INVESTIGATION OF FACTORS ASSOCIATED WITH MORTALITY AND VACCINATION EFFECTIVENESS DURING AN OUTBREAK OF *STREPTOCOCCUS SUIS* DISEASE IN A SWINE NURSERY

by
Danielle Hopkins

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INVESTIGATION OF FACTORS ASSOCIATED WITH MORTALITY AND VACCINATION EFFECTIVENESS DURING AN OUTBREAK OF *STREPTOCOCCUS SUIS* DISEASE IN A SWINE NURSERY

Danielle Catherine Hopkins
University of Guelph, 2017

Co-advisors: R. Friendship and Z. Poljak

An investigation was conducted into the potential risk factors associated with mortality during an outbreak of *Streptococcus suis* disease in weanling pigs. Retrospective data were used to identify both sow- and pig-level factors that could result in increased risk of mortality. A Cox’s hazard regression model identified pigs originating within certain litters that appeared to be at increased risk. Risk for individual pigs increased if a littermate died or if the pig originated from a litter where the sow’s previous litter had low mortality. During the outbreak, a proportion of litters were vaccinated using an autogenous bacterin. Direct, indirect, total, and overall measures of vaccine effectiveness were evaluated using Cox’s hazard and binomial regression models. Measures of total (~27%) and overall (~21%) vaccine effectiveness had the largest estimated magnitude, with no evidence of direct effectiveness. Overall, there is potential for benefits of vaccination despite lack of evidence of direct effectiveness.
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1.0 Chapter 1

1.1 General Introduction

*Streptococcus suis* is a common cause of disease in swine particularly in weanling pigs. It typically causes clinical disease in a small proportion of nursery pigs (0-5%), however, outbreak situations also occur. It is not well understood what triggers these outbreaks but the presumption is that most pigs carry *S. suis* in the respiratory tract and there is an unknown event that occurs causing the pig to be susceptible to systemic infection. It is therefore important to direct research towards what triggers systemic infection and outbreak scenarios, and to determine whether the trigger is a single factor or a mixture of factors. Research evidence could lead to potential control measures to prevent the outbreaks from occurring.

The unpredictable nature of the disease caused by *S. suis* makes it a difficult disease to control through prevention or treatment methods. Prevention through the use of farm management is the preferred approach, because the current treatment approaches are not widely successful. Farm management strategies fit basically into two broad categories. Firstly, there are strategies to reduce the bacterial challenge, for example all-in/all-out pig flow, good sanitation and minimizing mixing of pigs. The second general approach is to ensure the pig is robust and has strong immunity, for example minimizing weaning stress, low stocking density, and possibly using a vaccine. Prevention through the use of vaccination alone has not proven to be effective or at least not consistently in field trials.

This literature review will provide background information on bacterial-viral relationships, focusing primarily on the potential for interaction of *S. suis* with influenza A virus (IAV). It will also include a review of control measures for *S. suis* and a brief review on current
vaccination procedures and vaccination protocols. The literature review will provide the necessary knowledge base for my second and third chapter analyzing *S. suis* outbreak in a commercial nursery and vaccination strategies to control this outbreak, respectively.

The risk factors associated with mortality during an outbreak of *S. suis* disease in a 300-sow operation were evaluated in Chapter 2. Sow-level and pig-level risk factors were analyzed based on their potential to increase or decrease the risk of mortality in the nursery. The research was based on retrospective records from 2011 and 2012, where an outbreak of *S. suis* caused nursery mortality as high as 30% in some litters. Risk factors of interest included: parity, pre-weaning mortality, same-sow mortality levels in consecutive litters, within litter mortality, age of weaning, and the number of piglets weaned to each sow.

A vaccination study that was conducted during the same *S. suis* outbreak is described in Chapter 3. An autogenous bacterin was given to 75% of pigs to evaluate different levels of vaccine effectiveness. The different levels of vaccine effectiveness including direct, indirect, total and overall were then compared using two statistical models. Direct effectiveness was measured using a Cox’s hazard regression model, while indirect, total and overall effectiveness were evaluated using binomial regression models.

Research on *S. suis* is increasing due to its constant presence and economic impact on nursery pigs. This literature review and research described in this thesis will further our knowledge of this complicated swine disease.
1.2 LITERATURE REVIEW
1.2.1 *Streptococcus suis*

*Streptococcus suis* is a Gram-positive facultative anaerobic bacteria and microscopically appears as coccoid or ovoid, and it can occur in pairs or in short chains (Lun et al., 2007).

*Streptococcus suis* was first identified in the 1950s, and affects swine herds around the world creating economic losses, especially during the nursery phase of development (Gottschalk, 2012). As of 1995, serotypes 1-34 and 1/2 have been identified based on the presence of a capsular polysaccharide (cps), a carbohydrate coat that encapsulates the bacterial surface (Goyette-Desjardins et al., 2014). *Streptococcus suis* also has zoonotic potential and can cause meningitis in humans, the documented cases usually involve humans who have direct contact with pigs such as pork producers or abattoir employees (Goyette-Desjardins et al., 2014).

1.2.1.1 Clinical diagnosis

*Streptococcus suis* causes clinical disease most frequently in pigs 4-8 weeks of age, but has the potential to cause disease in pigs of all ages (Brisebois et al., 1990). Newborn piglets are immunologically naïve and rely on colostrum to provide passive immunity for the first few weeks of life. If pigs are weaned at 3 to 4 weeks of age their immune system is still immature and maternal antibody protection is decreasing, leaving them susceptible to infection (Staats et al., 1997). Often, *S. suis* remains colonized in the tonsils of pigs and does not cause clinical disease (Gottschalk, 2012). Diagnosis is based on clinical signs of acute meningitis which presents as: loss of appetite, reddening of the skin, fever, depression, loss of balance, lameness, paralysis, paddling, convulsing, and even sudden death (Cloutier et al. 2003). The bacterium can also cause chronic disease due to endocarditis, septicaemia, and arthritis (Staats et al., 1997).

In a recent study Bi et al. (2014), experimentally infected piglets via posterior auricular muscle injection with a virulent strain of *S. suis* serotype 2 isolated from fatal human cases. They
proposed a 3-phase classification system for progression of disease caused by *S. suis*. Firstly, in
the early phase, affected piglets displayed joint swelling and crouching approximately 1-2 days
post infection (d.p.i). Secondly, in the acute phase, piglets experienced clinical signs consistent
with toxic shock between 1 d.p.i. to 4 d.p.i. Pigs also experienced meningitis-like clinical signs
during the early and acute phases with histopathological analysis revealing meningeal damage in
all clinically affected cases. Thirdly, in the convalescent period, most piglets that had survived
the first two phases continued to exhibit joint swelling, crouching, lameness, and action
impairment up to 3 months post-infection. Final diagnosis of clinical disease due to *S. suis*
infection is based on combining the observed clinical signs with laboratory confirmation of the
bacteria (Gottschalk, 2012).

1.2.1.2 Pathological diagnosis

Although there are multiple serotypes and various clinical presentations of *S. suis*
infection, the pathological findings tend to remain restricted to the brain, heart and joints
(Gottschalk, 2012). Upon gross examination, the brain can have numerous multifocal lesions,
signs of edema, fibrinopurulent or suppurative lesions, and other characteristic signs of
inflammation (Reams et al., 1994). Histological analysis shows bacteria in the cytoplasm of
neutrophils, macrophages in meningeal lesions, and in some cases, interstitial pneumonia as a
consequence of septicaemia (Ye et al., 2009). It is important to combine pathological findings
with laboratory analysis to confirm the presence of *S. suis*, as many pyogenic bacteria can cause
similar lesions (Gottschalk, 2012).

1.2.1.3 Pathogenesis
The exact pathogenesis of *S. suis* is currently unknown (Lun et al., 2007). Aspects of this bacterium that make it difficult to study include the fact there are multiple serotypes and potentially multiple virulence factors, and this might explain why there are various clinical presentations. To add to the complexity, the bacteria can infect several species, requiring multiple animal models that do not always allow for a transfer of knowledge from one species to the next (Fittipaldi et al., 2012). For clinical disease to occur, the bacteria must cross mucosal barriers and disseminate in the blood, then cross the blood-brain barrier (BBB) and gain access to the subarachnoid space resulting in systemic infection (Gottschalk, 2002).

There are numerous ways by which *S. suis* could create systemic infection. There is strong evidence that the production of the cps is up regulated after bacterial colonization. This up regulation allows the bacteria to be protected from phagocytosis and remain in circulation (Fittipaldi et al., 2012). However, there is evidence of highly encapsulated strains being excreted from the host as soon as 48 hours after infection (Gottschalk, 2002).

Another theory is that *S. suis* is taken up by monocytes and travels within the blood intracellularly. This hypothesis is also known as the “Trojan horse” method of systemic infection (Gottschalk and Segura, 2000). Alternatively *S. suis* may travel within the blood stream by attaching to monocytes extracellularly, or have the ability to travel as free bacteria (Gottschalk, 2002).

Once *S. suis* has entered the blood stream, in order to cause meningitis it must pass the blood-brain-barrier (BBB). It may adhere to the brain microvascular endothelial cells (BMEC), epithelial cells that form the choroid plexus surrounding the BBB, and secrete toxic factors indirectly causing clinical disease (Higgins and Gottschalk, 1990). The toxins have the potential to create an increased inflammatory profile and increased permeability at the BMEC, leading to
edema and eventually to meningitis. Dominguez et al. (2007) used a mouse model to show how prolonged sepsis as a result of *S. suis* infection, leads to an increased inflammatory profile, swelling of the brain and ultimately bacterial meningitis and death.

A major roadblock for researchers is understanding what causes the bacterium to transition from being restricted to the tonsils of healthy carriers, to causing septicemia and clinical disease. The general consensus is that a multifactorial set of circumstances ranging from genetics, environment, bacterial properties or concurrent infection with other pathogens is responsible for the transition to clinical disease (Gottschalk and Segura, 2000).

1.2.1.4 Virulence factors

Most virulence studies using *S. suis* focus on the virulence factors of serotype 2. Fittipaldi et al. (2012) has discovered and documented over 60 virulence factors associated with *S. suis*. The main virulence factors of interest for serotype 2 as described by Gottschalk and Segura (2000) are; the cps, muramidase-released proteins (*mrp*), extracellular protein factor (*ef*), haemolysin and adhesions.

The cps moiety has been classified as the main virulence factor responsible for adhering and colonizing epithelial cells (Lun et al., 2007). The majority of clinical cases of *S. suis* are encapsulated strains with the exception of clinical cases of endocarditis, which are usually caused by non-encapsulated strains. The absence of cps in *S. suis* results in increased hydrophobicity, phagocytosis, and overall increased bacterial clearance. There does not seem to be a consistent connection between clinical cases of *S. suis* and encapsulated bacterium, therefore the presence of a cps moiety should not be single-handedly used to confirm a virulent or avirulent strain.
Mrp and Ef are extracellular proteins and cell wall components with virulent capabilities. Mrp+ EF+ strains of S. suis can show increased virulence in some geographical areas, including North America. However, it is not known if this is because these proteins are secreted along with an already virulent strain, or if these proteins are the virulence factors themselves.

Another potential virulence factor is haemolysin or suilysin, a thiol-activated toxin produced by S. suis. Suilysin is toxic to epithelial, endothelial, and phagocytic cells and may play a role in S. suis serotype 2’s ability to cause clinical disease (Gottschalk, 2012). Suilysin protects S. suis from phagocytosis, facilitating its circulation in the blood stream (Gottschalk, 2012).

Virulent suilysin positive strains are consistently found in Europe and very few, if any, avirulent strains have been documented to be suilysin-positive. However, this virulence factor alone is again not sufficient to declare a strain of S. suis as virulent.

Adhesions adhere to albumin, increasing the virulence of S. suis and its ability to circulate and survive within a host. It has been shown that virulent strains of serotype 2 with adhesions present had increased virulence, although, avirulent strains also may contain adhesions.

Presently, there isn’t one specific bacterial characteristic that is responsible for high virulence (Fittipaldi et al., 2012). The pathogenesis relies on bacterial characteristics combined with the host’s immune response and if the set of factors is in favour of bacterial invasion, clinical disease can occur.

