucks picking up animals on a regular basis can be an important source of virus coming from other herds. It has been previously shown that trucks are able to mechanically transport the virus (Dee et al., 2002). It is currently recommended that rendering trucks should be avoided at all times, while composting or incinerating carcasses on the farms would be more appropriate (Pitkin et al., 2009).

**Presence of other diseases:** It is known that dual infections can cause more severe disease compared to single infections (Zimmerman et al., 2012); and this is not different with PRRS. For example, a study reported that animals infected with *Mycoplasma hyopneumoniae* had potentiated PRRS-induced disease and lesions (Thacker et al., 1999). Interestingly, in regards to PRRS infection, co-infection with this same pathogen (*M. hyopneumoniae*) did not seem to aggravate shedding and persistence of PRRS infection itself according to a study conducted by Fano et al. (2007).

### 1.4.4. Risk factors at the regional level

Area spread corresponds to herd-to-herd transmission for which precise modes of transmission are unknown but factors such as aerosol transmission, spread via avian or insect vectors and contaminated fomites are hypothesized to be involved (Larochelle et al., 2003). A few mechanisms that might be involved on area spread of PRRS are explored below.

**Overall dynamics of modern swine production:** The modern swine production system is characterized by a high degree of connectedness between different sectors of the industry and the
concentration of swine herds in production systems. The vast majority of herds are part of a highly connected system characterized by a relatively small group of service providers that are in different ways connected to many herds (e.g. feed companies, trucks handling dead stock and culled pigs, semen companies, gilt sources, and others), and these herds are located on fewer premises and belong to a small number of owners. This situation might facilitate indirect spread of PRRSV from infected to susceptible herds through subtle ways that could sometimes go unrecognized.

Multi-site production: The above-mentioned production systems have routine intense pig movement among herds, not only facilitating disease spread but also introducing pools of susceptible animals (nursery pigs, gilts, boars) on a weekly or monthly basis into the herds. These multi-site production systems are involved in a “chain of infection”, therefore PRRSV status of a herd will depend on the status of the site it is receiving or sending pigs from/to.

Pig density: There is a consensus that farms located in pig dense areas are at increased risk of being infected or re-infected with PRRSV, even though the mechanisms of transmission of PRRSV between farms are still not completely understood (Mondaca-Fernandez et al., 2006). A study conducted in Quebec reported that sites located ≤ 2.5 km from the closest pig site were more likely to be positive than farms located farther away from other farms (Lambert et al., 2012).

Sharing of transport vehicles between herds: As briefly mentioned above, trucks are important in connecting herds, and a study conducted in Minnesota investigated the mechanical transmission of PRRSV in a sequence of events in cold weather (temperatures below 0°C), such as contamination of the transport vehicle and vehicle’s cab by footwear, contamination of the swine facility by contaminated footwear and vehicle; and survival of the virus and contamination of
fomites entering the animal space. Results validated PRRSV viability outside the host in field conditions, proving accidental contamination of vehicles and PRRSV transmission from herd to herd in cold weather conditions can easily occur (Dee et al., 2002). Supporting these findings, Lambert et al. (2012) also showed that PRRS infected sites had higher odds of allowing access to their main entrance by the rendering truck (Lambert et al., 2012).

**Aerosol transmission:** Herd-to-herd transmission of PRRSV by aerial spread has been extensively explored in the past. Findings from a study using 62 different herds show that the greater the distance between farms, the less genetic homology among PRRSV isolates (Mondaca-Fernandez et al., 2006), which would support the possibility of aerial spread of PRRSV. Another study, however, reported not being able to establish a correlation between geographic proximity and genomic similarity, and suggested that other mechanisms might explain PRRSV introduction into neighboring herds (Goldberg et al., 2000a). Otake et al (2010) demonstrated that some strains of the virus can travel in the air for up to 9 km from an infected herd, and still be viable.

1.5. **Diagnosis**

Porcine reproductive and respiratory syndrome can be diagnosed in the individual animal or herd using different tools. The choice of detection method depends on the goal of disease detection. Porcine reproductive and respiratory syndrome should be suspected in any herd with reproductive and/ or respiratory disease in pigs of any age, but the absence of clinical signs is not enough to declare freedom of disease (Zimmerman et al., 2012). As previously mentioned, PRRSV infection varies widely between herds and pigs. Subclinical infections are common, and there are no pathognomonic macro or microscopic lesions for PRRSV infection (Botner, 1997;
Nodelijk, 2002). For these reasons, a definitive demonstration of PRRSV and/or antibodies is required for definitive diagnosis. These two diagnostic approaches should be used in combination, since they will be dependent on stage of infection and herd dynamics.

1.5.1. Antibody detection

Serology is a powerful tool that is commonly conducted using serum or oral fluid samples. Antibodies are known to appear as early as 2-4 weeks after infection (Lopez and Osorio, 2004). Serological assays indicate whether the individual animal or population of animals were exposed to PRRSV, but they do not differentiate among strains, vaccine type or maternal immunity (Zimmerman et al., 2012), neither do they provide information on whether clinical disease was present in a herd (Christopher-Hennings et al., 2002). It is also important to note that the high degree of variation in the immune response of individual animals may at times lead to “over-interpretation” of results (Christopher-Hennings et al., 2002). Lastly, since antibodies do not persist for the lifetime of an animal, it is recommended that young pigs rather than breeding stock be tested to determine a herd’s PRRSV infection status (Yoon et al., 2003).

Immunoperoxidase monolayer assay (IPMA): This was the first serological test to be developed, and has been reported to have high specificity and sensitivity (Wensvoort et al., 1991), but is no longer routinely used in diagnostic laboratories in North America (Christopher-Hennings et al., 2002).

Enzyme-linked immunosorbent assay (ELISA): On a routine basis, the commercially available serum ELISA test-kit HerdCheck® X3 PRRS ELISA (IDEXX Laboratories, Inc., Westbrook, ME) is definitely the most commonly used serological test. It can detect antibodies as early as 9 to 13 days post-infection, peaking between 30 and 50 days and declining to negative levels 4 to 12 months post-infection. According to the manufacturer, the sensitivity and specificity of the
test are 100% and 99.5%, respectively, when a sample-to-positive (S:P) ratio cut-off of ≥ 0.4 for a positive sample is used. Advantages of using the ELISA test include; reproducibility, easy interpretation of test results and availability of the test. The limitations include; a high degree of variation among individual animals (therefore, the test is subject to over-interpretation at the individual level) and requires a certain degree of precision from the technician performing the test (Christopher-Hennings et al., 2002). In the last few years, an ELISA test has been developed and optimized for oral fluid samples and it is currently available in North America (IDEXX Laboratories, Inc., Westbrook, ME). Pen-based oral fluid sampling has been shown to improve antibody detection over single animal serum testing, and it has other advantages such as practicality and being less labour intense (Olsen et al., 2013).

**Indirect fluorescent antibody (IFA):** The IFA test is a variant of the IPMA test, used to detect IgM and IgG by fluorescence (Nodelijk, 2002). This technique can be used as early as 7 to 14 days post-infection and maximum titres can be reached after 4 to 6 weeks and decline to low limits of detection around 6 to 12 months (Nodelijk, 2002). This is most commonly used as a supplementary test to rule out false positives with ELISA, since it has high specificity (Collins et al., 1996; Christopher-Hennings et al., 2002).

**Serum virus neutralization (SVN):** The SVN assay detects antibodies that are capable of neutralizing PRRSV in cell culture. Antibodies detected by this assay are slow to appear, peak around 60 to 90 days post-infection and are undetectable after one year, but it is considered a specific test (Collins et al., 1996; Yoon et al., 2003). It is not used in laboratories routinely (Zimmerman et al., 2012), and some of the reasons include that it is not as sensitive as the other assays, it is more expensive and technically complex, and not very well standardized among different laboratories (Christopher-Hennings et al., 2002). This assay is more used as a tool in
research settings.

1.5.2. Virus detection

Virus detection is an indicator of virus circulation in a herd. The presence of the virus can be demonstrated using cell cultures, by direct detection of viral antigen in tissues, or by detection of virus-specific RNA (Nodelijk, 2002). In general, specimens should be collected as early as possible in the course of the disease, but virus detection techniques also allow for late detection of the virus in carrier animals. Virus detection and subsequent characterization are an essential part in strain differentiation for better epidemiological understanding of transmission dynamics. The techniques highlighted below should be considered for use in combination with serology in order to monitor disease trends in herds.

**Virus isolation using cell cultures:** Virus can be isolated with best results from serum, thoracic fluid and lung tissue (Nodelijk, 2002) using cells such as the porcine alveolar macrophages (PAMs) and the MARC-145 and can be visualized through immunostaining using specific antiserum (Botner, 1997). The tissues must be fresh, but serum is the preferred specimen because pigs can be viremic for prolonged times (Collins et al., 1996), however in older animals, viremia is of shorter duration and PRRSV may be found in tissues longer than in serum (Christopher-Hennings et al., 2001). An advantage of this technique is that the isolated virus might be used for the production of autogenous vaccines.

**Histopathology and fluorescent antibodies (FA):** These techniques are commonly used in association with each other. They are inexpensive and can be conducted with frozen tissues. They have been shown to be specific but not very sensitive, and the results are highly affected by sample quality (Yoon et al., 2003).

**Histopathology and immunohistochemistry (IHC):** These techniques are also commonly used in
association, and virus can be detected in tissues such as lungs and tonsils that are frozen or fixed with formalin. Viral antigens and lesions are best seen during acute infection, 4-14 days post-infection (Zimmerman et al., 2012). This test is not very sensitive but it is very specific (Collins et al., 1996), and it is more expensive and time-consuming than FA (Yoon et al., 2003).

**Reverse-transcriptase polymerase chain reaction (RT-PCR):** This assay is very commonly used to detect genetic material of the virus using serum, oral fluids, semen, lung lavage and tissue samples. It is based on RNA extraction from the sample followed by conversion of RNA to DNA and DNA amplification using a thermocycler (Christopher-Hennings et al., 2002). The amplification process is guided through the use of primers that target a conserved part of the RNA sequence. Genetic material can be detected for long periods of time (>200 days post-infection in tonsil according to Wills et al., 2003). Advantages of PCR include the rapid and standardized method, as well as high sensitivity and specificity. Some limitations on the other hand include false positives due to contaminated samples, the fact that use of certain primers may limit detection of different genetic variants (false negatives), and the failure in differentiating infectious and non-infectious virus (Zimmerman et al., 2012).

It is common that pooled samples are submitted by veterinarians for PCR testing, but it is important to note that RT-PCR performed on pooled serum samples or blood swabs have reduced sensitivity as the number of samples in the pool increases (Rovira et al., 2007), even though RT-PCR performed in oral fluid samples has been proved quite effective (Kittawornrat et al., 2010).

**Loop-mediated isothermal amplification (LAMP):** This technique has been developed as a cheaper option compared to PCR since it is done using a water bath and visualized by the amount of turbidity in the reaction tube that is correlated with the amount of amplified DNA. It
has been shown to be 99% specific, simple and inexpensive, but with lower sensitivity when compared to RT-PCR (Rovira et al., 2009).

**Restriction fragment length polymorphism (RFLP):** This technique is based on digestion of the ORF5 gene using three restriction enzymes (MUuI, HincII and SacII) and assignment of a three-digit code based on the cutting patterns (Christopher-Hennings et al., 2002). The technique was created during vaccination trials in order to differentiate vaccine and field strains, and was then largely applied in the field. However, it is now recognized that it is not a sensitive or reliable method for differentiation of PRRSV or for characterizing relatedness (Cha et al., 2004), and it should not be used to assess relative virulence or for selection of vaccines (Christopher-Hennings et al., 2002).

**Sequencing:** Sequencing of the ORF5 gene has become very popular over the last few years for better characterizing relatedness of virus strains over time and within/between herds. This part of the genome is used since it shows extensive variability but is yet surrounded by conservative sequences that are used as primer targets. It corresponds to the exact nucleotide sequence of a selected part of the genome, which can be aligned with other known sequences (such as previous PRRSV on the herd, vaccine strains, etc.) and provide insightful information regarding genetic difference and similarity among PRRSV strains and evolutionary relationships. Phylogenetic trees and/or similarity matrices are commonly used for easy visualization. An advantage of sequencing includes identification of nucleotide insertions and deletions, however it is important to be aware that it is not the whole genome that is sequenced on a routine basis, and the role of genomic regions in PRRSV related to pathogenesis is yet to be defined (Christopher-Hennings et al., 2002).

1.5.3. *Sample size considerations*
The number of animals to sample in a herd will depend on the goals of the sampling, which might involve making a clinical diagnosis, estimating prevalence or investigating whether the herd is free of PRRSV infection. For making clinical diagnosis or pathogen isolation, it is recommended that risk-based sampling is done, targeting ‘high risk’ individuals, such as animals presenting clinical signs and as early as possible. It has been reported that shortly after an outbreak it is easier to find animals that will seroconvert, therefore sample size for these purposes could potentially be small (Botner, 1997). A reduced sample size has also been suggested when collecting samples for serology in the grow-finish unit of a single-site, farrow-to-finish operation, since during that production phase seroprevalence is usually high compared to other stages of production (Collins et al., 1996). It has been shown that for detection of virus circulation in swine herds without clinical signs of PRRS, testing pigs at 9 and 16 weeks of age yielded greater PRRSV detection by PCR when compared to testing sows in early or late gestation, as well as finisher pigs of 22 weeks of age (Duinhof et al., 2011).

For the purpose of estimating prevalence, however, the sample size will depend on the size of the herd, the level of confidence and it needs to be a random sample of animals in the herd so that it is representative of the target population you aim to estimate prevalence from. Finally, for detecting freedom of infection, it is generally accepted in the North American swine veterinarian community that a sample size of 30 should be aimed for when collecting samples (Perez et al., 2015). This allows for detection of at least 10% prevalence in a large population (‘infinite’) with 95% confidence of detecting at least one positive animal. It is interesting to note that in this case, the expected prevalence and level of confidence play an important role in determining sample size, while herd size might be considered a minor factor, since its impact is the lowest compared to the other factors. Finally, the type of herd, circumstances for sampling and consequences of
PRRSV status should be considered to determine frequency of sampling. As an example, it has been previously reported that monitoring should be done repeatedly over time for acutely infected breeding herds that aim to produce negative piglets using load-close-expose programs in order to increase the confidence that the herd is producing PRRSV negative piglets (that will be potentially moved downstream) (Linhares et al., 2014).

1.6. Control and Elimination

1.6.1. Control

Treatments for PRRS are not available, and therefore several management strategies have been developed over the years in order to prevent and control PRRS in swine herds (Zimmerman et al., 2012). The main goal of control measures is to wean negative pigs from sow herds (Corzo et al., 2010). Among these measures; vaccination, gilt isolation and acclimatization, elimination of contaminated semen, use of “McRebel” to minimize within herd spread, use of strict biosecurity measures and potentially genetic improvement, can be cited and will be explored in the paragraphs to follow.

Vaccination

There are currently three vaccines commercially available in Canada, and all of them are composed by modified-live attenuated viruses. The three vaccines are commonly differentiated by their different RFLP patterns: 1-4-2 (ATP vaccine, Boehringer Ingelheim Vetmedica Inc.), 2-5-2 (MLV vaccine, Boehringer Ingelheim Vetmedica, Inc.) and 1-3-2 (FOSTERA™, Zoetis Animal Health). In the early 2000s, it was reported that approximately 33% of herds in Ontario were using a PRRSV vaccine (Young et al., 2010), and a recent descriptive study showed that this percentage might be currently lower (Arruda et al., 2015). However, it is noticeable that the
use of vaccine changes as veterinarians explore control strategies in swine sites, considering factors such as pig density within the region, historical information, regional PRRS incidence, among others. The data available in regards to vaccine efficacy are somewhat contradictory. On one hand, the vaccines have been associated with issues that include shedding of vaccine virus, persistent infections, incomplete protection, and reversion to virulence (Huang and Meng, 2010). Another issue with the vaccines is that antibodies from animals exposed to the vaccine strains cannot be differentiated from those from animals infected with a field strain, which compromises interpretation of disease status by routine serology (Lillie et al., 2008).

On the other hand, a field trial showed that a commercial live attenuated vaccine could improve reproductive performance in gilts and sows from endemically infected herds, increasing the number of pigs born alive per sow and reducing irregular return to estrus (Pejsak and Markowska-Daniel, 2006). In populations of infected growing pigs, the vaccine did not improve growth performance or alter viremia levels, viremia duration, or antibody response, but it reduced duration of shedding of the virus in oral fluids and bio-aerosols (Linhares et al., 2012a). Another field trial showed that vaccination of 6- to 8-week-old PRRSV-infected pigs with a heterologous strain reduced clinical signs and enhanced pig growth, but it did not prevent infection, nor did it eliminate the initial virus (Cano et al., 2007).

Inactivated and autogenous vaccines have been used in the field with inconsistent results (Corzo et al., 2010; Papatsiros et al., 2006). Darwich et al. (2010) pointed out some of the challenges or gaps in the knowledge that prevent the development of protection by current available vaccines, and some of these are the different virulence among virus strains, the ability of the virus to alter the innate immune response from the host and the delayed adaptive response from the hosts (delay in the production of neutralizing antibodies and cell-mediated immune responses).
Gilt isolation and acclimatization

Introducing replacement animals that have been previously exposed to PRRSV is essential to control the disease in sow herds, therefore quarantine facilities with frequent PRRSV testing are highly recommended. Exposure of replacement animals at 2 to 4 months of age provides enough time for them to develop immunity to the PRRSV that resides in the herd and no longer be viremic at the time of breeding (Zimmerman et al., 2012). There are several ways to expose the animals to the virus including vaccination, serum inoculation and providing biofeedback. Commercially available MLV vaccines can be used but their limitation is that they have limited cross-protection against field viruses, and the vaccine virus will be shed, therefore all animals should be exposed at the same time and managed AIAO (Zimmerman et al., 2012). Serum inoculation refers to exposure of animals to a herd-specific strain of PRRSV through the injection of serum obtained from viremic animals from the same herd. This method brings risks of spread of other pathogens and increased mortality (Corzo et al., 2010), but it has been shown effective in leading to production of negative weaned pigs (Fano et al., 2005). Biofeedback corresponds to the offer of contaminated material that may include contaminated tissues from weak-born piglets and stillbirths. Exposing gilts to viremic nursery pigs or cull sows is another option to get animals exposed to the virus, but these last two options are less consistent since the producer can’t be confident whether all animals were actually exposed.

Elimination of contaminated semen

Contaminated semen has long been identified as a risk factor for PRRSV transmission. Currently the vast majority of boar studs in North America maintain a negative PRRSV status and routinely tests their animals and semen to be shipped for PRRSV (Corzo et al., 2010).
The McRebel (Management Changes to Reduce Exposure to Bacteria to Eliminate Losses) concept was introduced by McCaw (2000). Measures aim to limit the spread of the virus among litters, and include: restriction of cross-fostering for the first 24 hours of life, euthanasia of poor-doing piglets, use of AIAO by room pig flow and change of needles between litters (McCaw, 2000). These practices have shown to improve pre-weaning and nursery mortality and growth performance levels even while PRRSV infection is still in the acute phase in the herd, and is an alternative when vaccination or depopulation are not feasible (McCaw, 2000), while being also commonly recommended and used as an important component during PRRS elimination projects and/or long-term control of infectious diseases within a swine site.

Biosecurity

Current protocols that are recommended for PRRS control include use of quarantine facilities with regular testing, sanitation and drying protocols for incoming trucks and supplies, personnel entry protocols such as shower-in facilities and Danish entry, implementation of AIAO pig flow, and insect control programs (Pitkin et al., 2009). The basis of AIAO is strict control over animal movement, so that mixing of older, slower-growing, poor-doing pigs with younger animals does not occur (Dee et al., 2003). Air filtration systems have been shown to be effective in reducing introduction of PRRSV (Dee et al., 2005; Alonso et al., 2013). Some of these measures will prevent PRRSV spread within the herds, but most importantly they will aid in preventing infection or re-infection coming from other herds.

Genetic improvement

The role of host genetics in PRRSV infection is unknown, but a study showed that animals can be divided into groups that have different susceptibility to PRRSV infection, and genome-wide analysis has shown that some chromosome regions might be associated with resistance (Rowland...
et al., 2012). This is a relatively recent area and if consistent results are found over the next few years with regard to genetic markers involved in virus tolerance, breeding programs might be able to incorporate that into their programs, improving immune response to the virus in the pig (Rowland et al., 2012).

1.6.2. Elimination

The control measures described above will help in preparing a population of pigs for eradication of PRRS, since a virus-free population is the goal for elimination of the virus (Zimmerman et al., 2012). A few commonly used measures to eliminate PRRSV from swine herds are the following:

Herd closure and rollover

This procedure refers to the confinement of herds uniformly exposed to PRRSV (by vaccination or homologous virus exposure) for 200 days or more without introduction of new animals. This procedure is based on the fact that PRRSV does not persist in an immune population (Torremorel et al., 2003). After the period of closure, negative animals can be introduced to the herd and seropositive animals will be eliminated over time through culling, until the entire herd has been replaced (rollover). Advantages of herd closure include the maintenance of genetic material and reduced labor. The main disadvantage is that the sow population becomes old and less productive without the introduction of replacement animals for such a long period of time (Dee, 2003). The use of off-site breeding herds can minimize the economic effects of herd closure (Zimmerman et al., 2012).

Test and removal

This procedure uses serological and virological testing of all breeding animals, to identify seropositive animals and remove these animals from the herd. This practice has been shown effective in the past (Dee et al., 2001) and can be recommended for herds with segregated
production and PRRS prevalence below 25% (Zimmerman et al., 2012), but its feasibility in vaccinated herds is complicated by the fact that vaccine and field strain antibodies cannot be differentiated at the present time (Dee, 2003). Disadvantages of this method include that it is labour-intensive, and expensive, and in addition there is the possibility of removing potentially immune animals that are seropositive but have cleared the virus or animals that are false positive (Dee et al., 2001; Corzo et al., 2010).

**Whole herd depopulation-repopulation**

Whole herd depopulation-repopulation is the removal of all animals in the herd, cleaning and disinfection of the premises and restocking with negative animals (Corzo et al., 2010). The advantage of this method is that the producer is able to eliminate more than one pathogen at the same time and potentially improve genetics and upgrade the facility, but disadvantages include the high cost and the fact that all current genetic material will be lost. This strategy is specifically appealing for farrow-to-finish herds where PRRSV is in the growing population and cannot be eliminated through other methods (Zimmerman et al., 2012).

**Partial depopulation**

This procedure is similar to the above method, but in this case only a certain population of the herd goes through the depopulation-repopulation process. It can be implemented when the breeding herd has eliminated PRRSV (absence of active viral shedding or recent exposure), but the virus can still be found in the growing pig population (Dee and Joo, 1997).

The methods described above can be used in combination, and overall have the objective of stabilizing and/or eliminating PRRS infection. Even though they have been proved feasible and successful, maintaining PRRSV negative status is an on-going challenge and requires region-level efforts. A study conducted using farrow-to-wean herds that went through either
depopulation-repopulation or a completely new start up in new facilities showed that approximately 40% of the herds became positive within one year from when they were established as free of the virus, and 85% of the sites became positive during the course of 5-9 years (Holtkamp et al., 2010).

1.6.3. Regional elimination (PRRS Area Regional Control and Elimination [ARC&E] Projects)

A broad assessment of PRRS dynamics at the regional level other than at the individual herd level has been the focus of current PRRS Area Regional Control and Elimination projects (ARC&E). In general, objectives of the programs include; to reduce prevalence of PRRSV in specific areas and therefore disease transmission risk, to monitor disease trends and to provide support on the design of adequate control strategies considering the type of farm and area/producer objectives. In order for these objectives to be accomplished, shared information and collective development of solutions are an essential part of the programs. Meetings usually occur regularly and involve participation of producers, industry, service providers and veterinarians. Even though PRRS ARC&E programs became very popular in the US and Canada over the last few years, reports on past or existing programs are very limited in the peer-reviewed literature. The few that are available support the idea that these programs can lead to the achievement of successful results. In the United States, the first PRRSV regional elimination program started in 2004 in west-central Minnesota (Corzo et al., 2010). The region had 87 voluntary participating farms and was successful in eliminating the virus from the herds in 2010. The authors emphasize, however, the fact that this specific county might have had great success due to the fact that most pigs from that region originated from sow herds located either in the county or outside but owned by the county’s producers, which limited pig movement (Corzo et al., 2010). In 2010, ARC&E projects started in Canada. In Quebec, four different regions were
participating in control programs in 2012, with participation rates ranging from 65 to 98% within regions and a total of more than 170 herds (Klopfenstein and Goulet, 2013). A considerable amount of investment came from producers for control strategies such as mass vaccination, better management of dead stock, change in transport routes and improvements in biosecurity. In the short term, the benefits of these projects have exceeded the expectations of the project organizers (Klopfenstein and Goulet, 2013). In Ontario, the first region to start an ARC&E project was the Niagara region, due to its relative low pig density, clear natural borders and strong industry and producer leadership. At the time of writing, there were seven regional projects, with high acceptance rates among swine producers and on-going discussions to become province-wide. Specifically, there has been great communication among producers with development of creative solutions regarding management of dead animals, transportation logistics, biosecurity, placement of disease positive pigs, etc. The program also includes the development of sampling protocols for sites with an unknown disease status, and development of low cost effective sampling methods for future surveillance based on clinical triggers and changes in events (manure spread, change of pig source, etc.), such as the use of ear vein blood swabs that can be conducted by producers themselves.

1.7. **New Epidemiological Tools for Better Understanding of PRRS**

1.7.1. *Spatial Analysis*

The use of spatial epidemiology in the study of infectious diseases is not new. It has been early recognized that transmission of infectious pathogens is more likely to occur when at-risk individuals (or, in a larger scale, swine sites) are spatially closer compared to individuals that are farther apart (Pfeiffer et al., 2008). Spatial analyses involve the use of multiple methods to
describe disease patterns and spread, and investigate disease clusters. Some of its uses include the prediction of disease risk based on location, and elucidation of cause-effect relationships (Pfeiffer et al., 2008). Particularly for the study of PRRS, spatial analysis is important because, as previously mentioned; area spread, often interpreted as airborne virus transmission, is commonly assumed in outbreaks or re-breaks. This epidemiological tool has been and should be further explored for PRRSV transmission investigations and regional descriptions or PRRSV occurrence and spread (Rosendal et al., 2014; Tousignant et al., 2015).

1.7.2. Network Analysis

The methodology for exploring and understanding networks has developed significantly in the last years due to the fact that social media has brought new challenges and opportunities to the area (Goldbeck, 2013). Within the human sociology context, network analyses can provide insightful information on the important players considering a certain group of people, on the connectedness of subgroups, and even on the best approach to control the spread of disease outbreaks (Goldbeck, 2013). Similarly, in the animal health area, these techniques can provide useful information on the connections between animals or farms, potential disease transmission routes, number of farms that we would expect to get infected in case of a disease introduction, and potential effectiveness of control measures (Danon et al., 2011). In the early 2000’s, contact tracing using animal movements was successfully used to investigate the spread of foot and mouth disease in Great Britain (Gibbens et al., 2001), and these techniques are becoming more popular with other diseases, and for the design of effective surveillance strategies (Frossling et al., 2012; Dorjee et al., 2013; Baudon et al., 2015).

1.7.3. Disease Modelling

Lastly, disease modelling is particularly attractive for planning of prevention and control
methods for numerous infectious diseases. According to Vynnycky and White (2010), “a model is just a simplified representation of a complex phenomenon”. The development of mathematical models aims to simplify complex situations and allow for investigation of specific scenarios, providing a range of outcomes that would otherwise be difficult to visualize. This approach can and usually is combined with other methodologies such as the ones described above. After the scenario of interest is built, simulations can be run varying important parameters and the outcomes of interest can be evaluated for relevant changes. Important applications in animal and public health have been the calculation of vaccine coverage necessary to reach herd immunity within a particular population, and the evaluation of disease control measures that would be unethical to carry out as field experiments. Mathematical models commonly carry important assumptions, and come in different types (e.g. deterministic, stochastic, agent-based, hybrid). The selection of best approaches should be based on the biological question, level of resolution needed, type of intervention to be tested or evaluated, besides others. The application of this and the previously mentioned epidemiological methods in animal health have been limited in the past by the lack of available and reliable data, and advances on the statistical techniques, but this picture is currently changing.

1.8. Thesis Overview and Research Objectives

This doctoral thesis is subdivided into seven chapters. This paragraph concludes the first chapter, which included a detailed description of the porcine reproductive and respiratory syndrome (PRRS), highlighting important areas of challenges in terms of the disease itself, as well as of our understanding of it. The last chapter presents a summary of the main results from this doctoral thesis and future directions for this area of research. Chapters two to six make use of one or more
of the novel methods briefly discussed on the last subheading of this literature review to address
the following objectives:

1) Describe swine sites from the PRRS area regional control and elimination projects of Ontario,
Canada, and spatially analyze how PRRSV positive and negative swine sites are distributed
across three different areas of the province (Chapter 2).

2) Investigate the occurrence of three distinct PRRSV genotypes using a combination of
molecular and spatial epidemiology and network analysis (Chapter 3).

3) Characterize important networks within the Ontario swine industry, including truck, feed,
boar, gilt and semen sources, and extract network-related parameters to investigate risk factors
for being positive for PRRS (Chapter 5).

4) Evaluate different control strategies for PRRS in a hypothetical breeding swine herd,
including vaccination with a modified-live attenuated vaccine and live-virus exposure, using a
within-farm hybrid stochastic model (Chapter 4).

5) Develop a framework of a regional disease agent-based model that aims for the elucidation of
a cost-effective surveillance methodology while accounting for important characteristics of the
current Ontario swine industry, which includes indirect connections between swine sites through
different networks, site demographics and biosecurity practices (Chapter 6).

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CHAPTER 2: Descriptive analysis and spatial epidemiology of porcine
reproductive and respiratory syndrome (PRRS) for swine sites participating
in area regional control and elimination programs from three regions of
Ontario, Canada

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2.1. Abstract

The objectives of this study were to describe demographics, basic biosecurity practices, ownership structure and prevalence of porcine reproductive and respiratory syndrome (PRRS) in swine sites located in three regions in Ontario, Canada, and investigate the presence of spatial clustering and clusters of PRRS positive sites in the three regions. A total of 370 swine sites were enrolled in PRRS Area Regional Control and Elimination (ARC&E) projects in Niagara, Watford and Perth from 2010 to 2013. Demographics, biosecurity and site ownership data were collected using a standardized questionnaire and site location was obtained from an industry organization. Porcine reproductive and respiratory syndrome virus (PRRSV) status was assigned on the basis of available diagnostic tests and/or assessment by site veterinarians. Spatial dependence was investigated using the D-function, the spatial scan statistic and the spatial relative risk method. Results showed that the use of strict all-in all-out (AIAO) pig flow and shower before entry (shower-in) are uncommon biosecurity practices in swine sites even though they are recommended for control of several infectious diseases. The prevalence of PRRS in the three regions ranged from 17% to 48% and localized high and low risk clusters were detected. Data from the PRRS ARC&E projects were characterized by membership in multiple and overlapping ownership structures and networks, which complicates the way the results of monitoring and disease management measures are communicated to the target population.

