Nursery pig health and performance

by

Karen Marie De Bruyn

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ABSTRACT

NURSERY PIG HEALTH AND PERFORMANCE

Karen Marie De Bruyn
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Advisor:
Robert Friendship

The growth performance and the presence of common respiratory pathogens were studied. Fifty nurseries were sampled including farms that routinely used in-feed antimicrobials (conventional), and farms that used no antimicrobials. These latter farms were classified as "raised-without-antibiotics" (RWA) and organic. No difference was found between the growth rates of RWA and conventional nursery pigs ($P > 0.05$), while organic pigs grew slower than conventional pigs ($P < 0.001$). Based on serology, approximately a third of nurseries were seropositive for porcine reproductive and respiratory syndrome virus and this had a negative impact on individual pig growth rates. 80 % of nurseries were seropositive for influenza A virus. It was observed that most farms vaccinated weaned pigs for *Mycoplasma hyopneumoniae* so seroprevalence was not possible to assess. Despite vaccination, 73 % of these nurseries were classified as seronegative based on samples taken from pigs at the end of the nursery stage.

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"I alone know the plans I have for you, plans to bring you prosperity and not disaster, plans to bring about the future you hope for." Jeremiah 29:11.

CONTRIBUTIONS

Karen De Bruyn contributed to the project planning, coordination of farm visits and data collection. Karen De Bruyn also organized, analyzed and interpreted data and was the primary author for this thesis.

Emily Hanna and Chris Almond contributed to project planning, coordinating farm visits and data collection for the first 30 farms in the study.

Elana Raaphorst and Bailey Fuller processed sera samples.

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ADG- Average daily gain

ELISA- Enzyme-linked immunosorbent assay

IAV- Influenza A virus

PRRS- Porcine reproductive and respiratory syndrome

PRRSv- Porcine reproductive and respiratory syndrome virus

RWA- Raised-without-antibiotics

CHAPTER 1: LITERATURE REVIEW

1.1 General introduction

The nursery stage is a challenging period in a pig`s life and impacts the overall health of the entire swine operation. Pigs are particularly prone to disease shortly after weaning because this is a time when passive immunity wanes and active immunity is not fully developed. Pathogens tend to cycle on a farm by continuing to infect pigs as they enter the nursery. It is generally accepted that if pigs fall behind during the nursery stage, then this setback will impact their performance in the grower-finisher barn as well. Because of the health risks at weaning and the additional stressors of changes in feed and environment, many farms use mass medication of antimicrobials during this period. With the growing concern from consumers about the use of antimicrobials in food animal production this practice is being questioned. There are production systems in Ontario that raise pigs without the use of antimicrobials, such as “raised-without antibiotics (RWA)” and certified organic, but there is little information regarding how the nursery pig performance and health status in these systems compares to conventionally raised pigs.

1.2 Importance of nursery pigs in the health of a swine herd

While it is important to carefully monitor the health of all swine in a herd, the monitoring and prevention of disease in nursery pigs is especially important to prevent disease outbreaks. Piglets are born with an underdeveloped immune system and rely on the colostrum from their dam for immunoglobulins that pass through the intestinal lining and into the blood stream during the first 24 hours of life (Rooke & Bland, 2002). In addition, they rely on a continuous supply of immunoglobulins in the sow`s milk to provide local immunity in the gut (Rooke & Bland, 2002). The circulating antibodies from colostrum gradually disappear within a few weeks of life and the

lactogenic immunity ends abruptly with weaning. On modern intensive farrowing operations in Ontario, piglets are commonly weaned at approximately 21 days of age, but the active immunity of these pigs has not fully developed at this time (Chase & Lunney, 2012). This lag in the complete development of their immune systems in addition to the stress of weaning, mixing of animals from different litters and possibly different sources, changes to their environment and transition from milk to solid feed, means that nursery pigs can be very susceptible to disease. Due to this, the nursery can become a reservoir of disease within a swine herd, depending on how it is managed (Chung et al., 1997). Thus, being able to monitor and control disease within the nursery can benefit the entire production of a swine herd.

1.3 Growth rates of Ontario nursery pigs

A key practice for monitoring the impact of disease on a herd's production is tracking pig growth rates. A commonly used measurement to determine a pig's growth is the average daily gain (ADG) measurement. This is determined by subtracting the final weight of a pig from the initial weight and dividing this difference by the number of days between weight measurements. Average daily gain provides the amount of weight a pig gained each day.

Cottrell (2005) analyzed nursery production data from 1995-2004, from three Canadian swine systems. The systems were based in Alberta, Manitoba and Ontario, respectively. The dataset included 8.37 million pigs within 3,527 batches from 248 barns. The mean average daily gain of nursery pigs from all three systems for their total time in the nursery was 370 g/day with a standard deviation of 50 g/day (Cottrell, 2005).

1.4 Antimicrobial-use in the nursery period

A commonly used practice to reduce disease during the nursery period and to optimize growth, is to include antimicrobials in feed and possibly treat groups of nursery pigs with antimicrobials in the water. In a review, Cromwell (2002) estimated that 70-80% of nursery starter diets over the past 25 years had contained antimicrobials. This practice has continued because the inclusion of antimicrobials in nursery diets has been effective at both reducing disease and improving growth rates. As outlined by Cromwell (2002) a summary by Maddock, containing 67 studies over 22 years, showed that in-feed antimicrobials reduced mortality in nursery pigs from 4.3% to 2.0%. The Cromwell review also summarized more than 1000 studies that showed that for pigs ranging between 7 and 25 kg, in-feed antimicrobials increased growth rates by an average of 16.4% and improved feed efficiency by 6.9%. Feeding antimicrobials can also have performance benefits during the growing and finishing phases but the most substantial effects are seen within the nursery period (Cromwell, 2002).

The exact method that allows antimicrobials to improve pig growth and reduce disease is still a topic of debate. Pluske (2017) reviewed the current research on the mode of action of antimicrobials. One theory is that antimicrobials reduce the load of gastrointestinal tract (GIT) bacteria, lessening the competition between GIT bacteria and the pig for nutrients and reducing microbial metabolites that depress growth. Another theory is that the reduction of GIT bacteria and subsequently microbial fermentation results in less short chain fatty acids being formed in the GIT. The reduction of fatty acid production is believed to cause the GIT walls to thin due to less cell proliferation in the mucosal lining. The thinner intestinal walls are thought to increase nutrient absorption. Other research indicates that antimicrobials allow a pig to grow more efficiently by inhibiting microbiota and thus increasing the amount of nutrients available to the

animal and reducing the maintenance needs of the GIT. Some researchers have also indicated that antimicrobials promote growth by restricting the production of catabolic metabolites from inflammatory cells in the intestines (Pluske, 2017).

1.4.1 Pressure to reduce antimicrobial use

Even though antibiotics have been shown to be extremely beneficial in livestock production, there are growing concerns about antimicrobial usage in agriculture contributing to the international problem of antimicrobial resistant bacteria and the implications this can have on human health. This concern isn't necessarily new; Cromwell stated in his 2002 review that measures were already being taken in the 1970s in Great Britain to reduce antimicrobial usage in livestock. Due to the concern of antimicrobial resistance, the use of antibiotics as growth promoting agents has been banned in many European countries since the 1990s (Jensen & Hayes, 2014).

A review commissioned by the UK Prime Minister in 2015, studied 192 scientific papers about antimicrobial use in agriculture and found that 114 or 59% of these papers suggested a link between antimicrobial use in livestock production and antimicrobial resistance in human infections. From the results found by this review, the chair of the review, Jim O'Neill, stated that he believed there was a concern that antimicrobials used in livestock were contributing to antimicrobial resistance in human infections, and that there should be actions taken worldwide to curtail agricultural use of antimicrobials (O 'Neill, 2015).