1.2.1.5 Laboratory analysis

1.2.1.5.1 Isolation and culturing

Streptococcus suis can be isolated from the tonsils, nasal cavity, meninges, serosal surfaces, and macroscopic infected areas (Pijoan, 1994). The bacteria are grown at 37°C for
approximately 48 h on sheep or horse blood agar, and become alpha- and beta-hemolytic, respectively (Staats et al., 1997). After culturing, matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) can be used as a highly sensitive and specific method to identify *S. suis* isolates (Pérez-Sancho et al., 2015). Higgins and Gottschalk (1990) have described a set of tests to confirm a *S. suis* diagnosis including; the isolates do not grow in 6.5% NaCl agar, they must have a negative Voges-Proskauer (VP) test, and an acid must be produced in trehalose and salicin broths. These tests are useful if there is a potential for another bacteria to be the source of infection or clinical disease. For example, pigs infected with *Haemophilus parasuis* the cause of Glässer’s disease, occasionally presents with clinical signs very similar to *S. suis* disease.

### 1.2.1.5.2 Co-agglutination

There are currently 35 serotypes of *S. suis* identified based on cps antigens. Co-agglutination reactions rely on cps antigens reacting with anti-sera for each of the 35 serotypes individually. A positive reaction occurs when the isolate agglutinates with the *Staphylococcus aureus* protein A contained in the anti-sera. If *S. suis* does not possess a cps moiety, no reaction occurs, and it is classified as untypable (Gottschalk et al., 1993). Auto-agglutinating strains are able to react with all, or the majority, of the 35 anti-sera. This method is popular in epidemiological studies and vaccine development, however, it can be time consuming and labour intensive as all 35 serotypes must be tested individually for each isolate (Gottschalk, 2012). This method is not always accurate as it is subject to bias by the interpreter, and cross-reactions can occur between serotypes leading to inconclusive results.

### 1.2.1.5.4 Polymerase Chain Reaction (PCR)
There are multiple PCR tests that have been developed as a rapid, highly sensitive and specific diagnostic tool to measure the presence of \textit{S. suis} and determine serotype. Okwumabua et al. (2003) developed a PCR test to determine the presence or absence of \textit{S. suis} based on the glutamate dehydrogenase gene (\textit{gdh}) that appears to be conserved among \textit{S. suis} serotypes. If the \textit{gdh} gene is detected this indicates the presence of \textit{S. suis}. The use of the \textit{gdh} test has consistently proven to be able to identify the presence of \textit{S. suis} regardless of serotype or region.

It is also important to determine the specific serotype for both vaccine development and monitoring of virulent strains on farms. The use of multiplex PCR tests is quickly becoming the method of choice for identifying serotypes of \textit{S. suis}. Liu et al. (2013) developed a PCR test that uses a series of 4 different multiplex PCR assays. The assays identify serotypes using the “\textit{wzy}” gene cluster, a serotype specific gene found within the \textit{cps} gene. These assays have the ability to identify all serotypes of \textit{S. suis} with the exception of serotypes 1 and 14, and serotypes 2 and 1/2, due to the similarity of the \textit{cps} moiety for these serotype groups.

An even quicker multiplex PCR method was develop by Okura et al. (2014), who created a two-step process. First, an assay detects the \textit{cps} gene and classifies the isolates into 7 groups then a second assay is used to identify the \textit{cps}-type specific strain. This test correctly identified 95% of the isolates tested, regardless of serotype, and region.

\subsection*{1.2.1.6 Epidemiology characteristics}
Isolates of \textit{S. suis} from clinical cases most frequently range from serotypes 1-8 (Fittipaldi et al., 2012). Serotypes 9-34 are more likely to be isolated from the tonsils of healthy carrier pigs (Gottschalk, 2012). Serotype 2 continues to show high prevalence and virulence among swine farms around the world (Goyette-Desjardins et al., 2014). Specific to North America, serotype 2 is the most common cause of clinical cases in swine in Canada (Gottschalk et al., 2013).
However, the prevalence of serotype 2 appears to be decreasing in recent studies (Higgins and Gottschalk, 1990). In the United States, Serotype 3 is most commonly isolated from clinical cases in swine, followed by serotypes 1/2, 7, and 8, overall (Goyette-Desjardins et al., 2014). The highest recovery rates of S. suis occur during the colder months of October to January (Reams et al., 1993).

*Streptococcus suis* can also be isolated from the tonsils and respiratory tract of most pigs. In a typical swine herd, clinical disease occurs in less than 5% of the population (Staats et al., 1997). In some cases, *S. suis* can transition into an outbreak situation where it can cause much higher levels of mortality (Lun et al., 2007). *Streptococcus suis* can cause clinical disease in pigs at all ages but they are most susceptible at 3-8 weeks of age (Reams et al., 1996).

### 1.2.1.7 Transmission

*Streptococcus suis* is present in the environment of almost all swine farms. It can colonize piglets within the tonsils 24 hours after birth (Amass et al., 1996). The sow may transmit *S. suis* to the piglets during farrowing via vulva, fecal matter, and body fluids (Gottschalk and Segura, 2000). Amass et al. (1996) confirmed colonization at birth when attempting to use segregated early weaning to prevent the bacteria from colonizing young piglets. During the study, early weaning was found to be unsuccessful as *S. suis* was isolated from piglets as early as one day after birth.

The major cause of *S. suis* transmission results from a colonized carrier being introduced into an immunologically susceptible herd. Colonized or non-clinical carrier pigs harbor *S. suis* in their tonsil and nasal cavities, spreading the bacteria through direct contact with susceptible pigs or airborne transmission (Gottschalk, 2012). It is unclear what causes *S. suis* to produce clinical disease in some pigs while remaining a harmless commensal organism in others (Fittipaldi et al.,
There is usually only one serotype that causes clinical disease within a pig, even though multiple serotypes can be isolated from a farm and within a single pig (Monter Flores et al., 1993). In addition, there does not appear to be cross-immunological protection between pigs affected by different serotypes (Reams et al., 1994).

Horizontal transmission between pigs can occur through direct contact and also indirectly via aerosol particles. Dekker et al. (2013) compared the direct and indirect spread of serotype 9 from infected pigs to susceptible pigs. Direct contact between a experimentally infected pig and a susceptible population resulted in 3.58 pigs per day becoming infected, compared to indirect transmission where 0.001 new infections per pig per day occurred. The frequency and intensity of transmission increases with sick pigs and can lead to outbreak situations. In Dekker et al. (2013) study, indirect transmission was able to occur at a 1 m distance. Indirect transmission can also occur via vectors such as flies, contaminated fomites, and especially feces (Dee and Corey, 1993). The general consensus to control the spread of S. suis disease is to decrease environmental stress experienced by pigs, use all-in/all-out farrowing systems for proper disinfection between batches, early treatment of animals with clinical signs and isolation of sick pigs (Blouin et al., 1994; Gottschalk, 2002).

1.2.2 Influenza A virus and S. suis interaction

Viral infections have been suggested as a factor in increasing the susceptibility of hosts to bacterial pathogens. Bacterial-viral relationships can follow additive, synergistic, or even uncooperative patterns that can lead to significant health consequences to the hosts that they infect (Hament et al., 1999). Bacterial-viral interaction commonly occurs within the respiratory tract of animals and humans (Avadhanula et al., 2006). A variety of mechanisms have been
proposed that facilitate this relationship including; damage to the nasopharyngeal passage which can allow the bacterial species to invade the hosts defenses, increased inflammation that can lead to a compromised immune system, and in some cases, a direct interaction between the viral and bacterial pathogens (Wang et al., 2013).

As an example of how viral infection might be a trigger for \textit{S. suis} disease outbreaks in the swine nursery this review will focus on influenza A virus (IAV). Influenza A virus is habitually present on swine farms around the world and has been associated with several other bacterial and viral co-infection diagnoses (Neumann et al., 2009). While interaction between IAV and \textit{S. suis} still needs to be confirmed under field conditions, \textit{S. suis} continues to be a common issue in the nursery phase of swine production. Therefore an additional objective of this review is to summarize information about factors contributing to disease development and potential control and prevention strategies.

1.2.2.1 Swine Influenza A Virus (IAV)

Swine influenza A viruses are members of the family \textit{Orthomyxoviridae} and is a negative sense RNA viruses with 8 gene segments that each code one or two viral proteins (Maeda and Uede, 2010). Influenza viruses encode up to 11 proteins on 8 segments of negative-sense RNA. These gene segments are what allow the virus to undergo continual genetic re-assortment (Taubenberger and Morens, 2008). There are three main subtypes of influenza -- A, B, and C -- based on their genomic composition; however, primarily influenza A subtypes consistently cause clinical disease in pigs (Taubenberger and Morens, 2008). The virus is then further characterized by its surface viral glycoproteins. The main glycoproteins used for classification are hemagglutinin (HA) and neuraminidase (NA) (Maeda and Uede, 2010). Hemagglutinin and NA each have 16 and 9 different groups that comprise the virus, respectively. They are the main
targets for the host immune response and are responsible for the binding and release of the virus into host cells, respectively (Brookes et al., 2010).

Influenza A virus is a self-limiting, respiratory virus with high morbidity, and generally low mortality (<1%). Pigs are susceptible to infection with IAV and S. suis at approximately the same age and same phase of development, 4-8 weeks of age within the nursery. Influenza A viruses in the presence of S. suis and other bacterial pathogens results in increased mortality rates (McCullers, 2006). A study done by Williamson et al. (2012) looked into the clinical and epidemiological presentation of IAV H1N1 in England. Several deaths were reported in pigs infected with IAV H1N1 as a result of an S. suis infection. The majority of cases of co-infection were in weaned pigs that experienced a progression of increased coughing, lethargy, sneezing, and ultimately meningitis or sudden death.

In swine, IAV is involved with multiple co-infections, described as the porcine respiratory disease complex (PRDC). The most notable bacterial pathogens associated with PRDC include: Staphylococcus aureus, S. pneumoniae, Neisseria meningitidis, Bordetella bronchiseptica and Haemophilus parasuis (Opriessnig et al., 2011). The most notable viruses beyond IAV include: porcine circovirus type 2 (PCV2) and porcine reproductive and respiratory syndrome virus (PRRSV) (Hament et al., 1999). The pathogens colonize in the lungs of pigs causing destruction of alveolar macrophages, increased inflammation, and decreased host defenses (Opriessnig et al., 2011).

Porcine respiratory disease complex is also associated with S. suis and other bacterial pathogens that may induce septicemia. Streptococcus suis can travel to the lungs via the lymphatic system, cause lung damage and potentially increase the severity of clinical signs associated with PRDC. Bacterial species which also induce septicemia and facilitate viral
Infection in pigs include: *Haemophilus parasuis, Actinobacillus suis, Arcanobacterium pyogenes, Salmonella enteric var. Choleraesuis*, and *Typhiumurium* (Opriessnig et al., 2011).

Influenza virus can also promote bacterial infection in pigs by compromising the immune system, damaging the epithelial layer of the respiratory tract and increase host receptors for bacteria (Loving et al., 2010). However, it is difficult to conclude whether or not IAV predisposes pigs to bacterial infection or vice versa. It is highly possible there is a unilateral and bilateral relationship between virus and bacteria, each contributing to their enhanced survival and reproduction within the host (Bosch et al., 2013).

Currently, the majority of evidence for an interaction between IAV and *S. suis* resides in a cell culture study. There is speculation IAV has the potential to facilitate the colonization and uptake of *S. suis* into porcine epithelial cells (Wang et al., 2013). This could explain why *S. suis* can remain colonized in some pigs and transition into clinical disease in others. Since there are multiple strains of *S. suis* and IAV, and numerous potential interaction sites and pathogenic routes, there is a need for additional co-infection studies to begin to understand this relationship. There is also limited *in-vivo* evidence involving IAV and *S. suis*, which limits our ability to extrapolate current information in a commercial setting.