2.2. Introduction

Porcine reproductive and respiratory syndrome (PRRS) was first recognized in the late 1980s in North America and Europe (Hill, 1990). In 1991 and 1992, the causative agent was identified as small single-stranded enveloped RNA viruses in Europe (Wensvoort et al., 1991) and North
America (Collins et al., 1992), and it was discovered that these so-called PRRS viruses (PRRSV) had properties similar to those of the family Arteriviridae, genus Arterivirus. A few characteristics of the virus are essential for its ability to maintain itself in pig populations, which is a constant challenge for disease control. The first one is persistence of the virus in lymphoid tissues of individual pigs for long periods of time (Wills et al., 2003). The virus is also able to mutate and recombine (Murtaugh et al., 2010), and is stable in cold and moist conditions (Dee et al., 2002). Additional challenges in PRRS control include the dynamics of modern swine production, which is characterized by a high degree of connectedness between sectors of the industry and the concentration of swine herds in production systems, further connected through various specialized service providers. Routine pig movements among herds within production systems not only facilitate disease spread but also regularly introduce pools of susceptible animals (nursery pigs, gilts, boars) into the herds (Dorjee et al., 2013). In addition, it is not uncommon for sites to be located close to feed resources, creating areas of high pig density that may facilitate disease transmission. Area spread is a common term that corresponds to site-to-site transmission for which precise modes of transmission are unknown, but it is hypothesized that factors such as aerosol transmission, spread via avian or insect vectors, and contaminated fomites are involved (Larochelle et al., 2003). Some strains of the virus are able to remain viable after travelling in the air for up to nine km (Otake et al., 2010).

Disease control programs focused on individual herds and production systems have limited effectiveness in long-term control of PRRS infection (Mondaca-Fernandez et al., 2005). Consideration of PRRS dynamics at the regional level has been the focus of current PRRS Area Regional Control and Elimination (ARC&E) projects. Even though PRRS ARC&E programs have recently become popular in North America, reports on past or current programs are very
limited in the peer-reviewed literature (Corzo et al., 2010), and thorough analysis of data from such programs is almost entirely lacking. Descriptions of such programs (including their implementation, infrastructure and infection control practices) would be valuable since they represent rare examples of approaches initiated by producers and the industry rather than imposed by the government towards control of infectious disease in animal populations with complex demographics (Mondaca et al., 2014). Furthermore, the investigation on the role of spatial proximity to other sites on PRRS status for this particular region might aid in future PRRS outbreak investigations. The objectives of the present study were first to describe demographics, biosecurity practices, ownership structure and PRRS prevalence in swine sites participating in PRRS ARC&E projects located in the regions of Niagara, Watford and Perth in Ontario (Canada) and secondly to investigate the presence of spatial clustering and clusters of PRRS-positive sites located in those regions.

2.3. Materials and methods

2.3.1. Selection of sites, questionnaire administration and data management

The source population for this cross-sectional study was the PRRS ARC&E database for the regions of Niagara, Watford and Perth. Regions were not necessarily defined according to county or city boundaries, but according to the extent of producer’s interest and geographical plausibility (kept to a reasonable distance). The only county-level project was Perth, the other two were considered regional-level projects. All swine sites enrolled in the control projects up to August of 2013 for Niagara sites, October of 2013 for Watford, and January of 2014 for Perth were included in the current study. Sites participating in such ARC&E projects were enrolled on a voluntary basis, and to be eligible the sites had to be located within the determined area and the
producer had to agree to share site location, attributes and PRRS status among participants in the same PRRS ARC&E. The producer also had to answer a 20-minute questionnaire over the phone.

The questionnaire applied for data collection contained up to 40 questions regarding basic site demographics (e.g., number of animals and type of system), ownership structure (e.g., contact information for the premises owner, pig owner and contract producer), sources (e.g., semen, boars, gilts and feed), networks (e.g., truck and feed companies), biosecurity (e.g., use of all-in all-out, shower-in and Danish entry), presumed PRRS status and producer’s opinion on PRRS control. Site location was obtained from an industry partner (Ontario Pork, Guelph, ON), and was based on the center point of the premises or parcel. A production system was defined as two or more sites linked by a common owner or management structure. It was possible for a single-site farrow-to-finish operation to be part of a production system if the owner had more than one facility.

2.3.2. Diagnostic testing

After enrollment, sites were assigned an unknown PRRS status until results of diagnostic testing became available. The sample size recommended for PRRS status definition was nine for oral fluids (composite sample) and eleven for individual animals, based on a sample size calculation to declare freedom of infection (detection of at least one positive sample). For oral fluids, the assumptions for sample size calculations were that an individual oral fluid test had low sensitivity (56%) and perfect specificity (Christopher-Hennings et al. (2002), expected within-herd prevalence of 10%, and that five pigs would contribute to one rope sample, with the confidence level set at 90%. For individual animal sample size, perfect test sensitivity and specificity were assumed (Christopher-Hennings et al. (2002), with expected within-herd
prevalence of 20%, and 90% confidence. Even though a high within-herd prevalence is expected for PRRS (Albina, 1997), prevalence levels were set to relatively low levels for sample size calculations to increase the ability to detect the virus in low prevalence situations. Serological testing was performed with the aim of assessing evidence of exposure to PRRSV. Additional sampling and/or diagnostic testing using PCR was performed if the site veterinarian had reason to believe, on the basis of epidemiological or clinical assessment that PRRSV was circulating on the site. All samples were submitted for testing to the Animal Health Laboratory at the University of Guelph. The immunofluorescent antibody assay (IFA) was used to rule out potential false positives on ELISA results.

PRRSV site status based on actual diagnostic testing was considered to be “confirmed” on a case by case basis, declared by the veterinary coordinator according to the use of recommended sample size being achieved and a combination of tests. A site was considered positive if at least one animal tested positive by ELISA (previous PRRSV exposure) or PCR (current PRRSV infection). As such, sites that were vaccinating animals (and therefore had positive ELISA results) were also considered positive. For cases where diagnostic test was not available, status was defined by the site veterinarian’s knowledge on pig flow (e.g., confirmed positive feeder pigs being moved to a downstream finisher site), and such sites were considered “presumed” (Mortensen et al., 2002). For the purposes of this study, confirmed and presumed status were combined to define the binary outcome of interest for spatial analysis (PRRSV positive or negative site).

2.3.3. Statistical Analysis

Descriptive Analysis

All captured data including geographical location, enrollment information, test submissions and
test results, were entered in an online-based software (Fluid Surveys™, Ottawa, ON) by project coordinators, investigators, and supervised technicians and were linked to a central database. In the later phase of the project, a Microsoft SQL 2008 database was constructed in collaboration with industry groups to maintain the data in a standardized form.

All descriptive analyses were conducted at the site level, stratified by region, using SAS version 9.3 (SAS Inst. Inc., Cary, NC), ArcMap version 10.1 (ESRI, Environmental Systems Resource Institute, 2012, Redlands, CA), R version 3.0.2 (R Development Core Team, 2013) and Stata-IC version 10 (StataCorp., 2007, College Station, TX). In SAS, initial descriptive statistics were determined and plots were generated for exploratory data analysis. ArcMap was used to visualize point data, create digital maps, and transform geographic coordinates from longitude/latitude to Cartesian coordinates. The contributed R package “spatstat v.1.33-0” (Baddeley and Turner, 2005) was used to create risk maps for each of the regions. Two raster surfaces were created: the first representing the intensity of PRRS-positive sites and the second representing the intensity of all swine sites. The ratio of the case population over the population at risk was then calculated for each grid in the map and smoothed using Gaussian kernel smoothing. Finally, Stata-IC was used to describe PRRS prevalence overtime for the region of Niagara.

**Spatial Analysis**

Spatial analysis was conducted through clustering and cluster analysis, the first being a global measure of spatial dependence and the second being a localized investigation of aggregation of positive sites.

Clustering analyses was conducted using R version 3.0.2 (R Development Core Team, 2013). Sites that clearly did not belong to the study are were excluded from the spatial analysis. The study area for the spatial analysis was based on the convex hull of the remaining sites in each
region. For clustering analysis, first the K-function was calculated using the “splan v. 2.01-34” package (Rowlingson and Diggle, 2013). It is a measure of the number of events of the same type occurring within a certain distance. As this information is not very informative on its own, the K-function calculated for positive sites was compared with the one calculated for negative sites, and the difference between the two, known as the D-function, is a measure of the extra aggregation of positive sites over and above that observed for the negative sites (Pfeiffer et al., 2008; Diggle and Chetwynd, 1991). Monte Carlo randomization was used to randomly permute locations of positive and negative sites, and values of the difference between the two were computed for each permutation. The 95% simulation envelope of these permutations, as well as 95% confidence limits calculated using a normal approximation, were plotted together with the observed D-functions (Pfeiffer et al., 2008) for each of the regions analyzed. Significant evidence of clustering was declared when the observed D-function deviated from the envelope formed by the upper and lower bounds calculated from the simulations (p < 0.05). The D-function was estimated over a distance of 20, 10, and 25 km for Niagara, Watford, and Perth, respectively. The presence of localized clusters in the three regions was also investigated. The SaTScan™ program uses the spatial scan statistic method (Kulldorff and Nagarwalla, 1995), and in this case the test was based on a purely spatial Bernoulli model and a circular shaped window that scans the region with gradually increasing sizes to include an increasing population of sites up to a limit of 50%. The risk of disease within each circle was compared to the risk outside using a maximum likelihood test and the window(s) with the maximum likelihood ratio function indicated the location of the most likely cluster(s), with significance being declared when p < 0.05. Both high and low risk clusters were investigated.

A different method for cluster detection was also applied for each area using the packages
“splanes v. 2.01-34”, “spatstat v.1.33-0” and “sparr v.0.3-4” on R (Davies et al., 2011). In this case, a spatial relative risk surface was constructed to represent the ratio of positive and negative densities estimated using quartic kernels with fixed or adaptive bandwidth, depending on which fitted better to the particular region. The areas were divided into grids and the observed surface relative risk was calculated for each grid. Case locations were randomly assigned to the grids, and a surface relative risk was simulated on the basis of 999 iterations. Finally, the function compared the observed with the expected spatial relative risk (simulations) for each grid and joined the ones with similar probabilities. Statistically significant high risk clusters were declared when \( p < 0.05 \).

2.4. Results

2.4.1. Descriptive Analysis

Demographics of regions and sites

A description of region and site characteristics is provided in Table 2.1. A total of 370 sites were enrolled in the three PRRS ARC&E projects in Southern Ontario during the years 2010 to 2013. For Niagara and Watford regions, participation rate was high (95% of all swine sites in the regions were enrolled for both projects). For the Perth region, participation rate was estimated at approximately 50%. Perth was the region with the largest study area (2,589 km\(^2\)), followed by Niagara (1,666 km\(^2\)) and Watford (510 km\(^2\)). Figure 2.1 shows a graphical representation of the boundaries for each region. Assuming 50% of swine sites in the Perth region participate in the control program and represent a random selection of all sites in the region, in terms of number of animals, the pig density is estimated to be twice as high as the one calculated with the current enrolled sites, or approximately 207 pigs/km\(^2\). Therefore the region of Perth had the highest pig
density (207 pigs/km$^2$), followed by Watford (153 pigs/km$^2$) and Niagara (61 pigs/km$^2$). The distribution of production types for the three regions is shown in Table 2.2.

**Biosecurity practices and herd immunity management**

Biosecurity practices available for these analyses were based on data from the Watford and Perth regions only and are shown in Table 2.3. For both regions, less than 50% of the swine sites reported having a shower-in facility at the entrance of the site. The sites that did not have a shower-in facility were asked whether there was a Danish entry, and most of those reported having at least this physical barrier at the entrance of their facilities. The majority of Watford and Perth swine sites had continuous-flow management (78% and 66%, respectively). The “continuous” category includes both sites that had continuous flow and AIAO by room, while the “AIAO” category includes sites reporting having AIAO by site and/or by building (Table 2.3). The question concerning type of pig flow was not asked for farrow-to-wean operations which were considered to be strictly continuous due to the inherently open nature of sites containing sows.

Herd immunity management differed between regions. In the Watford area less than 9% of the sites were exposing animals to PRRSV (in the last 6 months of questionnaire administration): six sites were vaccinating using either the Ingelvac ATP vaccine (Boehringer Ingelheim Vetmedica, Burlington, ON, n = 4) or the Ingelvac MLV vaccine (Boehringer Ingelheim Vetmedica, Burlington, ON, n = 2). In the Perth area, less than 20% of the sites were exposing their animals, either by using the FOSTERA™ vaccine (Zoetis Animal Health, Kirkland, QC, n = 1), the Ingelvac ATP vaccine (Boehringer Ingelheim Vetmedica, n = 3), the Ingelvac MLV vaccine (Boehringer Ingelheim Vetmedica, n = 28), or doing live virus inoculation (n = 11).

**Ownership structure**
The mean number of sites owned per premises owner was similar for the three regions (1.2 site/premises owner for Niagara, 1.3 site/premises owner for Watford and 1.4 site/premises owner for Perth). However, the mean number of sites per pig owner was somewhat higher for Perth (2.7 sites/pig owner) than for Watford (2.2 sites/pig owner). The premises owner was not the pig owner for 43.06% of the premises in Watford and 46.67% in Perth. The information regarding pig owner was not available for Niagara at the time of analysis. A relatively small number of veterinarians (18) were responsible for the farms located in the three regions, and 56% of the veterinarians were responsible for sites in more than one region. For the region of Niagara, there were approximately eight sites per veterinarian (range: 1-38, inter-quartile range [IQR]: 6.0), for Watford seven sites per veterinarian (range: 1-26, IQR: 6.5) and for Perth, 16 sites per veterinarian (range: 2-69, IQR: 10.0).

**Diagnostic testing**

A total of 335 laboratory submissions were available during the period examined herein for the three regions: 183 submissions for Perth, 71 for Niagara and 81 for Watford. Of those submissions, 194 submissions were based on serology only (either serum or oral fluids ELISA), 58 were based on virology (serum, oral fluids or tissue PCR) and 77 were based on both types of diagnostic tests. For six submissions, neither serology nor virology was performed (three submissions were for sequencing only and three had missing information). The 335 submissions were from 252 premises: 206 premises with only one submission, 25 premises with two submissions, 12 premises with three submissions and 9 premises with four or more submissions. Out of the 271 submissions tested using ELISA, all of them were tested individually (i.e. none were pooled for testing), and out of the 135 samples tested using PCR, 78% were pooled. For a total of 209 submissions, serum samples were submitted; for 108 submissions, oral fluids were
submitted; for 11 submissions, both serum and oral fluid samples were submitted; for one submission, fresh tissue was submitted; and for 2 submissions both serum and tissue samples were submitted. For four submissions, information regarding type of sample was missing (data entry error).

Porcine Reproductive and Respiratory Syndrome Regional Prevalence

The mean within-site prevalence of PRRS calculated using the serum ELISA data (n = 37 positive sites) was 71.41% (median = 90.91% and SD = 0.35). The mean overall prevalence of PRRS was lowest for the Niagara region, followed by Perth, and finally Watford (Table 2.4). For the region of Niagara, prospective data allowed for a temporal description of PRRS prevalence during the previous three years. Overall prevalence in the region of Niagara decreased over time (Figure 2.2). In February of 2012, PRRS was diagnosed on some sites in a few production systems, and control and elimination strategies such as site closure (herd closure) with or without homogenization (exposure of all animals in the herd to PRRSV) were put in place then. PRRS prevalence decreased to approximately 16% by August of 2013.

2.4.2. Spatial Analysis

Complete flows regarding exclusion of sites from spatial analysis for the three regions are shown in Figure 2.3. The D-functions estimated from the data did not drift outside the 95% confidence bands constructed from the Monte Carlo simulations; therefore, spatial clustering of PRRSV in swine sites could not be detected for the region of Niagara in August 2013 (p = 0.25), for the region of Watford in October 2013 (p = 0.91), and for the region of Perth in January 2014 (p = 0.11).

Cluster detection with the scan statistic method found one cluster where risk of disease was higher and one cluster where risk of disease was lower for both regions of Watford and Perth.
For the region of Watford, the high risk cluster had an observed number of 18 cases (expected
10.6 cases, p = 0.013), and the low risk cluster had 0 observed cases (expected 11.0 cases, p =
0.016). For the region of Perth, the high risk cluster had 58 cases (expected 37.8 cases, p < 0.01)
and the low risk cluster had 2 cases (expected 13.8 cases, p < 0.01). No clusters were detected in
the Niagara region. High risk clusters for the regions of Watford and Perth were mapped using R
and are shown with the risk maps in Figure 2.4. High risk areas were located on the south west of
the region of Watford, and the central-southern area of Perth, along with one smaller area located
on the north-eastern portion of the polygon (Figure 2.4). For the Niagara region, a high risk area
was located in the eastern part and southwest. The spatial relative risk method was applied in
order to detect high risk clusters. This method allowed for detection of clusters for all regions,
including for the Niagara region using adaptive bandwidth (p < 0.05). For the regions of Watford
and Perth, clusters in the same area were located using the two different methods.

2.5. Discussion
To the knowledge of the authors, this is the first study to describe swine sites participating in
PRRS ARC&E projects in Ontario and to explore spatial distributions of positive and negative
sites from three different projects. It is important to note not only the large sample size used,
representing approximately 15% of all Ontario swine sites (Statistics Canada, 2012), but also the
inclusion of three regions that contain different pig densities. The average number of pigs per site
for the three regions was very similar to Ontario’s average, 1,238 (Statistics Canada, 2012).

2.5.1. Biosecurity practices as contributors to regional disease control strategies
Even though biosecurity practices such as use of shower-in and AIAO are highly recommended
for infectious disease prevention and control (Pitkin et al., 2009), results of this study show that
implementation of these practices is not high for the analyzed regions. The percentage of sites with growing pigs managed by AIAO pig flow could have considerable implications for the design of disease control strategies, since for these sites PRRS status could rapidly change with strict age separation and thorough cleaning between batches. In contrast, elimination of infection from continuous flow sites is expected to be more challenging. The relatively high percentage of continuous flow sites, as defined in this study, is a reflection of the demographics of the Ontario swine industry, and it would be unrealistic to attempt to change it over a short time frame. It would therefore be worthwhile to develop and evaluate procedures for PRRSV elimination from continuous flow sites without complete site depopulation.

Dead stock removal may present an important risk factor for PRRSV infection. Regular visits by incoming trucks can be an important source of virus coming from other sites, as trucks may mechanically transport the virus (Dee et al., 2002). It is currently recommended that rendering trucks should not be allowed access to farms; composting or incinerating carcasses on farm would be a more appropriate control measure (Pitkin et al., 2009). The most appropriate procedure to manage deadstock, however, also needs to be considered on a herd-by-herd basis. The high percentage of sites using external trucking companies for outgoing pigs is another area where regional-level collaboration is needed, given the importance of transportation practices for risk of transmission between sites (Dee et al., 2004).

2.5.2. Ownership structure

For the regions of Watford and Perth, approximately 43% and 47% of the sites had a premises owner that was different from the pig owner, representing a challenge when determining who should be notified if needed. This becomes particularly important when the concept of surveillance comes into place: according to the Center for Disease Control and Prevention,
surveillance corresponds to “the ongoing systematic collection, analysis and interpretation of health-related data essential for the planning, implementation and evaluation of (public) health practice, (...) closely integrated with the timely dissemination of these data to those who need to know” (Lee et al., 2010).

Our findings also demonstrate the complex nature of the structure of modern swine systems in Canada, raising questions about effective methods of communicating major changes in the disease control regions to all involved stakeholders. The solution to this issue is not simple and requires effort from both producers and premises owners as well as from informers; an effective way of communicating is still under development.

2.5.3. Sampling and diagnostic strategies

The PRRS ARC&E projects in Ontario are producer-driven and focused on conducting disease surveillance, control and elimination when warranted; with minimum cost and maximum practicality for producers and veterinarians involved. When data are being utilized for research purposes, the inconsistency of sample collection for laboratory analysis among sites and regions might raise some concerns. Recognition that there is heterogeneity among sites supports the idea of an output-surveillance approach, where rather than prescribing what surveillance activities must be done, standards prescribe what must be achieved (Cameron, 2012). This provides more flexibility with regard to the use of different tests or test combinations, different sample sizes and different sampling strategies that will be defined according to factors such as historical information and multiple sources of surveillance, always with the aim of increasing efficiency (Cameron, 2012). This has been the approach of the on-going surveillance project from which data for this study were generated, with individual samples based on serum and rope samples based on oral fluids both allowed (and yielding similar theoretical confidence). This provides
accurate classification and is at the same time practical and more economically effective.

2.5.4. PRRS Prevalence

The overall PRRS prevalence found in this study was 37.1%, below that previously reported for Canada (Magar and Larochelle, 2004, Lambert et al., 2012), the United States (APHIS, 2009), Mexico (Batista et al., 2010), and Spain (Lopez-Soria et al., 2010). It is important to note that the region of Niagara contributed low site level prevalence since it has had control and elimination strategies previously implemented. With regard to PRRS within-site prevalence, assessments from all regions over time support a mean within-site prevalence above 70%. This is in agreement with previous reports that 80% to 95% of the pigs in a site experience seroconversion within 2 to 3 months of exposure (Albina, 1997).

It is important to note that for the region of Perth, the volunteer cohort used in the current study only represented approximately 50% of the swine sites in the region, therefore PRRS prevalence estimate for this region is less accurate compared to the prevalence on the other two regions.

2.5.5. Spatial clustering and clusters

Existence of spatial clustering should be reflective of area spread under certain conditions. Larochelle et al. (2003) report area transmission being suspected when PRRSV strains originated from sites belonging to different ownerships located within 3 km from one another. In our study, despite existence of spatial clusters, clustering analysis was not able to identify that distance to positive sites plays a significant role in the PRRS status of neighboring sites. These findings agree with what has been previously reported for Ontario by Rosendal et al. (2014), but disagree with a study conducted in Quebec that reported that sites located ≤ 2.5km from the closest pig site were more likely to be positive (Lambert et al., 2012), and could be a reflection of differences in the source population or unreported efforts already taken in disease control. There
was surprising complexity of the data collected from the producers, which included ownership
structure (premises owners, pig owners, contract producers) and related networks from service
providers (veterinarians, feed companies, semen and gilt sources, truck companies, etc.).
Observed data complexity and the absence of spatial dependence in the study findings suggest
that other factors may be involved in PRRS area transmission. Further analysis on the relative
contribution of network structure and location on PRRS spread are needed for better
understanding of PRRSV dynamics and development of efficient control and elimination
strategies at a regional level.

2.6. Conclusions
The current study described the demographics, biosecurity practices and ownership structure for
swine sites located in three regions participating in PRRS ARC&E projects in Ontario. These
programs have been well received by producers, with high participation rates, and are currently
expanding to be province-wide. For the Niagara region, coordinated control efforts resulted in a
25% decrease in PRRS prevalence over the last three years.
Patterns of disease spread were also investigated and results showed no evidence for spatial
dependence in the data. However, other visual representations, such as risk maps and cluster
analysis, suggest the presence of high risk areas within regions, and those might be an important
focus in situations where resource usage must be optimized. They could also be useful as
hypothesis generators and in further investigations. Future challenges include sustainability and
update of information on a regular basis, and the design of strategies to facilitate decision-
making processes when it comes to infectious disease prevention and control at the farm and
region levels.
2.7. Acknowledgements

Financial support for the PRRS ARC&E projects and for this particular study was provided by the Ontario Ministry of Agriculture, Food and Rural Affairs (OMAFRA), the Ontario Swine Health Advisory Board (OSHAB), the Canadian Swine Health Board (CSHB), Ontario Pork, the Agricultural Adaptation Council (AAC), the Animal Health Laboratory (AHL) of the University of Guelph, and the Natural Sciences and Engineering Research Council (NSERC). We would like to thank the participating premises owners, producers, area leaders and veterinarians who assisted with sampling and data entry.

2.8. References


Collins JE, Benfield DA, Christianson WT, Harris L, Hennings JC, Shaw DP, Goyal SM, McCullough S, Morrison RB, Joo HS. Isolation of swine infertility and respiratory syndrome
virus (isolate ATCC VR-2332) in North America and experimental reproduction of the disease in
Christopher-Hennings J, Faaberg KS, Murtaugh MP, Nelson EA, Roof MB, Vaughn EM, Yoon


Table 2.1. Region and site descriptors. The number in brackets correspond to number of sites with missing information.

<table>
<thead>
<tr>
<th>Descriptors</th>
<th>Niagara</th>
<th>Watford</th>
<th>Perth</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of sites enrolled as part of the region(^a)</td>
<td>75</td>
<td>72</td>
<td>223</td>
</tr>
<tr>
<td>Mean number of buildings per site(^b)</td>
<td>- Inventories for nurseries, wean-finish and finishers from the Watford and Perth regions only</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of veterinarians</td>
<td>9 (1)</td>
<td>11</td>
<td>14</td>
</tr>
<tr>
<td>Number of production systems</td>
<td>12</td>
<td>22</td>
<td>49</td>
</tr>
<tr>
<td>Number of premises owners</td>
<td>63 (1)</td>
<td>54 (1)</td>
<td>163</td>
</tr>
<tr>
<td>Number of sites part of a production system</td>
<td>56</td>
<td>56</td>
<td>191</td>
</tr>
<tr>
<td>Number of pig owners</td>
<td>- Inventories for nurseries, wean-finish and finishers from the Watford and Perth regions only</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of sites included in the polygon(^c)</td>
<td>73</td>
<td>66</td>
<td>218</td>
</tr>
<tr>
<td>Polygon area (km(^2))</td>
<td>1,666.30</td>
<td>509.83</td>
<td>2,589.10</td>
</tr>
<tr>
<td>Total number of animals in the polygon</td>
<td>100,050 (1)</td>
<td>77,800 (1)</td>
<td>267,725 (2)</td>
</tr>
<tr>
<td>Number of animals per site in the polygon- Mean</td>
<td>1,370.55</td>
<td>1,178.79</td>
<td>1,222.49</td>
</tr>
<tr>
<td>Minimum</td>
<td>75</td>
<td>154</td>
<td>52</td>
</tr>
<tr>
<td>Maximum</td>
<td>4,500</td>
<td>5,750</td>
<td>6,000</td>
</tr>
<tr>
<td>Number of nursery/ finishing animals in the polygon</td>
<td>87,165 (1)</td>
<td>66,240 (9)</td>
<td>237,995 (2)</td>
</tr>
<tr>
<td>Number of sows/boars in the polygon</td>
<td>12,885</td>
<td>11,560 (2)</td>
<td>29,730</td>
</tr>
<tr>
<td>Pig density (pigs/ km(^2))</td>
<td>60.04</td>
<td>152.60</td>
<td>103.41(^e)</td>
</tr>
<tr>
<td>Site density (sites/km(^2))</td>
<td>0.04</td>
<td>0.13</td>
<td>0.08(^e)</td>
</tr>
<tr>
<td>Number of sites included in the spatial analysis(^f)</td>
<td>65</td>
<td>56</td>
<td>197</td>
</tr>
<tr>
<td>Distance to closest neighbour- All sites (mean, km)</td>
<td>2.32</td>
<td>1.66</td>
<td>1.58</td>
</tr>
<tr>
<td>Positive sites (mean, km)</td>
<td>2.38</td>
<td>1.63</td>
<td>1.63</td>
</tr>
<tr>
<td>Negative sites (mean, km)</td>
<td>2.31</td>
<td>1.70</td>
<td>1.55</td>
</tr>
<tr>
<td>All sites (max, km)</td>
<td>8.20</td>
<td>8.15</td>
<td>13.52</td>
</tr>
<tr>
<td>All sites (min, km)</td>
<td>0.22</td>
<td>0.18</td>
<td>0.15</td>
</tr>
</tbody>
</table>

\(^a\)Includes all sites participating in the control program for the regions (those sites were included in all descriptive analysis regarding demographics and biosecurity practices)

\(^b\)Information collected for nurseries, wean-finish and finishers from the Watford and Perth regions only

\(^c\)Includes all sites that fit inside the created polygon (status positive, negative and unknown)

\(^d\)Area corresponds to the area of the polygon that was created for spatial analysis purposes and does not represent geographic or political boundaries

\(^e\)As we estimate that about 50% of the sites in the region are enrolled on the project, the reader should be aware that pig and site density are underestimated on this table

\(^f\)Includes only sites with known status and within the areas of the polygon
Table 2.2. Description of production types of swine sites participating in the PRRS ARC&E control programs by region, in % (number of sites).

<table>
<thead>
<tr>
<th>Production Type</th>
<th>Niagara</th>
<th>Watford</th>
<th>Perth</th>
</tr>
</thead>
<tbody>
<tr>
<td>Farrow-wean</td>
<td>5.33 (4)</td>
<td>6.94 (5)</td>
<td>16.14 (36)</td>
</tr>
<tr>
<td>Farrow-finish</td>
<td>10.68 (8)</td>
<td>12.50 (9)</td>
<td>7.62 (17)</td>
</tr>
<tr>
<td>Farrow-feeder</td>
<td>9.33 (7)</td>
<td>6.94 (5)</td>
<td>4.93 (11)</td>
</tr>
<tr>
<td>Nursery</td>
<td>9.33 (7)</td>
<td>4.17 (3)</td>
<td>15.25 (34)</td>
</tr>
<tr>
<td>Wean-finish</td>
<td>5.33 (4)</td>
<td>9.72 (7)</td>
<td>6.73 (15)</td>
</tr>
<tr>
<td>Finish</td>
<td>60.00 (45)</td>
<td>56.94 (41)</td>
<td>46.64 (104)</td>
</tr>
<tr>
<td>Isolation/ Acclimatization</td>
<td>0.00 (0)</td>
<td>2.79 (2)</td>
<td>2.24 (5)</td>
</tr>
<tr>
<td>Dry sows</td>
<td>0.00 (0)</td>
<td>0.00 (0)</td>
<td>0.45 (1)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>100.00 (75)</td>
<td>100.00 (72)</td>
<td>100.00 (223)</td>
</tr>
</tbody>
</table>
Table 2.3. Description of biosecurity practices for sites located in the Watford and Perth regions. Numbers are expressed as % (number of sites that had the practice in place/ total at risk).