Consumers are concerned about antibiotic usage in livestock production as well. A study conducted in 2006 at grocery stores in Stillwater, Oklahoma, allowed consumers to make a series of choices about buying pork that was raised antibiotic-free vs. conventionally. The study

concluded that consumers do place a significant premium on pork that is raised antibiotic-free (Lusk et al., 2006).

1.4.2 Antibiotic-free production systems in Ontario

To fulfill consumer demand, different systems that market pork as being raised antibiotic-free have formed. These differ from conventional farms, which do not sell their pork in niche markets that require specific production rules beyond those mandated for every swine producer. Three of the antibiotic-free systems that exist in Ontario are described below.

1.4.2.1 Raised-Without-Antibiotics (RWA):

According to the Canadian Food Inspection Agency (CFIA), for meat and animal products to claim to have been "raised without the use of antibiotics", "the animal may not have been treated with antibiotics, administered by any method, from birth to slaughter or harvest". This includes any substance that is categorized as an antimicrobial according to Health Canada's "Categorization of Antimicrobial Drugs Based on Importance in Human Medicine" document. Furthermore, lactating animals with offspring that are being marketed as antibiotic-free, cannot be administered any form of antimicrobials (CFIA, 2019a).

While animals that have been treated with antimicrobials are not allowed to be marketed as RWA, in some systems producers do have the option of treating a sick animal and then marketing it as conventional. Besides the prohibition of the use of antimicrobials, RWA farms generally follow the same practices as conventional farms.

1.4.2.2 Organic:

For any product to be labelled and sold as organic in Canada, it must contain 95 % organic material, as per the CFIA regulations (CFIA, 2019b). Labelled organic products must have been certified by a certification body that is accredited through the CFIA (CFIA, 2019b). To be certified as organic, livestock production must follow specific management practices outlined by the Canadian General Standards Board committee on organic agriculture (CFIA, 2019b). As outlined in the Organic Production Systems document written by the Canadian General Standards Board Committee on Organic Agriculture, the goal of organic production is to "develop operations that are sustainable and harmonious with the environment." One of the key pillars of organic production is that antibiotics are not allowed to be administered to animals unless no other treatment options remain (Canadian General Standards Board, 2015a). If an animal is given antibiotics, it can no longer be marketed as organic (Canadian General Standards Board, 2015a).

Other regulations for organic livestock production include; animals being allowed access to the outdoors when the weather permits, animals being fed only organic feed which consists solely of organic crops and products, and all animals having freedom of movement (Canadian General Standards Board, 2015a). Specific regulations for swine include; the prohibition of tail docking, using farrowing pens rather than crates to allow sows freedom of movement, and a minimum weaning age of 4 weeks of age (Canadian General Standards Board, 2015a). Vaccines are permitted in organic production (Canadian General Standards Board, 2015a).

1.4.2.3 Certified Humane:

Certified humane is a production system that was founded in 2003 by duBreton, a Quebec abattoir, and is sold in Sobey's stores (DuBreton, 2019). Unlike the organic and RWA systems, there is no specific oversight by the CFIA on the certified humane system except what is required of every labelling claim, that the labelling is correct and accurate (CFIA, 2019a). For producers to be accredited as Certified Humane, they must meet the Humane Farm Animal Care (HFAC) Standards (HFAC Scientific Committee, 2014). HFAC is a non-profit organization that states their "mission is to improve the lives of farm animals by providing viable, credible, duly monitored standards for humane food production and assuring consumers that certified products meet these standards" (HFAC Scientific Committee, 2014). The Humane Farm Animal Care Standards are determined by the HFAC scientific committee (HFAC Scientific Committee, 2014).

The specific standards for swine production in the Certified Humane system are very similar to those of organic swine production. Pigs are not permitted to be given subtherapeutic antibiotics through feed or water (HFAC Scientific Committee, 2014). They are also not allowed to be fed protein sourced from animals (HFAC Scientific Committee, 2014). A noticeable difference in this system compared to other antibiotic-free systems is that animals can be individually treated with antibiotics (HFAC Scientific Committee, 2014). Piglets in the Certified Humane program are not allowed to be weaned before 4 weeks of age (HFAC Scientific Committee, 2014). They also must be fed creep feed while they are in the farrowing barn (HFAC Scientific Committee, 2014). Pigs of all ages must be given access to bedding and must have freedom of movement (HFAC Scientific Committee, 2014). Sows are not permitted to be kept in farrowing crates but instead farrow in pens that allow nesting behaviours to occur (HFAC

Scientific Committee, 2014). Pigs do not need to be given access to the outdoors, but it is encouraged (HFAC Scientific Committee, 2014).

There were 1,179 pork producers in Ontario in 2018 (Ontario Pork, 2018). Of these nearly 1200 producers, only 69 were part of an antibiotic-free program (Burlatschenko et al., 2019, personal communication). Of these 69 producers, 54 of them are part of the RWA program and the remaining 15 are organic or humane producers (Burlatschenko; Canning; Dimmers; Tenbergen & Scorgie, 2019, personal communication). Thus, the proportion of producers in the province that raise pigs without antibiotics is relatively small at only 5.9%.

Another way to view the Ontario swine industry is through the numbers of sows. According to Statistics Canada, there were approximately 313,200 sows at the end of the first half of 2018 (Statistics Canada, 2018). Of these 313,200 sows, approximately 27,000, or 9 %, were from herds that are part of antibiotic-free programs (Burlatschenko et al., 2019, personal communication). Of the 27,000 sows in antibiotic-free programs, 25,500 of these sows, or 94 %, are from RWA systems and 1,700, or 6%, are from organic or humane herds (Burlatschenko et al. 2019, personal communication).

1.5 Current literature on antibiotic-free swine production

The research on antibiotic-free swine production systems is limited. A study by Dee et al. (2018), exposed 3 groups of pigs that had been vaccinated against PRRS to a strain of the virus. The first group of pigs had been mass-treated twice with antibiotics before the exposure and therapeutically treated after the exposure, the second was mass treated once with antibiotics and selectively treated with antibiotics after the exposure and the third was given no antibiotics at any time. The study concluded that pigs in the first two groups, that had been exposed to and

treated with antibiotics, had significantly better feed conversion rates and growth rates in the finishing period compared to the pigs that were never exposed to antibiotics. The pigs in the first two groups also had significantly lower mortality rates than pigs in the third group (Dee et al., 2018).

In general, there are various references in the literature that emphasize that to raise pigs successfully without the use of antimicrobials, other environmental and husbandry factors need to be emphasized. Strategies for optimizing health and production during the nursery phase in an antibiotic-free system include; maintaining strict vaccination protocols, ensuring ventilation is working properly and that rooms are kept at optimal temperatures, and that strict biosecurity protocols are followed. Alternatives to antibiotics such as feeding nutraceuticals and pro- and pre-biotics are also often employed (Greiner, 2017). Additional advice includes increasing weaning age to four-weeks of age, culling failing pigs, maintaining a clean environment with thorough washing and disinfecting protocols, cleaning and flushing water lines, supplementing the water with electrolytes, feeding essential oils, and increasing vitamins in the feed in times of high stress or low immunity (van Donkersgoed, 2015). A key strategy to successful management in an antibiotic-free system is being proactive rather than reactive (Terpstra, 2018).