1.2.2.2 Pathogenesis of co-infection of IAV and *S. suis*

These pathogens are ubiquitous on swine farms and pigs are frequently identified to be subclinical carriers of both IAV and *S. suis*. In addition both pathogens colonize the respiratory tract, allowing for close proximity of pathogens and a potential for interaction. Studies using PRDC as a model for co-infection have hypothesized several methods surrounding bacterial-viral relations within the respiratory tract. It is believed that damage to mucociliary apparatus and respiratory tract, depressing the immune system, altering inflammatory profiles, and direct
bacterial-viral relationships could all lead to increased susceptibility for secondary bacterial infection (Opriessnig et al., 2011). Wang et al. (2013) reviewed these proposed mechanisms for IAV, specifically IAV H1N1 facilitating the uptake of S. suis, serotype 2, in a cell culture study.

Viral damage or colonization of the epithelial barrier / mucosa of the lungs damages the primary line of defense against harmful pathogens. Influenza damages epithelial layers and undergoes replication in regions such as the nasopharyngeal passage, bronchial and bronchiolar epithelial cells, pneumocytes, and alveolar macrophages (Thanawongnuwech et al., 2001). Damage to the epithelium can expose surface molecules and cell receptors for bacterial pathogens, such as S. suis, to attach and invade the host (Pittet et al., 2010). Infection with IAV also impairs clearance mechanisms in the respiratory tract, enhancing bacterial invasion and survival. In cell culture, Wang et al. (2013) examined the kinetics of S. suis invasion into porcine epithelial cells and discovered a 100-fold increase in adhesion of the bacteria with cells that had been pre-infected with H1N1. The ability of influenza to increase host susceptibility has been reproduced with other bacterial pathogens such as: Bordetella bronchiseptica, S. pneumonias, Staphylococcus aureus (Avadhanula et al., 2006; Zhang et al., 2013).

Infection with IAV H1N1 is associated with an increase in inflammatory mediators including cytokine and chemokine production (Brookes et al., 2010). This is particularly important in terms of secondary S. suis infection, as an increased inflammatory profile can create an environment for S. suis to cross the BBB and ultimately cause septicemia and clinical disease. This optimal environment was replicated in cell culture where co-infection with classical IAV H1N1 and S. suis serotype 2 had a 100 to 300 fold increase in mRNA proteins for CCL4, and CCL2 (Wang et al., 2013). Conversely, infection with S. suis up-regulated the production of
TNF-α and IL6 and IL8, which provided ideal conditions for H1N1 to transition into the cell, replicate and survive.

Optimal survival within a host relies on a pathogen’s ability to colonize and compete for a certain niche, however in some cases, pathogens can co-inhabit the same niche if a mutualistic or synergistic relationship favours their survival (Opriessnig et al., 2011). Wang et al. (2013) discovered a relationship between the cps of S. suis and IAV H1N1 in cell culture. Results of the interaction study showed an increased adhesion and invasion rate of S. suis with serotypes 2 and 14, and not with serotype 3. This was attributed to the sialic-acid side chain on the cps moiety of S. suis that is conserved in both serotype 2 and 14, but not 3. This concept was further confirmed after inhibiting the cps, the increase in adherence of S. suis after IAV pre-infection was not observed (Hament et al., 1999).

Influenza A virus could also facilitate S. suis adherence and colonization of epithelial cells through the HA glycoprotein, which may provide receptors for serotype 2 of S. suis to bind (Bosch et al., 2013; Wang et al., 2013). This concept was replicated in cell culture when H1N1, H1N2 and H3N2 were bound to a well-encapsulated S. suis strain. Each virus was unable to perform its haemagglutinin activity since the receptor was bound to S. suis and unable to perform its function (Wang et al., 2013). A great interest in bacterial-viral relationships also lies with NA of IAV in the literature. However in the study by Wang et al. (2013) NA was found not to have interacted with S. suis. This is surprising as other co-infection studies involving IAV show NA attaching to epithelial cells and providing an entry point for secondary bacterial infection (McCullers and Bartmess, 2003).

**1.2.3 Treatment and control of IAV and S. suis**

The control of IAV and S. suis within a herd is very important as both pathogens are commonly present in swine nurseries and can result in clinical disease with significant economic
impact. Unfortunately, the control of these pathogens is difficult. Vaccination as a control
measure is costly in terms of vaccine development and labour costs (Kitikoon et al., 2006;
Vincent et al., 2008). Development of vaccines requires constant monitoring, sampling and
laboratory analysis. In addition, there does not appear to be cross-immunological protection
against the bacterial or viral strains making vaccine development a constant effort as the
infecting strains of *S. suis* and IAV are subject to change (Vincent et al., 2008). Given the
economic investment, farmers may choose to not vaccinate and shift their control efforts to farm
management strategies. However, this leaves the farm susceptible to outbreak scenarios and
significant losses. Finally, there is the option for broad range treatment in water or feed as a
preventative measure for *S. suis* disease. This approach also has its drawbacks however, as farms
using broad range antibiotics are at an increased risk of developing antimicrobial resistance
issues.

1.2.3.1 Vaccination against IAV
Commercial vaccines against classical H1N1 were previously effective at reducing
clinical signs on farms until the late 1990s, when there was a shift to an increased presence of
H3N2 strains (Kitikoon et al., 2006). Since there can be multiple strains of virus on individual
farms, there is an increased demand for farm-specific, autogenous vaccines (Vincent et al.,
2008). The majority of farmers who choose to vaccinate against IAV prefer to vaccinate sows
using autogenous vaccinations. Sow vaccination is a cost effective and convenient vaccination
protocol that can provide protection to piglets through maternally-derived antibodies (MDA) and
decrease the level of clinical disease in the nursery (Neumann et al., 2009). However, sow
vaccination is not perfect, Kitikoon et al. (2006) discovered that MDA protection alone was not
enough to provide prolonged protection from heterologous IAV strains. There is also evidence
that if piglets are vaccinated when MDA are present, this can affect the efficacy of the vaccine and lead to an increase in susceptibility to secondary infection (Kitikoon et al. 2006). Therefore, the timing and type of vaccine used are critical to its effectiveness.

Swine face potential IAV transmission from human, avian and swine origin. Pigs are therefore able to generate novel strains, as well as contribute to the antigenic drift that contributes to the evolution of IAV (Brown, 2000). Therefore, even though many pigs remain subclinical or have self-limiting clinical signs, it may still be beneficial to vaccinate and frequently monitor circulating IAV strains on farms. This can reduce the economic losses associated with clinical IAV infection in pigs and the potential public health risks associated with the introduction of novel strains.

1.2.3.2 Vaccination against S. suis

In North America, commercial vaccines for S. suis is not common practice on swine farms and at least in Canada commercial vaccines aren’t available (Baums and Valentin-Weigand, 2009). This may be a result of the multiple serotypes affecting farms across North America along with inconsistent experimental results surrounding vaccine efficacy. Despite the inconsistency, some farmers do choose to vaccinate their pigs using autogenous bacterins (Gottschalk et al., 2013). Autogenous bacterins are prepared by isolating a particular serotype of S. suis from clinically ill pigs and using that isolate to make a vaccine against that specific serotype (Baums et al., 2010). There are limitations associated with autogenous bacterins in general. By their very nature, each vaccine is different because it is produced by an isolate from the specific farm for which it is intended to be used (Segura, 2015). There is little safety and no efficacy testing performed before the vaccine is used on the farm. Given this, results reported in the literature regarding autogenous vaccines for the control of S. suis disease are inconsistent.
The majority of vaccine studies are currently based on S. suis serotype 2 in nursery pigs, and their influence on morbidity and mortality rates remains uncertain (Gottschalk, 2012). Additionally, a sufficient sample size of clinically ill pigs to identify the virulent strain on farm is necessary as there can be multiple virulent serotypes present on an individual farm and these serotypes may change over time (Reams et al., 1996). The proper timing of vaccine administration is unclear because maternal antibodies may be interfering with vaccine efficacy (Blouin et al., 1994; Rooke and Bland, 2002).

Vaccination of sows may provide a more economic and effective method of vaccination against S. suis. However, sow vaccination studies also yield conflicting results. Blouin et al. (1994) vaccinated sows using a formalin-killed vaccine against serotype 2 and 14, three weeks before farrowing. This resulted in a slight increase in maternal antibody transference to pigs and a non-significant impact on nursery mortality rates (Blouin et al., 1994). Another study following the same vaccination protocol, indicated an increase in opsonizing antibodies that were transferable to pigs through maternal antibodies and remained within pigs until approximately 6 weeks of age (Baums et al., 2010). Although this method has the potential to increase the survival of pigs within the nursery, there is a risk that active immunity will be less developed in pigs as a result, and leave pigs at increased susceptibility to infection in the grower/finisher phase of development (Segura, 2015). Therefore, it may be a good idea to either identify the optimal age for long term vaccination effects, or vaccinate again later on in development.

If there are severe morbidity and mortality rates due to S. suis infection, or an outbreak situation on a farm, it may be beneficial to implement vaccination protocols despite their inconsistent results. If a farmer chooses to vaccinate pigs, it is recommended by Haesebrouck et al. (2004) to vaccinate the pigs twice, 2-3 weeks apart, and 2 weeks prior to the risk period which
is generally 3-8 weeks of age. If vaccinating sows, Haesebrouck et al. (2004) recommend vaccination at 6 weeks and again at 2 weeks pre-farrowing to allow for effective transference of maternal antibodies within the colostrum.

1.2.3 Population-level vaccination studies

The majority of studies for commercial animal vaccines focus on the direct impact of a vaccine on an individual and its ability to confer protection in that individual (Halloran et al., 1991). However, vaccination of individual pigs can lead to a “herd immunity” effect, where a population of unvaccinated pigs benefit from the presence of vaccinated pigs (Rose et al., 2016). This concept has been widely proven in human influenza studies, but less so in swine IAV vaccine studies, and even less so with respect to S. suis vaccines (Plans-Rubió, 2012). Vaccination protocols that are aimed at population-level effects may lead to more timely and cost-effective farm management strategies against clinical infection and disease. Vaccination on a population-level or herd-level is essentially a balancing act between these factors to generate an appropriate vaccination strategy that will maximize the number of pigs protected against infection. Although more studies are needed in this area, this vaccination strategy could be beneficial for a bacterium such as S. suis. Providing protection against clinical disease at a population level could help prevent the rapid transmission and subsequent mortality seen with S. suis during these outbreaks.

1.2.3.4 Farm management strategies
It is almost impossible to eradicate S. suis from a swine herd and difficult to eradicate IAV. The majority of efforts focus on reducing the incidence of clinical disease by decreasing transmission rates and spread of both pathogens. It is possible to reduce transmission of both pathogens by keeping the stocking density of pigs in a single room or pen low (Dekker et al., 2013; Grøntvedt et al., 2011). Increasing the stocking density can increase stress, and increase the direct contact between pigs, and therefore contribute to the overall transfer of pathogens among pigs.

Specific to S. suis, it is very beneficial to isolate sick pigs to avoid them shedding a virulent strain to other susceptible pigs (Dekker et al., 2013). Individual pigs can be treated with an injectable antibiotic such as penicillin or trimethiprim-sulfa and an anti-inflammatory agent, however treatment isn’t always effective at preventing mortality (Baums and Valentin-Weigand, 2009). Dead pigs should be promptly removed from the pen and properly disposed because S. suis can survive for up to 10 days in porcine tissue and bodily fluids (Dee and Corey, 1993).

It is recommended to practice all-in/all-out farrowing and pig movement instead of continuous flow, to be able to implement proper cleaning in between batches and reduce the overall pathogen load. Furthermore, IAV has been shown to survive and circulate on farms for a prolonged period of time in continuous flow systems (Williamson et al., 2012). Dee and Corey (1993) found S. suis could be eliminated from the environment if proper disinfectant protocol was implemented between batches.