<table>
<thead>
<tr>
<th>Biosecurity practice</th>
<th>Region</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Watford</td>
</tr>
<tr>
<td>CSHB (a)</td>
<td>78.26 (54/69)</td>
</tr>
<tr>
<td>Air filter</td>
<td>0.00 (0/69)</td>
</tr>
<tr>
<td>Shower in</td>
<td>47.69 (31/65)</td>
</tr>
<tr>
<td></td>
<td>(Breeding sites</td>
</tr>
<tr>
<td></td>
<td>Growing pig sites</td>
</tr>
<tr>
<td>Danish entry (b)</td>
<td>80.65 (25/[62-31])</td>
</tr>
<tr>
<td>Use of an external truck- all (c)</td>
<td>75.00 (54/72)</td>
</tr>
<tr>
<td></td>
<td>(Incoming pigs</td>
</tr>
<tr>
<td></td>
<td>Outgoing pigs</td>
</tr>
<tr>
<td>Dead stock disposal (c)</td>
<td>26.39 (19/72)</td>
</tr>
<tr>
<td></td>
<td>(Compost/ burial/ incineration within CAZ (d)</td>
</tr>
<tr>
<td></td>
<td>Third party pick up within CAZ</td>
</tr>
<tr>
<td></td>
<td>Third part pick up outside CAZ</td>
</tr>
<tr>
<td></td>
<td>Deliver to rendering</td>
</tr>
<tr>
<td>Pig Flow- Summary</td>
<td></td>
</tr>
<tr>
<td>Continuous (e)</td>
<td>77.94 (53/68)</td>
</tr>
<tr>
<td>AIAO (f)</td>
<td>22.06 (15/68)</td>
</tr>
<tr>
<td>Pig Flow- Detailed</td>
<td></td>
</tr>
<tr>
<td>Continuous</td>
<td>30.90 (21/68)</td>
</tr>
<tr>
<td>Continuous and AIAO by room (g)</td>
<td>4.41 (3/68)</td>
</tr>
<tr>
<td>AIAO by room</td>
<td>35.29 (24/68)</td>
</tr>
<tr>
<td>AIAO by room and AIAO by building (g)</td>
<td>0.00 (0/68)</td>
</tr>
<tr>
<td>AIAO by building</td>
<td>13.23 (9/68)</td>
</tr>
<tr>
<td>AIAO by site</td>
<td>8.82 (6/68)</td>
</tr>
<tr>
<td>F-W (h)/dry sows operations (not asked)</td>
<td>7.35 (5/68)</td>
</tr>
<tr>
<td>Missing information</td>
<td>4</td>
</tr>
</tbody>
</table>

(a) Sites that completed the Canadian Swine Health Board National Biosecurity Training Program
(b) Only sites that did not have a shower facility were at risk for this outcome
(c) More than one of these answers might have been chosen by one site
(d) CAZ correspond to Controlled Access Zone
(e) Combines continuous flow (including F-W and dry sow operations), AIAO by room and any site that any of those combined or with other flows
(f) AIAO corresponds to all-in all-out practice; this category includes AIAO by site or by building. Missing information was excluded from the denominator
(g) Sites had a combination of those two pig flows
(h) Farrow-to-wean operations
Table 2.4. Mean prevalence of PRRS for the three different control regions and stratification on presumed versus confirmed status.

<table>
<thead>
<tr>
<th>Region</th>
<th>Prevalence % (n(^a)/N(^b))</th>
<th>95% CI (%)</th>
<th>Exact 95%CI (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Niagara</td>
<td>Watford</td>
<td>Perth</td>
</tr>
<tr>
<td></td>
<td>16.92 (11/65)</td>
<td>48.21 (27/56)</td>
<td>40.61 (80/197)</td>
</tr>
<tr>
<td></td>
<td>7.81, 26.04</td>
<td>35.13, 61.30</td>
<td>33.75, 47.47</td>
</tr>
<tr>
<td></td>
<td>8.76, 28.27</td>
<td>34.66, 61.97</td>
<td>33.69, 47.82</td>
</tr>
</tbody>
</table>

Presumed status (n)

<table>
<thead>
<tr>
<th></th>
<th>Niagara</th>
<th>Watford</th>
<th>Perth</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>0</td>
<td>9</td>
<td>30</td>
</tr>
<tr>
<td>Negative</td>
<td>0</td>
<td>2</td>
<td>21</td>
</tr>
<tr>
<td>Total</td>
<td>0</td>
<td>11</td>
<td>51</td>
</tr>
</tbody>
</table>

Confirmed status (n)

<table>
<thead>
<tr>
<th></th>
<th>Niagara</th>
<th>Watford</th>
<th>Perth</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>11</td>
<td>18</td>
<td>50</td>
</tr>
<tr>
<td>Negative</td>
<td>54</td>
<td>27</td>
<td>96</td>
</tr>
<tr>
<td>Total</td>
<td>65</td>
<td>45</td>
<td>146</td>
</tr>
</tbody>
</table>

\(^a\)Number of sites with the outcome  
\(^b\)Total number of sites with known status in the region
Figure 2.1. Map of southern Ontario showing the 3 regions participating in porcine reproductive and respiratory syndrome (PRRS) Area Regional Control and Elimination (ARC&E) projects that are described in this study.
Figure 2.2. Temporal trend of porcine reproductive and respiratory syndrome (PRRS) for the Niagara region.
Figure 2.3. Flow diagram of the porcine reproductive and respiratory syndrome (PRRS) Area Regional Control and Elimination (ARC&E) projects in Ontario.
Figure 2.4. Risk maps and high risk clusters detected by the spatial scan statistic method for the regions of (A) Niagara in August 2013, (B) Watford in October 2013, and (C) Perth in January 2014.
CHAPTER 3: Investigation of the occurrence of porcine reproductive and respiratory virus in swine herds participating in an area regional control and elimination project in Ontario, Canada

Published in Transboundary and Emerging Diseases: 2015, doi: 10.1111/tbed.12343
3.1. Abstract

The main goal of the present study was to investigate the occurrence of porcine reproductive and respiratory syndrome virus (PRRSV) specific genotypes in swine sites in Ontario (Canada) using molecular, spatial and network data from a porcine reproductive and respiratory syndrome (PRRS) regional control project.

For each site, location, animal movement service provider (truck companies), PRRSV status and sequencing data of the open reading frame 5 (ORF5) encoding the major glycoprotein were obtained. Three kilometer buffers were created to evaluate neighborhood characteristics for each site. Social network analysis was conducted on swine sites and trucking companies to assemble the network and define network components. Three different PRRSV genotypes were used as outcomes for statistical analysis based on the region’s phylogenetic tree of the ORF5.

Multivariable exact logistic regression was conducted to investigate the association between being positive for a specific genotype and two main exposures of interest: 1) having at least one neighbour within three kilometers also positive for the same genotype outside the production system, and 2) having at least one positive site for the same genotype in the same truck network component outside the production system. Results showed that the importance of area spread and truck network on PRRSV occurrence differed according to genotype. Additionally, the Ontario PRRS database appears suitable for conducting regional disease investigations. Finally, the use of relatively new tools available for network, spatial and molecular analysis could be useful in investigation, control and prevention of endemic infectious diseases in animal populations.
3.2. Introduction

Porcine reproductive and respiratory syndrome (PRRS) is a widespread disease that causes detrimental effects on swine health and production in most countries in the world. The PRRS virus (PRRSV) is able to persist in individual animals and in pig populations for long periods of time. Outbreaks of acute disease are common and linked to introduction of new virus and/or lack of immunity in the herd (Rowland and Morrison, 2012). The PRRSVs are highly heterogeneous, and the open-reading frame 5 (ORF 5) encoding the major glycoprotein has shown to be the most diverse, being often used for phylogenetic analysis (Shi et al., 2010). This type of analysis becomes particularly important during PRRSV outbreak investigations since discriminatory power facilitates virus tracking and source attribution.

Methods for PRRS prevention, control and elimination have been developed over the last few years, however biosecurity programs that have tried to address infections from indirect sources (ex: fomites, needles, personnel, insects, flies and vehicles) have frequently been unsuccessful at preventing introduction of the virus into swine barns (Desrosiers, 2011). The investigation of risk factors for PRRS outbreaks is complex and requires information that is not always readily available to herd veterinarians. Often during outbreak investigations, a source cannot be identified and “area spread” is assumed, which includes but is not restricted to airborne transmission (Rosendal et al., 2014).

In a cross-sectional study from a high density area in Quebec (Canada) it was shown that PRRSV transmission was likely to occur between sites that belong to the same owner, or through area spread within a five km distance (Lambert et al., 2012). While area spread has been confirmed to happen between pig sites (Otake et. al, 2010), other studies point towards transmission by common sources of animals and other herd inputs (Rosendal et al., 2014). Monitoring data and
Bayesian analysis were used to determine the relative importance of different networks, spatial proximity and specific management factors to the spread of PRRSV type 1-18-4 in Ontario during 2004-2007 (Kwong et al., 2013). Results showed that market truck network was among the three most important networks in PRRSV spread, the other two networks being ownership and gilt source.

Considering these factors, a broad assessment of PRRS dynamics at the regional level has been the focus of current PRRS Area Regional Control and Elimination projects (ARC&E). Objectives of these programs include (1) reducing prevalence of PRRSV in specific areas and therefore disease transmission risk; (2) monitoring disease trends; and (3) providing support on the design of adequate control strategies considering the type of farm and area/producer objectives (OSHAB, personal communication). There are limited reports in the peer-reviewed literature on PRRS regional control projects (Corzo et al., 2010), and even fewer on the direct use of data coming from such projects for disease investigation, control and prevention. It has been suggested that organizing a region into systems of linked clusters based upon pig flow and/or spatial proximity to other sites could facilitate logistics and improve the likelihood of a disease control plan succeeding (Mondaca-Fernandez and Morrison, 2007). Furthermore, diagnostic data, including sequence information, is commonly used for making decisions at the herd, system, and region level. The availability of such molecular data from surveillance systems, combined with new analytical approaches and development in bioinformatics allow for new epidemiological models and investigations (Muellner et al., 2011).

In mid-2014, the project coordinator of the PRRS ARC&E projects in Ontario (so-called “Watford project”) reported concerns with regard to the spread of particular strains of PRRSV. The main goal of the present study was to make use of molecular, spatial and network data
available from this particular region’s project to conduct a disease investigation. The objectives were: 1) to describe the PRRSV genotypes present in the area from 2012-2014, and 2) to investigate spatial proximity and animal transportation network as potential risk factors for the spread of particular PRRSV genotypes.

3.3. Materials and Methods

3.3.1. Site selection and enrollment

The specific project was selected purposively due to concerns of unusual PRRSV circulation in the area. Sites participating in the Watford PRRS ARC&E project were selected on a voluntary basis. Area leaders and swine producers played an important role in the implementation of the project, and participation rates were estimated to be above 80% (OSHAB, personal communication). Inclusion criteria were that all swine producers enrolled in the regional project had to sign a consent form agreeing to share site location and PRRS status information among other producers in their region, and answer a questionnaire. The 20-minute questionnaire with approximately 40 questions was administered over the phone and included questions regarding basic herd demographics, ownership structure, sources, biosecurity, networks, besides others. The questionnaire is available from the authors upon request.

3.3.2. Definition of PRRSV status and sequencing data

After enrollment, site PRRSV status was assigned according to a combination of diagnostic testing and assessment of a herd veterinarian related to movement of PRRSV-positive animals. Diagnostic testing included ELISA and PCR; where the recommended sample size was nine for oral fluids and 11 for serum samples. Both strategies allow for detection of 20% within-herd prevalence with a confidence of 90%. Samples were processed and tested at the Animal Health
Laboratory, University of Guelph. The immunofluorescent antibody assay (IFA) was used to rule out potential false-positive ELISA results on serum. Gene sequencing (ORF 5) was attempted on positive serum PCR results, and all available sequences from the specified region during the years 2012-2014 were used for analysis.

Sequences were aligned using the MUSCLE algorithm using the MEGA 5.2 software. Genetic analysis was conducted using the packages ape (Paradis et al., 2004) and adegenet (Jombart and Ahmed, 2011) in R (The R Program, version 3.0.2). An unrooted neighbour-joining tree was constructed based on pairwise genetic distances using Tamura and Nei’s distance. Cases were divided into clusters, where cases were members of the same cluster if their nucleotide difference was 4% or less (24 nucleotides). Three of those clusters, that were considered homogenous and had a reasonable sample size, were used as the outcomes for statistical analyses. It is important to note that, for simplicity reasons, these clusters were named after the predominant restriction fragment length polymorphism (RFLP) pattern, but the definition of the groups was determined using sequence data (therefore the precedent letter ‘G’ was included before the RFLP pattern).

Site PRRSV genotype status was determined on the basis of the sequence analysis, and could be confirmed (for sites where virus was successfully sequenced) and presumed from pig flow. A similar approach has been used previously for investigation of spread of North American PRRSV in Denmark (Mortensen et al., 2002). Sites were considered presumed positive with a certain genotype only if the following two conditions were met: 1) sequence information from the sow herd where pigs were coming from was available and confirmed (same production system), and 2) test date in the sow herd was more recent than that of the grower pig population (if not, then PRRSV genotype status was based on the most recent information from the site itself). Approximately 16.9% of sites (n=23) had unknown PRRS status at the time data were
retrieved for analysis and were therefore considered negative for all PRRSV genotype analysis.

3.3.3. Location data

Longitude and latitude were obtained for each site from an industry partner (Ontario Pork, Guelph, ON) and used for data point visualization and construction of three kilometer buffers using ArcMap 10.1. Neighbourhood descriptive statistics were calculated using SAS (version 9.3: SAS Inst. Inc., Cary, NC). For the purpose of this study, area spread was defined as transmission of PRRSV from herd to herd in space (radius of three km) by unknown factors, which may include, but is not exclusive to, aerosol transmission (Rosendal et al., 2014).

3.3.4. Animal transportation network data

Trucking network information was obtained through the questionnaire administered at enrollment. Each swine site could name a maximum of three truck companies that they used for animal transportation. The type of service (e.g.: transport of market pigs, nursery pigs, breeding herd, etc.) was not specified. During data processing, the names of all truck companies and production systems were examined, checked for accuracy and standardized. The network structure was constructed using Gephi 0.8.2 (Bastian et al., 2009). Nodes were defined as swine sites or truck companies, and an edge (also known as “link”) was defined as a connection between a site and a truck company (as reported by producers in the questionnaire). Network statistics were calculated using Gephi and the network was exported for use in UCINET 6 (Borgatti et al., 2002), where identification of network components was conducted. A “trucking component” was defined as a group site and trucking company that were directly or indirectly connected. The ‘scale-free’ nature of the network was evaluated and tested using the R version 3.0.2 package ‘poweRlaw’ (Gillespie, 2014).
3.3.5. Definition of exposures

In order to investigate spatial proximity to other sites as a risk factor for being positive for a specific genotype, the exposure of interest for each study site was defined as having at least one neighbour within the three km buffer that was positive for that specific genotype, but was not in the same production system as the study site (spatial exposure). To investigate being a member of a specific “truck network component” as a risk factor, the exposure of interest for each study site was defined as having at least one site positive in the same component of the transportation network that was positive for that specific genotype, but was not in the same production system as the study site (transportation exposure). The decision to exclude swine sites from the same production system from the definition of the main exposure variables was based on the fact that disease status of sites within the same production system is expected to be the same, since many times they share management practices, personnel and animals; and could be clustered in space (Rosendal et al., 2014).

Other variables considered for statistical analysis included number of swine sites within a three km distance, number of PRRSV positive sites (sequenced or not) within a three km distance, number of swine sites that were part of the same network component, and heat producing unit (HPU) at the site level. The latter variable combines production type and number of pigs, and it was calculated using the following formula:

\[ \text{HPU} = 0.17 \times (\text{weaners and finishers}) + 0.30 \times (\text{gilts and sows}) \] (Lambert et al., 2012).

3.3.6. Statistical Analysis

Statistical analyses were conducted at the site level using SAS version 9.3. Three binary outcomes were evaluated separately: being positive for “G 1-3-2”, “G 1-8-4” and “G 1-22-2”. Descriptive statistics were generated for each variable and correlation was tested using the
Spearman correlation coefficient. Two variables were considered correlated if correlation > 0.70. All of the aforementioned variables were captured as continuous variables except for the two main exposures of interest, and univariable analysis was conducted between each of them and the three dependent variables of interest separately. A multivariable exact logistic regression model was constructed using a forward stepwise approach with the two variables of interest being offered primarily. Final statistical significance was declared if \( p < 0.05 \), and \( P \)-values between 0.10 and 0.05 are described as ‘trends’. Models were compared during the model-building process using the Akaike Information Criteria (AIC) and Pearson and deviance residuals were plotted and evaluated. For each outcome, population attributable fractions (PAF) were estimated for each risk factor in the final multivariate model. The PAFs were obtained from the logistic regression model using a sequential approach as originally presented by Ruckinger et al. (2009), which includes calculation of predicted probabilities of the outcome for each individual if the risk factor (s) were absent from the population; followed by the subtraction of expected minus observed cases.

3.4. Results

3.4.1. Descriptive Analysis

Description of swine sites

Region boundaries are shown in Figure 3.1. A total of 136 swine sites were included in the study, from 69 different production systems. The maximum number of sites per production system was seven. The region comprehended an area of 3,990 km\(^2\), with an approximate density of pigs of 40 pigs per km\(^2\).

The majority of swine sites enrolled in the project were finisher sites (48.53%), followed by
farrow-to-finisher (16.91%), wean-to-finisher (10.29%), farrow-to-weaner (8.82%), farrow-to-feeder (7.35%), nursery (4.41%) and isolation/ acclimatization (3.68%).

Approximately 37% of all sites reported having a shower-in facility in place (five percent missing information), and from those that did not report having one, 65% reported having a bench entry, which corresponds to a separation between “clean” and “dirty” area before entering the barn, change in coveralls and washing hands.

None of the sites reported having air filters, and only 17% of the sites reported having all-in all-out (AIAO) pig flow (for the purpose of this descriptor, an AIAO facility was defined as AIAO by building or AIAO by site only; sites with AIAO by room were considered continuous flow). Sixteen different veterinarians were responsible for all sites enrolled in the project, with five veterinarians being responsible for ten or more sites and 11 veterinarians being responsible for nine sites and below.

**PRRSV status and sequencing**

Fifty ORF5 sequences were available from the Animal Health Laboratory. Two sequences were not used because they were from 2007/2009, one sequence was omitted from analysis due to poor quality of sequencing data, and one was omitted because the corresponding site was outside the study area. Forty-six sequences from 42 unique sites were used for phylogenetic tree construction.

All sequences were 603 bases long. There were 273 polymorphic sites in the sample, and the polymorphism appeared to be distributed randomly across the gene. Eighteen clusters of cases were found in the dataset, showing evidence of very high diversity considering that only 46 sequences were available from this region. In approximately 50%, 14% and 17% of the times, RFLP type did not correlate with genotype determined by sequence data for RFLPs 1-3-2, 1-8-4.
and 1-22-2, respectively.

Figure 3.2 shows the phylogenetic tree and the selected outcomes that were separately used for analysis. Cluster G 1-3-2 was composed of eight sequences from eight different sites. They were used to assign status for those eight sites and to presume status for downstream pig flows (growing pig sites) for another ten sites. For G 1-8-4, out of the ten sequences shown in the cluster, six were used to assign status for six different sites (four of them were not used—three were repeated cases from the same site, and were not used because a more recent sequence was available; and for one sequence the site was not yet enrolled in the project). These six sequences were also used to presume status for six other sites. Finally, for G 1-22-2, eight sequences were available but only six could be used to assign status for six sites (two were not used because there were more recent sequences available). Those six sequences were also used to presume status for one downstream site.

**Spatial component**

Table 3.1 shows a description of spatial proximity of sites. The mean number of swine sites positive for G 1-3-2 within three km outside the production system was 0.39 (median:0, SD: 0.66, min:0, max:2). The mean number of sites positive for G 1-8-4 within three km outside the production system was 0.21 (median:0, SD: 0.55, min:0, max:2), and the mean number of sites positive for G 1-22-2 within three km outside the production system was 0.07 (median:0, SD: 0.25, min:0, max:1). Figure 3.3 shows the distribution of number of neighbours within and outside each site’s production system.

**Truck network component**

The truck network was composed of 175 nodes (136 sites and 39 truck companies) and 134 edges (Figure 3.4). The average degree was 1.53, the network diameter was 11, the average path
length was 4.20 and network density was 0.01.

The network of swine sites and transportation companies identified in this region could be characterized as scale-free network (Figure 3.5), characterized by a “right skewed, long-tailed, power-law distribution of the number of links (degrees) to nodes, where a large number of nodes have a few links, but a few nodes have relatively large numbers of links” (Dorjee et al., 2013). Additional statistical analysis revealed that indeed the number of degrees in the sites and companies could be coming from a power-law distribution ($P=0.85$, null hypothesis is that the distribution is not different from a power-law distribution).

Small-world networks are characterized by clusters of nodes that are connected to each other through a few long-range links. For those networks, disease tends to spread slower, but can eventually reach more distant clusters compared to random networks (Rahmandad and Sterman, 2008). The network did not meet this requirement, since the clustering coefficient was zero.

Twenty-one network components were identified. Approximately 75% of all sites were part of a component, while 15% did not name any transporter. Interestingly, 49.26% of all sites ($n = 67$) were part of one component, which was identified as the weak giant component. All others were composed of one to eight sites. The mean size of the trucking network component was 40.87 nodes (median: 12, SD: 37.80, min: 0, max: 79).

The mean number of swine sites within the same component outside the production system was 35.04 (median: 20, SD: 28.88, min: 0, max: 66) and the mean number of swine sites positive for PRRSV within the same component outside the production system was 26.39 (median: 11, SD: 23.59, min: 0, max: 53).

The mean number of swine sites positive for G 1-3-2 within the same component outside the production system was 5.65 (median: 1, SD: 5.58, min: 0, max: 12). The mean number of sites
positive for G 1-8-4 within the same component outside the production system was 5.27 (median:1, SD: 5.17, min: 0, max: 11), and the mean number of swine sites positive for G 1-22-2 within the same component outside the production system was 1.90 (median:1, SD: 1.95, min: 0, max: 4).

3.4.2. Multivariable exact logistic regression analysis

A description of outcome distribution for the different variables considered for analysis is shown in Tables 3.2 and 3.3. Exact logistic univariable and multivariable analysis for the three outcomes are shown on Tables 3.4 and 3.5. The type of analysis selected for this study (exact logistic regression) is computationally demanding, which limited model construction. A combination of the correlation matrix, a causal diagram and AIC values guided decisions for final model construction. During forward selection of variables, only the two main predictors could fit in the model, otherwise the model would become unstable.

The proportion of cases that could be attributed to having at least one site within the truck component positive with G 1-3-2 outside the production system was 45.60%, and the proportion of cases that could be attributed to having at least one neighbour within the three km buffer positive with G 1-3-2 outside the production system was below zero (which indicates the factor is protective). For G 1-8-4, these percentages were 70.26% and 15.05%, respectively, and for G 1-22-2, 0.1% and 23.49%, respectively.

3.5. Discussion

The most important finding of this study was that the existence of PRRSV-genotype specific positive swine sites in the transportation network that were located outside the same production system represented a significant risk factor for the spread of PRRSV G 1-8-4 and a potential risk
factor for the spread of PRRSV G 1-3-2. In contrast, such exposure could not be identified as a significant risk factor associated with positivity for PRRSV G 1-22-2. The same set of multivariable models also identified that spatial exposure to herds that harboured the same PRRSV genotype and did not belong to the same production system could not be detected as statistically significant for G 1-3-2 and G 1-22-2 but was a potential risk factor for 1-8-4.

Overall, findings regarding the importance of transportation agree with results from other studies conducted in the same source population at different times (Kwong et al., 2013, Rosendal et al., 2014). In the first study, spatial location could not be identified as an important contributor to spread; but herd ownership, gilt source and market trucks played an important role. The second study investigated ownership and the presence of spatial clusters of RFLP 1-8-4 using laboratory submissions in Ontario, and results showed no spatial dependence but suggested common source of animals and herd ownership could be important. Ontario multi-site production systems are characterized by intense movement of animals amongst sites with frequency of off-farm shipments from 1 to 6 per week, which corresponds to a high contact rate (Dorjee et al., 2013). This information, combined with results from the present study, suggest that transportation could be a constant high risk for disease spread in swine populations.

Importance of transportation in the spread of PRRSV has previously been confirmed in experimental studies (Dee et al., 2002, Dee et al., 2004). Nonetheless, the exact circumstances and the chain of events under which such transmissions occur are more difficult to elucidate. The transmission could be easy to justify when transportation is conducted using unwashed trucks. One recent industry study identified gaps in the biosecurity of trucks and mentioned that one of the biggest concerns for transporters was the cost of washing and disinfection procedures (OSHAB, 2012). This is particularly of concern given the economic volatility of swine
production. Despite efforts, the chain of events that would lead to successful virus transmission could still exist in these situations particularly under harsh winter conditions.

In our study, the truck network was characterized as “scale-free”, which has been reported in previous studies conducted using animal movement within the swine industry (Bigras-Poulin et al., 2007, Dorjee et al., 2013, Thakur et al., 2014). For this type of network the speed of disease spread is faster compared to non-free-scale networks due to the presence of super-spreaders (Rahmandad and Sterman, 2008). The trucking network did not have the characteristic of a “small world” in this study population, which suggests that a disease probably would not spread between distant clusters (trucking components). This finding was due to the fact that, even though one of the identified truck components was somewhat connected, the others were not as much, and many nodes had a clustering coefficient of zero (sites transporting their own animals/sites part of a small component). Further exploration of trucking networks needs to be conducted using actual movement networks. The weak giant component contained almost half of all swine sites in the region, which needs to be considered since this could potentially be an estimate of an upper size of a potential epidemic under certain conditions (Dube et al., 2009).

The lack of a strong spatial component that would be indicative of local spread is in agreement with previous studies (Goldberg et al., 2000, Badaoui et al., 2013, Rosendal et al., 2014) and in contrast with several findings from other source populations (Lambert et al., 2012, Mondaca-Fernandez et al., 2006, Otake et al., 2011). It is interesting to note that most statistically significant associations were detected for PRRSV 1-8-4. The authors speculate that this is due to the timing of disease emergence in the region, since this PRRSV strain was most recently detected in the study population. This has been previously hypothesized by other authors for porcine circovirus (Vigre et al., 2005): clusters can be more easily identified in early phases in
the development of an outbreak, and in the later phases local spread is observed. Such
description would be consistent with these data because the G 1-8-4 was in the emerging phase,
whereas G 1-22-2 had been circulating in this area since 2009 and had opportunities to
disseminate through a variety of mechanisms. There is however, one important difference: PRRS
is endemic in Ontario, and due to the existence of partial immunity in many herds, not every
virus introduction will result in a major epidemic in a swine site. This will continue to be a
limitation of many PRRSV field investigations.
Attributable fractions assess the proportion of cases in a population attributable to certain risk
factors (considering excess cases due to that exposure). In this case, these fractions were
calculated for both trucking membership and neighborhood exposures accounting simultaneously
for one another. For both G 1-3-2 and G 1-8-4, the proportion of cases that could be attributed to
having at least one site within the same trucking component for the respective genotypes outside
the production system was quite high. This suggests that interventions at the truck company level
would potentially diminish disease prevalence and spread, a few examples being avoiding the
visit to multiple sites between car washes, planning visits considering health status and
production type of sites, reducing contact between service providers and animals whenever
possible, use of disposable boot covers, besides others. Since this exposure was generated at the
trucking component level, another strategy would be to fragment the network by targeting sites
that act as bridges (Buttner et al., 2013).
One important novelty of this study is in how the two main exposures of interest were defined. It
is common that the number of sites in the near proximity is considered as a potential risk factor
for different diseases, but it is uncommon that this is adjusted for the ownership. The authors
argue that this is a necessity in today’s swine industries, where production systems could
spatially cluster, and disease spread due to movement of animals could be erroneous identified as local spread. A similar analogy is equally valid for the trucking components. Finally, the binary classification of exposures also facilitates communication to stakeholders (who are responsible for making changes), and allows for the calculation of PAFs as an additional measure of population-level effects.

This study showed that regional disease control programs such as the PRRS ARC&Es can be used effectively as the primary source of data for disease investigations. It is, however, essential that swine producers and herd veterinarians are committed to providing real-time updated accurate data, and that diagnostic data are readily available. The collaboration between researchers, herd veterinarians and diagnosticians should improve the speed of interventions and improve disease prevention, control and surveillance of infectious disease. A dataset such as this facilitates quick identification of potential outbreaks and how viruses spread, allowing for more effective, cost-effective interventions in a timely matter, and ideally preventing major losses and economic damages. Also, the collaboration between experts from diverse fields, such as molecular and social epidemiology, might be extremely valuable. The strength of using, for example, networks, is that they allow for visualization of relationships that may lead to paths for direct disease transmission (Bigras-Poulin et al., 2007), and could otherwise go unrecognized.

A few limitations are recognized in this study. As a cross-sectional study, causation cannot be established; the main aim of this observational study was to generate hypotheses and describe static associations. The study is based on prevalent cases of PRRSV positive herds, and not incident cases, therefore it cannot be ascertained whether the exposures investigated herein occurred prior or after the herd became PRRSV positive. Sample size was an issue because analysis was done at the site level and in response to spread of PRRSV genotypes and this
further impacted the models, since only a limited number of variables could be offered when performing the needed statistical analysis. This, however, does not concern the authors for two reasons: first, the main confounder, production system, was controlled for when the exposures were being defined, and second, the variables that were not able to be offered to the models were antecedent and highly correlated to the main exposures of interest, therefore keeping them would only reduce the effect of the direct variables (variables closest to the outcomes). Area-based disease investigations based on classification into discrete PRRSV genotype clusters commonly involves low number of cases, rarely providing sufficient power for typical analytical epidemiological studies. Another limitation of the present study was the possibility for misclassification bias for approximately 30% of the sites, for which a PRRSV status and/or ORF5 sequence, was not directly available. This bias would likely lead to the estimates being towards the null, since it is a non-differential misclassification bias. Finally, it is important to acknowledge that the ORF5 corresponds to only 4% of the PRRSV genome (Murtaugh et al., 2010), therefore whole genome sequencing would be ideally used and could be possibly explored in the near future, when whole genome sequencing becomes more widely available.

3.6. Conclusions

In conclusion, the Ontario PRRS database contains extensive data that can be utilized for regional disease investigations. In this specific area, proximity to another swine site that was positive with the same genotype was not identified as a significant risk factor. Being part of a truck network that has at least one site positive for a specific genotype might increase the odds of a herd being positive for that genotype (particularly for G 1-8-4). Results showed that the importance of area spread and network connections were dependent on PRRSV genotype.
The uses of relatively new tools such as network analysis and a combination of other epidemiological fields could aid in investigation, control and prevention of infectious diseases in animals.

3.7. Acknowledgements

The authors would like to acknowledge Ontario swine producers and herd veterinarians participating in the PRRS ARC&E project of the Watford region, and funding provided by the Ontario Ministry of Agriculture, Food, and Rural Affairs, the Ontario Swine Health Advisory Board, Ontario Pork, the Canadian Swine Health Board, the Natural Sciences Engineering Research Council, the Agricultural Adaptation Council and the Animal Health Laboratory (University of Guelph).

3.8. References


Goldberg, T. L., E. Hahn, R. M. Weigel, G. Scherba, 2000: Genetic, geographical and temporal


Table 3.1. Swine site descriptors and neighborhood characteristics for the 136 swine sites enrolled in the study (region of Watford)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean</th>
<th>Median</th>
<th>Standard Deviation</th>
<th>Range</th>
<th>Missing observations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of pigs</td>
<td>1138.00</td>
<td>800.00</td>
<td>1173</td>
<td>(55 – 7000)</td>
<td>3</td>
</tr>
<tr>
<td>HPU$^1$</td>
<td>226.69</td>
<td>153.00</td>
<td>211.27</td>
<td>(16.5 – 1190)</td>
<td>3</td>
</tr>
<tr>
<td>N 3km$^2$</td>
<td>2.68</td>
<td>2.00</td>
<td>2.23</td>
<td>(0 – 9)</td>
<td>0</td>
</tr>
<tr>
<td>N positive 3km$^3$</td>
<td>1.78</td>
<td>1.00</td>
<td>1.80</td>
<td>(0 – 7)</td>
<td>0</td>
</tr>
<tr>
<td>N outside 3km$^4$</td>
<td>2.11</td>
<td>2.00</td>
<td>2.16</td>
<td>(0 – 9)</td>
<td>0</td>
</tr>
<tr>
<td>N positive outside 3km$^5$</td>
<td>1.40</td>
<td>1.00</td>
<td>1.69</td>
<td>(0 – 7)</td>
<td>0</td>
</tr>
</tbody>
</table>

$^1$HPU is a measure that combines production type and number of pigs, and was calculated using the following formula: \( \text{HPU} = 0.17 \times \text{(weaners and finishers)} + 0.30 \times \text{(gilts and sows)} \) (Lambert et al., 2012).