Since organic farming is still a relatively new trend in North America, the literature for organic swine production is nearly non-existent. In Europe, however, organic production is more established and has continuously been growing in popularity (Früh et al., 2014). At the end of 2011, there were 290,000 organic European farms that managed 10.6 million hectares of land (Früh et al., 2014). Germany has the most organic pigs in Europe, followed by Denmark, France and Austria, respectively (Früh et al., 2014).

While there are no internationally accepted rules for organic agriculture, in Europe, the Standards Committee of the International Federation of Organic Agriculture Movements (IFOAM), provides rules that are intended to be guidelines for national programs around the world (Früh et al., 2014). Specific practices for organic swine farms vary depending on country. The most extreme example of this is that organic pigs in the UK constantly live outside while pigs in Germany live inside with limited access to the outdoors (Früh et al., 2014).

A European review published in 2014 summarized the challenges that newly weaned organic pigs face on European farms (Leeb et al., 2014). While organic farming practices strive to enhance animal welfare and reduce stress on animals, it was determined in the review that the process of weaning is still a stressful time in a piglet's life (Leeb et al., 2014). Weaning can be particularly stressful on organic farms where piglets are transitioned from indoor farrowing units to outdoor nurseries (Leeb et al., 2014). It was stated in the review that respiratory diseases, diarrhea, and endoparasitic infestations are the most commonly seen diseases at weaning in organic farms in Denmark, Sweden, the Netherlands, and Germany (Leeb et al., 2014).

Another European review by Kijlstra and Eijck, stated how specific health problems in organic production are often related to the outdoor housing systems that animals are raised in (Kijlstra & Eijck, 2006). Animals being reared outside are exposed to various viral, bacterial and parasitic pathogens that animals raised totally indoors might not encounter (Kijlstra & Eijck, 2006). The review also states that solutions to health issues in organic production include improving animal breeds, feeding pro- and pre-biotics, enhancing housing conditions, proper implementation of vaccines and acidifying drinking water (Kijlstra & Eijck, 2006).

The differences in key management practices between the conventional, RWA, organic and humane production system types are shown in Table 1.1.

1.6 Respiratory disease

1.6.1 Introduction

Pigs in the nursery stage of production are vulnerable to disease in general, but among the most economically significant diseases affecting post-weaned pigs are respiratory pathogens. Often the true impact of these diseases is not realized until after the pigs leave the nursery. This makes the nursery period an important time for immunization and preparing pigs so that they can successfully fight off the potentially devastating impact of respiratory diseases.

1.6.2 Significance of respiratory diseases in the swine industry

Respiratory diseases are among the most important diseases affecting nursery pigs. They are a serious concern for swine producers worldwide, as they can result in significant economic losses (VanAlstine, 2012). In this review, three common respiratory diseases that affect swine; porcine reproductive and respiratory syndrome (PRRS), enzootic pneumonia, and influenza, are outlined and their impact on nursery pig performance is discussed.

1.7 Porcine Reproductive and Respiratory Syndrome

1.7.1 History

Porcine reproductive and respiratory syndrome (PRRS) is an internationally endemic disease affecting swine. The first clinical signs of the disease were seen in the United States in 1987 (Hill, 1990). In 1990, similar clinical signs were exhibited in pigs in Germany (de Jong et al., 1991). Originally, the disease was known under a variety of names in several locations around the world including; mystery pig disease, blue-eared pig disease, swine infertility and respiratory syndrome, and swine reproductive failure syndrome (Corzo et al., 2010).

The cause of the disease was unknown until 1991 when researchers in Lelystad, Netherlands used Koch's postulates to identify the etiologic agent of the disease to be an enveloped RNA virus (Wensvoort et al., 1991). The researchers named the virus the Lelystad virus and suggested that the disease be called porcine epidemic abortion and respiratory syndrome or PEARS (Wensvoort et al., 1991). Shortly afterwards, researchers in the United States isolated a similar virus that fulfilled Koch's postulates to prove that it was the cause of the disease that they called swine infertility and respiratory syndrome (SIRS) (Collins et al., 1992). This virus isolate was named ATCC VR-2332 (Collins et al., 1992). At the 1992 International Symposium on Swine Infertility and Respiratory Syndrome, it was decided that the disease resulting from the Lelystad and ATCC VR-2332 virus isolates would be called porcine reproductive and respiratory syndrome (PRRS) and the virus would be called the PRRS virus (Corzo et al., 2010).

1.7.2 Economic importance

In 2005, it was estimated that PRRS costs the American swine industry $560 million dollars each year (Neumann at al., 2005). Given that PRRS is found amongst swine herds worldwide (Zimmerman et al., 2012), the disease and thus its control has a great significance to the swine industry across the globe. A 2014 study determined that PRRS costs Ontario nurseries $ 2,485,831 annually or a loss of $4.50 per nursery pig during the year following an outbreak on affected farms. A total annual cost of $1,464,012 was determined for the grower-finisher stage on Ontario farms. This translates to a loss of $1.87 in the year following an outbreak for each grower-finisher pig on affected farms (Zorzolan et al., 2014).

1.7.3 Etiology

The PRRS virus (PRRSV) is an enveloped, single stranded RNA virus (Cavanagh et al., 1995) that is non-hemagglutinating and only grows in specific cell lines (Benfield et al., 1992; Wensvoort et al., 1991). PRRSV is classified as a member of the genus Arterivirus, the family Arteriviridae and the order Nidovirales (Zimmerman et al., 2012). The virus can be further classified into two distinct genetic lineages based on the origination of the disease: type 1 genotype, or Lelystad virus, and type 2 genotype, or VR-2332 (Zimmerman et al., 2012). The two genotypes vary considerably, with 44 percent of the nucleotide sequence differing between them (Zimmerman et al., 2012). While both genotypes are found throughout the world, they are still each predominantly found near their points of origin with type 1 being predominant in Europe and type 2 predominant in North America as well as Asia (Zimmerman et al., 2012).

Within both main genotypes of the virus, there is extensive genetic diversity (Zimmerman et al., 2012). There have been multiple methods used to further type PRRSV isolates, including using cleavage patterns of restriction endonucleases and serotyping, as an attempt to understand the variation within each genotype of the virus (Zimmerman et al., 2012). However, due to the capability of the virus to easily mutate, no typing method has been universally accepted as being completely effective (Zimmerman et al., 2012).

1.7.4 Epidemiology

Porcine reproductive and respiratory syndrome virus has multiple routes of transmission. The virus can be transmitted vertically from a sow to her offspring through the placenta during gestation or through milk during nursing (Pileri & Mateu, 2016). A study conducted by Christopher-Hennings et al. (1995) determined that boars that are infected with PRRSV can

transmit live virus through their semen. The virus is also transmitted horizontally through aerial transmission, insemination or sexual contact, ingestion, direct contact, and percutaneously (Pileri & Mateu, 2016). Methods of direct contact include secretion of the virus through saliva and urine (Wills et al., 1997). Indirect transmission can also occur via fomites (Pileri & Mateu, 2016).

A distinguishing characteristic of the PRRSV is its ability to cause persistent infections in pigs. Wills et al. (1997) found that PRRSV could still be isolated from oropharyngeal swabs of infected animals up to 157 days after their infection date. In another study, 51 out of 59 infected pigs tested positive for the PRRSV between 63 and 105 days post-infection (Horter et al., 2002). In their study, Allende et al. (2000), concluded that most pigs can be infected with PRRSV until 150 days post-infection. Similarly, in another study it was determined that infected pigs tested positive for PRRSV until 130 days post-infection (Fangman et al., 2007).