Reduction of overall stress of pigs by minimizing overcrowding, reducing mixing of pigs, and ensuring proper ventilation and limited temperature fluctuations can also be effective in reducing S. suis disease outbreaks (Sanford et al., 2004).
1.2.4 Antimicrobial resistance

To control a persistent *S. suis* issue, group-level treatment in feed or water can be an effective measure, however, this introduces concerns for development of antimicrobial resistance. High prevalences of resistance among *S. suis* (up to 85% of isolates) to antibiotics including lincosamides, macrolides, sulphonamides, and tetracyclines have been reported (Varela et al., 2013). These antibiotics are used worldwide in the swine industry and for decades in many countries they have been used at low levels for growth promotion and prophylaxis (Varela et al., 2013). Specific to Canada, the majority of *S. suis* isolates taken from swine farms have been found to be resistant to clindamycin and erythromycin (Glass-Kaastro et al., 2014; Gottschalk, 2012). The prevalence of penicillin resistance is generally low across the globe. Tetracycline resistance appears to be conserved worldwide, and has even begun to appear in *S. suis* isolates from humans (Xu et al., 2010). Given the rise in resistance on farms, susceptibility testing may be warranted particularly when response to treatment is poor.

1.5 Summary and research objectives

*Streptococcus suis* is an important swine pathogen and outbreaks of disease in swine nurseries are common. It is generally assumed that outbreaks of *S. suis* disease are triggered by factors such as stress, and possibly concurrent viral infection, but risk factors are not fully understood. Likewise control strategies have not been consistently effective and the most common approach of using prophylactic mass medication of antibiotics via feed or water is coming under public pressure and may become less accessible due to legislative changes concerning on-farm drug use. Vaccination is a promising approach for the control of *S. suis* disease but reports in the literature suggest this has not produced consistently positive results.
The objectives of this research are: I) to assess a previous *S. suis* outbreak and determine potential sow- and pig-level risk factors associated with the risk of *S. suis* related mortality II) to assess the results of a vaccination trial conducted during an outbreak of *S. suis* disease in order to determine both the direct effectiveness and population level effects through analysis of the indirect, total, and overall effectiveness of vaccination. Through this we will aim to illustrate potential risk factors for *S. suis* mortality within a nursery, and identify targets for intervention and control strategies either through specific farm management strategies or potential vaccination protocols.
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FACTORS CONTRIBUTING TO MORTALITY DURING A *STREPTOCOCCUS SUIS* OUTBREAK IN NURSERY PIGS
The objective of this study was to investigate the association between sow- and litter-level factors on mortality in a swine nursery barn experiencing a severe *Streptococcus suis* disease outbreak. All-cause mortality data from a 300-sow farrow-to-finish herd was analyzed using a Cox’s regression model. The data were recorded over 6 months and included 24 cohorts, 297 sows, 295 litters, and 2,779 piglets with an average of 14.4% post-weaning mortality. If the sows had 2 litters within the study period and pigs from their first litter experienced mortality, then pigs from their subsequent litter had a decreased risk of mortality (HR=0.34, *P*=0.024). Pigs were more likely to experience mortality if at least one additional littermate experienced mortality (HR=9.22, *P*=0.001). Under conditions observed in this study, the results suggest mechanisms related to sow immunity and within-litter spread could have contributed to the risk of mortality during the *S. suis* outbreak.
2.1 Introduction

The early nursery phase is stressful for pigs due to a variety of novel environmental factors, in combination with the pig’s immature immune system that leaves them susceptible to infection with multiple pathogens and possibly leading to clinical disease. *Streptococcus suis* is considered to be one of the most important pathogens affecting nursery pigs (1). There are currently 35 serotypes of *S. suis* identified based on capsular polysaccharides (cps), in addition to untypable strains that are un-encapsulated, or do not contain a cps moiety (2). The bacteria may present as a commensal bacteria or as an opportunistic pathogen in the nasal cavity and tonsils of the majority of pigs (2). In most cases, the bacteria remains colonized in healthy pigs, however it can sometimes transition into a systemic infection and cause clinical disease (3,4). Although it is unknown what causes this transition in some pigs and not others, nursery pigs seem to be at a higher risk, in part due to the stress associated with this transitional period of development (5).

Infections caused by *S. suis* in nursery pigs are usually characterized by a low incidence (0-5%) of clinical cases that show a variety of signs related to septicemia, arthritis, pericarditis, and meningitis. The onset of clinical signs is typically rapid and sudden death can also be reported as a common finding (2). Treatment with penicillin or broad spectrum β-lactams such as ampicillin and amoxicillin can be somewhat effective, however, even with early treatment prognosis is often poor (6). Outbreaks involving a large proportion of at risk-animals (>20%) can occur, demonstrating the severe impact this bacterial disease can have on swine populations. The pathogenesis of *S. suis* is not fully understood, adding an additional layer of complexity when attempting to determine what triggers an outbreak of clinical disease (7).

Control of *S. suis* disease using individual animal or farm level treatments is challenging
and vaccination trials provide inconsistent results, making prevention strategies within the
nursery a very important area of research (8). There is a great interest in what factors cause the
transition from healthy commensal bacteria to clinical disease in pigs. A few of these risk factors
may include the influence of a sow’s passive immunity, co-infection, environmental or genetic
factors that leave some pigs more susceptible to *S. suis* disease than others.

The objectives of the current study were to identify sow- and litter-level risk factors
associated with the nursery pig mortality observed during a prolonged outbreak of clinical
disease caused by *S. suis*, and to use this information to help guide potential prevention and
control strategies for future outbreaks.
2.2 Materials and Methods

The data used were the production records from a 300-sow farrow-to-finish farm experiencing an outbreak of *S. suis* within the nursery over a 6-month period, October 2011 to March 2012. Mortality data for this period included overall mortality due to any reason, however the majority of deaths were assumed to be due to *S. suis* based on clinical signs of acute meningitis. In addition, a subset of sick pigs had diagnostic post mortem examinations conducted to confirm *S. suis* as the cause of the disease.

During the months of February and March, a vaccination trial was conducted on pigs in 4 weaning cohorts in an attempt to control the outbreak of *S. suis*. The trial included data on the overall mortality and mortality consistent with clinical disease due to *S. suis*. However, for the purpose of this investigation only the all-cause (overall) mortality for the entire 6-month period was taken into account. The dataset used for analysis was a compilation of data from two sources (Figure 2.1): I) Pig-mortality data indicative of all-cause mortality recorded by the farm staff during the 6-month study period. This included date of weaning, date of death, sow ID, and litter ID. II) Individual sow production records for all sows that farrowed during the study period. The latter dataset was downloaded from an online database that served as farm management software and was regularly updated by the farm staff. The sow dataset included information on sow ID, parity, date of weaning, number of pigs born alive, and number of pigs weaned. For the sow-level dataset, sow records were excluded if information about the weaning date or the number of pigs weaned was missing (Figure 2.1), after examining the corresponding paper records available. For the pig-level mortality dataset, records were excluded if mortality occurred before or after time at risk during nursery phase of production (Figure 2.1). The time at risk was defined
to be 63 days, 9 weeks, or the approximate time a pig spent in this farm’s nursery and the period immediately following the nursery phase. Thus, mortalities that occurred prior to or after the period of risk were excluded from the pig-level records (Figure 2.1). After removal of all missing observations the datasets were merged based on sow ID and further processed so that it represented pig-level data from specific litters that entered the nursery between October 2011 and March 2012.

Three new variables were generated from the expanded data set including pre-weaning mortality, previous-litter mortality, and within-litter mortality. The variable pre-weaning mortality was generated by subtracting the number of pigs weaned from the number of pigs born alive, then divided by the number of pigs born alive; previous litter mortality was generated as a nominal variable with 3 levels based on nursery mortality levels in each litter in the following way: I) reference category - the first litter of sows with repeated farrowing’s had 0% nursery mortality, II) the risk factor of interest - the first litter of sows with repeated farrowing’s had >0% nursery mortality, III) for sows with only 1 litter in the data set, their records were classified into a third category as they could not contribute to evaluation of this risk factor of interest. Mortality within a litter was generated as a binary variable for each individual pig, where the value of 1 was assigned to each pig that originated from a litter where at least 1 additional pig from the same litter experienced mortality during the nursery phase, or 0 otherwise.

Parity was grouped into 6 categories, where category 6 contains parities 6, 7, and 8 as there were only 6 sows in the dataset with a parity greater than 6. Pre-weaning mortality was organized into 6 groups based on percentiles mortality within litters. The first category represents cross-fostered pigs, where a litter received pigs from another sow and therefore yielded a
percentage pre-weaning mortality <0% if no mortality was experienced by those pigs. The rest of
the observations were grouped into 10% mortality categories up until 50%. Then pre-weaning
mortality between 50-100% was grouped together as few litters experienced pre-weaning
mortality levels in this range.

Frequency count of mortality, categorized on the basis of percentage mortality within
litters, calendar time, and time in the nursery were visualized as histogram plots. Descriptive
statistics were then preformed on each predictor variable of interest including sow parity
(Parity), the month of weaning (Month-wean), age of weaning (Age), the number of piglets
weaned (Number wean), pre-weaning mortality (Pre-wean-mort), previous litter mortality (Prev-
mort), and mortality within litters (Litter-mort).

Descriptive statistics for categorical and continuous explanatory variables was
determined using appropriate statistics at the pig- and sow-level. Observations collected on pigs
between February 2, 2012 and March 15, 2012 when the vaccination trial occurred were
excluded from descriptive statistics to avoid potentially altering mortality patterns experienced at
that time (Figure 2.1).

For the inferential analysis, the full dataset of 2,779 observations coming from the
merged sow- and pig- level datasets was used. The categorical variables were assessed using
Kaplan-Meier survival curves, followed by a log-rank test for statistical significance (P<0.05).
Univariable analysis was conducted using Cox’s proportional hazard model on each of the
categorical and continuous predictor variables of interest with a liberal P-value cut-off of P<0.20
for inclusion in the main effects model. All continuous variables were additionally assessed for a
significant quadratic term (P<0.05) using Cox’s regression. The time to event variable for the
Cox’s proportional hazard model was the time it takes for mortality to occur once a pig entered the nursery.

The Cox’s-proportional hazard model was built based on the causal diagram composed for this dataset and biologically plausibility (Appendix). For the purpose of this study, biological plausibility is defined as factors that could increase or decrease mortality due to *S. suis* within the nursery based on previous literature including age, parity, pre-weaning mortality, the number of pigs weaned, within-litter mortality, mortality in previous litters from the same sow, and the month of weaning (3,12,13). A cut-off point of *P* ≤ 0.05 was established for the coefficients in the main effects model. Independent variables that did not meet the liberal p-value cut off point of (*P* < 0.20) during univariable analysis and did not have confounding effects, were eliminated from the main effects model. The proportional hazard model was built using manual forward stepwise model building approach. The statistical significance of variables was assessed using a likelihood ratio test (LRT). Potential final models were compared using Akaike’s information criteria (AIC) to identify the more parsimonious model. This process was continued until all factors were statistically significant, or remained in the model as confounders based on biological plausibility.

The interaction terms were tested based on the causal model (Appendix). The main relationships of interest involved the age, number weaned, and preweaning mortality based on their potential to represent overall litter health and subsequent impact on mortality within the nursery (14). Once the final model was identified, the assumption of proportional hazards was evaluated for each variable using Schoenfeld residuals as well as the statistical significance of the time-varying effect. Deviance residuals were used to identify potential outlying observations and the overall fit of the model was evaluated using Cox-Snell residuals. Due to the potential of
clustering within litters, the model was evaluated using robust standard errors to adjust for this effect.

2.3 Results

Descriptive statistics of categorical and continuous variables provided at the sow- and pig-levels are presented in Tables 2.1 and 2.2, respectively. Over the duration of the 6-month period, 12 pigs showing signs for acute meningitis were submitted for full post-mortem analysis and all 12 were confirmed cases of S. suis disease (Appendix). They were confirmed in the laboratory based on positive growth from meningeal swabs plated on Columbia agar, and confirmed via matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF) technology.

Descriptive statistics revealed a few important mortality trends within the nursery prior to the vaccination trial. Approximately 40% of litters did not experience any mortality (Figure 2.2). In the litters where mortality occurred, the most common level was between >0% and 30% (Figure 2.2). The mortality occurred most frequently during the 2nd - 4th week after entry into the nursery, followed by a rapid decline in reported mortality after the 4th week (Figure 2.3). In addition, Figure 4 shows high levels of mortality were seen during the colder months of January and December. The full data set containing 2,779 pigs, evaluated over the 6-month study had 358 pigs or 13% experienced mortality within the nursery and average within-litter mortality was 14.4%.