$^2$Number of swine sites within 3km

$^3$Number of swine sites positive for PRRSV within 3km

$^4$Number of swine sites outside the system within 3km

$^5$Number of swine sites positive for PRRSV outside the system within 3km
Table 3.2. Distribution of continuous variables for the three selected outcomes; means (standard deviation) are shown.

<table>
<thead>
<tr>
<th></th>
<th>Positive(^1)</th>
<th>Negative(^1)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>G 1-3-2(^2)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N 3km(^3)</td>
<td>3.39 (1.75)</td>
<td>2.57 (2.28)</td>
</tr>
<tr>
<td>N positive 3km(^4)</td>
<td>2.33 (1.24)</td>
<td>1.70 (1.86)</td>
</tr>
<tr>
<td>Truck component size(^5)</td>
<td>53.67 (36.88)</td>
<td>38.92 (37.71)</td>
</tr>
<tr>
<td>HPU(^6)</td>
<td>306.3 (209.7)</td>
<td>214.2 (196.70)</td>
</tr>
<tr>
<td><strong>G 1-8-4(^2)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N 3km(^3)</td>
<td>3.42 (2.94)</td>
<td>2.60 (2.15)</td>
</tr>
<tr>
<td>N positive 3km(^4)</td>
<td>2.92 (2.19)</td>
<td>1.68 (1.73)</td>
</tr>
<tr>
<td>Truck component size(^5)</td>
<td>72.42 (22.80)</td>
<td>37.82 (37.62)</td>
</tr>
<tr>
<td>HPU(^6)</td>
<td>315.1 (194.5)</td>
<td>217.9 (211.6)</td>
</tr>
<tr>
<td><strong>G 1-22-2(^2)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N 3km(^3)</td>
<td>1.86 (1.46)</td>
<td>2.72 (2.26)</td>
</tr>
<tr>
<td>N positive 3km(^4)</td>
<td>1.29 (0.95)</td>
<td>1.81 (1.84)</td>
</tr>
<tr>
<td>Truck component size(^5)</td>
<td>46.71 (40.28)</td>
<td>40.56 (37.80)</td>
</tr>
<tr>
<td>HPU(^6)</td>
<td>288.8 (211.60)</td>
<td>223.2 (207.30)</td>
</tr>
</tbody>
</table>

\(^1\)Positive/ Negative for the specific outcome (genotype).
\(^2\)For G 1-3-2, total positives was 18, and total negatives was 118. For G 1-8-4, total positives was 12 and negatives was 124. For G 1-22-2, total positives was 7 and negatives was 129.
\(^3\)Number of swine sites within 3km
\(^4\)Number of swine sites positive for PRRSV within 3km
\(^5\)Number of swine sites within the same truck component
\(^6\)HPU is a measure that combines production type and number of pigs, and was calculated using the following formula: HPU = 0.17 × (weaners and finishers) + 0.30 × (gilts and sows) (Lambert et al., 2012).
Table 3.3. Distribution of the main variables of interest for the three selected outcomes; frequency % (n/N) are shown.

<table>
<thead>
<tr>
<th></th>
<th>Positive(^1)</th>
<th>Negative(^1)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>G 1-3-2(^2)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spatial exposure positive</td>
<td>12.19 (5/41)</td>
<td>87.80 (36/41)</td>
</tr>
<tr>
<td>Spatial exposure negative</td>
<td>13.68 (13/95)</td>
<td>86.32 (82/95)</td>
</tr>
<tr>
<td>Transportation exposure positive</td>
<td>18.84 (13/69)</td>
<td>81.16 (56/69)</td>
</tr>
<tr>
<td>Transportation exposure negative</td>
<td>7.46 (5/67)</td>
<td>92.54 (62/67)</td>
</tr>
<tr>
<td><strong>G 1-8-4(^2)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spatial exposure positive</td>
<td>20.00 (4/20)</td>
<td>80.00 (16/20)</td>
</tr>
<tr>
<td>Spatial exposure negative</td>
<td>6.90 (8/116)</td>
<td>93.10 (108/116)</td>
</tr>
<tr>
<td>Transportation exposure positive</td>
<td>12.64 (11/87)</td>
<td>87.36 (76/87)</td>
</tr>
<tr>
<td>Transportation exposure negative</td>
<td>2.04 (1/49)</td>
<td>97.96 (48/49)</td>
</tr>
<tr>
<td><strong>G 1-22-2(^2)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spatial exposure positive</td>
<td>22.22 (2/9)</td>
<td>77.78 (7/9)</td>
</tr>
<tr>
<td>Spatial exposure negative</td>
<td>3.94 (5/127)</td>
<td>96.06 (122/127)</td>
</tr>
<tr>
<td>Transportation exposure positive</td>
<td>5.80 (4/69)</td>
<td>94.20 (65/69)</td>
</tr>
<tr>
<td>Transportation exposure negative</td>
<td>4.48 (3/67)</td>
<td>95.52 (64/67)</td>
</tr>
</tbody>
</table>

\(^1\)Positive/ Negative for the specific outcome (genotype).
\(^2\)For G 1-3-2, total positives was 18, and total negatives was 118. For G 1-8-4, total positives was 12 and negatives was 124. For G 1-22-2, total positives was 7 and negatives was 129.
Table 3.4. Univariable analysis for each of the three outcomes

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Variable</th>
<th>Estimate (SE)</th>
<th>OR (95% CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>G 1-3-2</td>
<td>Spatial exposure¹</td>
<td>-0.13 (0.56)</td>
<td>0.88 (0.22, 2.87)</td>
<td>&gt;0.99</td>
</tr>
<tr>
<td></td>
<td>Transportation exposure²</td>
<td>1.05 (0.56)</td>
<td>2.86 (0.88, 10.90)</td>
<td>0.09</td>
</tr>
<tr>
<td></td>
<td>N 3km³</td>
<td>0.16 (0.11)</td>
<td>1.17 (0.94, 1.96)</td>
<td>0.17</td>
</tr>
<tr>
<td></td>
<td>N positive 3km⁴</td>
<td>0.18 (0.13)</td>
<td>1.20 (0.91, 1.54)</td>
<td>0.20</td>
</tr>
<tr>
<td></td>
<td>Truck component size⁵</td>
<td>0.01 (0.007)</td>
<td>1.01 (0.99, 1.02)</td>
<td>0.15</td>
</tr>
<tr>
<td></td>
<td>HPU⁶</td>
<td>0.002 (0.001)</td>
<td>1.002 (1.00, 1.004)</td>
<td>0.11</td>
</tr>
<tr>
<td>G 1-8-4</td>
<td>Spatial exposure¹</td>
<td>1.20 (0.66)</td>
<td>3.33 (0.66, 14.31)</td>
<td>0.15</td>
</tr>
<tr>
<td></td>
<td>Transportation exposure²</td>
<td>1.93 (1.06)</td>
<td>6.88 (0.94, 305.15)</td>
<td>0.06</td>
</tr>
<tr>
<td></td>
<td>N 3km³</td>
<td>0.16 (0.13)</td>
<td>1.17 (0.89, 1.51)</td>
<td>0.26</td>
</tr>
<tr>
<td></td>
<td>N positive 3km⁴</td>
<td>0.32 (0.15)</td>
<td>1.38 (1.02, 1.87)</td>
<td>0.04</td>
</tr>
<tr>
<td></td>
<td>Truck component size⁵</td>
<td>0.03 (0.01)</td>
<td>1.03 (1.01, 1.07)</td>
<td>0.04</td>
</tr>
<tr>
<td></td>
<td>HPU⁶</td>
<td>0.002 (0.001)</td>
<td>1.002 (0.99, 1.004)</td>
<td>0.16</td>
</tr>
<tr>
<td>G 1-22-2</td>
<td>Spatial exposure¹</td>
<td>1.91 (0.91)</td>
<td>6.77 (0.55, 52.04)</td>
<td>0.14</td>
</tr>
<tr>
<td></td>
<td>Transportation exposure²</td>
<td>0.27 (0.78)</td>
<td>1.31 (0.21, 9.30)</td>
<td>&gt;0.99</td>
</tr>
<tr>
<td></td>
<td>N 3km³</td>
<td>-0.20 (0.20)</td>
<td>0.82 (0.51, 1.20)</td>
<td>0.37</td>
</tr>
<tr>
<td></td>
<td>N positive 3km⁴</td>
<td>-0.19 (0.26)</td>
<td>0.82 (0.44, 1.32)</td>
<td>0.55</td>
</tr>
<tr>
<td></td>
<td>Truck component size⁵</td>
<td>0.004 (0.001)</td>
<td>1.004 (0.98, 1.03)</td>
<td>0.71</td>
</tr>
<tr>
<td></td>
<td>HPU⁶</td>
<td>0.001 (0.001)</td>
<td>1.001 (0.99, 1.004)</td>
<td>0.41</td>
</tr>
</tbody>
</table>

¹Corresponds to a site having at least one neighbour within 3km positive for the genotype of interest, outside the production system
²Corresponds to a site having at least one other site within the same truck component positive for the genotype of interest, outside the production system
³Number of swine sites within 3km
⁴Number of swine sites positive for PRRSV within 3km
⁵Number of swine sites within the same truck component
⁶HPU is a measure that combines production type and number of pigs, and was calculated using the following formula: HPU = 0.17 × (weaners and finishers) + 0.30 × (gilts and sows) (Lambert et al., 2012).
Table 3.5. Final model from multivariable exact logistic regression analysis for each of the three outcomes

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Variable</th>
<th>Estimate (SE)</th>
<th>OR (95% CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>G 1-3-2</td>
<td>Intercept</td>
<td>-1.99</td>
<td>&lt;0.01</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Spatial exposure(^1)</td>
<td>-0.12 (0.28)</td>
<td>0.88 (0.44, 1.61)</td>
<td>0.89</td>
</tr>
<tr>
<td></td>
<td>Transportation exposure(^1)</td>
<td>0.53 (0.28)</td>
<td>1.70 (0.95, 3.34)</td>
<td>0.08</td>
</tr>
<tr>
<td>G 1-8-4</td>
<td>Intercept</td>
<td>-2.46 (0.53)</td>
<td>&lt;0.01</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Spatial exposure(^1)</td>
<td>0.72 (0.35)</td>
<td>2.05 (0.88, 4.48)</td>
<td>0.10</td>
</tr>
<tr>
<td></td>
<td>Transportation exposure(^2)</td>
<td>1.04 (0.53)</td>
<td>2.82 (1.03, 18.98)</td>
<td>0.04</td>
</tr>
<tr>
<td>G 1-22-2</td>
<td>Intercept</td>
<td>-2.11 (0.45)</td>
<td>&lt;0.01</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Spatial exposure(^1)</td>
<td>0.94 (0.47)</td>
<td>2.57 (0.72, 7.40)</td>
<td>0.15</td>
</tr>
<tr>
<td></td>
<td>Transportation exposure(^2)</td>
<td>0.001 (0.40)</td>
<td>1.001 (0.38, 2.75)</td>
<td>&gt;0.99</td>
</tr>
</tbody>
</table>

\(^1\)Corresponds to a site having at least one neighbour within 3km positive for the genotype of interest, outside the production system

\(^2\)Corresponds to a site having at least one other site within the same truck component positive for the genotype of interest, outside the production system
Figure 3.1. Map of southern Ontario showing the region of interest.
Figure 3.2. Phylogenetic tree constructed using the neighbour-joining method.
Figure 3.3. Distribution of number of neighbours for swine sites from the region of Watford, considering neighbours within (black) and outside (grey) the production system.
Figure 3.4. Network structure for swine sites and truck companies participating in the PRRS ARC&E project in the region of Watford. Triangles correspond to truck companies, diamonds correspond to sow sites and squares correspond to growing pig sites. Network components are represented with different colors.
Figure 3.5. Distribution of degree number and betweenness centrality measure for sites and truck companies (combined) from the Watford region.
CHAPTER 4: Characterization of multiple swine site networks, cluster and risk factor analyses for porcine reproductive and respiratory syndrome

Submitted to Preventive Veterinary Medicine, Sept 2015
4.1. Abstract

The objectives of this study were to describe networks of Ontario swine sites and their service providers (including trucking, feed, semen, gilt and boar companies); to categorize swine sites into clusters based on network measures, and to investigate risk factors for porcine reproductive and respiratory syndrome (PRRS) using information gathered from the above-mentioned analyses. Swine sites selected for this project were all 816 sites enrolled in the PRRS area regional control and elimination projects in Ontario. Demographics, biosecurity and network data were collected using a standardized questionnaire and PRRS status was determined on the basis of available diagnostic tests and assessment by site veterinarians. Two-mode networks were transformed into one-mode dichotomized networks. Cluster and risk factor analyses were conducted separately for breeding and growing pig sites. Besides the clusters obtained from cluster analyses, other explanatory variables of interest included production type, type of animal flow, use of a shower facility, number of neighbors within 3km, besides others. Unadjusted univariable analysis were followed by two types of adjusted models (adjusting for production systems): a generalizing estimation equation model and a generalized linear mixed model (GLMM). Results showed that the gilt network was the most fragmented network, followed by the boar and truck networks. Considering all networks simultaneously, approximately 94% of all swine sites were indirectly connected. Unadjusted risk factor analyses showed significant associations between almost all predictors of interest and PRRS positivity, but these disappeared once production system was taken into consideration. Finally, the vast majority of the variation on PRRS status was explained by production system according to GLMM models, which shows the highly correlated nature of the data, and raises the point that interventions at this level could potentially have the highest impact in PRRS status change and/or maintenance.
4.2. Introduction

The importance of networks in infectious disease epidemiology has been recognized in the last decade for several animal diseases such as foot and mouth disease and bovine tuberculosis (Mansley et al., 2003; Gilbert et al., 2005). Mansley et al. (2003) describes the role of livestock markets in the spread of foot-and-mouth disease in Great Britain during the early stages of the 2001 outbreak, pointing out that the virus had already spread considerably through animal, vehicle and people movement by the time the first case was confirmed. Similarly, researchers described a link between animal movement and the occurrence of bovine tuberculosis also in the UK over a period of approximately 20 years (Gilbert et al., 2005). Even though the risk of disease transmission via animal movement is well understood and many countries have measures in place to mitigate such risk (e.g. quarantine of infected farms), Fevre et al. (2006) argue that movements of both domestic and wild animals continue to be an important part of the spread of infectious diseases.

Animal movements contribute to disease across regions and even countries, but other types of networks have shown to be similarly important. More specifically for swine, a link has been established between the introduction of porcine epidemic diarrhea (PED) virus and the exposure to contaminated feed in Canadian swine herds (Pasick et al., 2014). Another study showed that being part of particular truck networks can be associated with the occurrence of specific porcine reproductive and respiratory syndrome (PRRS) virus genotypes in a defined area (Arruda et al., 2015a). Diseases can also be disseminated via semen of infected boars, with a few examples being reviewed by Althouse et al. (2011). Buttner et al. (2013) suggests that even though trading activities are a major risk factor, there are numerous indirect manners by which disease can be transmitted. Despite such potential impacts, characterization of networks involving movement of
other products and services (such as feed or semen companies) did not deserve much attention in the past.

The use of network analysis allows not only for characterization of networks as a whole, but also for the description of members of such networks (e.g. swine sites) in terms of how important they are to the network in spreading disease and in getting infected, aiding in identification of important players (Dube et al., 2011). Furthermore, it allows for the capture of indirect connections amongst swine sites that would otherwise go unnoticed. This becomes particularly important when considering the current North American swine industry, which is characterized by a high degree of connectedness of swine sites clustered within production systems and a limited number of specialized service providers that focus on specific parts of the system (e.g. animal transporters and feed suppliers). This knowledge could also be of great use when designing cost-effective monitoring and risk-based surveillance strategies (Baudon et al., 2015) within regions or even countries.

Porcine reproductive and respiratory syndrome is an infectious disease caused by an RNA virus, and it is currently the most costly disease for the swine industry (Holtkamp et al., 2013). It is endemic in North America, with regional attempts to control, prevent and eliminate the disease from swine herds (Corzo et al., 2010; Mondaca-Fernandez et al., 2014, Arruda et al., 2015b). The objectives of the current study were to provide a detailed description of static connections between swine sites and their service providers (including transportation, feed, semen, gilt and boar companies) and to conduct cluster and risk factor analyses for PRRS virus positivity using parameters extracted from network analysis.
4.3. Materials and Methods

4.3.1. Descriptive analysis

All swine sites voluntarily participating in the area control and elimination (ARC&E) projects in the province of Ontario, Canada, were enrolled in this study. Demographics (site type, number of animals, production system, and production type), biosecurity (animal flow, and use of shower) and network information was collected using a standard questionnaire. Site location was determined as the center point of the premises, and was obtained from an industry organization (Ontario Pork, Guelph, ON). The software ArcMap 10.1 was used to map swine sites and construct a 3 km radius, from which the number of neighbors for each site was extracted. Swine sites were classified as PRRS positive or negative, according to definitions used and standardized in the control program (Arruda et al., 2015b). In summary, status could be confirmed negative or positive when recent and sufficient diagnostic test was available, or presumed when notified by herd veterinarians (based on animal movement). For the purpose of the current analysis, those two types (confirmed and presumed) were combined. For sites that did not have laboratory results or the veterinarian was unsure about the PRRS status, the site status was considered ‘unknown’, and excluded from risk factor analyses.

All descriptive analyses were conducted using the statistical program STATA 13 (College Station, TX: StataCorp LP).

4.3.2. Network analysis

Network analyses were conducted using UCINET 6 (Borgatti et al., 2002). Swine producers or managers were able to name up to three companies that provided feed and truck services to the site. In addition, for breeding herds, three semen, boar and gilt sources were also included. Swine sites that had no information on networks were considered as “missing information”, due to the
fact that these sites also tended to have missing values for other variables in the questionnaire, and the authors were not confident to assume the lack of answer was related to lack of connections necessarily. For the current manuscript, the relationships/connections between swine sites and service providers are referred to as being ‘static’ because detailed information on the frequency, timing and direction of contact between the two were not recorded. All networks were explored in two steps: first, the so-called ‘two-mode’ networks (affiliation/membership network) were visualized, and in this case both companies and swine sites were considered a node and a link was defined as a swine site having nominated a certain company. Second, these networks were transformed to ‘one-mode’ networks, where companies were eliminated and sites (nodes) that were indirectly connected through a common company were connected, thus a link was defined as an indirect connection between two swine sites through a company. For both types of networks, edges were considered undirected. Singletons corresponded to swine sites that reported having one or more connections but, after the mentioned transformation, resulted in not being connected to any other site. Furthermore, one-mode networks were dichotomized in order to simplify the calculation of whole network and individual node parameters; in that case a link was defined as an indirect connection between sites through one or more companies. All five networks were examined separately, and a network was created that was the combination of all networks. This network will be referred to as the “combined network”.

Whole network parameters extracted from each examined network included average degree, density, number of components, component ratio, clustering coefficient, average geodesic distance, fragmentation, closure, and compactness. A brief description of each parameter is given in the sentences to follow. The network’s average degree refers to the average of individual node’s degrees; density can be interpreted as the likelihood that any randomly chosen pair of
nodes is connected, and it uses the total number of ties in the network in its calculation (Borgatti et al., 2013). A network component is defined as a group of at least two nodes that are connected, and component ratio can be calculated by \((c-1) / (n-1)\), where \(c\) is the total number of components, and \(n\) is the total number of nodes, in a network. This measure reaches a value of one when every node is an isolate, therefore the smaller the measure, the more cohesive the network (Borgatti et al., 2013). The clustering coefficient is a measure used for undirected networks and its aim is to capture the amount of low and high dense areas of a network, or, in other words, the “clumpiness” (Borgatti et al., 2013), and the average geodesic distance refers to the average distance (in terms of number of links) between two nodes in a network, considering the shortest paths. Connectedness refers to a similar measure, but considered more sensitive- it captures the proportion of pairs of nodes that are within the same component; the opposite of such measure is called fragmentation, referring to the proportion of nodes that cannot be reached by others. The measure of compactness is a variation of connectedness, but it considers and weights the paths connecting nodes inversely by their length.

At the site level, four parameters were extracted from each dichotomized network and included degree centrality, eigenvector centrality, betweenness centrality, and closeness centrality. The four selected centrality measures can describe, yet in different ways, how nodes can be important in a network (Borgatti et al., 2013). Degree centrality is simply the number of links a site has with other sites, and eigenvector centrality is a variation of such measure, but one that gives consideration to the centrality of adjacent nodes. Betweenness centrality refers to how often a certain site falls within the shortest path between two other sites (Freeman, 1979). This latter measure is important for aiding in the identification of sites that are important in connecting otherwise scattered groups and possibly transmitting events (e.g. infectious pathogens), sites with
high betweenness centrality are commonly targets during control strategies. They can be interpreted as a quantification of “brokeage” each node has between all other nodes in the network (Borgatti et al., 2013). Finally, closeness centrality refers to how close a node is to all other nodes in the network, being a measure of how long it takes for something to spread from a certain node to all other sequentially. It is important to note that nodes were examined within the transformed one-mode networks, therefore indirect connections through service providers are being considered and described.

4.3.3. Cluster and risk factor analysis

Cluster and risk factor analyses were conducted separately for breeding (included farrow-to-wean, farrow-to-feeder, farrow-to-finish, isolation/acclimatization, boar studs and dry sow sites) and growing pig sites (included wean-to-finish, nurseries and finisher sites).

Multivariate analysis in the form of cluster analysis was conducted using STATA 13 (College Station, TX: StataCorp LP) as an exploratory tool for grouping swine sites into similar groups in regards to site-level network parameters extracted in the step described above. The average linkage method was used, with a continuous dissimilarity measure and based on L2 or Euclidean distance. The threshold number of clusters (and therefore, the cut-off point for dissimilarity) to be created varied based on the ability to reach a reasonable number of sites per cluster and on the creation of a maximum of seven clusters so that they could be offered for risk factor analysis.

Two different sets of site-level network parameters were subject to cluster analyses: one of them considered the continuous variables degree centrality and betweenness centrality for each individual network (feed and truck networks for growing pig sites, feed, truck, boar, gilt and semen networks for breeding sites), and the other set corresponded to all four parameters (degree centrality, betweenness centrality, eigenvector centrality and closeness centrality) extracted from
the “combined network”. These sets will be referred as “groupings” from this point forward, where groupings 1 and 2 refer to breeding sites (1: multiple networks and 2: combined network) and groupings 3 and 4 refer to growing pig sites (3: multiple networks and 4: combined network).

Risk factor analyses were conducted using STATA 13 (College Station, TX: StataCorp LP). Explanatory variables of interest included the “groupings” described above, production type (categorized), type of animal flow (for growing pig sites, categorized into continuous or all-in, all out), use of a shower facility (yes or no), number of neighbors within 3km (continuous), and heat-producing unit (HPU). The latter predictor was a continuous variable calculated using the variable number of animals, according to the equation: 

\[ HPU = 0.17 \times (\text{weaners and finishers}) + 0.30 \times (\text{gilts and sows}) \] (Lambert et al., 2012). Furthermore, swine sites were clustered within production systems, which were defined as two or more sites linked by a common owner or management structure. Information on whether there was a sow herd positive for PRRS within the site’s production system was collected for growing pig sites, and information on whether there was another sow herd positive for PRRS within the site’s production system was collected for sow herds. In both cases, this variable was captured as binary (yes or no) and offered as a predictor.

Initially descriptive statistics and plots were generated for exploratory analysis. Basic diagnostic techniques were used to evaluate normality and presence of outliers. The continuous variables did not meet the assumption of linearity and were categorized based on descriptive statistics and plausibility. Multicollinearity was assessed using the Spearman correlation coefficient and a cut-off of 0.80. Unadjusted univariable analyses using logistic regression were conducted between each predictor of interest and the outcome, PRRS status, followed by two types of adjusted
univariable models (adjusted for production type): a generalizing estimation equation model (GEE) and a generalized linear mixed model (GLMM). The GEE model employed a binary structure, a logit link, and robust standard errors were estimated. The exchangeable correlation structure was first attempted, but the model did not converge, therefore the independent structure was used even though it is recognized that this structure type would not completely control for all clustering effects. The GLMM had a random effect to account for the clustering of swine sites within production systems, which allowed for the estimation of an intraclass correlation coefficient (ICC) using the latent variable approach (Dohoo et al., 2010). Finally, an attempt was made to construct a multivariable generalized linear mixed model using a forward stepwise approach, including predictors that had $p < 0.20$ in the GLMM analysis. Demographic variables were used to test for confounding during model building. However, due to the fact that there were no variables that met the criteria of a statistical significance of $p < 0.05$ in the final model, univariable models were considered final and are therefore presented. First-order biologically plausible interactions including production type and animal flow were also considered with no success, and confounders were assessed using a cut-off of 20% for changes in the coefficients from the model once the confounding variable was added.

4.4. Results

4.4.1. Descriptive analysis

A total of eight hundred and sixteen sites were participating in the ARC&E projects in the province of Ontario, Canada, at the time of data completion for this study. This represents approximately 32% of Ontario swine sites considering a total of 2,556 farms reported in the most recent agricultural census (Brisson, 2014), and approximately 45% (136,000) of Ontario sows,
considering a total of 302,800 sows described by Kulasekera (2015).

There were a total of 97 production systems represented amongst the 816 sites, with 195 sites (approximately 24%) reporting not being part of a system. One production system had 125 sites (approximately 15% of all sites), two production systems had 30 sites each, nine production systems had between 10 and 19 sites, and finally 85 production systems had between 2 and 9 sites. Considering breeding sites only, approximately 57% (from a total of 259) reported to be part of a production system, while for growing pig sites this percentage was much higher, approximately 85% (from a total of 557).

For breeding sites (n = 259), PRRS status was unknown for 26.25% (n = 68). Among positive sites (n = 80), 61.25% were confirmed and 38.75% were presumed, and for negative sites (n = 111), 51.35% were confirmed and 48.65% were presumed. Finally, for growing pig sites (n = 557), PRRS status was unknown for 27.83% (n = 155). Among positive sites (n = 159), 53.46% were confirmed and 46.54% were presumed, and for negative sites (n = 243), 43.21% were confirmed and 56.79% were presumed. Table 4.1 contains descriptions of site characteristics for breeding and growing pig sites.

4.4.2. Network analysis

Two-mode and one-mode networks are shown on Figures 4.1 and 4.2, respectively. The descriptions to follow are considering one-mode networks. The largest network, as expected, was the combined network. A total of 93.87% of sites were part of the largest network component, which connected 766 sites. There was only one other component, composed of two swine sites, and three singletons. Approximately 5% of all sites (n = 45) did not report having any connections.

Among breeding herds, the gilt network was the most fragmented network (Table 4.2). It was
composed of a total of 19 components, 29 singletons, and 33.33% of breeding sites (n = 84) did not name any gilt sources. The boar network was similarly fragmented (Table 4.2), with a total of 15 components, 16 singletons, and 54.76% of sites (n = 138) did not name any boar sources. Finally, the semen network was much more connected (Table 4.2), being composed of five components, seven singletons, and a smaller percentage of sites (11.11%, n = 28) did not name any semen sources compared to the other breeding sites networks. For this network, the largest network component connected 82.54% (n = 208) of all breeding sites.

The feed and truck networks, shared amongst all production types, were characterized by a low level of fragmentation, especially the feed network (Table 4.2). The truck network was composed of 12 components, with the largest component connecting 69.24% of all sites (n = 565), 23 singletons, and 24.14% of all sites did not report any truck company as service providers. The feed network was composed of only five components, with the largest component connecting 86.52% of all sites, 6 singletons and 11.15% of all sites (n = 91) did not report any feed company as a feed provider to the site. A complete description of the whole networks is presented in Table 4.2.

4.4.3. Cluster and risk factor analysis

Cluster analysis identified five clusters for grouping 1 (breeding sites in regards to boar, gilt, semen, truck and feed networks), six clusters for grouping 2 (breeding sites in regards to the combined network), six clusters for grouping 3 (growing sites in regards to truck and feed networks), and seven clusters for grouping 4 (growing sites in regards to the combined network). A detailed description of each cluster considering all network-related variables is shown on Table 4.3.

**Grouping 1** – clusters within this grouping could be differentiated by a few aspects. Cluster 1
was characterized by an overall modest median number of indirect connections (degree centrality) across all networks, even though there was considerable variation in the number of indirect connections within the feed network. Cluster 2 was noted to have a higher median degree centrality for the boar, truck, gilt and semen networks compared to other clusters, as well as a higher median betweenness centrality for the truck network. Clusters 3 and 4 were similarly characterized by a higher median degree centrality for the feed network, with cluster 4 being more extreme. Cluster 5 corresponded to all other sites combined, therefore there is considerable variation in the spread of the majority of the variables.

Grouping 2- when considering the combined network, swine sites within cluster 1 were characterized with reduced median eigenvector and betweenness centralities when compared to the overall and other cluster’s median values. On the other hand, clusters 2, 3 and 4 showed a higher median eigenvector centrality, with clusters 3 and 4 also showing an overall higher median value for both degree and betweenness centrality. Cluster 4 showed more extreme values for all mentioned variables. Cluster 5 corresponded to all other sites combined that did not belong to any of the other clusters, and cluster 6 was a small cluster in which swine sites had missing information and therefore, could not be characterized.

Grouping 3- within this group, cluster 1 was characterized with high median number of indirect connections (degree centrality) for both feed and truck networks. Cluster 2 had similar high number of degree centrality for the feed network, but reduced median degree centrality for the truck network. Cluster 3 was characterized by an overall reduced number of indirect connections for both truck and feed networks; and cluster 4 with a reduced degree centrality for feed network but moderate truck network degree centrality. Finally, cluster 5 showed moderate truck degree centrality when compared to other clusters, and cluster 6 was composed by all sites that did not
fit within any of the other clusters.

Grouping 4 - the grouping considering all networks for growing pig sites produced the larger number of clusters, seven. For this group, cluster 1 presented relatively high degree, eigenvector and betweenness centralities; and a reduced closeness centrality. Cluster 2 was characterized by a reduced eigenvector and betweenness centralities, and clusters 3 and 4 by reduced degree and eigenvector centralities, but cluster 4 presented high betweenness centrality. Cluster 5 was characterized by overall low degree and eigenvector centralities. Finally, cluster 6 was composed by all other sites, expressing large variation, and cluster 7 corresponded to a group of sites that had missing information and therefore could not be characterized.