Susceptible animals (e.g. newborn or newly weaned pigs) are constantly present in a breeding herd which allows the virus to be continuously transferred from carrier animals to susceptible pigs (Zimmerman, 2012). Thus, the ability of animals to act as carriers of the virus for long periods of time allows the disease to become endemic in herds (Zimmerman et al., 2012). This makes control of the disease a challenge.

1.7.5 Immunity

Sows have been shown to pass maternal immunity to the PRRSV onto their offspring (Chung et al., 1997). These maternal antibodies protect piglets until they reach 6-9 weeks of age (Chung et al., 1997). At this age, when maternal antibodies have receded, nursery pigs may become susceptible to infection with the PRRSV (Chung et al., 1997).

While some herds do vaccinate against PRRSV, the numerous strains of the pathogen can make this a challenge (Zimmerman et al., 2012). To be effective, vaccines must provide cross-protection by evoking an immune response against multiple strains of the virus (Zimmerman et al., 2012). Whether this can be successfully accomplished may depend on specific herd dynamics.

1.7.6 Pathogenesis

After a pig is infected with PRRSV, the virus replicates within the macrophages in the lung and lymph nodes (Zimmerman et al., 2012). Thus, most lesions from the disease occur within these tissues (Zimmerman et al., 2012). Viremia occurs in infected swine within 12-24 hours with the titres of virus peaking between 7-14 days (Zimmerman et al., 2012). The acute phase of viremia lasts for about 28 days (Chand et al., 2012). The virus damages the respiratory tract by causing apoptosis, stimulating inflammatory cytokines, activating polyclonal B cells, and increasing an animal's susceptibility to septicemia by reducing key defence mechanisms (Zimmerman et al., 2012). Indications of a pig being infected with the virus include enlargement of the lymph nodes and regions of the lobes within the lung appearing purple, soft and wet (Rossow et al., 1995).

1.7.7 Clinical signs

The clinical signs of PRRS can vary depending on factors such as the strain of virus, immune status of the host and the environment (Zimmerman et al., 2012). As the name of the disease implies, PRRS is associated with two types of clinical signs: reproductive and respiratory. Anorexia and pyrexia are common signs in breeding animals (Hill, 1990), and sudden death of sows has occasionally been seen (de Jong et al., 1991). Sows have also shown

cyanosis and occasional paralysis or a stumbling gait (de Jong et al., 1991). Increased numbers of abortions are also common, and sows often show irregular oestrous cycles which result in issues with re-breeding (Hill, 1990). Some farms have also experienced a decrease in semen production from infected boars (de Jong et al., 1991). Sows have been shown to farrow early and produce piglets with low viability, as well as have increased numbers of stillbirths and mummified pigs (Hill, 1990). Pre-weaning mortality increases due to poor milk production from anorexic sows and low viability of piglets (Hill, 1990; de Jong et al., 1991). Conjunctivitis has also been observed in animals suffering from the disease, particularly in newborn piglets (de Jong et al., 1991).

Endemic PRRSV infections can also occur. A herd usually becomes endemically infected with the virus after an epidemic infection. Strains of virus with low virulence can also result in endemic infections. Herds that are endemically infected can also undergo a PRRS epidemic if a new strain of virus infects the herd. Usually with endemic PRRS, outbreaks of clinical signs occur only in the most vulnerable animals. These outbreaks typically include nursery pigs that have just lost their maternal antibodies against the virus, or newly introduced breeding animals that have not been previously exposed to the pathogen (Zimmerman et al., 2012).

1.7.8 Significance to nursery pigs

Due to their high susceptibility to infection with the PRRSV, nursery pigs have been shown to be a reservoir of the virus within farrow to finish herds (Chung et al., 1997). Infected nursery and finishing pigs experience anorexia and pyrexia (Hill, 1990) but generally of greater concern is respiratory disease. Clinical signs of PRRS in nursery pigs include; coughing, distressed breathing known as “thumping”, and increased mortality (Hill, 1990). Secondary bacterial infections also commonly occur (de Jong et al., 1991; Harper, 1991). A great concern to

swine producers is that there is a significant reduction in the growth of infected nursery and finishing pigs (Harper, 1991).

1.7.9 Prevention and treatment

Due to the severity of PRRSV infections and the difficulty in controlling the disease, the literature on treatment and prevention of the disease is vast. In PRRSV-negative herds, the ideal strategy is to prevent exposure to the virus (Zimmerman et al., 2012). This involves following good biosecurity protocols such as isolating new animals entering a herd, washing trucks that transport animals, restricting people entering the barn and in pig dense areas, using air filters on barn ventilation (Zimmerman et al., 2012).

For herds that are infected with PRRSV, there are numerous strategies to eradicate the disease (Corzo et al., 2010). These include depopulating and repopulating a herd or adopting the "McRebel" system to reduce transmission of pathogens within the farrowing room (Corzo et al., 2010). A popular strategy to eradicate the PRRSV from a sow herd is to close the herd for at least 6 months by not adding any new animals to the barn (Corzo et al., 2010). In addition, after closing the herd, breeding animals should be exposed to homologous virus or a modified live vaccine (Corzo et al., 2010). This ensures that all animals within a herd have the same immunity against PRRSV (Corzo et al., 2010). Once the sows in a herd have stopped producing viremic piglets, the nursery should be depopulated. By following key management strategies, it is possible to eradicate PRRSV from a sow herd.

1.8 Enzootic pneumonia

1.8.1 History

In 1965, researchers at Iowa State University, isolated the etiologic agent for what was then called virus pneumonia of pigs (Mare & Switzer, 1965). They determined that the agent causing the characteristic microscopic and gross lesions in the lungs of swine was a small coccobacillus bacterium (Mare & Switzer, 1965). The bacterium that was isolated was determined to be in the genus *Mycoplasma* due to the morphology, dimensions and staining of the colonies and individual bacteria (Mare & Switzer, 1965). The other distinguishing characteristic that led the researchers to classify the new organism as part of the genus *Mycoplasma* was its resistance to penicillin (Mare & Switzer, 1965). Mare and Switzer decided to name the newly found *Mycoplasma* species, *Mycoplasma hyopneumoniae* (Mare & Switzer, 1965). The organism is now found worldwide and is a serious concern for pork producers everywhere (Thacker & Minion, 2012).

1.8.2 Economic importance

Since the impact of *M. hyopneumoniae* on individual animals varies and infection with the organism is known for resulting in secondary infections, it is difficult to estimate the economic impact of enzootic pneumonia and thus studies estimating economic losses vary (Maes et al., 1996). The costs associated with enzootic pneumonia arise from reduced growth rates and thus longer times to slaughter, increased mortality rates and increased medication costs (Maes et al., 2018).

Most studies that have tried to quantify losses due to enzootic pneumonia compare the growth rates of pigs to the prevalence of lung lesions at slaughter. The results of these studies

can vary from differences in the methods of scoring lesions, ages of animals at slaughter and types of statistical analysis conducted (Maes et al., 1996). While some studies have reported 6-16% reductions in growth rates of finishing pigs that exhibit slaughter lesions indicative of *M. hyopneumoniae*, other studies have shown no relationship between lung lesions at slaughter and a decrease in growth rates (Maes, 2018).

Regula et al. (2000) took a different approach to estimating the cost of the disease by comparing growth rates between pigs that were seropositive and seronegative for *M. hyopneumoniae* rather than solely relying on lung lesions to indicate infections. Seronegative pigs had growth rates of 38 g/day more than seropositive pigs. Lung lesions at slaughter were also evaluated in the study and were determined to be associated with decreased average daily gain values.