The complete univariable analysis results can be found in Table 2.3. Univariable analysis based on Cox’s proportional hazard model revealed parity to be non-statistically significant ($P>0.05$), in addition log-rank test indicated there was no significant difference between the categories ($P>0.05$), resulting in the removal of parity from the model building process.
The final Cox’s regression model can be found in Table 2.4. Age of weaning had time-varying affects based on Schoenfeld residuals and was left in the model due to its potential biological impact on mortality and statistically significant impact on the Cox’s regression model \((P<0.05)\) (Appendix). The model that was adjusted for the effect of clustering within litters using robust standard errors was adopted as the final model. Cox’s-Snell and Deviance residuals revealed the model fit the data well and did appear to have no significant outliers, respectively (Appendix).

The factors that remained in the final model were the number of piglets weaned, age of weaning as well as its time varying component, within litter mortality, previous litter mortality, and month of weaning. There were no significant interaction terms identified (Appendix). Number of pigs weaned per litter had a protective effect on nursery mortality, specifically; the hazard of mortality was decreased if the number of pigs weaned increased (HR=0.9 \(P<0.05\)). Increasing the age of weaning increased the hazard of mortality within the nursery (HR=1.1, \(P<0.05\), Table 2.3). However, as the time in nursery progressed, a higher weaning age started to demonstrate protective effect as suggested by the time-varying effect of the weaning age (HR=0.9, \(P<0.05\)). This concept is further illustrated in Figure 2.6, which estimates that increasing weaning age increases the hazard of dying early in the nursery phase, and this hazard decreases in the later nursery phase. Litter-mort is illustrated in Figure 2.7. If an additional pig within a litter experienced mortality there was a significant increased hazard of mortality for another pig within that same litter to experience mortality (HR=9.2, \(P<0.05\)). Previous-mort had a significantly protective effect, indicating if a sow’s first litter experienced greater than 0% nursery mortality, a pig from a second litter would have a decreased risk of experiencing mortality in the nursery (HR=0.3 \(P<0.05\)). Finally, month-wean was found to have confounding
effects on previous litter mortality and age of weaning, and a significant difference between 
mortality rates were observed between the months of October and January ($P<0.05$).


**2.4 Discussion**

This *S. suis* outbreak investigation was able to provide important information on common mortality patterns as well as identify potential risk factors that may have led to an increase or decrease in nursery mortality levels. Descriptive statistics identified a common pattern of mortality occurring in the nursery, where pigs generally experienced mortality due to *S. suis* in the second, third or fourth week after entry. Other studies have also reported that mortality related to *S. suis* commonly occurs at a specific age within the nursery, although the age of mortality may vary between farms (3,5,15). One explanation for this pattern of mortality may be the transition from passive to active immunity that pigs experience generally corresponds to the time pigs have been relocated from the farrowing room into the nursery (7). Pigs can be colonized at birth from the vaginal canals of sows and rely on the passive immunity received from the sow’s colostrum to protect them from the clinical disease causing bacteria (16). After the initial colostrum intake, the maternal antibodies within the piglet begin gradually declining, while the pig mounts its own active immunity. Unfortunately at weaning, the disease exposure may increase during this time of stress due to transition from the farrowing room to the nursery, leaving pigs susceptible to systemic infection with *S. suis* within these first few weeks post-weaning (7).

According to this dataset, if pigs are able to survive past the first 2-4 weeks within the nursery, they were most likely able to survive the entire duration of the nursery. However, more investigation into this pattern should be conducted as some pigs are able to remain healthy carriers of the bacteria throughout this transitional period, while others that appear to experience the same set of risk factors develop clinical disease due to *S. suis* infection (15). In addition, this
dataset focuses on all-cause mortality and although a subset of piglets underwent post-mortem
analysis and was confirmed cases of *S. suis*, the mortality patterns observed during this outbreak
situation likely contain a small proportion of deaths due to other causes.

Another important factor to consider due to this stressful transition into the nursery is the
optimal age to wean to ensure a healthy transition. The results of the Cox’s regression model
showed age having a significant effect on the hazard of mortality. According to this outbreak
investigation, increasing the weaning age by 7 days increases a pig’s hazard of dying on day 1
within the nursery and then the longer the pig survives within the nursery the hazard of mortality
gradually reduces relative to the pig that was weaned earlier. If the pig survives until
approximately day 21 in the nursery, it then appears that an increased weaning age has a
protective affect on a pig’s survival. These conclusions are however not necessarily directly a
result of the age of weaning, and may be indirectly related to the high mortality levels seen in the
early phase of the nursery. Therefore, older weaned pigs are expected to have a higher risk of
mortality in the early phase of the nursery, but lower risk in the later phase.

Age of weaning is a widely debated topic due to multiple factors involved with the
transition into the nursery (5). Davis *et al.* (2006) conducted a study comparing the overall health
and growth of older (21 days) and younger weaned piglets (14 days), discovering a significant
difference in growth performance and immunological responses to stress between the 2 groups.
The younger weaned pigs demonstrated a weaker immunological response to stress and had
lower average daily gains throughout their time in the nursery (5). Although there is currently no
defined age to wean pigs that can help prevent *S. suis* outbreak situations, previous literature
suggests weaning between 21-28 days of age to be an appropriate target for strong healthy pigs
that are best prepared to handle the stress of weaning (5).
The number of pigs weaned per sow also appears to have an impact on survival in the nursery. In this study, increasing the number of pigs weaned resulted in a significantly decreased risk of mortality within the nursery. This was hypothesized to be a result of the overall health status of the litter. More specifically, if there is an increase in the number of pigs born alive and surviving until weaning; it is likely this is representative of a healthier litter and subsequently these pigs should have greater success within the nursery. However, due to the potential influence of cross fostering and factors that could not be controlled, this finding was treated as a potential confounder to the data and further investigation into this risk factor is necessary.

Once a pig has entered the nursery there are a few factors that can lead to significantly increased mortality during an S. suis outbreak. Specific to this study, there was a significantly increased hazard of mortality for pigs within a litter if at least one additional pig from the same litter died. It is possible that this increased hazard may be attributed to within pen transmission of S. suis. As stated previously, studies on transmission of S. suis have demonstrated both direct and indirect spread of S. suis within litters (10). A direct transmission model showed all pigs within a pen being infected with S. suis at a rate of 3.58 pigs per day following the introduction of a S. suis carrier (10). The indirect transmission model had infected pigs at a 1m distance from non-infected pigs and after approximately 7–25 days the non-infected pigs in the neighboring pens became colonized with the bacteria (10). The exact rate and intensity of transmission between pigs with these models was attributed to the type of contact the pigs experienced, the virulence or serotype of S. suis, and the susceptibility of the pigs (10). If a pig is exposed to a pig that has died due to S. suis within a pen, the transmission of bacteria occurs at an even higher rate (17). To decrease within litter transmission of S. suis, it would likely be effective to quickly treat and isolate sick pigs when possible.
It is important to also consider that littermates experience similar risk factors such as having similar passive immunity, genetics and pathogen exposures. Therefore, these pigs may share a similar set of risk factors that have led them to be more susceptible to disease than other litters, which is supported in the findings for this outbreak data set with respect to the large hazard ratio calculated for within litter mortality (17).

Although the risk factors at the pig-level are very important for implementing prevention measures for mortality occurring during S. suis outbreaks, it is also important to explore sow-level factors on the survival of pigs within the nursery. This investigation revealed an interesting risk factor when comparing the mortality patterns experienced within litters from the same sow over multiple parities. Only 15% of the sows in the study had multiple litters during the study period, however, within those sows there was a significant protective effect on the survival of pigs within a litter if pigs from a previous litter experienced mortality during the post-weaning stage. Based on this dataset, it is hypothesized that the sow was able to provide greater immunological protection from infection with S. suis if they had a previous litter that experienced mortality due to S. suis. While this finding is not of the confirmatory nature, it points that interaction between the health of sows and their offspring perhaps need to be tracked over time in multiple litters. There has been evidence that vaccination of sows during gestation with an autogenous vaccine can increase the opsonizing antibodies in pigs in both the suckling and nursery phase. This increase in antibodies did not translate to any significant impact on the morbidity and mortality of the pigs but indicates there may be potential for intervention at the sow-level (6). Overall, this is an important area for further investigation as prevention measures at the sow-level can be both an economically viable and time effective form of intervention (6).
Finally, season was identified as potentially a confounding observation based on the Cox’s regression model. During the colder months of January and December there were consistently higher mortality rates than compared to October. Previous studies have shown different geographical regions experiencing increased mortality during more extreme weather time periods, indicating temperature may serve as an additional stressor in the nursery which may leave piglets at an increased susceptibility to systemic infection with *S. suis* (18–20). Overall, the results surrounding seasonal affects are varied and *month-wean* was considered to be a confounding observation to include when analyzing the other identified risk factors.
There were a few limitations to consider when interpreting the results of this outbreak investigation. The major limitation of the study was that not all mortalities included were confirmed cases of *S. suis* based on post mortem analysis and laboratory confirmation. Out of the 358 mortalities experienced during the 6-month duration of the trial, only 12 of those were submitted for post-mortem analysis and confirmation of *S. suis* infection. However, all the samples submitted were confirmed *S. suis* cases, therefore it appears that clinical signs for acute meningitis used as a tool for diagnosis was an effective tool. In addition, there was missing data as the online source didn’t have information on pre-weaning mortality or parity for a proportion of the sows within the study (5% of sows, or 15 of 297 sows), therefore these risk factors should be explored further as they still may be important to consider during these outbreak scenarios.
2.6 Conclusions

In conclusion, the combination of sow- and pig-level factors all contribute in their own respective ways to the high mortality levels observed during this outbreak of *S. suis*. Although *S. suis* infection is a complicated bacterium requiring further research to understand its full impact in the nursery, we can use outbreak situations such as this to establish certain prevention measures aimed at minimizing the effects of *S. suis*. For example, further research on the influence that vaccinating sows could have on the immune status of piglets, has the potential to create highly efficient prevention strategies; focusing efforts on treating and isolating sick pigs as quickly as possible to control bacterial transmission; and decreasing the stress that pigs experience when entering the nursery through efficient husbandry practices could all prove to be useful in the prevention and control strategies against *S. suis* outbreaks in the nursery.

2.7 Acknowledgements

Funding for this research project was received from Ontario Pork and the University of Guelph research partnership with Ontario Ministry of Agriculture, Food, and Rural Affairs OMAFRA. Animal Health Laboratory at the University of Guelph conducted the laboratory analysis.
2.8 References


Table 2.1: Descriptive analysis of each categorical variable of interest based on the number of observations and % of the total observations at the pig-level and the sow-level.