Unadjusted risk factor analyses showed significant associations between all predictors of interest and PRRS positivity, except for the predictors number of neighbors for breeding sites and production type for growing pig sites (Tables 4.4 and 4.5). The variable ‘animal flow’ was eliminated from analysis for breeding herds due to the large percentage of missing information and lack of variability (Table 4.1). Most of the significant associations disappeared when a GEE model was used to account for some of the clustering effects, and all associations except for one (for both breeding and growing pig models) disappeared when accounting for clustering effects using a GLMM. For breeding sites, being part of cluster 5 (sites that did not fit in any other cluster) within grouping 1 was positively and statistically associated with being positive for PRRS compared to being part of cluster 1 (OR = 4.02, P = 0.03; Table 4.4). For growing pig sites, being part of cluster 3 within grouping 4 was negatively and statistically associated with being positive for PRRS compared to being part of cluster 1 (OR = 0.15, P = 0.03; Table 4.5). Finally, the use of random effects allowed for the estimation of the intraclass correlation coefficient, which was 0.73 for breeding sites and 0.86 for growing pig sites. This indicates the
highly correlated nature of the data, and the difficulty in identifying plausible risk factors as statistically significant.

4.5. Discussion

To the knowledge of the authors, this was the first study to provide detailed description of multiple networks of service providers within the swine industry of Ontario, Canada. Dorjee et al. (2013) previously described networks for shipment of swine for 245 herds in southern Ontario, but there was no separate characterization of different networks, and the investigation of disease-related outcomes was not amongst the study objectives. The most important insight from the current study was that there is incredible complexity in the contact structure of the Ontario swine industry, which likely leads to complexity in the spread of infectious pathogens among swine herds, including PRRS virus.

Generalized linear mixed models showed that the vast majority of the variation in PRRS status (75% and 86% for breeding and growing pig sites, respectively) could be explained at the production system level. Surprisingly, this level of dependence is, in many cases, not thoroughly considered during statistical analysis for PRRS risk factors, even though it is known that sites within the same ownership structure have higher chances of becoming infected in case of a disease introduction compared to sites outside the system (Martinez-Lopez et al., 2009). This finding also suggests that interventions such as the ones aimed at prevention, control and elimination strategies would have the highest impact at the production system level.

There is remarkable difficulty in assessing meaningful risk factors for PRRS considering the given data, and it was expected that traditional risk factor analysis might provide questionable results for two main reasons. First, some sites deliberately choose to expose or vaccinate their
animal population against PRRS virus in order to maintain a partially immune herd. Second, high similarity in terms of biosecurity practices and demographics for sites within the same production system provides little variability within predictors of interest after accounting for such level of dependence. Interestingly, analysis from GLMM for breeding herds showed that the odds of being PRRS positive were 4 times higher for sites grouped in cluster 5 compared to sites grouped in cluster 1 (P = 0.03). Even though the overall P-value of this variable was not significant, it is noticeable that cluster 5 was composed by sites that could not fit in any other cluster, and these were characterized by an extremely high median betweenness centrality for the feed network (however with high variation; Figure 4.3A). Sites with high betweenness centrality could be interpreted as sites that are responsible for indirectly connecting other sites in the network that could otherwise be isolated. Traditionally this measure raises awareness in terms of disease spread within the network, but the observed association suggests that it might be the case that these sites have also higher chances of being infected, since they are indirectly connected to different parts of the networks, therefore increasing the chances of PRRS virus exposure through these indirect connections. The authors do not have a definite explanation to the fact that the feed network alone would be particularly important, but it is plausible that this measure is a proxy for overall contact rate, or number of visits to swine sites through various means. Unfortunately, information was not available at such level for further investigation.

For growing pig sites, there was likewise one significant association to note, even though the overall P-value for the variable was not significant. Sites that belonged to cluster 3 within grouping 4 had 0.15 odds of being PRRS positive compared to sites from cluster 1 (P = 0.03). That cluster was specifically characterized by having a limited overall median number of indirect
connections (degree centrality), as well as reduced betweenness and eigenvector centralities (Figure 4.3B), and had a sufficient number of observations (sites) to allow for demonstration of the significant association (unlike cluster 5 of the same grouping). This finding aligns with the idea that having fewer indirect connections, being connected to sites that have fewer connections, and being less important in connecting ‘distant groups’ may have a protective effect in being positive for infectious diseases, in this case, PRRS. This association remained significant after further GLMM models were built controlling for the presence of a positive sow herd within the same production system, HPU and production type (variables identified as potential confounders).

Although not statistically significant in the analysis based on GLMM, a few associations from the models intrigued the authors, as an example for breeding sites among grouping 1, cluster 2 had reduced odds of being PRRS positive compared to cluster 1, even though that group of sites were characterized by a higher median number of indirect connections for the truck, boar, gilt and semen networks. This association becomes plausible, however, as demographics for those particular clusters are examined more closely (Appendix, Table 4.6). Cluster 2 had all sites characterized with as smaller HPU (< 170) when compared to cluster 1, for which approximately 50% of sites presented a high HPU. Furthermore, cluster 2 contained fewer farrow-to-feeder and farrow-to-finish sites and considerably more farrow-to-wean sites, and it might be that the first two types of sites have higher frequency of contact with service providers involved in slaughtering and deadstock removal, which could be considered as being “risky” connections in terms of infectious pathogen spread. It might also be the case that these two herd types are “older” and more “family owned”, therefore not built with specific biosecurity measures in mind (Bottoms et al., 2013). Similarly, for growing pig sites among grouping 3, cluster 2 had higher
odds of being PRRS positive compared to cluster 1, even though the median number of indirect connections for the truck network was considerably smaller. However, once more, as demographics within these clusters are further investigated (Appendix, Table 4.6), cluster 2 was characterized with a higher percentage of sites with greater HPU and sites with continuous animal flow compared to cluster 1, and these factors have been previously reported as predictors for PRRS status in a similar way (Bottoms et al., 2013). This unexpected finding brings, nonetheless, an important point: it might not necessarily be solely the number of connections that characterizes risk, but the type of connection, and service provider/ company-level characteristics. Future studies should consider collection of data at the service provider level, and inclusion of such unmeasured covariates in risk factor models. Important variables could include the frequency of cleaning of transportation, presence or absence of a disease-based schedule for pick-up and drop-offs, frequency of service deliver, amount of contact between service provider personnel and swine site personnel, besides others.

This study had some limitations. First, there was a limited sample size for risk factor analysis after eliminating swine sites that had “unknown” disease status. This becomes particularly important when dealing with predictors characterized by lack of variability and high levels of dependence. Furthermore, even though correlation coefficients between explanatory variables were below 50%, the authors believe the presence of predictors such as HPU and production type within the same model could produce unstable results. These factors limited the ability to construct multivariable models. The GEE models produced coefficients that were similar to the unadjusted model coefficients because an exchangeable correlation structure was not supported (models would not converge). However, the robust standard errors produced from those models provided a better representation of the data structure, and therefore the authors opted for
including those models for the purposes of comparison.

Finally, the cross-sectional nature of the data should be recognized, as well as the possibility for reverse causation associations and misclassification of both outcome and predictors of interest. Information was collected when producers were enrolled in the regional disease control programs and therefore a systematic update of site demographics and biosecurity information is not currently in place. Reverse causation between predictors such the presence of a shower facility could have happened, since measures such as this could had been taken after the site became PRRS positive in past circumstances. This could explain the unexpected finding that the use of shower facilities might increase the odds of being positive for PRRS virus. Ideally, information would be captured and used in a longitudinal way, but this could not be achieved considering the current structure of the program and the way information is being collected. The authors also recognize that all PRRS viruses were grouped, even though it is known that there is considerable virus genetic diversity. In the case that molecular data from a large number of sites were to become available, separate analysis considering virus phylogenetic groups would have provided better insights on virus spread and discriminatory transmission pathways. Lastly, it is important to note that even though the regional disease control program from which data was obtained is possibly representative of the Ontario swine industry, static network information was not available for all swine sites of the province. This limitation is commonly recognized in social network analysis (Martinez-Lopes et al., 2009), but the authors believe there was no systematic bias introduced during site selection, which would diminish the impact associated with this issue.

The development of target-based surveillance and ability to timely detect new cases of PRRS or other infectious diseases using network data appears to be an attractive method, but complete information from all swine sites would be needed, which is a challenge for many cases (Ribeiro-
It is important to consider the static nature of relationships described in the current manuscript. There is an important underlying assumption that indirect contacts are the same and independent of service provider type, and due to the dichotomized nature of one-mode networks, it was assumed that the level of indirect connections between two swine sites was the same regardless of the number of shared service providers.

4.6. Conclusions
The current study described static networks including Ontario swine sites participating in area regional control projects and multiple service providers including trucking, feed, semen, gilt and boar companies. The analyses of such networks separately and combined revealed an extremely indirectly connected swine industry. Network-related findings from this study should be considered to mitigate within-herd PRRS transmission risk, and results support the fact that interventions at the production system level would provide the greatest impact on PRRS status.

4.7. Acknowledgements
The authors would like to acknowledge graduate student funding provided by the Natural Sciences Engineering Research Council and the Ontario Graduate Scholarship (OGS). Financial support for the PRRS ARC&E projects was provided by the Ontario Ministry of Agriculture, Food and Rural Affairs (OMAFRA), the Ontario Swine Health Advisory Board (OSHAB), the Canadian Swine Health Board (CSHB), Ontario Pork, the Agricultural Adaptation Council (AAC), and the Animal Health Laboratory (AHL) of the University of Guelph. The authors would also like to extend their appreciation to the participating swine producers, veterinarians
and area leaders who assisted with sampling and data entry.

4.8. References


Table 4.1. Description of all swine sites included in the current project

<table>
<thead>
<tr>
<th>Continuous variables, mean (SD)</th>
<th>Breeding sites (n = 259)</th>
<th>Growing pig sites (n = 557)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of animals</td>
<td>1,062.35 (1,321.99)</td>
<td>1,570.94 (1,514.71)</td>
</tr>
<tr>
<td>% missing</td>
<td>0.00</td>
<td>4.13</td>
</tr>
<tr>
<td>Number of neighbours within 3km</td>
<td>2.71 (2.55)</td>
<td>2.83 (2.58)</td>
</tr>
<tr>
<td>% missing</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>HPU(^1)</td>
<td>249.06 (278.67)</td>
<td>267.06 (257.5)</td>
</tr>
<tr>
<td>% missing</td>
<td>0.00</td>
<td>4.13</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Dichotomous variables, %</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Use of continuous flow(^2)</td>
<td>22.22</td>
<td>42.73</td>
</tr>
<tr>
<td>% missing</td>
<td>58.33(^3)</td>
<td>10.95</td>
</tr>
<tr>
<td>Use of shower</td>
<td>39.00</td>
<td>27.65</td>
</tr>
<tr>
<td>% missing</td>
<td>5.40</td>
<td>12.75</td>
</tr>
<tr>
<td>Part of a production system</td>
<td>57.14</td>
<td>84.92</td>
</tr>
<tr>
<td>% missing</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>PRRS positive</td>
<td>30.89</td>
<td>28.55</td>
</tr>
<tr>
<td>% missing</td>
<td>26.25</td>
<td>27.83</td>
</tr>
</tbody>
</table>

\(^1\)Heat-producing unit  
\(^2\)For breeding herds, AIAO was defined as herds that reported having AIAO by room only. For growing pig herds, AIAO was defined as herds that reported having AIAO by building or by site only. Sites reported as AIAO by room or any flow combined with continuous flow was considered continuous.  
\(^3\)The authors believe missing information correspond to continuous flow sites.
Table 4.2. Characterization of whole networks in a study of Ontario swine sites participating in regional PRRS regional control and elimination programs

<table>
<thead>
<tr>
<th>Network</th>
<th>Average degree</th>
<th>Density</th>
<th>Component Ratio</th>
<th>Clustering coefficient</th>
<th>Average distance</th>
<th>Fragmentation</th>
<th>Closure</th>
<th>Compactness</th>
</tr>
</thead>
<tbody>
<tr>
<td>All¹</td>
<td>129.38</td>
<td>0.17</td>
<td>0.005</td>
<td>0.77</td>
<td>2.0</td>
<td>0.01</td>
<td>0.68</td>
<td>0.55</td>
</tr>
<tr>
<td>Truck</td>
<td>58.57</td>
<td>0.09</td>
<td>0.05</td>
<td>0.96</td>
<td>2.8</td>
<td>0.17</td>
<td>0.94</td>
<td>0.36</td>
</tr>
<tr>
<td>Feed</td>
<td>103.46</td>
<td>0.14</td>
<td>0.014</td>
<td>0.92</td>
<td>2.2</td>
<td>0.05</td>
<td>0.83</td>
<td>0.49</td>
</tr>
<tr>
<td>Boar</td>
<td>11.07</td>
<td>0.01</td>
<td>0.26</td>
<td>0.99</td>
<td>1.1</td>
<td>0.84</td>
<td>0.00</td>
<td>0.07</td>
</tr>
<tr>
<td>Gilt</td>
<td>19.11</td>
<td>0.11</td>
<td>0.26</td>
<td>1.00</td>
<td>1.0</td>
<td>0.89</td>
<td>1.00</td>
<td>0.11</td>
</tr>
<tr>
<td>Semen</td>
<td>48.89</td>
<td>0.22</td>
<td>0.05</td>
<td>0.97</td>
<td>2.1</td>
<td>0.14</td>
<td>0.90</td>
<td>0.49</td>
</tr>
</tbody>
</table>

¹Corresponds to the combined network containing all others
Table 4.3. Site-level network characterization of swine sites, in median (inter-quartile range) from cluster analysis. D: degree centrality, B: betweenness centrality, E: eigenvector centrality, C: closeness centrality. Values are color-coded according to being considerably above (red) and below (green) other clusters/all sites

<table>
<thead>
<tr>
<th>Cluster</th>
<th>Variable</th>
<th>All sites</th>
<th>1 (reference)</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grouping 1</td>
<td>Number of sites</td>
<td>252</td>
<td>118</td>
<td>38</td>
<td>10</td>
<td>16</td>
<td>77</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>D, boar</td>
<td>0 (8)</td>
<td>0 (3)</td>
<td>44 (0)</td>
<td>0 (1)</td>
<td>0 (1)</td>
<td>0 (4)</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>B, boar</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>D, feed</td>
<td>137 (71)</td>
<td>73 (120)</td>
<td>144 (0)</td>
<td>136 (26)</td>
<td>152 (64)</td>
<td>142 (105)</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>B, feed</td>
<td>0 (326)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>123.5 (81.3)</td>
<td>520.6 (51.3)</td>
<td>1,138.6 (2245)</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>D, truck</td>
<td>17 (50)</td>
<td>1 (18)</td>
<td>114 (2)</td>
<td>0.5 (18)</td>
<td>0 (4)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>B, truck</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>41.3 (41.3)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>D, gilt</td>
<td>2 (21)</td>
<td>0 (9)</td>
<td>49 (0)</td>
<td>0 (0)</td>
<td>0 (1)</td>
<td>2 (11)</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>B, gilt</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>D, semen</td>
<td>2 (21)</td>
<td>0 (9)</td>
<td>49 (0)</td>
<td>0 (0)</td>
<td>0 (1)</td>
<td>0 (0)</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>B, semen</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Grouping 2</td>
<td>Number of sites</td>
<td>252</td>
<td>119</td>
<td>53</td>
<td>29</td>
<td>21</td>
<td>33</td>
<td>4²</td>
<td>0</td>
</tr>
<tr>
<td>D, all</td>
<td>150 (77)</td>
<td>144 (66)</td>
<td>166 (77)</td>
<td>195 (49)</td>
<td>224 (82)</td>
<td>213 (100)</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>C, all</td>
<td>1,468 (143)</td>
<td>1,523 (81)</td>
<td>1,405 (83)</td>
<td>1,373 (57)</td>
<td>1,348 (81)</td>
<td>1,361 (112)</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>E, all</td>
<td>0.02 (0.04)</td>
<td>0.01 (0.01)</td>
<td>0.05 (0.04)</td>
<td>0.05 (0.03)</td>
<td>0.06 (0.03)</td>
<td>0.04 (0.03)</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>B, all</td>
<td>230.4 (640.3)</td>
<td>47.8 (44.8)</td>
<td>332.3 (149.4)</td>
<td>649.4 (153.3)</td>
<td>1,108.7 (135.3)</td>
<td>2,262.4 (2,536)</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Grouping 3</td>
<td>Number of sites</td>
<td>557</td>
<td>110</td>
<td>54</td>
<td>195</td>
<td>25</td>
<td>36</td>
<td>137</td>
<td>0</td>
</tr>
<tr>
<td>D, feed</td>
<td>73 (124)</td>
<td>144 (7)</td>
<td>137 (15)</td>
<td>12 (34)</td>
<td>0 (24)</td>
<td>73 (0)</td>
<td>136 (97)</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>B, feed</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>218.2 (1138.6)</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>D, truck</td>
<td>37 (92)</td>
<td>112 (17)</td>
<td>1.5 (16)</td>
<td>15 (36)</td>
<td>95 (18)</td>
<td>77 (0)</td>
<td>36 (88)</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>B, truck</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (317.3)</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Grouping 4</td>
<td>Number of sites</td>
<td>557</td>
<td>81</td>
<td>149</td>
<td>125</td>
<td>25</td>
<td>19</td>
<td>117</td>
<td>41²</td>
</tr>
<tr>
<td>D, all</td>
<td>128 (87)</td>
<td>174 (60)</td>
<td>145 (23)</td>
<td>64 (76)</td>
<td>78 (43)</td>
<td>24 (6)</td>
<td>183 (142)</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>C, all</td>
<td>1,611 (239)</td>
<td>1,412 (87)</td>
<td>1,611 (42)</td>
<td>1,726 (55)</td>
<td>1,552 (35)</td>
<td>1,871 (39)</td>
<td>1,434 (234)</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>E, all</td>
<td>0.02 (0.04)</td>
<td>0.05 (0.02)</td>
<td>0.01 (0.03)</td>
<td>0 (0.02)</td>
<td>0.01 (0.01)</td>
<td>0 (0)</td>
<td>0.04 (0.05)</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>B, all</td>
<td>33.1 (220.4)</td>
<td>212.7 (59)</td>
<td>0.22 (0.22)</td>
<td>14.7 (32)</td>
<td>194.2 (60.7)</td>
<td>0 (0)</td>
<td>531 (679.2)</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

¹Groupings 1 and 2 refer to breeding sites; grouping 3 and 4 refers to growing pig sites; ²Cluster 6 for grouping 2 and cluster 7 for grouping 4 refers to sites with missing information.
Table 4.4. Univariable analysis using three different types of models for the association of different predictors on PRRS status for breeding sites.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Unadjusted OR, logistic</th>
<th>P-value</th>
<th>Adjusted OR, GEE</th>
<th>P-value</th>
<th>Adjusted OR, GLMM</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grouping 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cluster 2</td>
<td>0.051 *</td>
<td>&lt; 0.01</td>
<td>0.051</td>
<td>&lt; 0.01</td>
<td>0.22</td>
<td>0.26</td>
</tr>
<tr>
<td>Cluster 3</td>
<td>8.5</td>
<td>0.06</td>
<td>8.5</td>
<td>0.06</td>
<td>22.43</td>
<td>0.09</td>
</tr>
<tr>
<td>Cluster 4</td>
<td>1.7</td>
<td>0.39</td>
<td>1.7</td>
<td>0.41</td>
<td>1.60</td>
<td>0.67</td>
</tr>
<tr>
<td>Cluster 5</td>
<td>3.23 *</td>
<td>&lt; 0.01</td>
<td>3.23</td>
<td>0.004</td>
<td>4.02 *</td>
<td>0.03</td>
</tr>
<tr>
<td>Grouping 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cluster 2</td>
<td>1.53</td>
<td>0.29</td>
<td>1.53</td>
<td>0.56</td>
<td>0.26</td>
<td>0.27</td>
</tr>
<tr>
<td>Cluster 3</td>
<td>5.83 *</td>
<td>&lt; 0.01</td>
<td>5.83</td>
<td>0.03</td>
<td>6.65</td>
<td>0.16</td>
</tr>
<tr>
<td>Cluster 4</td>
<td>4.11 *</td>
<td>0.01</td>
<td>4.11</td>
<td>0.08</td>
<td>1.43</td>
<td>0.77</td>
</tr>
<tr>
<td>Cluster 5</td>
<td>2.47</td>
<td>0.07</td>
<td>2.47</td>
<td>0.25</td>
<td>0.89</td>
<td>0.93</td>
</tr>
<tr>
<td>Cluster 6</td>
<td>0.74</td>
<td>0.80</td>
<td>0.75</td>
<td>0.83</td>
<td>0.08</td>
<td>0.35</td>
</tr>
<tr>
<td>HPU &gt; 180</td>
<td>3.66 *</td>
<td>&lt; 0.01</td>
<td>3.66 *</td>
<td>0.03</td>
<td>3.66</td>
<td>0.11</td>
</tr>
<tr>
<td>Use of shower</td>
<td>2.81 *</td>
<td>&lt; 0.01</td>
<td>2.82</td>
<td>0.08</td>
<td>1.66</td>
<td>0.47</td>
</tr>
<tr>
<td>Production type</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Farrow-to-feeder</td>
<td>3.27 *</td>
<td>0.01</td>
<td>3.27</td>
<td>0.11</td>
<td>2.49</td>
<td>0.46</td>
</tr>
<tr>
<td>Farrow-to-finish</td>
<td>4.08 *</td>
<td>&lt; 0.01</td>
<td>4.08</td>
<td>0.03</td>
<td>6.46</td>
<td>0.10</td>
</tr>
<tr>
<td>Isolation + dry sows + boars</td>
<td>3.36</td>
<td>0.06</td>
<td>3.36</td>
<td>0.13</td>
<td>3.13</td>
<td>0.43</td>
</tr>
<tr>
<td>Number of neighbours</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-2</td>
<td>0.96</td>
<td>0.93</td>
<td>0.96</td>
<td>0.94</td>
<td>0.53</td>
<td>0.54</td>
</tr>
<tr>
<td>3-5</td>
<td>1.54</td>
<td>0.33</td>
<td>1.54</td>
<td>0.37</td>
<td>1.36</td>
<td>0.76</td>
</tr>
<tr>
<td>&gt;5</td>
<td>0.88</td>
<td>0.80</td>
<td>0.88</td>
<td>0.77</td>
<td>1.48</td>
<td>0.61</td>
</tr>
<tr>
<td>Positive sow herd</td>
<td>0.35</td>
<td>&lt; 0.01</td>
<td>0.35</td>
<td>0.24</td>
<td>10.77</td>
<td>0.12</td>
</tr>
</tbody>
</table>

\* Significant associations, P < 0.05
1 References: Cluster 1 (for both groupings 1 and 2), smaller HPU, no use of shower, farrow-to-wean sites, and having zero neighbours
2 Heat-producing unit, categorized in the median (180)
3 Having a positive sow herd within the same production system
Table 4.5. Univariable analysis for the association of different predictors on PRRS status for growing pig sites.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Variable Description</th>
<th>Unadjusted OR, logistic</th>
<th>P-value</th>
<th>Adjusted OR, GEE</th>
<th>P-value</th>
<th>Adjusted OR, GLMM</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Grouping 3</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cluster 2</td>
<td></td>
<td>3.35*</td>
<td>&lt; 0.01</td>
<td>3.35</td>
<td>0.20</td>
<td>1.16</td>
<td>0.88</td>
</tr>
<tr>
<td>Cluster 3</td>
<td></td>
<td>1.35</td>
<td>0.33</td>
<td>1.35</td>
<td>0.77</td>
<td>0.44</td>
<td>0.38</td>
</tr>
<tr>
<td>Cluster 4</td>
<td></td>
<td>12.18*</td>
<td>&lt; 0.01</td>
<td>12.18</td>
<td>0.05</td>
<td>22.98</td>
<td>0.12</td>
</tr>
<tr>
<td>Cluster 5</td>
<td></td>
<td>2.79*</td>
<td>0.03</td>
<td>2.79</td>
<td>0.31</td>
<td>0.22</td>
<td>0.30</td>
</tr>
<tr>
<td>Cluster 6</td>
<td></td>
<td>3.59*</td>
<td>&lt; 0.01</td>
<td>3.59</td>
<td>0.20</td>
<td>1.12</td>
<td>0.89</td>
</tr>
<tr>
<td><strong>Grouping 4</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Cluster 2</td>
<td></td>
<td>0.16*</td>
<td>&lt; 0.01</td>
<td>1.16*</td>
<td>0.02</td>
<td>0.90</td>
<td>0.90</td>
</tr>
<tr>
<td>Cluster 3</td>
<td></td>
<td>0.15*</td>
<td>&lt; 0.01</td>
<td>0.15*</td>
<td>0.001</td>
<td>0.15*</td>
<td>0.03</td>
</tr>
<tr>
<td>Cluster 4</td>
<td></td>
<td>0.27*</td>
<td>0.04</td>
<td>0.27</td>
<td>0.08</td>
<td>0.14</td>
<td>0.25</td>
</tr>
<tr>
<td>Cluster 5</td>
<td></td>
<td>0.22*</td>
<td>0.04</td>
<td>0.22</td>
<td>0.17</td>
<td>0.25</td>
<td>0.48</td>
</tr>
<tr>
<td>Cluster 6</td>
<td></td>
<td>0.37*</td>
<td>&lt; 0.01</td>
<td>0.37*</td>
<td>0.01</td>
<td>0.27</td>
<td>0.06</td>
</tr>
<tr>
<td>Cluster 7</td>
<td></td>
<td>0.51</td>
<td>0.17</td>
<td>0.51</td>
<td>0.20</td>
<td>0.82</td>
<td>0.10</td>
</tr>
<tr>
<td><strong>HPU &gt; 170</strong></td>
<td></td>
<td>2.11*</td>
<td>&lt; 0.01</td>
<td>2.11</td>
<td>0.12</td>
<td>2.21</td>
<td>0.05</td>
</tr>
<tr>
<td><strong>Animal flow</strong></td>
<td></td>
<td>2.94*</td>
<td>&lt; 0.01</td>
<td>2.94*</td>
<td>0.03</td>
<td>2.21</td>
<td>0.07</td>
</tr>
<tr>
<td><strong>Use of shower</strong></td>
<td></td>
<td>1.60*</td>
<td>0.04</td>
<td>1.60</td>
<td>0.35</td>
<td>2.46</td>
<td>0.09</td>
</tr>
<tr>
<td><strong>Production type</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nursery only</td>
<td></td>
<td>0.55</td>
<td>0.15</td>
<td>0.55</td>
<td>0.27</td>
<td>1.001</td>
<td>0.99</td>
</tr>
<tr>
<td>Finish only</td>
<td></td>
<td>0.70</td>
<td>0.30</td>
<td>0.70</td>
<td>0.38</td>
<td>1.56</td>
<td>0.52</td>
</tr>
<tr>
<td><strong>Number of neighbours</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-2</td>
<td></td>
<td>0.98</td>
<td>0.94</td>
<td>0.98</td>
<td>0.94</td>
<td>0.62</td>
<td>0.49</td>
</tr>
<tr>
<td>3-5</td>
<td></td>
<td>1.95*</td>
<td>0.04</td>
<td>1.95</td>
<td>0.07</td>
<td>1.36</td>
<td>0.64</td>
</tr>
<tr>
<td>&gt;5</td>
<td></td>
<td>1.75</td>
<td>0.13</td>
<td>1.75</td>
<td>0.26</td>
<td>1.93</td>
<td>0.38</td>
</tr>
<tr>
<td><strong>Positive sow herd</strong></td>
<td></td>
<td>0.38</td>
<td>&lt; 0.01</td>
<td>0.38</td>
<td>0.27</td>
<td>14.41</td>
<td>0.08</td>
</tr>
</tbody>
</table>

*Significant associations, P < 0.05

1References: Cluster 1 (for both groupings 3 and 4), smaller HPU, continuous animal flow, no use of shower, wean-to-finish sites, and having zero neighbours

2Heat-producing unit, categorized in the median (170)

3Having a positive sow herd within the same production system
Appendix

Table 4.6. Site-level demographical characterization of swine sites from cluster analysis. Values are color-coded according to being considerably above (red) and below (green) other clusters/all sites.

<table>
<thead>
<tr>
<th>Variable</th>
<th>All sites</th>
<th>1 (reference)</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of sites</td>
<td>252</td>
<td>118</td>
<td>38</td>
<td>10</td>
<td>16</td>
<td>77</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>% F-W</td>
<td>47.49</td>
<td>49.15</td>
<td>89.47</td>
<td>30.00</td>
<td>31.25</td>
<td>29.87</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>% F-Feeder</td>
<td>11.97</td>
<td>11.02</td>
<td>0</td>
<td>30.00</td>
<td>31.25</td>
<td>12.99</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>% F-Fin</td>
<td>33.59</td>
<td>27.12</td>
<td>10.53</td>
<td>40.00</td>
<td>37.50</td>
<td>53.25</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>% iso/ boars</td>
<td>6.95</td>
<td>12.70</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3.90</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>HPU &gt; 180 (%)</td>
<td>50.19</td>
<td>53.39</td>
<td>0</td>
<td>50.00</td>
<td>75.00</td>
<td>64.94</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Grouping 2

| Number of sites           | 252       | 119           | 53 | 29 | 21 | 33 | 4² | 0 |
| % F-W                     | 47.49     | 60.50         | 37.74 | 44.83 | 38.10 | 27.27 | 25.00 | - |
| % F-Feeder                | 11.97     | 6.72          | 15.09 | 6.90  | 23.81 | 18.18 | 50.00 | - |
| % F-Fin                   | 33.59     | 21.85         | 43.40 | 48.28 | 33.33 | 48.48 | 25.00 | - |
| % iso/ boars              | 6.95      | 10.92         | 3.78 | 0   | 4.76 | 6.06 | 0   | - |
| HPU > 180 (%)             | 50.19     | 25.21         | 79.58 | 82.76 | 76.19 | 60.61 | 25.00 | - |

Grouping 3

| Number of sites           | 557       | 110           | 54 | 4.32 | 195 | 25 | 36 | 137 | 0 |
| % nurseries               | 17.77     | 25.45         | 35.19 | 14.36 | 4.00 | 22.22 | 10.95 | - |
| % W-F                     | 8.62      | 4.55          | 9.26 | 5.13  | 8.00 | 0   | 18.98 | - |
| % finishers               | 73.61     | 70.00         | 55.56 | 80.51 | 88.00 | 77.78 | 70.07 | - |
| HPU > 170 (%)             | 51.35     | 21.82         | 46.30 | 61.03 | 52.00 | 66.67 | 59.12 | - |
| Continuous flow (%)       | 47.98     | 17.17         | 57.90 | 42.68 | 69.57 | 27.78 | 76.92 | - |

Grouping 4

| Number of sites           | 557       | 81            | 149 | 125 | 25 | 19 | 117 | 41² |
| % nurseries               | 17.77     | 25.93         | 22.15 | 18.40 | 8.00 | 26.32 | 8.55 | 12.20 |
| % W-F                     | 8.62      | 14.81         | 4.03 | 1.60  | 0   | 15.79 | 21.37 | 0   |
| % finishers               | 73.61     | 59.26         | 73.83 | 80.00 | 92.00 | 57.89 | 70.09 | 87.80 |
| HPU > 170 (%)             | 51.35     | 56.79         | 28.19 | 56.80 | 44.00 | 52.63 | 66.67 | 68.29 |
| Continuous flow (%)       | 47.98     | 69.01         | 34.27 | 33.94 | 68.12 | 50.00 | 68.18 | 23.08 |

1 Groupings 1 and 2 refer to breeding sites; grouping 3 and 4 refers to growing pig sites.
2 F-W: farrow-to-wean sites; F-Feeder: farrow-to-feeder sites, F-Fin: farrow-to-finish sites, iso/ boars: sites that are isolation/acclimatization or boar stud sites (combined due to small sample size); W-F: wean-to-finisher sites
3 Cluster 6 for grouping 2 and cluster 7 for grouping 4 refers to sites with missing information.
Figure 4.1. Visual representation of two-mode networks; swine sites shown in red and service providers in blue

A. Combined network; B. Truck network; C. Feed network; D. Boar network; E. Gilt network; F. Semen network.
Figure 4.2. Visual representation of one-mode networks, network components are represented by different colours.