Haden et al. (2012) conducted a study using diagnostic and close-out reports of finishing barns within a large American production system. Using average daily gain values, cull, mortality and fallback percentages, the difference between loss per head placed was determined between barns infected with one or more respiratory pathogens and the baseline levels of healthy barns in the system. The barns infected with *M. hyopneumoniae* had a $0.63 increase in the loss per head from the baseline barns. Barns that were infected with *M. hyopneumoniae* and PRRSV had a loss of $9.69 above the baseline barns. Lastly, barns that were infected with *M. hyopneumoniae* and influenza A virus had an additional loss of $10.12 per head placed (Haden et al., 2012).

1.8.3 Etiology

Mycoplasma hyopneumoniae is a small bacterium that, like all species in the genus *Mycoplasma*, has no cell wall (Thacker, 2004). Since mycoplasmas lack a cell wall, they are resistant to the types of antimicrobials that work by disrupting the mechanisms of bacteria cell walls (Maes et al., 1996). Organisms in the genus *Mycoplasma* are the smallest known cells on earth (Pollack et al., 1997). *Mycoplasma* are classified in the genus *Mycoplasma*, family *Mycoplasmataceae*, order *Mycoplasmatales* and class *Mollicutes* (Razin, 1992). *Mycoplasma* colonies are known for having a circular morphology where the centre of the circle is denser than the outer area of the circle which has a translucent appearance (Smith, 1971). Since *Mycoplasmas* lack cell walls, the true morphology of individual cells is a point of debate and varies depending on the specific species of *Mycoplasma* (Smith, 1971).

1.8.4 Epidemiology

Mycoplasma hyopneumoniae can be transmitted horizontally from pig to pig. There has been no evidence to show transmission of the bacterium *in-utero*, thus pigs are considered naive to the disease at birth (Maes et al., 2018). Direct nose to nose contact is believed to be the most common route of transmission (Sibila et al., 2009; Thacker & Minion, 2012). Horizontal transmission can occur when dams lacking immunity against the bacterium infect their piglets through direct contact (Calsamiglia & Pijoan, 2000; Maes et al., 1996). This pathway can be a constant source of reinfection for a herd (Sibila et al., 2009). Originally it was believed that only gilts or low parity sows, with lower levels of exposure to the organism, could infect their piglets (Goodwin, 1965; Maes et al., 1996; Sibila et al., 2009), however, more recent research has shown evidence of sows up to parity-seven being persistently infected with the organism and

able to transmit the disease to their offspring (Calsamiglia & Pijoan, 2000). Horizontal transmission of the organism often occurs in the nursery when animals from different litters are mixed together in a new environment (Meyns et al., 2004). *Mycoplasma hyopneumoniae* has been shown to have a long incubation period, with shedding of the organism lasting as long as 240 days post-infection (Pieters et al., 2009).

A case-control study conducted by Goodwin (1985) determined risk factors that resulted in enzootic pneumonia in British pig herds that were formerly classified as being free of the disease. The study excluded all herds that could pinpoint pig movements to or near their farm that occurred around the same time as the determined time of infection. This left only herds in the study where the source of infection could not be determined. Goodwin determined a risk score for each case in the study using a variety of risk factors. As the risk score increased, the number of farms that were not infected with *M. hyopneumoniae* decreased. It was determined that the distance a herd is from an infected herd was the most important factor that influenced whether a naïve herd became infected. This was the first evidence that *M. hyopneumoniae* could be transmitted through the air. Since this study, other research has suggested that aerosol transmission of the bacterium can occur (Morris et al., 1995; Stärk et al.,1998). Airborne transmission occurs through the release of infectious microorganisms in small, dry or liquid particles (Stärk, 1999). These particles generally arise from sneezing or coughing animals and travel in the air before infecting naïve pigs (Stärk, 1999).

Climate has also been shown to play a role in *M. hyopneumoniae* transmission. Lower temperatures and higher levels of precipitation have been shown to be associated with higher percentages of pigs in a herd being infected with the bacterium (Goodwin, 1985).

The bacterium is also believed to spread via fomites. Goodwin (1985) observed the survival rate of cultured *M. hyopneumoniae* on cloth material and determined that the organism could survive for up to 76 hours but upon drying, the number of live bacteria would dramatically drop. What was of more concern was that the organism was found to survive in tap and rain water for at least a month. However, a study conducted in 2004 found that if people exposed to *M. hyopneumoniae* followed common biosecurity protocols, they did not transfer the disease to naïve swine (Batista et al., 2004).

1.8.5 Immunity

Wallgren et al. (1998) determined that sow antibody levels for *M. hyopneumoniae* decrease within the 4 weeks before farrowing and reach their lowest level at the time of farrowing. The theory for this is that antibodies move from a sow's blood to her udder to be transferred through her colostrum. As aforementioned, sows have been shown to transmit *M. hyopneumoniae* to their young (Calsamiglia & Pijoan, 2000; Maes et al., 1996), thus farrowing sows are at a higher risk of being infected by the bacterium and consequentially infecting their offspring as well. In the herd studied by Wallgren et al., dry sows that were healthy showed clinical signs of *M. hyopneumoniae* at farrowing as the disease spread through the herd. However, sows that are not newly infected with the bacterium and remain immune to *M. hyopneumoniae* can pass on maternal immunity against the bacterium to their piglets (Wallgren et al., 1998).

Interestingly, it has been shown that in an endemically infected herd, the level of antibodies against *M. hyopneumoniae* in sows can vary (Rautiainen & Wallgren, 2001). The duration of maternal immunity within piglets has also been shown to vary in relation to the levels of antibodies within dams (Wallgren et al., 1998).

A study comparing vaccinated and non-vaccinated sows for *M. hyopneumoniae* found that piglets from vaccinated sows had significantly higher titers, at weaning and two weeks after weaning, for the bacterium than piglets from non-vaccinated sows (Ruiz et al., 2003). In the same study, PCR tests at weaning showed that there were also significantly fewer positive piglets in the vaccinated sow group compared to piglets from non-vaccinated sows. This study provides evidence to support vaccinating sows prior to farrowing to provide maternal immunity to piglets. Contradictorily, a study in 2013 determined that as piglets, from both *M. hyopneumoniae* positive and negative sows, approached weaning, the likelihood of the piglets testing positive for the bacterium, increased by 10% every additional day that piglets were suckling (Nathues et al., 2013). Thus, the relationship between sows transferring passive immunity against *M. hyopneumoniae* and being able to transmit the bacterium to their offspring, is still a topic of discussion.

Wallgren et al., determined that 4 to 7-week-old pigs exposed to *M. hyopneumoniae* and exhibiting clinical signs of the disease, had low levels of antibodies against the bacterium. The researchers believed this was due to a combination of the loss of maternal antibodies and pigs of this age having a poor ability to produce antibodies for the bacterium (Wallgren et al., 1998). Morris et al. determined the half-life of maternally-derived *M. hyopneumoniae* antibodies in piglets to be 15.8 days (Morris et al., 1994).

Many commercial adjuvanted whole cell vaccines exist that can be administered to nursery pigs. Single dose and double dose administration of these vaccines have both been shown to be effective. Vaccinating nursery pigs against *M. hyopneumoniae* is a common management strategy. In fact, according to the USDA's National Animal Health Study, 72 percent of sites within the study vaccinated their nursery pigs against *M. hyopneumoniae*

(USDA, 2012). The role of sow vaccination in the protection to piglets through maternal immunity has been discussed in the literature, however, it is still a point of controversy (Thacker & Minion, 2012).