<table>
<thead>
<tr>
<th>Covariate</th>
<th>Category</th>
<th>Pig-level (N observations, % total)</th>
<th>Sow-Level (N observations, % total)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Month of weaning</strong></td>
<td>November 2011</td>
<td>524, 19</td>
<td>52, 17</td>
</tr>
<tr>
<td></td>
<td>December 2011</td>
<td>378, 14</td>
<td>39, 13</td>
</tr>
<tr>
<td></td>
<td>January 2011</td>
<td>484, 17</td>
<td>54, 18</td>
</tr>
<tr>
<td></td>
<td>February 2011</td>
<td>377, 14</td>
<td>43, 14</td>
</tr>
<tr>
<td></td>
<td>March 2011</td>
<td>447, 16</td>
<td>46, 15</td>
</tr>
<tr>
<td></td>
<td>November 2011</td>
<td>569, 20</td>
<td>51, 15</td>
</tr>
<tr>
<td><strong>Parity</strong></td>
<td>Parity 1</td>
<td>672, 24</td>
<td>74, 30</td>
</tr>
<tr>
<td></td>
<td>Parity 2</td>
<td>809, 29</td>
<td>82, 28</td>
</tr>
<tr>
<td></td>
<td>Parity 3</td>
<td>459, 17</td>
<td>54, 18</td>
</tr>
<tr>
<td></td>
<td>Parity 4</td>
<td>445, 16</td>
<td>51, 17</td>
</tr>
<tr>
<td></td>
<td>Parity 5</td>
<td>224, 8</td>
<td>24, 8</td>
</tr>
<tr>
<td></td>
<td>Parity 6,7,8</td>
<td>170, 6</td>
<td>12, 4</td>
</tr>
<tr>
<td><strong>Pre-weaning mortality</strong></td>
<td>Cross Fostered</td>
<td>475, 23</td>
<td>52, 26</td>
</tr>
<tr>
<td></td>
<td>≥0-10% Mortality</td>
<td>302, 14</td>
<td>29, 15</td>
</tr>
<tr>
<td></td>
<td>&gt;10-20% Mortality</td>
<td>506, 24</td>
<td>52, 26</td>
</tr>
<tr>
<td></td>
<td>&gt;20-30% Mortality</td>
<td>351, 17</td>
<td>34, 18</td>
</tr>
<tr>
<td></td>
<td>&gt;30-40% Mortality</td>
<td>311, 15</td>
<td>33, 17</td>
</tr>
<tr>
<td></td>
<td>&gt;40-50% Mortality</td>
<td>121, 6</td>
<td>14, 7</td>
</tr>
<tr>
<td></td>
<td>&gt;50-100% Mortality</td>
<td>45, 2</td>
<td>8, 4</td>
</tr>
<tr>
<td></td>
<td>Missing</td>
<td>668, 24</td>
<td>107, 35</td>
</tr>
<tr>
<td><strong>Nursery mortality within the same litter</strong></td>
<td>0-1 pigs dead</td>
<td>971, 35</td>
<td>96, 35</td>
</tr>
<tr>
<td></td>
<td>&gt;1 or 1 additional pig died within the same litter</td>
<td>1808, 65</td>
<td>188, 65</td>
</tr>
<tr>
<td><strong>Previous litter mortality</strong></td>
<td>Sow with previous litter having 0% mortality</td>
<td>285, 10</td>
<td>25, 8</td>
</tr>
<tr>
<td></td>
<td>Sow with previous litter having &gt;0% mortality</td>
<td>177, 7</td>
<td>17, 6</td>
</tr>
<tr>
<td></td>
<td>Sows with only 1 litter in data set</td>
<td>2,317, 83</td>
<td>252, 83</td>
</tr>
</tbody>
</table>
Table 2.2: Descriptive analysis of the continuous variables including information on the range and mean of each factor of interest

<table>
<thead>
<tr>
<th>Covariate</th>
<th>Range [min, max]</th>
<th>Mean±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of final piglets weaned</td>
<td>[0,18]</td>
<td>10.27±2.17</td>
</tr>
<tr>
<td>Age of weaning</td>
<td>[8,57]</td>
<td>27.63±3.16</td>
</tr>
</tbody>
</table>
Table 2.3: Univariable analysis representing each factor of interests LRT p-value, partial LRT p-value, and hazard ratios after each variable was run individually through a cox-proportional hazard regression model. The referent categories are listed below the table.

<table>
<thead>
<tr>
<th>Predictor</th>
<th>LRT P-value</th>
<th>Covariate Category</th>
<th>Hazard Ratios</th>
<th>Partial LRT P-value</th>
<th>95% Confidence Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Month of weaning</td>
<td>0.001</td>
<td>January</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>February</td>
<td>0.727</td>
<td>0.074</td>
<td>0.51, 1.03</td>
</tr>
<tr>
<td></td>
<td></td>
<td>March</td>
<td>0.387</td>
<td>0.001</td>
<td>0.26, 0.57</td>
</tr>
<tr>
<td></td>
<td></td>
<td>October</td>
<td>0.225</td>
<td>0.001</td>
<td>0.14, 0.36</td>
</tr>
<tr>
<td></td>
<td></td>
<td>November</td>
<td>0.735</td>
<td>0.098</td>
<td>0.51, 1.06</td>
</tr>
<tr>
<td></td>
<td></td>
<td>December</td>
<td>1.15</td>
<td>0.392</td>
<td>0.84, 1.56</td>
</tr>
<tr>
<td>Parity</td>
<td>0.635</td>
<td>Parity 1</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Parity 2</td>
<td>0.962</td>
<td>0.803</td>
<td>0.71, 1.30</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Parity 3</td>
<td>1.240</td>
<td>0.202</td>
<td>0.89, 1.72</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Parity 4</td>
<td>1.052</td>
<td>0.773</td>
<td>0.74, 1.48</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Parity 5</td>
<td>0.964</td>
<td>0.876</td>
<td>0.61, 1.51</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Parity 6</td>
<td>1.247</td>
<td>0.344</td>
<td>0.78, 1.97</td>
</tr>
<tr>
<td>Pre-weaning Mortality (%)</td>
<td>0.034</td>
<td>Cross Fostered</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pre-weaning Mortality 0-10</td>
<td>0.584</td>
<td>0.020</td>
<td>0.37, 0.91</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pre-weaning Mortality 10-20</td>
<td>1.166</td>
<td>0.348</td>
<td>0.84, 1.61</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pre-weaning Mortality 20-30</td>
<td>0.837</td>
<td>0.367</td>
<td>0.56, 1.23</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pre-weaning Mortality 30-40</td>
<td>0.782</td>
<td>0.238</td>
<td>0.51, 1.18</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pre-weaning Mortality 40-50</td>
<td>0.674</td>
<td>0.208</td>
<td>0.36, 1.25</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pre-weaning Mortality 50-100</td>
<td>0.940</td>
<td>0.885</td>
<td>0.41, 2.17</td>
</tr>
<tr>
<td>Nursery mortality within the same litter</td>
<td>0.001</td>
<td>0 or 1 death within litter</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>&gt;1 or 1 additional pig died within the same litter</td>
<td>10.342</td>
<td>0.001</td>
<td>7.79, 13.72</td>
</tr>
<tr>
<td>Previous litter mortality</td>
<td>0.001*</td>
<td>Sow’s previous litter &lt;1% mortality</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sow’s previous litter having &gt;1% mortality</td>
<td>0.553</td>
<td>0.016</td>
<td>0.36, 0.86</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sows with only 1 litter in data set</td>
<td>0.126</td>
<td>0.009*</td>
<td>0.04, 0.39</td>
</tr>
<tr>
<td>Age of weaning</td>
<td>0.002</td>
<td>Age at weaning</td>
<td>0.943</td>
<td>0.002</td>
<td>0.91, 0.98</td>
</tr>
<tr>
<td>Number of piglets weaned</td>
<td>0.001</td>
<td>Final # pigs weaned</td>
<td>0.897</td>
<td>0.001</td>
<td>0.85, 0.94</td>
</tr>
</tbody>
</table>

*a month weaned= January

*b parity= parity 1

*c pre-weaning mortality = <0% or crossfostered

*d nursery mortality in the same litter= 0 or 1 death

*e previous litter mortality = previous mortality 0%

* non-relevant p-value’s
**Table 2.4:** Final cox regression model illustrating the hazard ratio for each factor of interest before and after adjustment for the effect of clustering within litters

<table>
<thead>
<tr>
<th>Covariate</th>
<th>Category</th>
<th>Cox regression model</th>
<th>Cox regression mode using robust standard error</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Hazard Ratio</td>
<td>P-value</td>
</tr>
<tr>
<td>Number of weaned piglets</td>
<td></td>
<td>0.913</td>
<td>0.001</td>
</tr>
<tr>
<td>Age at weaning</td>
<td></td>
<td>1.077</td>
<td>0.002</td>
</tr>
<tr>
<td>Nursery mortality within the same litter</td>
<td>&gt; 1 or 1 additional pig in litter died</td>
<td>9.216</td>
<td>0.001</td>
</tr>
<tr>
<td>Previous litter mortality</td>
<td>Sow with previous litter having &gt;0% mortality</td>
<td>0.337</td>
<td>0.082</td>
</tr>
<tr>
<td></td>
<td>Sows with only 1 litter in data set</td>
<td>1.158</td>
<td>0.623</td>
</tr>
<tr>
<td>Month of weaning</td>
<td>October</td>
<td>0.349</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>November</td>
<td>0.822</td>
<td>0.295</td>
</tr>
<tr>
<td></td>
<td>December</td>
<td>0.768</td>
<td>0.103</td>
</tr>
<tr>
<td></td>
<td>February</td>
<td>0.767</td>
<td>0.143</td>
</tr>
<tr>
<td></td>
<td>March</td>
<td>0.925</td>
<td>0.771</td>
</tr>
<tr>
<td>Ageweaning*TVC</td>
<td></td>
<td>0.996</td>
<td>0.001</td>
</tr>
</tbody>
</table>

*referent category
Figure 2.1: Flow chart showing the step-by-step data manipulation that occurred starting with an online data base and mortality records from a single farm over a six-month period up until the point of survival analysis.
Figure 2.2: Mortality within litters in the nursery organized into 10 percent mortality categories from October 2011 to January 2012, prior to vaccination trial. The majority of litter level mortality resides in the 10-30th percentage.
Figure 2.3: Mortality within litters organized by the week of death within the nursery from October 2011 to January 2012, prior to vaccination trial. The time within the nursery or time for potential mortality to occur is 63 days or 9 weeks where weeks 2, 3, 4 are showing increased mortality levels.
**Figure 2.4:** Overall mortality within litters in the nursery organized by 10 percent mortality categories and by the month of weaning for seasonal analysis. The data included is from October 2011 to January 2012, prior to vaccination trial.
**Figure 2.5:** Hazard ratio associated with age of weaning vs time within the nursery. The hypothetical scenario depicted here involves increasing a piglet’s weaning age by seven days and after accounting for the time varying effects of age, the graphical results indicate a decreasing hazard over time within the nursery, protective effect of increased weaning age occurring at approximately 21 days within the nursery.
**Figure 2.6:** Cox’s survival curve comparing within litter mortality during the nursery, demonstrates a significantly decreased survival within the nursery if at least one additional pig within a litter experience mortality (red line).
Chapter 3

EVALUATION OF THE DIRECT, INDIRECT, TOTAL, AND OVERALL EFFECTIVENESS OF *STREPTOCOCCUS SUI* AUTOGENOUS VACCINATION IN NURSERY PIGS USING A RANDOMIZED CLINICAL TRIAL
3.0 Abstract

A randomized vaccination trial was conducted on a 300-sow farrow-to-finish farm experiencing mortality due to *Streptococcus suis* infection. In order to evaluate the direct vaccine effectiveness (VE) on mortality due to *S. suis*, 75% of pigs in 4 out of 24 cohorts received a farm-specific autogenous vaccine, while the remaining 25% were left unvaccinated. The remaining 20 cohorts were not vaccinated to assess indirect, total, and overall VE on all-cause mortality. A Cox’s regression and binomial regression model were used to evaluate vaccine effectiveness. Estimates of risk ratio were converted to measures of VE. There was no evidence of direct VE, however, total and overall VE were 27% and 21%, respectively. These findings indicate that there is potential for benefits of vaccination despite lack of evidence of direct effectiveness. However, further study using a larger population is necessary to investigate vaccine effectiveness more thoroughly.
3.1 Introduction

*Streptococcus suis* is considered to be one of the most important pathogens affecting nursery pigs (1,2). There are currently 35 serotypes of *S. suis* identified based on the capsular polysaccharide (cps), in addition to untypable strains that are un-encapsulated, or do not contain a cps moiety (3). The bacteria may present as a commensal bacteria or as an opportunistic pathogen in the nasal cavity and tonsils of the majority of pigs (2). In most cases, pigs that are colonized with *S. suis* remain healthy, however, on occasion systemic infection and clinical disease can occur (4,5).

Systemic infections caused by *S. suis* in nursery pigs are usually characterized by a low incidence (less than 5%), with clinical signs associated with one or more of the following conditions; septicemia, arthritis, pericarditis, and meningitis (6). Most commonly, disease presents as acute meningitis (7). Treatment may involve injection with procaine penicillin or a more broad spectrum antibiotic, however, even with early treatment prognosis is often poor (2,8). Occasionally, severe outbreaks of *S. suis* disease can occur, affecting a large proportion of at risk-animals (9). Clinical disease caused by *S. suis* occurs on most swine farms and can result in significant economic losses and therefore there is a need for the development of effective prevention and control methods.