A. Combined network; B. Truck network; C. Feed network; D. Boar network; E. Gilt network; F. Semen network.
Figure 4.3. Boxplots of cluster network characteristics for groupings that showed significant associations in the univariable generalized linear mixed models.

A. Boxplot for betweenness centrality for clusters 1-5 from grouping 1 (breeding sites); B, C, D, E. Boxplots for betweenness, closeness, degree and eigenvector centralities (respectively) for clusters 1-7 from grouping 4 (growing pig sites).
CHAPTER 5: Evaluation of control strategies for porcine reproductive and respiratory syndrome in swine breeding herds using a hybrid stochastic model

Submitted to PLOS One, Oct 2015
5.1. Abstract

The objective of this study was to develop a hybrid stochastic model that would explore the likelihood of the occurrence of outbreaks in swine herds with different porcine reproductive and respiratory syndrome (PRRS) control measures in place. The control measures evaluated included vaccination with a modified-live attenuated vaccine (MLV) and live-virus inoculation (LVI) of gilts, and both were compared to a baseline scenario where no control measures were in place. A typical North American 1,000-sow farrow-to-wean swine herd was used as a model, with production and disease parameters estimated from the literature and expert opinion. The model constructed herein was not only able to capture PRRS virus immunity and shedding heterogeneity within animals, but also the dynamic animal flow and contact structure typical in such herds under field conditions. The outcomes included in the model were the maximum number of females infected per simulation, and time at which that happened and the incidence of infected weaned piglets during the first year of challenge-virus introduction. Results showed that the baseline scenario produced a larger percentage of simulations resulting in large outbreaks compared to the control scenarios, and interestingly some of the outbreaks occurred over one year after virus introduction. The LVI scenario showed promising results, with fewer simulations resulting in large outbreaks than the other scenarios, but the negative impacts of maintaining a PRRS-positive population should be considered. Finally, under the assumptions of the current model, neither of the control strategies prevented the infection from spreading to the piglet population, which highlights the importance of maintaining internal biosecurity practices at the farrowing room level.
5.2. Introduction

Despite the progress in porcine reproductive and respiratory syndrome (PRRS) research in the last 25 years, this remains the most significant endemic swine disease in North America, and its eradication is extremely complicated. Major knowledge gaps still exist with regard to this important disease (Perez et al., 2015), not only at the individual animal level but also at the herd level. Control and elimination methods have been developed in the last few years, and one of the major impediments to PRRS control and possible eradication is the persistence of PRRS infection in individual pigs (Fangman et al., 2007). Even though control and elimination methods have been previously described (Corzo et al., 2010) and are commonly used in field settings (Dee et al., 1997), results of the comparative effectiveness of different methods are scarce (Linhares et al., 2014). An important reason for the lack of head-to-head studies is that it would be unethical to compare PRRS control methods using intentional virus challenge in a field trial. Furthermore, it is extremely challenging to reach a counterfactual state between swine herds in order to obtain valid comparisons and conclusions.

Mathematical modelling is becoming more common in the veterinary sciences, and is an attractive alternative for situations where traditional epidemiological studies are unfeasible under field conditions. It allows for the assessment of potential consequences of disease introduction and for the testing of control strategies on simulated outbreaks. There are two main types of mathematical models: deterministic and stochastic models. The difference between them is that deterministic models describe what happens on average to a particular population, while stochastic models embrace the variability between individuals in such a population and incorporate chance, allowing for the description of outliers (Vynnycky and White, 2010). Stochastic compartmental models have been previously developed for PRRS (Nodelijk et al.,
Nodelijk et al. (2000) used stochastic “SIR” (susceptible-infected-recovered, referring to the compartments in which the population is stratified) models to explore PRRS virus (PRRSV) transmission and time-to-extinction using a 115-sow breeding herd as a case study. In addition, Evans et al. (2010) investigated within-herd transmission of PRRS focusing on infection persistence and fade-out of infections, while accounting for different contact patterns among ages. Both publications used European farrow-to-finish swine facilities as examples. More recently, Jeong et al. (2014) developed models to evaluate the effectiveness of control strategies that included herd closure with or without gilt acclimation, and single or repeated mass immunization with a modified-live vaccine (MLV), using a typical Midwestern US farrow-to-wean herd as a case study.

Even though models such as the ones described above are able to describe dynamics of infection in populations, hybrid models, in particular, are especially useful for describing complex diseases such as PRRS for three main reasons. Firstly, they allow for the inclusion of agent-based approaches. Agent-based models allow for consideration of biological variability between individuals, local interactions, life cycles, and behavior adapting to the individual’s changing internal and external environment (Grimm et al., 2006). These individuals can be given a range of parameters in order to better reflect the variability of the population in terms of, for example, immune status. Secondly, discrete events can also be incorporated into these models, and spatial location, for example, can be explicitly taken into account. Thirdly, evolving connectedness between agents can also be modeled and updated as animals move through different locations and stages of production. The use of individual-based approaches has never been previously explored for PRRS to the knowledge of the authors.

The goal of the present study was to develop a hybrid stochastic model to evaluate control
strategies for PRRS in the case of a field virus re-introduction, using a typical North American
carrow-to-wean swine herd as a case study. Control strategies explored herein included
vaccination of gilts with a modified live-attenuated vaccine (MLV), and live-virus inoculation of
gilts (LVI). Secondary objectives were to explore how the number of infected animals
introduced into the herd, and how immunization efficacy would change the likelihood of large
outbreaks occurring on a swine farm. Both of these objectives are aligned with a frequently
asked question about the likelihood of introducing a novel genotype and major outbreak in a
swine herd that is purposively kept in the state of high herd immunity against PRRSV (i.e., the
so-called stable herd).

5.3. Materials and Methods

5.3.1. Purpose

A stochastic hybrid model was developed using the software Anylogic® version 7.1.2 (XJ
Technologies, St Petersburg, Russia). The main purpose of the model was to evaluate different
PRRS control strategies (Table 5.1) for previously infected PRRS sites using a 1,000-sow
carrow-to-wean North American herd as an example. The model description outlined in the next
paragraphs follows general reporting guidelines from Grimm et al. (2006).

5.3.2. State variables and scales

The hierarchical levels considered in this model were individuals (two types of so-called
‘agents’: female pigs and their offspring; piglets) and the environment. Individual adult female
pigs were characterized by mutually exclusive PRRS immunological status: ‘susceptible’,
‘infected’, or ‘recovered’. In cases where the vaccination scenario was evaluated, female pigs
could also be characterized as ‘vaccinated’ or ‘recovered by vaccination’. Immunity against both
vaccine strain and field strain viruses was not considered to be life-long, but assumed to wane over time. Individual piglets were similarly characterized by mutually exclusive PRRS status: ‘susceptible’, ‘infected’, or ‘maternally immune’. There was no waning of immunity for piglet agents. All possible PRRS immunological statuses for each control scenario are shown on Figure 5.1.

The environment was composed of six rooms, which were modeled as discrete locations where individuals would spend varying amounts of time. The first room was the isolation/acclimation room, where all breeding female pigs arrived in the herd and spent their first days; the second room was the breeding unit, where female pigs were bred and gestated; and finally four farrowing rooms where female pigs gave birth to the piglet agents, and where those agents would spend all their time. The four farrowing rooms followed an “all-in, all-out” schedule, which means that a group of female pigs would move into one of these rooms and remain there for a fixed period of three weeks. Hence, only one of the four rooms per week would be receiving new female pigs, and available rooms would rotate on a weekly basis.

5.3.3. Process overview and scheduling

The model proceeded in daily time steps, with a time frame of 1,730 days. A total of 1,000 days was allocated for an initial model run, and the following 730-day period corresponded to the herd follow-up after challenge (virus introduction), a period of approximately two years.

Replacement of the herd female pigs was considered in the model, with incoming gilts introduced into the herd once monthly (45 animals per time) through the isolation/acclimation unit. Individuals remained in this physical space for a period of 30-60 days (depending on the control measure being evaluated), and proceeded to the breeding/gestation unit, where they remained for a fixed period of 120 days. After this period, animals proceeded to one of four
farrowing rooms, according to the previously described sequential “on-off” room schedule, where they farrowed (gave birth to piglet agents). Piglet agents stayed exclusively in the farrowing room, where they were born, for a fixed period of 21 days, when they then left the herd. At this same point, female pigs returned to the breeding/ gestation unit and re-started their reproductive cycle. Female pigs were removed from the system at a constant rate, and at all times removal was conducted from the breeding/ gestation unit. A schematic of pig flow and room locations is presented on Figure 5.2.

5.3.4. Design concepts

Interactions between agents were modeled based on agent’s location. Animals within the same physical location (one of the six available rooms) were assumed to contact each other at the same rate. However, to account for the fact that there is heterogeneity in the number of contacts made per day amongst animals, contact rate was a stochastic parameter following a triangular distribution (Table 5.2). Due to the fact that PRRSV transmission between rooms is realistic to assume (e.g. through movement of animals, people, airborne transmission, among others; Wills et al., 1997), animals located in different physical locations had the possibility of contacting others and potentially spreading PRRS infection. This transmission probability was considered to be 10,000 times smaller compared to the transmission between animals housed in the same physical space, as previously assumed by Evans et al. (2010).

For the purpose of this manuscript, it is important to make the distinction between infection and disease; the first refers to the moment when the virus invades the host while the second refers to the development of clinical signs. Infectiousness, therefore, is directly linked to virus shedding and, therefore, transmissibility; while virulence is reflective of how likely the agent is to cause severe disease. In the current manuscript, infection was being modeled, and not disease. The
rationale was that control measures were focused on reducing transmission and duration of persistence of the virus in a herd, and not on reduction of clinical signs. Stochasticity was incorporated not only in contact rate between animals, but also in the rate at which animals recover from disease (duration of infectiousness, drawn from a triangular distribution), and in the rate at which immunity wanes (duration of immunity, drawn from a Pert distribution). These parameters were selected to be stochastic because duration of PRRSV shedding and duration of immunity to a field PRRSV strain are highly variable and uncertain.

In order to account for vertical transmission of PRRSV between sows and piglets, it was assumed that all susceptible animals would give birth to susceptible piglets, piglets born to immune sows were born with maternal immunity, and piglets born to infected sows were born infected (Jeong et al., 2014). Finally, piglets born to recovered vaccinated sows were assumed to be born immune, and piglets born to vaccinated sows that were still infectious were assumed to be born susceptible. As previously mentioned, waning of maternal immunity was not considered due to the fact that piglets only remained in the premises for a short period of time.

5.3.5. Initialization

The evaluation of each disease control scenario began after the challenge-virus introduction (in a randomly selected environment of the site) on day 1,000 for each simulation. Before the infectious animal was introduced into the herd, the model ran for 1,000 days to assure demographic equilibrium. The time of initialization was the same for all scenarios, but immunological statuses amongst animals differed for the vaccination, live virus exposure and naïve herd scenarios, due to the nature of each control measure. For the vaccination scenario, a percentage of the population was partially immune, because a certain percentage (depending on the scenario) of animals entering in the herd were vaccinated with a heterologous virus strain,
which offered some immunity against the field virus. For the virus exposure scenario, part of the population was completely immune, because a large proportion of animals entering the herd were exposed to a homologous virus strain, and for the naïve herd scenario, all animals within the population were susceptible.

5.3.6. Inputs

Model inputs were obtained from the peer-reviewed literature on previous challenge studies, field observations and, when not available, obtained from discussions with experts in the area of swine production. A list of inputs used in the model, as well as their references, is shown in Table 5.2.

5.3.7. Model calibration and statistical analysis of outcomes

Since empirical information was unavailable, the model was calibrated to reproduce plausible values, and those were reviewed by a panel of three experts that included field veterinarians and researchers. Data processing was conducted using Excel® and Stata 13. Descriptive statistical analyses were conducted using Stata 13 and SAS version 9.3.

5.3.8. Simulation experiments

Each scenario consisted of 1,000 iterations. Outcomes measured included the maximum number of females infected per simulation and time at which that happened, and the incidence of infected weaned piglets during the first year of challenge virus introduction. For boxplot graphs, the log 10 of the incidence rates was used. In total, ten scenarios were investigated, including changes in specific parameters for sensitivity analysis (Table 5.1). Further explanations of the three main scenarios are detailed in the following paragraphs.

Baseline scenario

As previously mentioned, the baseline scenario was developed to describe the dynamics of
infection in a situation where no PRRS control measures were in place, reflecting a completely naïve (susceptible) population of pigs. Such scenario is likely to occur in newly established herds, or herds that had a PRRSV introduction and conducted a rollover strategy to eliminate the virus without complete depopulation. A total of one, five or ten infected animals were introduced into the herd, respectively, in separate simulations to allow for the evaluation of the secondary objective.

Modified live-virus vaccination (MLV) scenario

For the MLV scenario, it was assumed that animals receiving vaccination had a decreased probability of infection with live virus by half compared to naïve animals, and that vaccination reduced duration of infectiousness by 30% for cases where animals became infected (Linhares et al., 2012a; Table 5.2). Vaccination occurred as soon as animals entered the isolation/ acclimation room, and they received the vaccine only once. Sensitivity analysis was run using duration of acclimation of 30 and 60 days separately, and using vaccine efficacies of 95%, 80% and 70%. There was no discrimination between vaccine coverage or vaccine effectiveness for the purpose of this differentiation, therefore ‘immunization efficacy’ can be interpreted as a combination of both.

Live-virus inoculation (LVI) scenario

Similarly to the scenario described above, for the LVI scenario all incoming animals (replacement gilts) were exposed to the virus as soon as they entered the herd, with an immunization efficacy of 95% and administered once. For sensitivity analysis, two scenarios were run using duration of acclimation of 30 and 60 days separately, and both using duration of infectiousness following a triangular distribution; and one scenario was run using duration of acclimation of 60 days, and a fixed duration of infectiousness of 56 days. This last simulation
was constructed because under field conditions, it is commonly assumed that animals will shed the virus for an approximate duration of 50-60 days. It is important to note that, for this scenario, even though a constant PRRS positive population is maintained in the herd at all times, the animals intentionally exposed via live-virus inoculation are not counted as the animals infected by introduction of challenge virus during collection of outcome measures. A complete list of production and disease parameters used in the model is shown in Table 5.2.

5.4. Results

During a period of one year, across all scenarios, a mean minimum of 19,452 and a mean maximum of 25,911 piglets were produced for the 1,000 simulations. This is within the expected value considering a herd size varying between 900 – 1,200 sows, and the fact that a sow produces between 2 and 3 litters per year, with 6-10 piglets weaned per litter (expected value lies between a minimum of 10,800 and a maximum of 36,000).

Baseline scenario

As expected, the baseline scenarios produced a greater percentage of simulations that resulted in large outbreaks compared to the other scenarios. This percentage increased as the number of infected animals introduced into the herd increased (Table 5.3, Figures 5.3A, 5.3B, 5.3C). On average, the maximum number of female pigs infected at one-point-in-time (prevalence measure), considering a period of approximately two years, was 99.5 (SD: 273.6), 321.2 (SD: 424.1) and 489.4 (SD: 445.2) when one, five and ten infected females were introduced into a totally naïve herd, respectively. For all scenarios, but most evident for the scenario where one infected animal was introduced, the distribution was highly right skewed, with the 50th percentile being 5, 75 and 143 female pigs, respectively (Figures 5.5A, 5.5B and 5.5C).
For all baseline scenarios, the median yearly incidence of PRRS positive weaned pigs was relatively small: 0.004%, 0.63% and 1.44% when one, five and ten infected females were introduced into the herd (Table 5.4, Figures 5.4A, 5.4B and 5.4C). Maximum incidences reached 20.44%, 27.70% and 29.76% for the scenarios where one, five and ten infected females were introduced into the herd (Table 5.4).

Interestingly, when introducing only one infected female into the herd, only a small number of the simulations resulted in more than 100 infected females (9.20%), but for a considerable percentage of those (27/92, 29.35%), the outbreak only occurred after one full year after virus introduction. This was also true for the scenarios where five or ten animals were introduced, even though the percentage of simulations where more than 100 animals were infected was considerably higher (35.4% for the 5-infected animal scenario, for which 30.50% reached the maximum after one year of virus introduction and 65.8% for the 10-infected animal scenario, for which 22.04% reached the maximum after one year after virus introduction). Due to the fact that introducing a total of five infected animals produced a reasonable number of simulations where more than 10% of the population became infected in the baseline scenario, this number of infected animals was chosen to evaluate the MLV and LVI control strategies.

**MLV scenario**

For the MLV scenario, as vaccine efficacy increased, the number of outbreaks occurring in the herd following virus introduction decreased (Table 5.3, Figures 5.3G, 5.3H and 5.3I). The increase in the percentage of simulations resulting in large outbreaks was especially evident when vaccination efficacy dropped from 80 to 70%. As duration of acclimation was shortened from 60 to 30 days, the overall number of large outbreaks increased, and they tended to occur earlier after virus introduction (Figures 5.3G and 5.3I).
Interestingly, despite the changes described above, the average maximum number of females infected remained relatively similar amongst scenarios with different immunization efficacies: 268.03 animals (SD: 399.12) for 95% efficacy, 279.99 animals (SD: 406.09) for 80% efficacy, and 295.32 animals (SD: 418.51) for 70% efficacy. Using duration of acclimation of 30 days, the mean maximum number of females infected was 255.22 animals (SD: 365.72).

The median yearly incidence of infected weaned piglets was 0.45% (maximum 25.79%) for the 95% efficacy scenario, 0.47% (maximum 23.83%) for the 80% efficacy, and 0.49% (maximum 24.65%) for the 70% efficacy. For the scenario with 30 days of acclimation, the median yearly incidence of infected weaned piglets was 0.50% (maximum 22.85%; Table 5.4). The data were highly skewed (Figures 5.4G, 5.4H, 5.4I and 5.4J).

Finally, simulations for all MLV scenarios showed that many large outbreaks occurred after one year of initial virus introduction, with approximately 30% of simulations resulting in more than 100 animals becoming infected for all evaluated scenarios.

**LVI scenario**

There were no simulations that resulted in the majority of the herd becoming infected for the live-virus exposure scenario. For scenarios using acclimation durations of 30 and 60 days, the maximum number of female pigs infected was never more than 300, and only 6% and 5% of the simulations resulted in more than 100 animals infected for each scenario, respectively (Table 5.3, Figures 5.5D, 5.5E and 5.5F). However, as pointed out in the methodology section, it is important to note that herds using such control measure have an underlying population of virus-exposed animals that are possibly acting as carriers of the resident virus used for LVI during the acclimation stage. These animals were not captured as infectious during outcome measure. For the scenario where the young female pigs were being moved to the main herd at 30 days, there
appeared to be more outbreaks in the early phase after virus introduction compared to the scenario where those animals were being moved after 60 days of acclimation (Figures 5.3G and 5.3J). As duration of infectiousness was fixed at 56 days, however, the percentage of simulations resulting in outbreaks increased, even though those outbreaks were not too large (maximum 500 females infected).

The median yearly incidence of infected weaned piglets was around 0.50% for all vaccination scenario simulations (Table 5.4). The maximum incidence was approximately between 22 and 25% (Table 5.4).

5.5. Discussion
The hybrid model developed herein allows for evaluation of different PRRS control strategies in farrow-to-wean swine facilities. The main question addressed herein for both LVI and MLV scenarios was the frequency by which a re-introduction of PRRSV would result in infection of a large number of animals in swine herds that are considered stable.

The model was not only able to mimic pig flow and animal replacement as seen in breeding herds under field conditions, but allowed for the incorporation of stochasticity for PRRS parameters that are subject to variability and uncertainty, such as duration of infectiousness. The current model could be modified as necessary, and scenarios for specific conditions and interventions could be created, for example with changes in herd size, contact patterns, disease, and production parameters. The model also allows for evaluation of different control scenarios, such as multiple vaccination schedules, which were not explored in the current project.

An interesting finding from the current model was that small or large outbreaks can occur after long periods of initial virus introduction into the herd. This has been previously reported (Bierk
et al., 2001), and is a reasonable explanation for situations where high health swine herds have PRRS outbreaks after long periods of presumably successful virus elimination programs and monitoring, which intrigue field veterinarians. The authors hypothesize that such phenomena might not necessarily be due to breaks in internal site biosecurity, but simply due to the existence of animals that are able to act as virus reservoirs for long periods of time. Furthermore, this finding raises important points, such as the importance of herd monitoring and use of sensitive and risk-based sampling methodologies when the goal is to detect low-prevalence of PRRSV in a herd that has invested time and resources in virus elimination. This observation could also have important implications for outbreak investigation. Typically, during disease investigation, a period of contact tracing is measured over several weeks. Nonetheless, such finding could mean that the duration of such period should extend, perhaps more in line with what has been reported during PRRS incursion in Sweden (Carlsson et al., 2009). Such investigation would require extensive resources and the decision should be carefully considered. Perhaps in areas where PRRS is endemic and resources are limited, such extension of the contact tracing period should be conducted for cases where PRRSV introduction is difficult to trace.

Another important observation from the results of the model is that when small loads of virus are introduced to the herd, as exemplified by the introduction of one infected animal, large outbreaks occur with lower frequency. This observation reinforces the idea that internal and external biosecurity, and regular and adequate testing of replacement animals are extremely important, especially when other factors that may alter PRRSV spread remain out of the producers or veterinarian’s control (e.g. weather conditions, pig flow, and area pig density).

A somewhat unexpected observation for the LVI scenario was that accounting for uncertainty appeared to be more conservative compared to using a fixed duration of infectiousness. This
could be attributed to the stochastic nature of the process, and to the fact that using the distribution allows for animals with short duration of shedding, which contributes to faster extinction of a potential epidemic.

The current model has limitations that also should be acknowledged. Firstly, the model does not include indirect introduction of PRRSV, for example via fomites. The authors argue that such introduction would be more short-lived than the introduction of an infected animal, and therefore the number of outbreaks produced would probably be reduced significantly compared to the introduction of infected live animals. Another limitation of this study is that different PRRSV strains were not considered, even though the authors recognize that there are indications that virus strain can play an important role in the minimum amount of infectious dose, and likely the probability of transmission (Hermann et al., 2005). Furthermore, statements with regard to clinical disease cannot be made from the current model, since infection was modeled. The dynamics of infection are important for propagation of the microbe and for herd health, while clinical disease is mostly important from an economic and animal welfare standpoint. However, clinical manifestation of disease could be incorporated into the present model and could provide insights on economic losses for producers. Finally, it is important to note that the MLV and LVI scenarios are structurally different models, therefore direct comparison of outcomes should be made with caution. Even though the LVI scenario seemed to produce promising results with the lowest percentage of simulations resulting in outbreaks being produced, the reader should consider that, under such a control strategy, swine producers would always have to deal with having an underlying infected population (“exposed to the virus”) in the herd. This could have an impact on piglet production (Linhares et al., 2014), besides increasing the risk of “virus leakage” to growing pig populations on or off-site. Both scenarios could damage the swine producer
financially, but such quantification was not conducted in the current research. The MLV scenario showed that vaccination efficacy rates have an impact in the likelihood of major outbreaks occurring in swine herds, therefore ensuring high coverage, correct vaccine administration and adequate vaccine storages are important recommendations for producers that decide to use such control strategy.

Surprisingly, the incidence of PRRS positive piglets produced for all scenarios examined herein was, on average, relatively small. The authors believe this may be explained by two main reasons. Firstly, it could be a result of the assumptions for the immunological states for piglets, such as the assumption that infected female pigs give birth to half the number of piglets compared to non-infected females. Secondly, due to the dynamic nature of the process, it could be that the virus is actually getting to the farrowing rooms (where piglets are) after a longer period of virus introduction, as opposed to early on. Even though the incidence of PRRS positive piglets was relatively small, under the assumptions of this model, not a single strategy guaranteed absence of piglet infection. This finding supports piglet-level control strategies such as the Management Changes to Reduce Exposure to Bacteria and Eliminate Losses (McRebel™), (McCaw. 2000). The impact of this and other strategies, however, were not evaluated in the current model.

5.6. Conclusions

In conclusion, the model developed herein showed that both immunization strategies (LVI and MLV), with different success, would decrease the likelihood of large outbreaks in a farrow-to-wean swine herd. However, none would be successful in completely ceasing virus circulation in the herd. This supports the role of additional infection control methods in the farrowing rooms,
such as McRebel™ and effective surveillance to detect prevalence at potentially low levels in herds that implement such immunization strategies.

Finally, the developed model is promising especially for PRRS and other animal diseases for which parameters are uncertain or many times unknown. Mathematical models can provide the users with a range of expected outcomes and insights on how to decrease detrimental disease effects. Intuitively, estimates would be more precise as more information becomes available. Therefore the development and use of such models would be even more helpful for diseases for which epidemiology and pathogen characteristics are well described in the literature. The model can be adjusted for other herd types, for example growing pig populations, as well as other animal species.

5.7. Acknowledgments

The authors would like to acknowledge graduate student funding provided by the Natural Sciences Engineering Research Council (NSERC), the Ontario Graduate Scholarship (OGS) and The Ontario Ministry of Agriculture, Food and Rural Affairs (OMAFRA), and swine production experts consulted for parameter estimations.

5.8. References


Fangman, T.J., Kleiboeker, S.B., Coleman, M., 2007. Tonsilar crypt ulexate to evaluate shedding and transmission of porcine reproductive and respiratory syndrome virus after inoculation with live field virus or vaccination with modified live virus vaccine. JSHAP 15, 219-223.


Table 5.1. Description of porcine reproductive and respiratory syndrome control scenarios investigated in the developed hybrid stochastic model

<table>
<thead>
<tr>
<th>Scenario</th>
<th>Immunization method</th>
<th>Duration of isolation/acclimation</th>
<th>Immunization efficacy</th>
<th>Number of infected animals introduced in the herd</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline(^2)(_1)</td>
<td>None</td>
<td>60 days</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>Baseline(^2)(_5)</td>
<td>None</td>
<td>60 days</td>
<td>-</td>
<td>5</td>
</tr>
<tr>
<td>Baseline(^2)(_10)</td>
<td>None</td>
<td>60 days</td>
<td>-</td>
<td>10</td>
</tr>
<tr>
<td>LVI(^3)(_{60_95_a})</td>
<td>LVI, I = distr(^4)</td>
<td>60 days</td>
<td>95%</td>
<td>5</td>
</tr>
<tr>
<td>LVI(^3)(_{60_95_b})</td>
<td>LVI, I = 56d(^5)</td>
<td>60 days</td>
<td>95%</td>
<td>5</td>
</tr>
<tr>
<td>LVI(^3)(_{30_95})</td>
<td>LVI</td>
<td>30 days</td>
<td>95%</td>
<td>5</td>
</tr>
<tr>
<td>MLV(^6)(_{60_95})</td>
<td>MLV</td>
<td>60 days</td>
<td>95%</td>
<td>5</td>
</tr>
<tr>
<td>MLV(^6)(_{60_80})</td>
<td>MLV</td>
<td>60 days</td>
<td>80%</td>
<td>5</td>
</tr>
<tr>
<td>MLV(^6)(_{60_70})</td>
<td>MLV</td>
<td>60 days</td>
<td>70%</td>
<td>5</td>
</tr>
<tr>
<td>MLV(^6)(_{30_95})</td>
<td>MLV</td>
<td>30 days</td>
<td>95%</td>
<td>5</td>
</tr>
</tbody>
</table>

\(^1\)Each scenario consisted of 1,000 simulations
\(^2\)No control strategy in place
\(^3\)Live-virus inoculation, assuming the field virus strain is known and can be isolated, and is homologous to future virus challenge
\(^4\)Duration of infectiousness follows a triangular distribution, with a mode of 56 days, a minimum of 7 days and a maximum of 250 days
\(^5\)Duration of infectiousness fixed at 56 days
\(^6\)Modified-live attenuated vaccination assuming heterologous immunity
Table 5.2. Definition of parameters and values used for model simulations, and their references

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value (unit)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Contact rate</td>
<td>Triangular distribution, mode 5 contacts/ day, min 2 contacts/ day and 15 max contacts/ day</td>
<td>Expert opinion</td>
</tr>
<tr>
<td>Duration of infectiousness</td>
<td>Triangular distribution, mode 56 days, min 7 days and max 250 days</td>
<td>Nodelijk et al., 2000; Wills et al., 2003, Expert opinion/Linhares et al., 2012</td>
</tr>
<tr>
<td>Duration of infectiousness for vaccinated animals</td>
<td>Triangular distribution, 30% reduction: mode 39 days, min 7 days and max 175 days</td>
<td>Expert opinion/Jeong et al. 2014, Expert opinion</td>
</tr>
<tr>
<td>Duration of infectiousness of vaccine virus strain</td>
<td>Triangular distribution, mode 56 days, min 7 days and max 250 days</td>
<td>Fangman et al., 2007</td>
</tr>
<tr>
<td>Duration of immunity</td>
<td>Pert distribution, mode 252 days, min 182 days and max 364 days</td>
<td>Evans et al., 2010, Expert opinion</td>
</tr>
<tr>
<td>Duration of immunity from the vaccine for vaccinated animals</td>
<td>120 days</td>
<td>Expert opinion, vaccine labels/Expert opinion</td>
</tr>
<tr>
<td>Probability of infection with a field strain for naïve animals</td>
<td>40%</td>
<td>Hermann et al., 2005</td>
</tr>
<tr>
<td>Probability of infection with a field strain for vaccinated animals</td>
<td>50% reduction of 40% = 20%</td>
<td>Expert opinion</td>
</tr>
<tr>
<td>Replacement Rate</td>
<td>50%</td>
<td>Expert opinion</td>
</tr>
<tr>
<td>Immunization efficacy</td>
<td>95%</td>
<td>Expert opinion</td>
</tr>
<tr>
<td>Number of piglets weaned per susceptible/immune/vaccinated sows</td>
<td>12 piglets</td>
<td>Jeong et al., 2014</td>
</tr>
<tr>
<td>Number of piglets weaned per infected sow</td>
<td>6 piglets</td>
<td>Jeong et al., 2014</td>
</tr>
</tbody>
</table>

1 Sensitivity analysis conducted for the modified-virus vaccination scenario
Table 5.3. Percentage of simulations and resulting maximum numbers of infected females (at one point-in-time) for all scenarios evaluated in the developed hybrid stochastic model, considering a period of two years. E.g.: for baseline scenario with one infected gilt being introduced, 8% of the simulations produced a maximum number of infected female pigs of 800 or more.