1.8.6 Pathogenesis

After inhalation by a pig, *M. hyopneumoniae* adheres to the cilia of the epithelium of the respiratory tract, namely the trachea, bronchi and bronchioles, and incubates in pigs for 10-16 days (Maes et al., 1996). The bacterium causes hyperplasia of the bronchial epithelium, clumping of the cilia, ceases cilia movement, and results in loss of cilia (DeBey & Ross, 1994; DeBey et al., 1992). Goblet cells in the bronchioles also change morphologically upon infection and their secretion patterns are altered resulting in changes to the type of mucus secreted (DeBey et al., 1992). The disruption of the respiratory tract, particularly the ability of the cilia to clear pathogens, allows other pathogens to invade the lungs, resulting in secondary infections (Thacker & Minion, 2012).

1.8.7 Clinical signs

Enzootic pneumonia can either be endemic or epidemic, but the endemic form of the disease is the most common (Thacker & Minion, 2012). All swine are susceptible to enzootic pneumonia, but the disease most commonly affects growing and finishing pigs (Maes et al., 1996). In all-in/all-out systems, clinical signs of the disease usually appear when pigs are between 12 and 20 weeks of age (Maes et al., 1996). In the epidemic form of the disease, where naïve animals are exposed to the bacterium, it is possible to see complete herd morbidity, with all animals affected (Thacker & Minion, 2012). Clinical signs of the epidemic form include; fever, coughing, laboured breathing, anorexia and mortality (Maes et al., 1996; Thacker & Minion,

2012). *Mycoplasma hyopneumoniae* colonization in the lungs results in consolidation of the antero-ventral region, which appears as purple to grey lesions (Maes et al., 2008).

A dry cough is one of the most characteristic clinical signs of endemic enzootic pneumonia (Maes et al., 1996). The coughing is maximized 4 weeks post-infection and can persist for a total of about 8 weeks (Maes et al., 1996). Subclinical infections, where no clinical signs of the disease are observed, can also occur (Maes et al., 1996). If secondary infections occur, as is common with this bacterium, more severe clinical signs like those of the epidemic form of the disease, such as fever, anorexia, laboured breathing, and weakness are exhibited (Maes et al., 1996; Thacker & Minion, 2012). Another sign of the disease is variability in the size of growing pigs (Thacker & Minion, 2012). Some pigs within a group will be small and unthrifty, have severely reduced growth rates and rough hairy coats (Maes et al., 1996).

1.8.8 Significance to nursery pigs

While clinical signs of enzootic pneumonia do not usually occur until the growing and finishing stages, piglets often become infected with *M. hyopneumoniae* during the nursery stage (Meyns et al., 2004; Sibila et al., 2009). Thus, efforts to prevent the transmission of the disease within the nursery can have a positive impact on the health and growth of growing and finishing pigs, and the profitability of an entire herd.

1.8.9 Prevention and treatment

Due to the nature of their cell wall, treating mycoplasmas can be a challenge. Antimicrobials, like penicillin, that disrupt the cell wall to destroy bacteria, are ineffective against *M. hyopneumoniae* (Maes et al., 1996). However, several other antimicrobials are available that are effective against the bacterium, including tetracyclines (Klein et al., 2017).

Since *M. hyopneumoniae* is notorious for occurring with secondary infections, treatment can still be a challenge (Maes et al., 1996). Prophylactic use of antimicrobials during periods of high risk is encouraged to prevent outbreaks of enzootic pneumonia (Maes et al., 1996).

Other methods to prevent enzootic pneumonia in a herd include having good management practices such as; following strict biosecurity protocols, having a closed herd, having an all-in/all-out flow system with minimum variation in the age of animals housed together, and using good sanitation practices (Maes et al., 1996). Having procedures to reduce parasite load and prevent lung lesions due to *Ascaris suum* larvae, as well as optimizing the housing and climate of the environment for animals is also recommended (Maes et al., 2008). Early weaning is another recommended strategy that has been shown to reduce the vertical transmission of *M. hyopneumoniae* (Maes et al., 2008).

Vaccination is probably the most widely used preventative measure. Although vaccination does not eradicate the disease it can greatly reduce the economic impact. Herds can be created and operated that are free of *M. hyopneumoniae* but because the disease spreads from farm to farm easily, maintaining a *M. hyopneumoniae* free herd is a challenge.

1.9 Influenza

1.9.1 History

The first indication of an influenza affecting swine was in 1918 in Iowa at the same time as the pandemic, Spanish influenza, was killing millions of people worldwide (Taubenberger, 2006). It is believed that the respiratory disease resulted from pigs becoming infected with the 1918 human influenza virus (Shope, 1936). Influenza was found in swine in the Midwestern states every year after the original discovery in 1918 (Taubenberger, 2006). As discussed in a

review by Taubenberger, in late 1918 and early 1919, disease outbreaks in swine resembling influenza were seen in Europe and China (Taubenberger, 2006). The etiologic agent of the disease, influenza A virus (IAV), was isolated in 1930 by Shope and Lewis (Taubenberger, 2006).

1.9.2 Economic importance

As outlined in the review by Torremorell et al. (2012) IAV is ubiquitous where pigs are found. Multiple studies have shown the virus is present in pigs in Europe, North America, and Asia. Influenza viruses have also been found in wild boar and pig populations within Europe and North America (Torremorell et al., 2012). Thus, the disease is one of concern for swine producers worldwide.

Holtkamp et al. (2007), surveyed 19 production systems in the U.S. that each produced more than 150,000 pigs annually. These systems represented 25 % of the pigs marketed annually in the United States. Each system was asked to rank the health challenges, by order of the most production losses they faced within their herd. In the study's ranking system, the lower the rank, the higher the productivity losses. Swine influenza was ranked by 18 of the 19 systems surveyed and was ranked to be the second highest for productivity losses in breeding herds, with an average ranking of 3.7. For the nursery herds, swine influenza was ranked by 18 of the 19 companies and had an average ranking of 4.3. In the finishing herds, swine influenza was ranked by 18 of the 19 systems and was given an average ranking of 3.1. Some systems also ranked the combination of swine influenza and porcine circovirus type 2 as being a health challenge (Holtkamp et al. 2007).

As previously mentioned, Haden et al. (2012) conducted a study using diagnostic and close-out reports of finishing barns within a large American production system. Using ADG values, cull, mortality and fallback percentages, the difference between loss per head placed was determined between barns infected with one or more respiratory pathogens and the baseline levels of healthy barns in the system. Barns infected with influenza had a loss of $3.23 per hog placed in the finishing barn above the base line. Barns infected with *M. hyopneumoniae* and IAV had a cost of $10.12 per hog placed while barns infected with IAV and PRRSV had a loss of $10.41 per hog placed. Meanwhile, another American study estimated the loss from IAV to be $10.31 per market hog, with 64% of the loss attributed to production losses and the other 36% attributed to additional veterinary costs (Donovan, 2008).

1.9.3 Etiology

Influenza viruses are classified within the family Orthomyxoviridae (Van Reeth et al., 2012). As with all viruses in this family, they consist of a genome of negative, single stranded, segmented RNA (Shaw & Palese, 2013). Influenza viruses are divided into types A, B and C, using matrix and nucleoproteins (Van Reeth et al., 2012). Influenza A is the only type of the virus that affects pigs (Van Reeth et al., 2012). Influenza A viruses have a pleomorphic viral envelope upon which hemagglutinin (HA) and neuraminidase (N) glycoproteins as well as M2 proteins project (Shaw & Palese, 2013). It is the distinctive shapes of spikes made by the HA and N proteins that are used to further classify influenza A viruses into subtypes, such as H3N2 and H1N1 (Shaw & Palese, 2013). The RNA of influenza A viruses is segmented into 8 different sections (Shaw & Palese, 2013). This segmentation allows different viruses that attach to the same host to exchange RNA, or as it is commonly known, reassortment (Van Reeth et al., 2012). Influenza in swine is usually caused by subtypes H1N1, H1N2 and H3N2 (Detmer, 2017).