Control of *S. suis* disease by vaccination is challenging because of several factors. Although the bacteria are present in the upper respiratory tract of almost all pigs, clinical disease only occurs sporadically. The multiple serotypes of *S. suis* all have their own set of virulence factors, and the exact pathogenesis of *S. suis* is still not fully understood (10). Overall, vaccination against *S. suis* is not a common practice on North American swine farms, and there
are no commercial vaccines licensed for use to control *S. suis* in Canada (10). If vaccines are used on a farm, they are typically serotype-specific autogenous bacterins delivered to individual pigs or sows via intramuscular (IM) injection (1). These vaccines are commonly generated from *S. suis* isolates taken from the meningeal swabs of clinical cases. However, the overall effectiveness of autogenous vaccines against *S. suis* have not yet been determined (2).

The overarching aim of the current study was to determine the effectiveness of vaccination with an autogenous vaccine in preventing mortality as a result of *S. suis* in nursery age pigs under field conditions. Most research studies have focused on testing the direct effects of vaccination at the animal level. However, under commercial conditions vaccines are often applied at the group level, which incorporates direct and indirect effects of vaccination. Hence, it is beneficial to apply other methods than calculation of direct effectiveness only (11). Therefore, the specific objectives of this study were to evaluate direct, indirect, total, and overall vaccine effectiveness on nursery pig mortality in a commercial setting.
3.2 Materials and methods

This project was approved by the Animal Care Committee at the University of Guelph.

3.2.1 Farm description

A vaccine trial was conducted on a 300-sow farrow-to-finish farm. The farm practiced weekly weaning with lactation length varying between 21-30 days. The facility had 6 farrowing rooms with 14 farrowing crates per room and operated on an all-in/all-out basis per room. There were 8 nursery rooms, which varied in size and pen conformation. Two rooms contained raised decks with 8 pens measuring 1.22 m x 2.44 m and typically held 12 pigs per pen. Two other rooms of similar size contained slightly larger pens at floor level (1.52 m x 2.44 m) with on average 13 pigs per pen. There were also 4 larger rooms containing 4 floor-level pens (2.7 m x 4.5 m) holding about 35 pigs each. The nursery rooms were also operated using an all-in/all-out flow. The herd was closed with breeding stock replacements produced from within. Further biosecurity measures included shower-in/shower-out, locked doors, and on-site composting of dead stock.

3.2.2 Farm history

The standard vaccination protocol on the farm was to vaccinate all pigs at weaning with a Mycoplasma hyopneumoniae, Hemophilus parasuis combination bacterin (Suvaxyn® MH/HPS, Zoetis, Kirkland QC), and a porcine circovirus vaccine (Ingelvac Circoflex®, Boehringer Ingelheim, Burlington, ON). The starter diet fed for approximately the first week after weaning contained 220 mg/kg of lincomycin (Lincomix 44, Bio Agri Mix, Mitchell, ON).

Prior to initiation of the study, the herd experienced high nursery room mortality with many of the pigs exhibiting neurological clinical signs consistent with a diagnosis of meningitis.
or pigs were found dead without previous signs of illness. Individual treatment for a suspected 
case of *S. suis* meningitis involved separation into a hospital pen, IM injection of 3 mL per 45 kg 
with trimethoprim-sulfadoxine (Trivetrin®, each mL contains 40 mg trimethoprim and 200 mg 
sulfadoxine, Merck Animal Health, Kirkland, QC), or humane euthanasia if animals showed 
advanced clinical signs. A convenience sample of nursery pigs that experienced mortality or 
were euthanized during the study period revealed that all animals had evidence of systemic 
infection with *S. suis*. These confirmations were based on meningeal swabs and swabs taken 
from internal organs or joints of pigs that displayed clinical signs consistent with *S. suis* 
infection. The presence of systemic infection with *S. suis* was confirmed in all 12 animals tested 
(Table 3.1).

### 3.2.3 Cohorts of pigs

This study included data from 24 weekly cohorts of weanling pigs that entered the 
nursery phase between October 2011 and March 2012. In 4 cohorts, a randomized pig-level 
study, blocked by litter, was conducted to evaluate vaccine effectiveness. Direct effectiveness 
focused on mortality due to clinical signs consistent with *S. suis*, or the outcome of interest. For 
the remaining 20 cohorts, only mortality due to any cause was available and was used to estimate 
indirect, total, and overall vaccine effectiveness. Case definitions are described in Table 3.2.

### 3.2.4 Randomized field trial

A vaccination study was conducted in 4 weaning cohorts between February 2\textsuperscript{nd} and 
March 1\textsuperscript{st}, 2012 (Figure 3.1). The study included the time and number of mortalities in each litter 
as a result of *S. suis* infection, diagnosed based on clinical signs (Table 3.2).
In the 4 cohorts, 73-75% of pigs from each litter were randomly selected to receive the autogenous serotype 2 vaccine using systematic random sampling. The other 25% of pigs were left unvaccinated. The vaccinated pigs received 2 mL of vaccine IM, a day prior to entry into the nursery and a 2 mL booster 3 weeks after entering the nursery. All pigs were ear-tagged for identification purposes.

The \textit{S. suis} vaccine used in the study was made at Gallant Custom Laboratory, Cambridge, Ontario, using one \textit{S. suis} serotype 2 isolate. The vaccine isolate was recovered from a meningeal swab sample collected from a previous clinical case of \textit{S. suis} on this farm.

### 3.2.5 Data management and data analysis

The data indicative of all-cause mortality recorded 24 unvaccinated cohorts during the 6-month study period by farm manager were used. These records also included information on the date of weaning, date of death, sow ID, and litter ID, number of pigs born alive, number of pigs weaned, pre-weaning mortality, and parity. Sows were excluded if information about the weaning date or the number of pigs weaned was missing. The vaccination study data set from February 2, 2012 to March 1, 2012 containing the 4 vaccination cohorts included information on sow ID, litter ID, date of weaning, weight of piglet at weaning, date of death, cause of death, gender, vaccination status, and cohort. The time at risk was defined as 9 weeks, or the approximate time a pig spent in this farm’s nursery and period immediately following the nursery phase. Thus, mortalities that occurred after the period of risk, were excluded from analysis. After removal of all missing observations the data sets were merged based on sow ID and further processed so that it represented pig-level data from specific litters that entered the nursery between October 2011 and March 2012.
Descriptive statistics were performed on vaccination status, the main variable of interest for vaccination effectiveness. Additional descriptive statistics were performed on potential confounding variables including sow parity, the month of weaning, age of weaning, the number of piglets weaned, pre-weaning mortality, previous litter mortality, mortality within litters, gender, weight at weaning, vaccination cohort. Frequency count of mortality categorized on the basis of percentage mortality within litters, calendar time, and time in the nursery was illustrated using histogram plots. Descriptive statistics for categorical and continuous explanatory variables were determined using appropriate statistics.

Univariable analysis was conducted using a Cox’s proportional hazard model on each of the categorical and continuous predictor variables of interest. A cut-off of $P<0.20$ was used for inclusion in the multivariable model. The linearity assumption between outcome and continuous independent variables was assessed by generating a quadratic term. The time to event variable was considered the time when a pig entered the nursery to the time when mortality occurred.

The hazard model was built using manual forward stepwise model building approach, with the vaccination status as the exposure of interest and other variables as possible confounders. The statistical significance of variables was assessed using a likelihood ratio test (LRT). A cut-off point of $P \leq 0.05$ was established for inclusion of independent variables in the final model. The potential interactions between vaccination status and the 4 vaccination cohorts were tested within the final model to assess their significance (Appendix). The model was additionally adjusted for clustering within litters using robust standard errors.

### 3.2.6 Vaccine effectiveness

An illustration of all measures of VE and their derivation can be found in Figure 3.2.
Direct effects were calculated by $VE = 1 - RR$ or

$$VE = 1 - \frac{Mortality \text{ due to } S. suis \text{ in } 75\% \text{ vaccinated pigs}}{Mortality \text{ due to } S. suis \text{ in } 25\% \text{ unvaccinated pigs}}.$$ Indirect vaccine effects look at the group-level effects by comparing mortality in non-vaccinated pigs of the partially vaccinated group to the mortality of non-vaccinated pigs in groups that did not receive a vaccine. Indirect vaccine effects were calculated by:

$$VE = 1 - \frac{Mortality \text{ in } 25\% \text{ unvaccinated pigs exposed to vaccinated pigs}}{Mortality \text{ in } 100\% \text{ unvaccinated pigs not exposed to vaccinated pigs}}.$$ Total vaccine effects are slightly different from indirect effects, as it focuses on the survival of pigs that did receive the vaccine in the population vaccinated. Total effects were calculated by:

$$E = 1 - \frac{Mortality \text{ in } 75\% \text{ vaccinated pigs}}{Mortality \text{ in } 100\% \text{ unvaccinated pigs not exposed to vaccinated pigs}}.$$ Overall vaccine effectiveness focuses on the complete herd-level mortality. It is described by Halloran et al. (10) as the weighted average of indirect effects compared to the weighted average of total effects. Overall effects were calculated by: $VE = 1 - \frac{Overall \text{ mortality in the vaccinated cohorts}}{Overall \text{ mortality in the } 100\% \text{ unvaccinated cohorts}}.$

The relative risks described above were calculated using two models in Stata 14 (StataCorp, Lakeway Dr, Texas). A Cox’s-proportional hazard model was used to test the direct vaccine effects with morality due to $S. suis$ as the outcome of interest. A binomial regression model was used to evaluate indirect, total and overall vaccine effectiveness on all-cause mortality. The hazard ratio (HR) from the Cox’s regression model evaluating direct VE was used as a substitute for RR during percentage VE calculations. Deviance residuals were used to identify potential outlier observations and the overall fit of the model was evaluated using Cox-Snell residuals. The model was evaluated using robust standard errors to adjust for the effect of clustering within litters.
Finally, the binomial logistic regression models were evaluated for the impact that any influential observations detected following deviance residual analysis had on the interpretation of the model. This was done by removing influential observations sequentially for each measure of VE and then assessing the influence their removal had on the resulting RR and calculated VE (Appendix).
3.3 Results

3.3.1 Overall observations

The full data set contained records of 2,977 pigs over the 6-month study; 358 (12%) pigs experienced post-weaning mortality and mean littermate mortality post-weaning was 14.4%. Of the 435 pigs included in the vaccination study, 39 (9%) of pigs died post-weaning, and 35 (90%) of those deaths were due to clinical signs consistent with S. suis disease. Post-weaning litter-mate mortality due to S. suis infection, in the 4 vaccination cohorts showed high variability (Figure 3.3), with the mean litter-level mortality of 14.0%.

All-cause mortality for all 24 cohorts involved in the study period, organized by their vaccination status, is illustrated using a histogram plot (Figure 3.4). Descriptive statistics of categorical and continuous variables that were used as explanatory variables are presented in the supplementary materials along with complete univariable analysis. Following univariable analysis and Cox’s hazard model building, the HR for vaccination status was not altered by any of the potential confounding variables including; sow parity, the month of weaning, age of weaning, the number of piglets weaned, pre-weaning mortality, previous litter mortality, mortality within litters, gender, and weight at weaning. In addition there was no significant interaction between “vaccination” and “cohort”. Therefore, the final models for the different measures of VE, contained “vaccination” and the vaccinated groups of pigs.

3.3.2 Vaccine effectiveness

The final model results for all measures of effectiveness, along with their calculated VE’s, are shown in Table 3.3. Direct VE was 1.6% but did not appear to have significant effects
on mortality due to *S. suis* (*P>*0.05). The final Cox’s hazard regression model was adjusted for
the affect of clustering within litters.

The binomial regression models also did not show any statistically significant measures
of associations; indirect VE was 7.6% (*P>*0.1), total VE was 27.6% (*P*=0.06) and overall VE
was 21.3% (*P*=0.08).

Deviance residual analysis revealed cohorts 9 and 20 to be potential outliers in the
binomial regression models. Removal of cohort 9 resulted in minor effects to all levels of VE
(Appendix). Removal of cohort 20, or the first vaccination cohort, increased all measures of VE
(Table 3.4). Most notably, total and overall VE became statistically significant (*P*<0.05). The
total VE increased from 27% to 54% and overall VE increased from 23% to 50%.