<table>
<thead>
<tr>
<th>Scenario</th>
<th>1-10</th>
<th>11-50</th>
<th>51-100</th>
<th>101-200</th>
<th>201-300</th>
<th>301-400</th>
<th>401-500</th>
<th>501-600</th>
<th>601-700</th>
<th>701-800</th>
<th>&gt;800</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline_1</td>
<td>53.3</td>
<td>30.0</td>
<td>7.5</td>
<td>1.2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>8.0</td>
</tr>
<tr>
<td>Baseline_5</td>
<td>3.0</td>
<td>25.3</td>
<td>36.4</td>
<td>8.2</td>
<td>0.1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>27.1</td>
</tr>
<tr>
<td>Baseline_10</td>
<td>0.1</td>
<td>4.7</td>
<td>29.4</td>
<td>20.9</td>
<td>0.3</td>
<td>0.3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.1</td>
<td>44.2</td>
</tr>
<tr>
<td>LVI_30_95</td>
<td>82.3</td>
<td>17.0</td>
<td>0.1</td>
<td>0</td>
<td>0.6</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>LVI_60_95_a</td>
<td>81.5</td>
<td>17.9</td>
<td>0.1</td>
<td>0.5</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>LVI_60_95_b</td>
<td>68.5</td>
<td>29.9</td>
<td>0.3</td>
<td>0</td>
<td>0.3</td>
<td>0.7</td>
<td>0.3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>MLV_60_95</td>
<td>5.4</td>
<td>30.0</td>
<td>33.6</td>
<td>9.0</td>
<td>0.1</td>
<td>0.2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>21.7</td>
</tr>
<tr>
<td>MLV_60_80</td>
<td>4.9</td>
<td>29.4</td>
<td>35.3</td>
<td>7.2</td>
<td>0.1</td>
<td>0.1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>23.0</td>
</tr>
<tr>
<td>MLV_60_70</td>
<td>5.7</td>
<td>31.3</td>
<td>32.9</td>
<td>5.1</td>
<td>0.1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>24.9</td>
</tr>
<tr>
<td>MLV_30_95</td>
<td>4.6</td>
<td>30.4</td>
<td>36.1</td>
<td>6.0</td>
<td>0.2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.2</td>
<td>22.5</td>
</tr>
</tbody>
</table>

1For scenario definitions please refer to Table 5.1.
Table 5.4. Median (inter-quartile range), maximum and minimum incidence of infected piglets, and average total number of piglets produced, considering a one-year period

<table>
<thead>
<tr>
<th>Scenario</th>
<th>Median incidence (IQR)</th>
<th>Maximum incidence</th>
<th>Minimum incidence</th>
<th>Mean total number of piglets produced</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline_1</td>
<td>0.004% (0.23)</td>
<td>20.44%</td>
<td>0%</td>
<td>23,236</td>
</tr>
<tr>
<td>Baseline_5</td>
<td>0.63% (0.96)</td>
<td>27.70%</td>
<td>0%</td>
<td>22,946</td>
</tr>
<tr>
<td>Baseline_10</td>
<td>1.44% (8.52)</td>
<td>29.76%</td>
<td>0%</td>
<td>22,585</td>
</tr>
<tr>
<td>LVI²_30_95</td>
<td>0% (0.03)</td>
<td>7.37%</td>
<td>0%</td>
<td>22,438</td>
</tr>
<tr>
<td>LVI²_60_95_a</td>
<td>0% (0.03)</td>
<td>7.58%</td>
<td>0%</td>
<td>22,231</td>
</tr>
<tr>
<td>LVI²_60_95_b</td>
<td>0% (0.07)</td>
<td>8.31%</td>
<td>0%</td>
<td>22,757</td>
</tr>
<tr>
<td>MLV_60_95</td>
<td>0.45% (0.69)</td>
<td>25.79%</td>
<td>0%</td>
<td>23,050</td>
</tr>
<tr>
<td>MLV_60_80</td>
<td>0.47% (0.74)</td>
<td>23.83%</td>
<td>0%</td>
<td>22,961</td>
</tr>
<tr>
<td>MLV_60_70</td>
<td>0.49% (0.72)</td>
<td>24.65%</td>
<td>0%</td>
<td>22,969</td>
</tr>
<tr>
<td>MLV_30_95</td>
<td>0.50% (0.61)</td>
<td>22.85%</td>
<td>0%</td>
<td>23,120</td>
</tr>
</tbody>
</table>

1Considered 1,000 simulations for each scenario. For scenario definitions please refer to Table 5.1.
2For LVI scenario, piglets were counted as infected if they were infected by the challenge virus only, therefore this measure might be underestimated.
Figure 5.1. Immunological state of animals (female pigs and piglets) for the different control scenarios, and baseline.

A. Baseline and live-virus inoculation scenario for female pigs; B. Modified-live vaccine scenario for female pigs and C. Immunological state for piglets (all scenarios).

1Duration followed a distribution, please refer to Table 5.2.
Figure 5.2. Schematic of the farrow-to-wean swine herd used as a model showing pig flow and spatial structure assumed in the developed hybrid stochastic model.
Figure 5.3. Porcine reproductive and respiratory syndrome dynamic as predicted by the model for different scenarios after virus introduction. Figures contain 100 simulations only for easier visualization.
A. Baseline 1; B. Baseline 5; C. Baseline 10; D. Live-virus inoculation, 30d acclimation; E. Live-virus inoculation, 60d acclimation; F. Live-virus inoculation, 60d acclimation, 56d for duration of infectiousness; G. Modified-live vaccine scenario, 95% immunization efficacy and 60 days of duration of acclimation; H. Modified-live vaccine scenario, 80% immunization efficacy and 60 days of duration of acclimation; I. Modified-live vaccine scenario, 70% immunization efficacy and 60 days of duration of acclimation; J. Modified-live vaccine scenario, 95% immunization efficacy and 30 days of duration of acclimation.
Figure 5.4. Boxplot graphs and histograms showing model piglet-level outcomes for different PRRSV control scenarios.

Left to right: Log of the incidence of infected weaned piglets for four cycles, with each cycle representing 3 months and log of yearly incidence of infected weaned piglets.
A. Baseline 1; B. Baseline 5; C. Baseline 10; D. Live-virus inoculation, 30d acclimation; E. Live-virus inoculation, 60d acclimation; F. Live-virus inoculation, 60d acclimation, 56d for duration of infectiousness; G. Modified-live vaccine scenario, 95% immunization efficacy and 60 days of duration of acclimation; H. Modified-live vaccine scenario, 95% immunization efficacy and 30 days of duration of acclimation; I. Modified-live vaccine scenario, 70% immunization efficacy and 60 days of duration of acclimation.
Figure 5.5. Boxplot graphs and histograms showing female-level outcomes for different PRRSV control scenarios.

Left to right: histogram and boxplot (log scale) of maximum number of female pigs infected over a period of two years.
A. Baseline 1; B. Baseline 5; C. Baseline 10; D. Live-virus inoculation, 30d acclimation; E. Live-virus inoculation, 60d acclimation; F. Live-virus inoculation, 60d acclimation, 56d for duration of infectiousness; G. Modified-live vaccine scenario, 95% immunization efficacy and 60 days of duration of acclimation; H. Modified-live vaccine scenario, 95% immunization efficacy and 30 days of duration of acclimation; I. Modified-live vaccine scenario, 70% immunization efficacy and 60 days of duration of acclimation.
CHAPTER 6: Development of a stochastic agent-based model to evaluate surveillance strategies for detection of emergent porcine reproductive and respiratory syndrome virus strains

Submitted to BMC Veterinary Research, Nov 2015
6.1. Abstract

The objective of the current study was to develop a stochastic agent-based model using empirical data from Ontario (Canada) swine sites in order to evaluate different surveillance strategies for detection of emerging porcine reproductive and respiratory syndrome virus (PRRSV) strains at the regional level. Four different strategies were evaluated, including (i) random sampling of fixed numbers of swine sites monthly; (ii) risk-based sampling of fixed numbers, specifically of breeding sites (high-consequence sites); (iii) risk-based sampling of fixed numbers of low biosecurity sites (high-risk); and (iv) risk-based sampling of breeding sites characterized as low biosecurity sites (high-risk/high-consequence).

The model simulated transmission of a hypothetical emerging PRRSV strain between swine sites through three important industry networks (production system, truck and feed networks) while considering sites’ underlying immunity due to past or recent exposure to heterologous PRRSV strains, as well as the different likelihood of infection due to demographic, geographic and biosecurity-related PRRS risk factors. Outcomes of interest included surveillance system sensitivity and time to detection of three first cases over a period of approximately two years. Results showed that surveillance system sensitivities were low and time to detection of three first cases were long across all examined scenarios. In conclusion, none of the four strategies compared herein appeared optimal for early detection of a novel infection at the regional level considering model assumptions, the underlying population of interest, and absence of other forms of surveillance. Traditional modes of implementing high-risk and high-consequence risk-based surveillance strategies based on site’s static characteristics do not appear to substantially improve surveillance system sensitivity; therefore, novel strategies need to be developed and considered for rapid detection of this and other emerging swine infectious diseases.
6.2. Introduction

Porcine reproductive and respiratory syndrome (PRRS) is an endemic infectious swine disease caused by an RNA virus and is responsible for considerable economic impacts in North America and many European countries (Holtkamp et al., 2013; Nieuwenhuis et al., 2012). The syndrome is characterized by decreased growth in pigs across all ages (mainly due to respiratory disease and secondary infections) and reproductive failure in adult female pigs. Even though strategies for PRRS control and elimination have been previously investigated and described (Corzo et al., 2010), it remains a challenge for the swine industry. Recognized factors related to the features of the current North American swine industry that contribute to the maintenance of PRRS within a country or region include the connectivity between swine sites through multiple dynamic networks, the constant exchange of subpopulations of animals that are at higher risk for disease transmission between sites (e.g. weaned piglets and culled sows), the high turnover rates of animals within farms, and the segregated nature of the different phases of production (Perez et al., 2015).

Currently in Canada the main approach for PRRSV surveillance is through submission of specimens from suspected clinical cases to diagnostic laboratories. This is commonly complemented by other activities that include: (i) detection of PRRSV cases through on-going monitoring when expected prevalence is low (e.g., nursery sites from specific production systems), (ii) certification of absence of infection using minimum pre-specified level, or (iii) specific regional studies or programs conducted to assess trends in disease prevalence or incidence over time. With the advent of regional disease control programs for PRRS, a frequently posed question relates to the design of effective surveillance strategies when one of the objectives is detection of cases due to circulation of a novel PRRSV strain. Even though the
use of random sampling is the only acceptable approach for proper estimation of disease frequency, risk-based surveillance strategies have been widely used and justified as effective and efficient strategies when the primary goal is to detect a pathogen. The main idea behind this approach is that it targets subpopulations of animals that are at increased risk for the occurrence of infection due to the presence of known risk factors (Stark et al., 2006). Although implementation of risk-based approaches to sampling in food veterinary medicine is relatively frequent under field conditions, its quantitative assessment has been relatively limited to cases of supporting declaration of freedom from infection in a jurisdiction, or for comparisons between alternative surveillance strategies before a recommendation is made. Typically, assessment of surveillance systems has been accomplished using stochastic scenario tree modelling (STM). This methodology models the process of disease detection while including all factors that affect probability of infection or detection of a surveillance system (Martin et al., 2007). Risk-based approaches could be considered when detection of new infections is important when dealing with diseases that are endemic in certain region(s). However, the framework for the quantitative assessment of alternative surveillance approaches for such situation is not well described in the relevant literature. Velasova et al. (2012) used a stochastic STM approach to evaluate the expected performance of a passive monitoring system for detection of novel strains of PRRSV in the United Kingdom. Even though STM seems like a logical extension when the main objective is detection of cases due to circulation of a novel PRRSV strain for endemic situations, its uniform application in all situations is limited due to two main reasons. Firstly, the goals and approaches to surveillance within endemic scenarios could vary greatly, and secondly the modern swine production systems are hierarchically structured and networks have been identified as important contributors to disease spread (Kwong et al., 2013; Arruda et al., 2015b),
representing yet another layer of risk factors. These risk factors would be difficult to incorporate in a typical STM, and an alternative is to develop agent-based models (ABM) with swine sites as agents. Such an approach is particularly appealing in the context of disease control projects because observed data on risk factors can be easily incorporated at the site level, together with contact structure among swine sites. In addition, performance of high-risk surveillance strategy could be compared to high-consequence surveillance strategies, as explained by Cameron (2012) in a natural manner since the directed flow of infected animals could be incorporated in such models explicitly.

The objective of the current study was to develop a stochastic ABM that would allow for the evaluation of different surveillance strategies for detection of emerging PRRSV strains at the regional level. Four different surveillance strategies were evaluated, including (i) random sampling of fixed numbers of swine sites monthly; (ii) risk-based sampling of fixed numbers of specifically breeding swine sites (high-consequence sites); (iii) risk-based sampling of fixed numbers of low biosecurity sites (high-risk); and (iv) risk-based sampling of breeding sites that are also characterized as low biosecurity sites (high-risk/ high-consequence sites). The main outcome of interest was the sensitivity of the surveillance systems evaluated, i.e. the probability of the systems in detecting infected sites. Furthermore, the time to detection of the three first cases was also described, and sensitivity analysis was conducted to evaluate the impact of the target design prevalence (level of disease that the system aims to detect) used for sample size calculation (1%, 2% or 5%) in the main outcomes of interest.

6.3. Materials and Methods

A stochastic agent-based model was created and implemented using the software Anylogic®
version 7.1.2 (XJ Technologies, St Petersburg, Russia). The model description outlined in the next paragraphs follows general reporting guidelines from the standardized overview, design concepts, and details (ODD) protocol as described by Grimm et al. (2006).

6.3.1. Purpose

The main purpose of the model was to simulate the dynamics of a representative sample of Ontario swine sites and the spread of PRRSV among those sites using empirical data from the PRRS ARC&E projects in Ontario, Canada. The model would allow for the evaluation of regional PRRS surveillance strategies to detect emerging PRRS virus cases within this population of interest.

6.3.2. State variables and scales

The agent modelled herein was the swine site therefore this was the unit of analysis. There were 816 swine sites characterized by mutually exclusive PRRS immunological status that included ‘completely susceptible’, ‘partially susceptible’, and ‘completely immune’. The nomenclature given to this so-called ‘immunity status’ refers to the likelihood of infection with the new virus strain that was purposely introduced as a ‘challenge’ in order to evaluate the surveillance strategies. Detailed rules for such classification at model start are presented on Table 6.1. In summary, the ‘completely susceptible’ compartment corresponded to swine sites containing a naïve animal population, characterized by being both seronegative and virus negative, therefore reflective of no previous or current PRRSV exposure. The ‘partially susceptible’ compartment corresponded to swine sites that were serologically positive or positive by virus detection, which meant that the population of animals within these sites was at a lower risk for infection with a new PRRSV strain compared to the naïve population due to partial immunity conferred by antibodies produced against a heterologous PRRSV strain. As a proportion of swine sites may
choose to eliminate PRRS viruses from their herds (i.e. move from the compartment ‘partially susceptible’ to ‘completely susceptible’), and as a proportion of negative sites get infected yearly according to a baseline PRRS incidence rate (i.e. move from the compartment ‘completely susceptible’ to ‘partially susceptible’), immunity statuses were allowed to change over time (Figure 6.1).

At time zero of model runs (model start), none of the sites were classified as ‘completely immune’ because the virus introduced into the population was considered completely new to the population of animals within sites. Swine sites would transition into that immunological compartment if animals within those sites were infected with the new virus strain as the model progressed. In this case, it was assumed that a sufficient percentage of animals were exposed to the new virus and sufficiently protected so that the site was considered ‘completely immune’ for a limited period of time, and therefore could not get re-infected while in this compartment. Sites could also opt for and successfully eliminate the virus and return to the ‘completely susceptible’ compartment at rates that varied according to production type (Table 6.2).

Swine sites were given characteristics, commonly referred to as parameters, which were extracted from the Ontario Swine Health Advisory Board regional control program database from a standardized questionnaire answered by swine producers as they enrolled in the project. These parameters, unlike the site immune status, were modelled as being static through the ‘Options list’ feature in Anylogic®, and therefore were not allowed to change over time.

Characteristics considered included animal flow (continuous animal flow or all-in all-out animal flow), number of neighbors (categorized into zero neighbors, one to five neighbors, and more than five neighbors), presence of a shower in facility (yes or no), number of animals (categorized into up to 500 animals, from 500 to 2,000 animals, and more than 2,000 animals) and production type.
type (breeding site, nursery site, and growing pig site, the last included wean-to-finish and
finishing operations). For a relatively small percentage of sites, the information regarding one or
more parameters was missing, and for those the model was set to randomly assign a category for
the parameters. This occurred for 85 sites (10%) for the parameter ‘presence of shower facility’,
23 sites (3%) for number of animals, and 217 sites (27%) for animal flow. These characteristics
were selected due to availability of information and because they have been previously reported
as risk factors for PRRS (Lambert et al., 2012; Pitkin et al., 2009). The increase or decrease in
the risk of PRRSV infection according to those risk factors was specified at model start
according to values found in Table 6.2. The risk of infection was calculated for each swine site
considering all site-level characteristics once the site was ‘exposed’ to the new virus.
Finally, swine sites were eligible to be part of up to three different networks- production system,
truck and feed networks. A site was considered to be connected to another site within each of
these networks if the swine producer had named a common ownership structure (for production
system), a common transportation company (for the truck network), and a common feed
company (for the feed network) as other(s) swine producer(s). This information was collected
during administration of the same questionnaire previously mentioned and corresponded to
‘static’ relationships (no frequency of contact information collected). A simplified visualization
of the model with site characteristics and network connections is shown in Figure 6.2.

6.3.3. Process overview and scheduling

The model proceeded in daily time steps, with the new PRRSV strain introduced on day 3 after
model start and a follow-up time of 700 days. It was assumed that this population of swine sites
was stable for the time evaluated, with no new swine sites being added or removed from the
population. At model start, surveillance system screenings were set to occur through Anylogic©
‘events’ triggered at regular intervals (monthly, or every 30 days) starting on day zero. The number of swine sites to be checked (inspected) for infection with the emerging PRRSV depended on the pre-determined design prevalence (Table 6.3).

6.3.4. Design concepts

Interactions between agents (swine sites) were modeled using the three above-mentioned networks. At model start, networks were loaded in Anylogic® as symmetric matrices extracted from network analysis using UCINET 6 (Borgatti et al., 2002). Networks were represented separately, and the frequency of indirect contact between swine sites through these networks was considered more intense for the production system network (a contact was assumed to occur one time per week), followed by the truck network (one contact every two weeks) and the feed network (one contact per month). The role of the production system network reflected the movement of people, personnel, equipment, and animals between sites, and two sites that were linked through this network could be connected directly or indirectly, depending on the direction of the movement (considering site type). For the truck and feed networks, however, connections between sites were always indirect, and estimation of their frequency is considerably challenging. Due to the type of service involved (movement of genetic stock, deadstock, slaughterhouse transportation, besides others for the truck network; and delivery of feed for the feed network), it was assumed that the truck network would be heavier in the “disease transmission scale” compared to the feed network.

Transmission of disease between sites was modelled through communications via the use of Anylogic® ‘messages’ reflecting exposure to the virus (opportunity for infection) that could result in infection or not. The virus was introduced into the population of sites through random selection of one site in the population to be infected. Following this, the infected site would
expose other sites within its networks at the frequencies established above. At receipt an ‘exposure’, non-infected swine sites characteristics were considered and a stochastic process was carried out using the baseline probability of infection of 10% per year to determine whether a site would be infected or not. This described process was modelled using compartments within a state chart as shown in Figure 6.1. It is important to note that, uniquely for the production system network, the direction of ‘exposure’ was taken into account. The production type for the site being the source of the ‘message’ (the infected site exposing others, or the sender of the ‘message’) was assessed, as well as the production type of site that was receiving the exposure (site at risk for infection, or receiver of the ‘message’). In cases where the exposure was between two sites of the same production type, the risk of infection was considered relatively small, since it would be reflective of indirect transmission through sharing of site personnel, equipment, etc. On the other hand, when the direction of exposure was from a breeding herd to a nursery or growing pig operation, as well as if it was from a nursery to a growing pig operation, the likelihood of infection was assumed to be higher due to the fact that there could be movement of animals involved in such direction, which would be indicative of direct PRRSV transmission.

6.3.5. Initialization

The evaluation of each regional surveillance strategy began after the challenge-virus introduction on day 3 for each simulation. The model ran for three days before virus introduction to assure loading of network and parameters datasets.

6.3.6. Inputs

Model inputs were obtained from the peer-reviewed literature whenever possible, and when not available, obtained from discussions with experts in the area of swine production. A list of inputs used in the model is shown in Table 6.2.
6.3.7. Model calibration and statistical analysis of outcomes

The model was calibrated to reproduce plausible values in terms of the underlying immunological status of the population for an endemic disease such as PRRS in North America. The aim was to produce a mean PRRS prevalence between 30 and 40% within the two-year period evaluated. There is no available information to the knowledge of the authors concerning the quantification of system sensitivity for any surveillance strategy; therefore, it was unfeasible to compare current model results with expected outcomes. The outcomes measured herein included daily total number of sites that were infected with the new PRRS virus strain, total number of infected sites that were detected and total number of non-infected sites. Data processing was conducted using Excel® and Stata 13; descriptive analyses, surveillance sensitivities, Kaplan-Meier survival functions and median survival times were estimated and calculated using Stata 13.

6.3.8. Simulation experiments

A total of 30 simulations were run for each of the evaluated scenarios, and this number was limited due to the fact that the ABM constructed was computationally demanding (likely due to extensive nature of multiple networks) and a maximum of three simulations could be run per time. Twelve scenarios were investigated, as described on Table 6.3. As previously mentioned under ‘process overview and scheduling’, site surveillance was programmed through cyclic events. Four main scenarios were investigated that are detailed in the following paragraphs, and the fixed number of swine sites to be sampled varied according to the desired design prevalence: 1% (n = 23 sites per month), 2% (n = 13 sites per month), or 5% (n = five sites per month). Sample sizes were calculated with the objective of demonstrating freedom of infection at the population level, and were calculated using the online tool “FreeCalc” (AusVet Animal Health
Services\textsuperscript{©} considering 100\% herd level specificity, 95\% herd level sensitivity, a population size of 816 and the modified hypergeometric exact calculation method. For all scenarios, it was assumed that, even when a swine site was detected with the new PRRSV strain, transmission to other sites was still possible which is reflective of field conditions for PRRSV and other endemic viral pathogens. It was further assumed that sampling size requirements for detection of PRRSV within herds was met in all cases (no false negatives among positive sites selected for sampling and sufficient number of animals sampled to detect pre-specified within herd design prevalence).

**Baseline scenario**

The baseline scenario consisted of random monthly sampling of fixed numbers of swine sites (varying according to desired design prevalence), based on sampling with replacement (if a swine site was not selected, it would be back in the pool and eligible for selection in the next sampling event).

**High-risk scenario**

The high-risk scenario targeted as sampling units swine sites that had low biosecurity measures: a combination of both continuous animal flow and the absence of a shower facility in the site. The sampling consisted of a random sampling of fixed numbers of swine sites (varying according to desired design prevalence) that met both criteria described above (n = 342, number is approximate due to the fact that sites with missing information were randomly assigned to an equal distribution of the possible responses).

**High-consequence scenario**

The high-consequence scenario targeted as sampling units swine sites that were considered to have high consequence if infected, i.e. they could potentially spread disease to multiple sites. This population of sites corresponded to breeding herds (n = 259).
High-risk/ high-consequence scenario

The final scenario corresponded to the targeted sampling of sites that met both high-risk/high-consequence criteria, therefore the eligible pool were sites that were breeding herds with low biosecurity (both absence of a shower facility and continuous animal flow, (number of eligible sites varied from 62 to 157, depending on how sites with missing information were assigned to characteristics during model runs). For all evaluated scenarios, once sites were detected as infected by the surveillance system, they were excluded from the pool of sites eligible for selection.

6.4. Results and Discussion

The model developed herein simulates transmission of a hypothetical emerging PRRSV strain between swine sites through three important industry networks; production system, truck and feed networks. It is particularly novel because it further accounts for sites’ underlying immunity due to past or recent exposure to heterologous PRRSV strains, and for the different likelihood of infection due to previously described demographic, geographic and biosecurity-related PRRS risk factors (Lambert et al., 2012; Pitkin et al., 2009). The present study fulfilled its main objective of development of a tool for the evaluation of surveillance systems for situations where emergent cases (e.g. emerging genotype) of a certain disease are to be detected within a specific area in which the disease is already endemic; in such the emphasis of the system should be on timely detection of new cases (Cano et al., 2012). Many times such evaluation is both financially and logistically unfeasible to complete under field conditions.

Results from the model were that, under the conditions specified, all evaluated surveillance strategies showed relatively low overall mean sensitivity in detecting a new emergent PRRSV
strain over an approximate two-year period (Table 6.4). It is, however, important to note that surveillance sensitivity distribution across simulations within the different surveillance strategies was highly right-skewed, with a small number of simulations yielding high sensitivity for almost all strategies (Figures 6.3 and 6.4). As expected, as design prevalence decreased (and number of sites sampled per month increased), system sensitivity also tended to increase, which is reasonable given the increase in the likelihood of sampling infected sites. Interestingly, this observation was not evident for the high-risk or high-risk/ high-consequence scenarios (Table 6.4, Figures 6.3 and 6.4). To the best knowledge of the authors, there is no available information on quantification of active surveillance system sensitivity for PRRS in endemic disease contexts that would allow for comparison of current study results.

While there are numerous arguments in favor of risk-based sampling for disease detection (Reist et al., 2012), the low surveillance system sensitivities found in this study for all the risk-based sampling scenarios, as implemented in this study, was not unexpected to the authors, considering what has been previously reported on the occurrence of PRRS within the Ontario swine industry. Previous work has shown that given the unique nature of such an industry, focusing on demographics or biosecurity characteristics of individual sites for risk-based surveillance would not yield the most impactful strategies because the most important determinant of PRRS status has been reported to be the production system, and not site characteristics on their own (Arruda et al., 2015b). The authors suggest that the target of risk-based sampling needs to be reconsidered and strategies need to be developed considering how production systems are connected, the importance of sites in the different networks, and the number of sites within network components. Additionally, it would be worthwhile to evaluate the manner by which downstream site status should be handled in cases where breeding sites are detected as infected. The current
approach for PRRS ARC&E projects in Ontario is to automatically declare downstream sites from positive breeding herds as positive by animal flow, a measure that can result in false positive classifications. An alternative would be to prioritize sampling of downstream growing pig sites, action which could result in rapid depletion of resources in the case of breeding sites from large production systems are infected. These additional scenarios were not evaluated herein, but are examples of a future research area that is very applicable under field conditions.

Kaplan-Meier survival functions showed that for both design prevalence of 1% and 2%, the random sampling surveillance strategy was the one for which detection of the three first cases was faster over an approximate time period of two years when compared to the other strategies, while the high-consequence strategy was the best for the 5% design prevalence scenario (Figure 6.5). The authors do not have a plausible explanation for this finding, and speculate that a higher number of simulations are necessary in order to separate this from chance variation. The median survival time, interpreted as the time by which 50% of the eligible simulations (simulations with at least three cases) achieved detection of a minimum number of three cases, however, was never reached for this last scenario, which supports the fact that, regardless of the strategy examined, if sample size is limited, prompt detection of potential outbreak cases will very likely not occur (considering such sampling is the only strategy in place to detect new infection cases). Under the 1% design prevalence scenario, median survival time was 301 days for the random sampling strategy and 481 days for both the high-consequence and high-risk scenarios. For the 2% prevalence scenario, the median survival time was 361 days for the random sampling strategy, 556 days for the high-consequence scenario, and was never reached for the high-risk scenario.

The high-risk/ high-consequence strategy never guaranteed that 50% of eligible simulations had at least three cases detected, independently of which design prevalence was employed. In
general, all survival times for examined scenarios were long, therefore we conclude that none of the strategies compared herein were optimal for early detection of this disease, considering model assumptions, the underlying population considered, and absence of other forms of surveillance.

There are important limitations in the current study that need to be acknowledged. Firstly, data were obtained from a limited portion of the Ontario swine industry (estimated at approximately 30% of all sites), and therefore the service providers network is not complete. Even though there is no reason to believe that the sample of sites are selectively biased in any form, the possibility exists that connections from absent sites could potentially change the structure in meaningful ways, and that could impact disease spread in unpredictable ways. Given this limitation, it is important to re-state that the focus of this study was primarily the development of the methodology and its prospects. Another important issue is the fact that a larger number of simulations could not be carried out due to computational constraints. A higher number of simulations would increase our confidence in the results and potentially decrease variability and the likelihood of finding certain results merely due to chance.

Passive surveillance systems were not taken into consideration in the current study, and in the case where the challenge PRRSV was a highly virulent strain, the role of passive surveillance might have been particularly important and increased disease recognition and control efforts. However, sensitivity of passive surveillance is difficult to estimate. In England, the probability of infected farms being detected through passive surveillance for PRRS was reported as low, with a mode of 7.4% assuming 35% active PRRS infection prevalence (Velasova et al., 2012). This varied when regional pig density and use of vaccination were considered, with farms in a low pig density area and not using a vaccine having the lowest detection probability. Sensitivity analysis
conducted in that study showed that an important parameter, as expected, was the probability that an infected pig would show clinical signs (Velasova et al., 2012). In a different context, during detection of the PRRS outbreak in Sweden in 2007 (Frossling et al., 2009), active surveillance had a major role, and that particular outbreak was detected from the annual surveillance program and not due to clinical suspicion, even though animals from the whole country were naïve to the virus. The authors of the current study were interested not in quantifying the passive surveillance system per se, but in evaluating which of other active surveillance strategies would optimize disease detection.

Lastly, an important assumption of the model was that transmission was possible even after swine sites were detected as infected by the surveillance systems. The authors acknowledge that this is to a certain extent contradictory to the whole point of surveillance for early detection, but argue that it is a plausible assumption given the current structure of this dynamic industry. Most of the time it is simply logistically impossible to stop animal movement or coordinate and schedule service providers according to PRRS status in a timely matter. However, it is important to note that if detection were to in fact prevent transmission and mitigate risk, the system sensitivities calculated herein would have been underestimated.

Future directions for prospective projects include the expansion of this tool for evaluation of different surveillance scenarios. For example, one could propose an active surveillance strategy based on monthly risk-based slaughterhouse sampling and evaluate how that compares to current methodologies, as well as assess the cost-benefit of such and other approaches. Finally, the mathematical modelling approach supports collaboration between multiple branches such as the private sector, swine veterinarians, academic researchers and governmental agencies, since input and feedback is needed from all involved parts to test new hypotheses and strategies that are
relevant at the same time as logistically and economically viable to all mentioned parties. 
Eventually, the development of the methodology could be applied for other emerging pathogens, 
and within different regions of the country or country-wide.

6.5. Conclusions

In conclusion, the model developed herein integrates the knowledge of the complex swine 
industry and characteristics of PRRSV transmission between herds to develop a comprehensive 
framework that could be used to test other hypotheses in the future regarding surveillance 
approaches for this and other emerging swine infectious diseases.