1.9.4 Epidemiology

Horizontal transmission of IAV, through nose to nose contact between pigs, is believed to be the most common transmission route of the disease (Van Reeth et al., 2012), however pigs can also transmit the virus indirectly within shared airspaces (Loving et al., 2013). New introductions of the virus into herds occurs through animal movements (Van Reeth et al., 2012). While multiple studies have indicated the possibility of airborne transmission of influenza virus in humans and other animal species (Tellier, 2009), there is little evidence to indicate the movement of influenza virus between swine barns through airborne particles (Van Reeth et al., 2012).

Some research has shown that sows and nursery pigs can circulate the disease in a herd by acting as a reservoir of the virus (Loeffen et al., 2003), resulting in finishing pigs in a farrow to finish operation having a higher risk of being infected with IAV than finishing pigs in an offsite barn (Detmer, 2017). Production systems that use continuous flow methods, with younger and older weaned animals being exposed to one another throughout the nursery and finishing period, are also associated with a greater incidence of influenza compared to all in/all out systems that segregate animals by age (Detmer, 2017). Other risk factors for a herd being infected with influenza virus include; low temperature set points in rooms, large pen sizes, short time between batches in farrowing rooms, high pig density and large numbers of pigs on one site (Fablet et al., 2013; Poljak et al., 2008).

As summarized by Van Reeth et al. (2012), historically, influenza was believed to be a seasonal disease in swine, with more infections occurring in the colder months of the fall and winter. More recent studies have shown that influenza can affect swine at any time of year, likely

due to the changes that have occurred in pig housing with the controlled climate of barns (Van Reeth et al., 2012).

1.9.5 Zoonosis

The ability of influenza A viruses to easily undergo reassortment, allows them to infect different species. Influenza viruses of swine have been shown to infect humans, waterfowl and turkeys (Van Reeth et al., 2012). Likewise, influenza A viruses can be transmitted to pigs from humans and birds (Van Reeth et al., 2012). Wild waterfowl are a reservoir of influenza A viruses and thus can circulate the virus by transferring it to other species (Van Reeth et al., 2012).

Previous pandemics of influenza virus are believed to be a result of animal-origin influenza viruses affecting humans (Cox et al., 2017). The transmission of influenza viruses from animals to humans can either occur directly or after the virus has undergone reassortment in another host (Cox et al., 2017). Previous research has indicated that swine act as a "mixing vessel" for influenza viruses by being the host where avian and mammalian strains of virus reassort (Van Reeth et al., 2012). More recent research however, has brought this theory into question (Van Reeth et al., 2012). Nevertheless, swine are the primary suspect for the reassortment of the 2009 H1N1 pandemic (Van Reeth et al., 2012). Thus, due to their versatility, influenza A viruses are not only a concern for swine and poultry health but also for humans worldwide.

1.9.6 Immunity

Pigs have a quick and efficient immune response to swine influenza viruses and are generally able to overcome infection within a week (Torremorell, 2017). After infection with an influenza virus, pigs will have future protection against this particular viral strain (Van Reeth et

al., 2012). The issue is that due to the nature of the virus, animals may be exposed and thus become infected multiple times to different strains of influenza virus because exposure to one strain does not produce protection to other strains (Van Reeth et al., 2012). Animals can be vaccinated against subtypes of influenza virus, with live, rather than killed, vaccines being recommended for stimulating an adequate immune response (Van Reeth et al., 2012).

Sows can transfer passive immunity to specific influenza virus strains to their piglets (Loeffen et al., 2003). This immunity however, has been shown to be incomplete as piglets with maternal antibodies can still show mild clinical signs upon infection (Loeffen et al., 2003). While pigs with maternally-derived antibodies can develop an active immunity against influenza virus, the development of this active immunity is hindered due to the presence of the maternal antibodies (Loeffen et al., 2003). As with active immunity, maternal antibodies are only effective against similar viral strains to those that the sows were originally exposed to (Loeffen et al., 2003).

1.9.7 Pathogenesis

After entry into the respiratory tract, the HA glycoproteins found on the viral envelope of influenza viruses, bind to the sialic acid receptors on the epithelial cells of the respiratory tract (Detmer, 2017; Van Reeth et al., 2012). The virus then replicates within respiratory epithelial cells (Van Reeth et al., 2012). The incubation period of IAV is 24-48 hours (Detmer, 2017). The sialic acid receptors are host specific, meaning that influenza viruses can only infect animals that have the corresponding receptors (Detmer, 2017). It is believed that IAV does not infect other regions of a pig but instead remains within the airways and lungs (Van Reeth et al., 2012). In experiments where pigs are inoculated with influenza virus, the severity of pneumonia exhibited varies depending on the inoculation route and the dose of virus administered (Van Reeth et al.,

2012). Thus, the higher the dose of virus a pig is exposed to, the more severe the clinical signs of disease will be.

The first lesions resulting from infection with influenza virus are seen within 24 hours post-infection (Detmer, 2017). Influenza virus causes death of the epithelial cells and the cilia that line the respiratory tract (Detmer, 2017). Inflammation in the cranioventral bronchi of the lungs also occurs and is a distinguishing feature of an influenza infection (Detmer, 2017). Infection with IAV can also result in collapsed regions of the lung (Detmer, 2017). Areas of an infected lung often appear dark red or purple, but this is associated with secondary bacterial infections (Detmer, 2017). The damage in the respiratory tract will heal within 14 to 21 days (Detmer, 2017).

1.9.8 Clinical signs

While pigs of any age can be infected, most clinical signs of influenza virus infections occur in pigs that are older than 10 weeks (Loeffen et al., 2003). Typical clinical signs of the disease are like many respiratory disease outbreaks and include, pyrexia, suppressed appetite, inactivity, huddling, coughing, dyspnea, tachypnea, nasal discharge, sneezing and reduced growth (Loeffen et al., 1999; Detmer, 2017; Van Reeth et al., 2012). Lung lesions have also been found in infected pigs (Loeffen et al., 1999). These lesions resemble those of typical viral pneumonia (Van Reeth et al., 2012). Generally, the disease has a low mortality and a high morbidity with most pigs recovering within 5-7 days (Van Reeth et al., 2012). However, secondary bacterial infections that commonly occur with the virus can compound the clinical signs exhibited (Van Reeth et al., 2012).

Like PRRSV and *M. hyopneumoniae*, IAV can be a component of the porcine respiratory disease complex (PRDC). As such, clinical signs exhibited with infection of influenza virus may also be a result of PRDC (Van Reeth et al., 2012). Since influenza virus is endemic in many swine herds, subclinical infections can also occur (Corzo et al., 2013).

1.9.9 Significance to nursery pigs

While the clinical signs of influenza infections usually arise during the finishing period, the nursery still has an important role to play in the transmission of the disease. It is within the nursery period that animals from different litters and possibly even different barns are mixed together. It is also during this period that pigs begin to lose their maternally derived antibodies (Chung et al., 1997). Nursery pigs can be exposed to a variety of pathogens including those involved in the PRDC (Sibila et al., 2009). Thus, proper management of pigs during the nursery period is essential for reducing the clinical signs of influenza later in a pig's life.