Removal of both influential observations (cohorts 9 and 20) resulted in total and overall
VE to become statistically significant (*P*<0.05) (Appendix). The indirect VE increased from
7.6% to 31.3% but remained non-statistically significant (*P>*0.05).
3.4 Discussion

Studies looking at different measures of vaccine effectiveness have the added advantage of identifying indirect vaccine benefits in addition to direct effects. Herd immunity, in a population is based on 3 main factors: the characteristics of pathogen transmission, the ability of the vaccine to confer protection, and the vaccination protocol or coverage in a population (12). Vaccination against infectious diseases can result in a reduction in the transmission probability and a reduction in the duration of infectiousness, subsequently altering the population dynamics involved with infectious agents (11). Most of the population level studies for vaccinations are conducted on human populations and we were unable to find any evidence of indirect analysis for *S. suis* in swine herds, making this a unique study.

With respect to study design and analysis, it is very common that vaccines are evaluated under controlled experimental conditions, which is a gold standard for evaluation of direct vaccine effects as well as a foundation for accurate assessment of clinical efficacy and safety. For at least some swine diseases such as porcine circovirus associated disease (PCVAD), pseudorabies and influenza, the indirect effect of vaccines has been recognized, but the attempts to evaluate total or overall vaccine effectiveness have not been made to the best of our knowledge (11, 12). Specifically with respect to *S. suis*, there is a lack of research surrounding population-level vaccine effectiveness in commercial settings.

In this study vaccine effectiveness differed considerably based on the level of analysis, both in terms of the magnitude of effect and its associated statistical significance. The direct effect of vaccination was not-statistically significant, however, the calculated total and overall VE showed potential protective effects.
Additionally, after the 2 outliers were removed from the analysis, all of these measures increased in magnitude. Specifically with respect to total and overall VE, their measures of VE increased dramatically with a statistically significant underlying measure of association. These were outliers based on unusual mortality levels for the observed outbreak. Cohort 9 had no mortality, or very low for this outbreak situation compared to the average 14.4% per litter. Cohort 20, or the first vaccination cohort, had very high mortality reaching >30%, compared to the average 14% mortality per litter experienced during the vaccination trial. Eliminating the cohorts that did not have the average levels of mortality in this relatively small data set led to significant differences being observed. Eliminating these cohorts and focusing on cohorts that experienced moderate to high levels of mortality (8-20%) over the entire outbreak duration provided evidence of total and overall benefits of vaccination (9). This means for the majority of cohorts involved in the study, there was evidence for protection from disease due to *S. suis* at the population level following vaccination.

The major concern to the validity of our results at the group level is that vaccination was attempted in 4 out of 5 consecutive cohorts. It is possible that mortality due to *S. suis* showed some cyclical variation and the effect of vaccination was therefore confounded. *S. suis* has the potential to cause severe to moderate outbreaks sporadically in nursery pigs and therefore there is a possibility that the high and low mortalities experienced could be attributed to the sporadic nature of the disease (9). Inclusion of one completely unvaccinated cohort within the vaccination period was driven by availability of the retrospective data, but this was not sufficient to have unbiased estimates of VE. In future studies, this should be improved by including a follow-up period post-vaccination, and restricting the outcome analysis to mortality strictly due to *S. suis*. 
Under a commercial setting, the overall vaccine effectiveness is commonly evaluated by
comparison of completely vaccinated and completely non-vaccinated cohorts. While the benefit
of such an approach is that the overall VE can be considered, the drawback is that the
commencement of the vaccination protocol could completely or partially overlap with other
control measures. This could create bias as unknown confounders could influence the mortality
levels during the vaccination period. These confounders could include: 1) the virulent strain
affecting the herd has the potential to change over time, 2) whether pigs were treated or untreated
after they showed clinical signs, and 3) a potential for maternal antibodies to influence the
effectiveness of the vaccine (16). Our study of retrospective data is not free from such bias and
future study is needed to have a better randomization of entire groups with respect to their
vaccination status.

Vaccination appeared to provide some beneficial overall effect despite lack of evidence
surrounding direct vaccine effectiveness. The complicated nature of the bacterium may be one
reason for inconsistent direct vaccine efficacy seen in other field studies (8, 18). Additionally,
there has not been an exact timing of administration for S. suis vaccines established as the
maternal antibody affect has not yet been quantified (16). At this time, the use of autogenous
vaccines in the field would require constant monitoring of affected herds, continual development
of farm specific vaccines, and potential safety concerns, making it an impractical disease
management solution if there continue to be inconsistent results in the field (15).

Experimental trials show promising potential for protection against S. suis through the
use of autogenous bacterins, however, replicating these results in the field has not been
successful (17). This study proposed the possibility for evaluating indirect, total and overall
vaccine effects, indicating alternative methods of studying vaccination effectiveness beyond direct effects.
3.5 Limitations

It is possible that unknown confounders influenced the observed vaccine effectiveness. This study was restricted by the retrospective nature of the data, and could have benefited from a follow-up period to better assess the full extent vaccination against *S. suis* contributes to controlling mortality levels in the nursery. A further weakness of the study was the relatively small sample size and the short duration of the intervention. Since *S. suis* appears to follow a pattern of dramatic increases and decreases in mortality rates, conducting the vaccination trial over a longer period would have strengthened the results. Taking into account these limitations, further studies conducted in a commercial setting aimed at developing a standardized vaccination protocol against *S. suis* could significantly advance the efforts to reduce mortality due to *S. suis*.
3.6 Conclusions

Taking into consideration all the limitations of retrospective analysis, this study demonstrates the potential for an alternate method for analyzing vaccines effectiveness in a commercial swine herd. By looking at different groups of vaccinated pigs, we were able to analyze the population level affects and compare and contrast vaccine effectiveness directly and indirectly with promising total and overall results. This farm experienced moderate to high total and overall vaccine effectiveness against \textit{S. suis} at the population level. Future studies should be aimed at developing a vaccination protocol on-farm that has the ability to evaluate these direct and indirect effects without the restrictions that accompanied this retrospective analysis.

3.7 Acknowledgments

Funding for this research project was received from Ontario Pork and the University of Guelph research partnership with Ontario Ministry of Agriculture, Food, and Rural Affairs OMAFRA. Animal Health Laboratory at the University of Guelph conducted the laboratory analysis.
3.8 References


Table 3.1: Results of the subset of samples taken from pigs that were clinical cases during the 6-month outbreak data set.

<table>
<thead>
<tr>
<th>Date</th>
<th>*Sample taken</th>
<th>*Streptococcus suis</th>
<th>Age (weeks)</th>
<th>Other pathogens</th>
<th>Clinical Signs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dec 7th, 2011</td>
<td>M</td>
<td>+</td>
<td>6</td>
<td></td>
<td>Septicemia / meningitis</td>
</tr>
<tr>
<td></td>
<td>L</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Jan 5th, 2012</td>
<td>M</td>
<td>+</td>
<td>10</td>
<td></td>
<td>Septicemia / meningitis /meningoencephalitis</td>
</tr>
<tr>
<td></td>
<td>H</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>S</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>March 14, 2012</td>
<td>M</td>
<td>+</td>
<td>8</td>
<td></td>
<td>Septicemia / meningitis</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td><em>Streptococcus porcinus</em></td>
<td></td>
</tr>
<tr>
<td>March 14, 2012</td>
<td>A</td>
<td>+</td>
<td>8</td>
<td></td>
<td>Septicemia / meningitis</td>
</tr>
<tr>
<td>March 14, 2012</td>
<td>M</td>
<td>+</td>
<td>8</td>
<td></td>
<td>Septicemia / meningitis</td>
</tr>
<tr>
<td></td>
<td>A</td>
<td>+</td>
<td></td>
<td>Actinobacillus minor</td>
<td></td>
</tr>
<tr>
<td>March 14, 2012</td>
<td>M</td>
<td>+</td>
<td>8</td>
<td></td>
<td>Septicemia / meningitis</td>
</tr>
<tr>
<td></td>
<td>A</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>March 15, 2012</td>
<td>M</td>
<td>+</td>
<td>10</td>
<td></td>
<td>Lesions consistent with sepsis</td>
</tr>
<tr>
<td></td>
<td>A</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>L</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>March 19, 2012</td>
<td>M</td>
<td>+</td>
<td>6</td>
<td></td>
<td>Meningitis / septicemia</td>
</tr>
<tr>
<td></td>
<td>A</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>March 19, 2012</td>
<td>M</td>
<td>+</td>
<td>6</td>
<td></td>
<td>Meningitis / septicemia</td>
</tr>
</tbody>
</table>

*M-Meningeal  
*A-Abdominal  
*L-Lung  
H-Heart  
S-Spleen
Table 3.2: The case selection and identification protocol for mortality due to *Streptococcus suis*.

Information gathered from convenience sample of pigs taken prior to vaccination study, and the samples taken from sick and dead pigs during vaccine study.

<table>
<thead>
<tr>
<th>Case Identification</th>
<th>Behavioral</th>
<th>Disease progression</th>
<th>Gross lesions</th>
<th>Laboratory testing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute meningitis, sudden death</td>
<td>Animal would exhibit head tilt, convulsions, ataxia, and eventually an inability to stand, paddling and death</td>
<td>Acute, 1-4 day disease progression was seen and in some cases sudden death</td>
<td>Lesions are often found in the respiratory tract, central nervous system, ear, and serosaes</td>
<td>During vaccine study meningeal swabs were tested to confirm <em>S. suis</em> - primarily by culturing method and MALDI-TOF technology</td>
</tr>
<tr>
<td></td>
<td>It is also common to see swollen joints and respiratory signs</td>
<td></td>
<td>Bronchopneumonia is also common finding in <em>S. suis</em> cases (18)</td>
<td></td>
</tr>
</tbody>
</table>

*MALDI-TOF (Matrix Assisted Laser Desorption/Ionization-Time of Flight)*
Table 3.3: Vaccine effectiveness (VE) estimations of an autogenous bacterin against *Streptococcus suis* in pigs during the nursery phase

<table>
<thead>
<tr>
<th>Measure</th>
<th>Relative Risk</th>
<th>95% CI</th>
<th>P-value</th>
<th>VE (%)</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Direct VE</td>
<td>0.98</td>
<td>0.46, 2.12</td>
<td>0.982</td>
<td>1.6</td>
<td>&lt;0*, 54</td>
</tr>
<tr>
<td>Indirect VE</td>
<td>0.92</td>
<td>0.61, 1.39</td>
<td>0.705</td>
<td>7.6</td>
<td>&lt;0*, 49</td>
</tr>
<tr>
<td>Total VE</td>
<td>0.72</td>
<td>0.52, 1.01</td>
<td>0.058</td>
<td>27.6</td>
<td>&lt;0*, 49</td>
</tr>
<tr>
<td>Overall VE</td>
<td>0.79</td>
<td>0.60, 1.03</td>
<td>0.082</td>
<td>21.3</td>
<td>&lt;0*, 40</td>
</tr>
</tbody>
</table>

*HR from Cox’s regression used as a substitute of RR

Table 3.4: Vaccine effectiveness (VE) estimations using an autogenous bacterin against *Streptococcus suis* in pigs during the nursery phase after removal of cohort 20, an influential observation determined through deviance residual analysis

<table>
<thead>
<tr>
<th>Measure</th>
<th>Relative Risk</th>
<th>95% CI</th>
<th>P-value</th>
<th>VE (%)</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Direct VE</td>
<td>0.93</td>
<td>0.30, 2.92</td>
<td>0.902</td>
<td>7.0</td>
<td>&lt;0*, 70</td>
</tr>
<tr>
<td>Indirect VE</td>
<td>0.75</td>
<td>0.45, 1.35</td>
<td>0.277</td>
<td>24.4</td>
<td>&lt;0*, 55</td>
</tr>
<tr>
<td>Total VE</td>
<td>0.45</td>
<td>0.28, 0.75</td>
<td>0.002</td>
<td>54.2</td>
<td>25, 72</td>
</tr>
<tr>
<td>Overall VE</td>
<td>0.50</td>
<td>0.40, 0.82</td>
<td>0.002</td>
<td>50.0</td>
<td>18, 60</td>
</tr>
</tbody>
</table>

*HR from Cox’s regression used as a substitute of RR