6.6. Acknowledgements

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(OSHAB), the Canadian Swine Health Board (CSHB), Ontario Pork, the Agricultural Adaptation 
Council (AAC), and the Animal Health Laboratory (AHL) of the University of Guelph provided 
financial support for the PRRS ARC&E projects. The authors would also like to extend their 
appreciation to the participating swine producers, veterinarians and area leaders who assisted 
with sampling and data entry.
6.7. References


Table 6.1. Definition of site immune status according to the area regional control program and mathematical model assumptions for underlying immunity

<table>
<thead>
<tr>
<th>Immunity level</th>
<th>OSHAB classification</th>
<th>Serology(^2)</th>
<th>Virus Detection(^3)</th>
<th>Breeding Sites Comments</th>
<th>Growing Pig Sites Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Completely susceptible</td>
<td>Confirmed negative</td>
<td>Negative</td>
<td>Negative</td>
<td>Series testing</td>
<td>Series testing</td>
</tr>
<tr>
<td>Partially susceptible</td>
<td>Confirmed positive</td>
<td>At least one positive</td>
<td></td>
<td>Parallel testing</td>
<td>Parallel testing</td>
</tr>
<tr>
<td>Completely susceptible</td>
<td>Presumed negative</td>
<td>-</td>
<td>-</td>
<td>Sample size not met, sample of downstream growing pig sites confirmed positive by diagnostic test, veterinarian assessment of site</td>
<td>Sample size not met, sample of upstream sow sites</td>
</tr>
<tr>
<td>Partially Susceptible</td>
<td>Presumed positive</td>
<td>-</td>
<td>-</td>
<td>Downstream sites confirmed positive by diagnostic test, veterinarian assessment of site</td>
<td>Upstream sites confirmed positive by diagnostic test, veterinarian assessment of site</td>
</tr>
</tbody>
</table>

\(^1\)As defined by the current model (underlying swine site immunity)

\(^2\)Evidence of previous exposure to porcine reproductive and respiratory syndrome virus (PRRSV), measured through ELISA testing for antibody detection in serum or oral fluids

\(^3\)Evidence of current virus infection, measured via PCR in serum, oral fluids or tissue samples
Table 6.2. Definition of parameters and values used for model simulations

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value (unit)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time to PRRSV(^1) elimination for breeding sites</td>
<td>385 (days)(^2)</td>
</tr>
<tr>
<td>Time to PRRSV elimination for AIAO(^3) nurseries</td>
<td>56 (days)</td>
</tr>
<tr>
<td>Time to PRRSV elimination for AIAO finishers/wean-to-finish sites</td>
<td>112 (days)</td>
</tr>
<tr>
<td>Baseline probability of infection with PRRSV (new strain)</td>
<td>10% per year</td>
</tr>
<tr>
<td>Percent of the swine site “completely susceptible”</td>
<td>63%(^4)</td>
</tr>
<tr>
<td>Relative Risks for getting infected with PRRSV</td>
<td></td>
</tr>
<tr>
<td>Relative risk of getting infected with PRRSV for nurseries compared to breeding sites</td>
<td>1.2</td>
</tr>
<tr>
<td>Relative risk of getting infected with PRRSV for finishers/wean-to-finish sites compared to breeding sites</td>
<td>1.5</td>
</tr>
<tr>
<td>Relative risk of getting infected with PRRSV for sites with continuous flow compared to sites with AIAO</td>
<td>1.5</td>
</tr>
<tr>
<td>Relative risk of getting infected with PRRSV for sites with medium number of neighbours compared to sites with no neighbours</td>
<td>1.5</td>
</tr>
<tr>
<td>Relative risk of getting infected with PRRSV for sites with high number of neighbours compared to sites with no neighbours</td>
<td>2.0</td>
</tr>
<tr>
<td>Relative risk of getting infected with PRRSV for sites with medium number of animals compared to sites with reduced number of animals</td>
<td>1.5</td>
</tr>
<tr>
<td>Relative risk of getting infected with PRRSV for sites with high number of animals compared to sites with reduced number of animals</td>
<td>2.0</td>
</tr>
<tr>
<td>Relative risk of getting infected with PRRSV for naïve sites compared to sites with complete immunity</td>
<td>2.0</td>
</tr>
<tr>
<td>Relative risk of getting infected with PRRSV for sites infected with other PRRSV strains (partial immunity) compared to sites with complete immunity</td>
<td>1.5</td>
</tr>
</tbody>
</table>

\(^1\) Porcine reproductive and respiratory syndrome virus  
\(^2\) Linhares et al., 2014  
\(^3\) All-in, all-out animal flow  
\(^4\) Arruda et al., 2015a
Table 6.3. Description of porcine reproductive and respiratory syndrome site-level surveillance scenarios investigated in the developed agent-based stochastic model

<table>
<thead>
<tr>
<th>Scenario</th>
<th>Type of sampling</th>
<th>Design prevalence (number of sites sampled)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Random_1</td>
<td>Random</td>
<td>1% (23)</td>
</tr>
<tr>
<td>Random_2</td>
<td>Random</td>
<td>2% (13)</td>
</tr>
<tr>
<td>Random_5</td>
<td>Random</td>
<td>5% (5)</td>
</tr>
<tr>
<td>HR_1</td>
<td>Risk based - High-risk^2</td>
<td>1% (23)</td>
</tr>
<tr>
<td>HR_2</td>
<td>Risk-based - High-risk^2</td>
<td>2% (13)</td>
</tr>
<tr>
<td>HR_5</td>
<td>Risk-based - High-risk^2</td>
<td>5% (5)</td>
</tr>
<tr>
<td>HC_1</td>
<td>Risk-based - High-consequence^3</td>
<td>1% (23)</td>
</tr>
<tr>
<td>HC_2</td>
<td>Risk-based - High-consequence^3</td>
<td>2% (13)</td>
</tr>
<tr>
<td>HC_5</td>
<td>Risk-based - High-consequence^3</td>
<td>5% (5)</td>
</tr>
<tr>
<td>HR/HC_1</td>
<td>Risk-based - High-risk and high-consequence^4</td>
<td>1% (23)</td>
</tr>
<tr>
<td>HR/HC_2</td>
<td>Risk-based - High-risk and high-consequence^4</td>
<td>2% (13)</td>
</tr>
<tr>
<td>HR/HC_5</td>
<td>Risk-based - High-risk and high-consequence^4</td>
<td>5% (5)</td>
</tr>
</tbody>
</table>

^1Each simulation consisted of 1,000 simulations
^2High-risk based sampling consisted of sampling of swine sites that were considered at the highest risk of being infected: sites that had low biosecurity (no shower-in facility and continuous animal flow)
^3High-consequence based sampling consisted of sampling of swine sites that were considered at the highest risk of infecting other sites: breeding sites
^4High-risk and high-consequence based sampling consisted of sampling of swine sites that had a combination of the highest risk of getting infected as well as the highest risk of infecting others: sites that had low biosecurity (no shower-in facility and continuous animal flow) and that were breeding herds.
Table 6.4. Mean (%), standard deviation (%), minimum (%) and maximum (%) estimated surveillance system sensitivities, according to the different surveillance scenarios evaluated

<table>
<thead>
<tr>
<th>Scenario</th>
<th>Surveillance System Sensitivity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
</tr>
<tr>
<td>Random_1</td>
<td>17.60</td>
</tr>
<tr>
<td>Random_2</td>
<td>7.50</td>
</tr>
<tr>
<td>Random_5</td>
<td>10.75</td>
</tr>
<tr>
<td>HR_1</td>
<td>14.11</td>
</tr>
<tr>
<td>HR_2</td>
<td>18.17</td>
</tr>
<tr>
<td>HR_5</td>
<td>6.94</td>
</tr>
<tr>
<td>HC_1</td>
<td>20.41</td>
</tr>
<tr>
<td>HC_2</td>
<td>7.95</td>
</tr>
<tr>
<td>HC_5</td>
<td>3.65</td>
</tr>
<tr>
<td>HR/HR_1</td>
<td>7.17</td>
</tr>
<tr>
<td>HR/HC_2</td>
<td>7.94</td>
</tr>
<tr>
<td>HR/HC_5</td>
<td>9.95</td>
</tr>
</tbody>
</table>

1Each simulation consisted of 30 simulations; please refer to Table 6.3 for detailed scenario definitions

2Calculated as the fraction of number of cases detected divided by the total of infected cases, for each simulation, considering in a period of 700 days
Figure 6.1. Compartmental states for swine sites.

A. New infections and new detections state chart, and B. Underlying immunity state chart considering infection with other porcine reproductive and respiratory syndrome virus (PRRSV) strains
Figure 6.2. Model scheme using ten hypothetical swine sites characteristics and locations in Southern Ontario.

Underlying immunity of swine sites are not shown due to their dynamic nature. A link between swine sites represented a common service provider within the specific network.
Figure 6.3. Surveillance sensitivity boxplots for the total of simulations, by surveillance strategy.

HC: high consequence, HR: high risk, HRHC: high risk- high consequence.
Figure 6.4. Surveillance sensitivity distribution for the total of simulations, by surveillance strategy.

HC: high consequence, HR: high risk, HRHC: high risk - high consequence.
Figure 6.5. Kaplan-Meier survival functions according to different sample sizes calculated for 1% (A), 2% (B) and 5% (C) design prevalence, stratified by surveillance strategies.

HC: high consequence, HR: high risk, HR/HC: high risk-high consequence.
CHAPTER 7: Summary, limitations and future directions

The main overall objective of this thesis was to apply novel quantitative epidemiological methods to shed light on porcine reproductive and respiratory syndrome (PRRS) spread, control and prevention in the context of regional disease control efforts. Interestingly, in recent years, one might argue that the swine industry in the province of Ontario (Canada) has distinguished itself from many places in North America due to the fact that a large proportion of swine producers have shown interest, initiative and willingness to participate in regional control projects. This involved not only swine producers being transparent in delicate issues such as infectious disease status, but also embracing the idea and recognizing the importance of a unique identifier (referred to as ‘premises ID’) as the means for referring to swine sites, which was essential for cross-referencing data from different sources (such as diagnostic laboratories, producer-driven organization responsible for site location e.g. Ontario Pork, Guelph, ON, etc.).

Control programs can serve for the purpose of data collection (Perez et al., 2015), and this thesis showed that such data can be used to address a wide variety of objectives, ranging from the description of molecular diversity of viruses within regions to the characterization of important service provider networks. Data from this program can be extracted and summarized at both the swine site and production system levels, which provides valuable information that is rarely available elsewhere. Furthermore, it serves by allowing for the extraction of important disease, health and production-related parameters that can then be applied to inform models that aim to evaluate disease prevention and control, and surveillance strategies.

Very importantly, immediate uses of databases obtained from regional control programs involve disease investigations, the possibility of improving the speed of diagnosis of emerging infectious
diseases, and their further spread and prevention. One of the greatest lessons learnt throughout
the development of this thesis was that the importance of on-going collaboration and
transparency amongst researchers, the private sector (including pharmaceutical companies),
veterinarians and producers can never be underestimated; these efforts require time and
commitment on a routine basis. During the development of the projects within this piece of
work, constant communication between all above-mentioned parts of the swine industry was
attempted, including inputs from experienced professionals and extension of study findings to
the public of interest; therefore, changes and evolution of the control projects within the province
are somewhat captured as the chapters progress.

This thesis began with relevant background information on PRRS, emphasizing the scientific
knowledge gaps and challenges for its control and prevention. The subsequent chapter (chapter
two) introduced the area regional control and elimination (ARC&E) projects, characterizing
participating swine sites at that particular point in time for three regions in Ontario, as well as
describing the presence of spatial clusters and areas within each region where the risk of being
PRRS positive was higher compared to other parts of the region. Besides providing detailed,
previously non-existent information that allowed for characterization of part of the Ontario swine
industry, the most important message from that chapter was that “area spread” did not seem
evident for any of the three analyzed regions, at least not when molecular data were not taken
into account.

These findings, coupled with an increase in the occurrence of particular PRRS virus genotypes
within one particular region of Ontario, led to the development of chapter three. Swine
veterinarians and area leaders played an important role on the timely accomplishment of this
disease investigation, where the main objective was to evaluate how area spread and truck
networks were associated with the occurrence of specific PRRS virus genotypes, while accounting for the crucial fact that swine sites are clustered within production systems (which intuitively tend to have a similar PRRS virus status). Findings from this investigation supported the fact that importance of area spread and truck network membership on PRRS occurrence but that this differed according to genotype (defined by sequencing of the ORF5 gene); however, the proportion of PRRS cases in the population of swine sites that could be attributable to trucking membership for two of the important genotypes (so-called RFLPs 1-8-4 and 1-3-2) was noticeably high.

This observation led to the development of the fourth chapter, where a detailed description of static service provider networks (including truck, feed, boar, gilt and semen sources) was conducted. The most revealing observation from this work was that indirect relationships (many times uncaptured during typical investigations) between swine sites (through one or more networks) were responsible for connecting over 90% of all sites enrolled in the project. Furthermore, a novel approach for the investigation of potential risk factors for PRRS was taken, where ARC&E participating swine sites were grouped into categorical clusters according to network-related parameters extracted from network analysis and these clusters were offered as covariates to generalized linear mixed models. These models accounted for the clustering of swine sites within production systems, and they showed that this important level was responsible for explaining the larger amount of variability in the outcome PRRS status (positive or negative): over 70% for breeding and 80% for growing pig sites. This suggested that interventions for change or maintenance of PRRS status in a swine site would have the greatest impact when implemented at the production system level.

Considering all information gained from the previous chapters, it became an attractive option to
develop mathematical models that would be supported by previously extracted data, and that would simulate real-life scenarios in order to provide a range of outcomes for questions that would otherwise be impossible to address. Chapter five arose from an unanswered question commonly asked by swine veterinarians and producers in regards to farm-level interventions that aim for PRRS control in breeding herds. More specifically, the interest relied on what was the likelihood of the occurrence of outbreaks such as the ones described on Chapter three into swine herds that implement certain control measures. A hybrid model combining agent-based with discrete event modelling approaches was created in order to simulate a farrow-to-wean swine site and evaluate different PRRS control strategies in the case of post-PRRS outbreaks. Strategies of interest included the use of live-virus inoculation (LVI) and the use of a modified-live vaccine (MLV) target at the gilt population. The insights resulting from this model were valuable: the first finding was that outbreaks could potentially occur after long periods following initial virus introduction. This observation raises important points in regards to disease monitoring and has implications on outbreak investigations. Another interesting finding of this chapter was that regardless of the control strategy applied at the site (LVI or MLV); neither was able to prevent novel virus circulation in the piglet population, even though that seemed to occur at low levels. This suggested that other farrowing room or piglet-level strategies should be considered in order to mitigate risk and should be evaluated in future studies.

Finally, the last research chapter (chapter six) arose from the industry need to improve surveillance strategies for PRRS at the regional level. The main purposes of surveillance in the context of PRRS being an endemic disease are to identify new PRRS cases, describe trends of disease over time, declare freedom from disease at a regional level, and advance the management of existing cases. The developed agent-based model aimed to build a framework to investigate
options for surveillance, ideally the perfect strategy would be cost-effective while prioritizing
detection of emerging infections (considering the endemic characteristic of PRRS in North
America). The model that was developed considered some important aspects of the complex
structure of the current North America swine industry; as a few examples diagnostic data
available from the regional control projects was used to account for underlying immunity of
swine sites, and networks were used to describe ways in which disease could potentially spread.
This proposed framework presents high flexibility potential and could be further developed and
modified as further information become available and as surveillance approaches change over
time. The main take home message from this regional model was that traditional modes of
implementing high-risk and high-consequence risk-based surveillance strategies based on site’s
characteristics do not appear to substantially improve surveillance system sensitivity; therefore,
novel strategies need to be developed and considered for rapid detection of this and other
emerging swine infectious diseases.

Even though these and all mathematical models inherently present a certain degree of
assumptions and limitations, important features of disease and herd dynamics could be
incorporated, and the author speculates that these models are to become more flexible and
realistic as modelling software develop, which suggests that their continued use in infections
disease research is promising in the near future.

It is important to acknowledge that because most chapters of this thesis relied on data captured
originally for a different main purpose, a few limitations were anticipated. Most importantly, the
lack of a systematic update on demographics, biosecurity and network information from ARC&E
participating swine site for various reasons (including those related to logistics, lack of resources
and time from involved personnel, etc.) made it difficult to investigate causal associations
between disease status and site-related predictors of interest. Similarly, the lack of diagnostic sampling for a large number of swine sites was an issue. In order to decrease the degree of lack of information at the same time as maintaining high levels of site recruitment, a ‘presumed’ status was introduced early during the projects, for which site veterinarians were allowed to presume a certain status (positive or negative) to swine sites based on their knowledge on the site history and/or available diagnostic testing from upstream or downstream sites. This approach presents obvious risks of misclassification of PRRS status, which cannot be ignored but at the same time is difficult to estimate. Overcoming this problem will be a challenge in the upcoming years.

Even though this particular voluntary program offered incentives for swine veterinarians and producers for testing their animals and establishing a PRRS virus status as herds were enrolled, high percentages of non-testing were observed. It is believed that the main reason for such fact was that field veterinarians for the most part aim for efficiency when sampling in the field. This means that in cases where sampling a specific site can actually provide diagnostic information for multiple sites, this would likely be the preferred choice. As a few examples, sampling animals at the end of the nursery would allow for the definition of status for different finisher sites that those animals might be sent to; or sampling animals that just arrived to a certain nursery from different breeding sites would potentially allow for the definition of PRRS status for those sites. This tends to occur especially in cases where there is a known link between sites, as shown in the examples with animal movement, or sharing of barn personnel/equipment.

Considering the fact that financial incentives currently available are likely not long-term, cost is a factor that is equally likely to play an important role. Finally, the lack of submission of available diagnostic results cannot be ruled out. The gathering of such information from
producers and laboratories, as well as data entry, require a certain amount of resources, which sometimes may be scarce or directed to other important areas. The recognition of the importance in maintaining accurate site statuses for disease control and prevention at the regional level must be a priority for the continuity of success from the control programs.

Even though, as mentioned, there was a large proportion of swine sites with ‘unknown’ or ‘presumed’ PRRS status (27% unknown, 36 % presumed), it is important to acknowledge that the Ontario ARC&E control program PRRS case definition (confirmed status) is relatively stringent (Appendix 1). Diagnostic data extracted from all sites enrolled in the projects showed that a great amount of diagnostic testing was conducted over the past five years (total of 848 test results were submitted). There were test results for 485 sites (59% of all sites); and the majority of those sites (323 sites, or 66% of 485) had one submission, 17% (80 sites) had two submissions, 14% (68 sites) had between three and five submissions, and 3% (14 sites) had between six to 14 submissions (Figure 7.1). Stratification of number of submissions per site according to PRRS status is provided on Figure 7.2. Interestingly, for sites with presumed status (either positive or negative), the mean number of submissions per sites was over 0.70 (Table 7.1), which indicates that, even though those sites did not meet the requirements for the definition of confirmed status (Appendix 1), a considerable number of diagnostic tests were submitted. The author speculates that the main reason for sites not meeting the requirement was the insufficient sample size.

Surprisingly, the mean time interval between date of sampling and date of test submission to the database was quite long, 59 days (standard deviation: 125 days; median: 27 days; Figure 7.3). For three submissions (0.3%), the interval was negative (which is an indication of errors in data entry), for five submissions (0.6%) the interval was zero, for 73 (9%) submissions the results
were entered within a week, for 171 (20%) submissions the results were entered between 8 days and a maximum of 14 days, for 229 (27%) submissions the test results were entered from 15 days to a maximum of one month from sampling date, for 348 submissions (41%) the interval was between one month to one year, and for 19 submissions (2%) the interval was over one year, up to 2,121 days (Figure 7.3).

The issues mentioned above in regards to status misclassification and interval from sampling to reporting become especially important when dealing with emerging diseases. The knowledge and rapid assessment of swine site’s connections and characteristics is extremely important when the goal is to build a global swine health initiative, where fast response to emergent diseases is crucial for mitigation of risk and impediment of disease spread. In order to be better prepared to face such challenges in the future, time and resources need to be allocated to this area. In the sense of these actions becoming global, it becomes equally important to increase participation of swine sites in these and future regional disease control projects. Even though the fact that only a percentage of sites were participating in the projects does not invalidate study findings because there is no reason to believe that systematic bias was introduced during the development of these projects, the industry as a whole would benefit from complete enumeration and characterization of swine herds and networks. Furthermore, future studies should focus on obtaining data at the service provider level in order to get a complete overview of the different networks and the service provider’s approach to disease control. Importantly, confidentiality issues always arise when such matters are discussed, and its assurance becomes indispensable when trust and credibility are at risk. There should be actions in place to guarantee privacy and protection of individual sites and producers, and this is especially important in times where social media and information is easily accessible in a global context.
In the case that a swine global health network is something the Ontario swine industry would like to pursue, the required expertise need to be in place to explore novel epidemiological tools as they become available. A few suggestions of future approaches to be considered include the use of whole genome sequencing for differentiation of PRRS genotypes and tracking of viruses within regions, and the use of more discriminatory networks, for example animal movements (including frequency and type of movement). Another area to be further developed and explored refers to communications and interconnectedness between diagnostic laboratories, databases and their use for the rapid creation of updated reports over time. The amount of data currently available through different organizations and groups can be extensive, duplicated and therefore overwhelming, and coordination and dedication is needed to identify ways of utilizing information in a way that brings benefits to the industry.

Lastly, an important issue was revealed during the development of the research last chapter, with the enumeration of the efforts for PRRS surveillance and control. It refers to the evident lack of coordination between numerous surveillance efforts that occur within the swine industry, and perhaps in the animal industry as a whole. The argument is that, if efforts are not communicated, coordinated, and conducted in a systematic way, the waste of valuable resources is almost inevitable. There should be more efforts to unify information at the industry level and create reliable, accurate data in order to gain knowledge that will inform common industry objectives, such as improving swine health and assuring an internationally competitive swine industry. Enhancement of real-time reporting of current swine site PRRS status, as well as incident cases, is a challenge that will need to be overcome in the near future.

As mentioned by Davies (2005), swine production is constantly being threatened by the emergence of infectious diseases, such as different PRRS virus genotypes, the porcine epidemic
diarrhea and, more recently, the Seneca Valley virus. It is impossible to predict when introductions or re-emergence of pathogens is going to happen, but it is important to be prepared so that damages to the industry are minimized. There is a need to embrace and be part of the development and application of communications technologies and information management as they refer to swine health (Davies, 2005). This could improve significantly issues such as the one identified in interval from sampling to test result submission. Furthermore, collaboration between professionals from different fields is warranted, and the role of swine industry professionals might need to be readjusted to meet expectations such as the ability to manage people, businesses, and guide logistics in case of emergence situations, besides the construction of skill sets that include the use of freely available software and applications, as well as understanding and critically appraising the scientific literature to make informed decisions in the field.

This thesis did not bring definitive answers to unanswered questions on the epidemiology of PRRS, nor did it solve swine industry issues in a permanent manner. Nonetheless, it has contributed to the understanding of the complex ways PRRS can be transmitted among swine herds, which has direct implications in its control and prevention at the regional level. This thesis has also served to raise awareness on the fact that persistent issues in the swine industry, such as the emerging and re-emerging of infectious diseases, need to be looked at from different perspectives, and using novel approaches and methodologies as they become available. Even though emerging issues will always appear in swine production globally, risk can be mitigated when different expertise are combined and policy makers make use of the best available knowledge at the current time to inform decisions. In the case that they turn out not to have been the best choices… we learn, and try to do better the next time around.
7.1. References

Perez, A. M., Davies, P. R., Goodell, C. K., Holtkamp, D. J., Mondaca-Fernandez, E., Poljak, Z.,
and knowledge gaps about the epidemiology and control of porcine reproductive and respiratory
syndrome virus in North America. JAVMA, 246: 1304-1317.

Davies, P. 2005. Regional control of swine disease - new tools for the future? Swine Disease
Eradication Center, University of Minnesota. http://www.extension.umn.edu/agriculture/
Table 7.1. Mean number of submissions per site according to site’s most recent porcine reproductive and respiratory syndrome (PRRS) status, stratified by type of sample (virology and serology)

<table>
<thead>
<tr>
<th>Site PRRS status</th>
<th>Total number of sites</th>
<th>Mean number of submissions containing any test type per site</th>
<th>Mean number of submissions containing PCR tests per site</th>
<th>Mean number of submissions containing serology tests per site</th>
</tr>
</thead>
<tbody>
<tr>
<td>Confirmed negative</td>
<td>162</td>
<td>1.58</td>
<td>0.46</td>
<td>1.47</td>
</tr>
<tr>
<td>Confirmed positive</td>
<td>134</td>
<td>2.24</td>
<td>1.65</td>
<td>1.33</td>
</tr>
<tr>
<td>Presumed negative</td>
<td>192</td>
<td>0.71</td>
<td>0.26</td>
<td>0.59</td>
</tr>
<tr>
<td>Presumed positive</td>
<td>105</td>
<td>0.72</td>
<td>0.48</td>
<td>0.45</td>
</tr>
<tr>
<td>Unknown</td>
<td>223</td>
<td>0.33</td>
<td>0.21</td>
<td>0.24</td>
</tr>
<tr>
<td>Total</td>
<td>816</td>
<td>1.03</td>
<td>0.54</td>
<td>0.77</td>
</tr>
</tbody>
</table>
Figure 7.1. Number of laboratory submissions per swine site enrolled in the Ontario area regional control and elimination projects
Figure 7.2. Number of submissions per swine site over the study period (2010-2015) according to most current site’s PRRS status.
Figure 7.3. Difference between sampling and database reporting dates for diagnostic results for swine sites participating in area control and elimination projects in the province of Ontario, Canada
Appendix I - Sampling and diagnostic testing in PRRS ARC&E Projects

(Source: Ontario Health Advisory Board, pages 1-5, last accessed: June 2013, http://prrsarce.ca/arce-resources/)

Version #3 08/03/2013

Sampling and Diagnostic Testing in PRRS ARC&E Projects

Goals
To take samples that will provide an accurate assessment of the serologic PRRS status of the pigs on a site using the most cost effective methods available.

Where appropriate, targeted sampling methods will be used to collect blood samples for the possibility of PRRSV sequencing.

The key to an ARC&E is the sustainable updating of site status based on clinical triggers or events. Knowing the site status will result in reduced risks for the area and impact decisions made for the area in which the site is located.

Methods

Initial Determination of Site Status
All sites with an unknown PRRS status and sites with a presumed negative PRRS status will be tested initially in a PRRS ARC&E project area using the protocols outlined below.

On-going Sampling Recommendations
Clinical triggers will be used as a key reason to resample project sites. These clinical triggers provide a sustainable way to ensure on-going monitoring of the areas.

Sow sites will utilize blood swab collection or serum samples from aborting sows for PCR testing. Growing herds will utilize serum or saliva ELISA testing with PCR and sequencing where appropriate.

Event based sampling will also be employed. Some examples of events are: manure spread, changing pig source, gilt introduction, area PRRS breaks etc.

NOTE: when visiting sites for sampling, producers will be trained on the clinical triggers and sampling techniques appropriate to their site(s). Two sampling kits should be provided to the producer with training.

Determining Initial PRRSV Status
All participating sites with an unknown PRRS status and sites with a presumed negative PRRS status will be tested in a PRRS ARC&E project area. Sites with a known PRRS status will not be tested; the most recent diagnostic results will be forwarded to project coordinator.
Sampling in sow herds to establish PRRS status

1. Presumed PRRS negative or unknown PRRS status sow herds
   a. Blood sample 1 suckling piglet from each of 11 litters and test for PRRS ELISA using serum.
      (The sample size of 11 is based on 90% confidence and 20% prevalence.)

2. Known PRRSV positive sow herds
   a. No testing will be done on these sites unless clinical triggers occur. Submit results to OSHAB.

3. Seropositive farrow to wean sow herds – see appendix A
Sampling in growing pig sites (nursery and finisher barns) to establish PRRS status

1. Presumed PRRS negative or unknown PRRS status grow/finish herds.
   a. Rope saliva sampling is the recommended method. (Protocol attached). In each site hang 9 ropes and collect saliva.Submit samples (do not pool) for PRRS ELISA testing.

2. Known PRRS positive herds
   a. No testing will be done on these sites unless clinical triggers occur. Submit results to OSHAB.

3. Targeted sampling
   Sample a growing pig population using targeted sampling methods either when clinical signs of respiratory disease are present or the history of the pig flow is suspicious. Sample the poorest pigs in inventory, particularly puffing pigs. Targeted sampling is to be used in addition to rope sampling when clinical signs or history suggest that additional sampling may be valuable.

   In the event that clinical signs are not present, target sample the age of pig when viral circulation would be most likely e.g. mid to late nursery or within the first month of placement into grow-finish barns. Clinical judgment is required to evaluate the specific circumstances for each growing pig population.

   Blood sample 20 pigs (targeted samples or age targeted). Store the samples until the results from the saliva testing confirm the population is seropositive. Submit the samples for PRRS PCR testing pooled 5:1
Sampling in Response to Clinical Triggers (On-going sampling recommendations)

Clinical Triggers in Sow Herds

Clinical triggers may include:

- Primary Triggers:
  - Aborting sows
  - Weak piglets
  - Premature farrowings
  - Mummies
  - Stillborns

- Secondary Triggers:
  - Diarrhea
  - Decreased appetite
  - Fever
  - Lethargy
  - Sow deaths

All sow herds (PRRS negative or positive) will use blood swabs or serum samples taken from aborting sows for monitoring PRRSV in the herd. The producer will be trained on the clinical triggers and the use of the blood swab collection technique. Refer to the protocol PRRS ARC&E Clinical Triggers – Sow Herds. The producer will submit samples for testing to his/her veterinarian. Upon submission to the AHL, request pooling of the blood swabs for PRRSV PCR at 5:1 (or less).

Clinical Triggers in Grow/Finish Herds

Clinical triggers may include:

- Hairy/razor backs
- Panting
- Puffing, coughing
- Reduced ADG
- Increased secondary bacterial infections
- Red eyes/black shadows
- Pale
- Increased mortality

Use clinical triggers above the normal herd level.

1. Target blood sample 10 pigs and using serum, pool 5:1 for PRRSV PCR. Sequence positive PCR test results.

   OR

2. Randomly place a minimum of 6 ropes (1 rope/pen) using targeted sampling in pens with pigs exhibiting the most clinical signs and/or the poorest looking pigs and test the saliva for PRRSV using PCR. The producer will submit samples for testing to his/her veterinarian.

Further testing may be required to determine the viral status of the population if this screening test does not yield positive results.
Appendix A

Determining Status of seropositive farrow to wean sow herds:

To determine if this site is producing PRRSV negative pigs:

a. At a “downstream” nursery hang 9 ropes and collect saliva for PRRS ELISA testing or blood sample 11 pigs submit serum samples for PRRSV ELISA testing (do not pool). Sample the oldest pigs in the nursery. If clinical triggers are present, instead sample 20 targeted pigs and submit and request 11 PRRS ELISA tests (from pigs weaned greater than 4 weeks), if any positive, request all samples pooled 5:1 for PRRS PCR testing. Or

b. If unable to test a “downstream” nursery and want to determine if PRRSV is present in the sow herd at low prevalence use targeted testing of the poorest piglets. Blood sample a minimum of 60 piglets (up to 120 piglets), starting with the oldest piglets. Using serum, pool 10:1 for PRRSV PCR. A minimum of 3 negative consecutive tests are required to have initial confidence that the PRRSV is no longer present in the sow herd.

c. Testing sentinel animals is often done in addition to the testing options outlined in a) and b) above. There are various herd specific strategies for the use of sentinel animals. Testing of sentinel animals only is not sufficient to declare the herd PRRSV negative.