1.9.10 Prevention

Since influenza viruses are relatively ubiquitous, biosecurity practices may be ineffective to prevent exposure to animals. The current most effective strategy to reduce clinical signs and the effect of the disease is thought to be vaccination. (Torremorell, 2017; Van Reeth et al., 2012). The issue with vaccinating, however, is that new strains and subtypes of the virus are continuously being formed (Torremorell, 2017). Producers combat this problem by using vaccines that target more than one subtype or by using autogenous vaccines but even these vaccines are not fully effective (Torremorell, 2017). While vaccines can help reduce the clinical signs of the disease, whether they can successfully prevent influenza virus transmission is a point of contention between researchers (Torremorell, 2017). Furthermore, vaccinating nursery pigs

can be problematic as maternally-derived immunity can interfere with the production of antibodies against the virus (Van Reeth et al., 2012). Generally, for producers that do vaccinate, the most effective strategy is to vaccinate sows so that they can pass maternal immunity to their offspring (Van Reeth et al., 2012).

Since IAV is one of the primary pathogens involved in PRDC, being able to prevent bacterial infections can also reduce disease resulting from an influenza infection (Torremorell, 2017). The administration of antimicrobials and practicing good biosecurity and sanitation practices are all strategies that can prevent bacterial infections (Torremorell, 2017).

1.10 Porcine respiratory disease complex

Since pigs infected with *M. hyopneumoniae* are extremely susceptible to secondary infections, the clinical signs observed post-infection with the bacterium can be due to a combination of pathogens. The combination of *M. hyopneumoniae* with a variety of viral pathogens, resulting in reduced growth, coughing and laboured breathing in 16 to 20-week-old pigs, is known as porcine respiratory disease complex, or PRDC (Done & White, 2003). The primary etiological agents of PRDC are PRRSV, porcine circovirus type 2, influenza A virus (IAV), and *M. hyopneumoniae* (Done & White, 2003). A variety of other viral, bacterial and parasitic pathogens can be involved in the complex as well (White, 2011).

1.11 Summary of respiratory diseases

Respiratory diseases like PRRS, enzootic pneumonia and influenza, are a serious threat for pork producers around the world. They constantly cause challenges and require excellent management strategies to control. A key component to these management strategies is diligent care and oversight within the nursery period. Being able to control disease challenges in the

nursery will improve the growth of animals during the finishing stage and ultimately improve the productivity of an entire swine production system.

1.12 Monitoring for disease

1.12.1 Key monitoring strategies

As with other types of disease, frequent monitoring is a key component to preventing respiratory disease. Early detection can restrict or prevent the transmission of respiratory pathogens and reduce the severity of disease (VanAlstine, 2012). There are a variety of monitoring strategies that can be employed, at the farm level and provincially and federally. These methods include having frequent visits from the herd veterinarian and conducting post-mortem examinations on animals that die with suspicious clinical signs (VanAlstine, 2012). Routine slaughter checks assessing lung lesions and air pathways are another strategy that can be used to check for common indications of respiratory disease (VanAlstine, 2012).

Taking frequent samples and performing diagnostic tests is the most common practice that is used to screen for pathogens of concern (VanAlstine, 2012). Some herds that have a high risk of transmitting disease, such as boar studs, follow strict sampling protocols to routinely screen for pathogens (Torrison, 2012). For other herds, sampling may be more sporadic and only occur in times of concern.

1.12.2 Industry databases

Frequent monitoring allows a producer to be aware of the health status of their herd for diseases of interest. Health statuses of herds can be recorded collectively for a region and used by producers and the industry to make management decisions. In Ontario, the Area Regional

Control and Elimination (ARC & E) program is a voluntary program that requires producers to provide their herd health status for PRRS and porcine epidemic diarrhea (PED) and in turn allows producers to access information about other participating producers (Swine Health Ontario, 2016). The program shows participating swine herds on a map with their health status for the two diseases of interest (Swine Health Ontario, 2016). Initiatives such as this one that promote industry cooperation have played a key role in controlling disease outbreaks in the province in the past and continually grow in importance as new threats of disease outbreaks arise. From an individual producer standpoint, knowing the status of nearby herds can change management decisions to further reduce biosecurity threats and prevent disease outbreaks.

1.12.3 Interpretation of test results

While other types of samples such as nasal swabs are occasionally used, swine veterinarians predominantly submit sera samples for diagnostic testing (Gardner, 2012). There are a variety of serological tests that exist, and proper selection of a test depends upon what question is to be answered from the submitted samples (Torrison, 2012). Some diagnostic tests have a higher sensitivity while others have a higher specificity (Gardner, 2012). Depending on the situation and the question being answered, one type of error, a false positive or a false negative, may be more important than the other and thus a test may be chosen for either its high sensitivity or specificity (Gardner, 2012). Tests such as polymerase chain reactions (PCRs) detect the presence of pathogens themselves while others such as indirect enzyme-linked immunosorbent assays detect the antibodies to pathogens of interest (Christopher-Hennings, et al. 2012). Thus, the interpretation of test results depends on the type of test conducted. For example, for tests that detect antibodies rather than specific pathogens, a positive result would indicate that the animal has been exposed to the pathogen at some point but the current clinical

signs being exhibited may not be a result of the indicated pathogen (Christopher-Hennings et al., 2012).

Since no diagnostic test has perfect sensitivity and specificity, it is important that multiple samples from a herd are tested (Christopher-Hennings et al., 2012). As well as using multiple tests, diagnoses should be made using test results as only a part of the evidence. Clinical signs, farm history and pathology should all be used in conjunction with test results to perform a diagnosis, and subsequently make management decisions (Christopher-Hennings et al., 2012).

1.13 ELISA testing

Enzyme-linked immunosorbent assays (ELISAs) are a common type of diagnostic testing available. ELISAs can test for antigens themselves or for the antibodies to antigens (Christopher-Hennings et al., 2012). There are ELISAs available that can test for many of the common pathogens causing disease in swine, including PRRS virus, *M. hyopneumoniae*, and IAV. The advantages of ELISA tests are that they are quick, generally have a high sensitivity and specificity and can be used for processing many samples at a time (Christopher-Hennings et al., 2012). A disadvantage to ELISA testing is that caution must be taken when using ELISA tests to interpret results for a single pig (Christopher-Hennings et al., 2012). Due to some issues with false-positives, it is advised to either perform multiple ELISA tests on one pig or to confirm with other types of testing to verify results (Christopher-Hennings et al., 2012).

The specific testing process varies depending on the type of ELISA, but the general principle of each ELISA test is the same (Christopher-Hennings et al., 2012). For example, with an antibody ELISA, the plate wells of the test kit are bound to antigens and then sera samples are incubated in the test kit (Christopher-Hennings et al., 2012). Sera that contain antibodies to the

bound antigens will bind to the plate wells (Christopher-Hennings et al., 2012). An enzyme-linked antibody that will bind to swine antibodies is then added to the test kit, followed by the substrate of this secondary antibody (Christopher-Hennings et al., 2012). The addition of the substrate results in a colour change to the plate wells, which is tested using the optical density (Christopher-Hennings et al., 2012). Each plate contains a positive and negative control (Christopher-Hennings et al., 2012). These controls are used to determine the sample to positive (S/P) ratio of each serum sample tested (Christopher-Hennings et al., 2012). A cut-off level for the S/P ratios is specified by each ELISA test kit that determines positive and negative samples (Christopher-Hennings et al., 2012).

1.13.1 ELISA tests for porcine reproductive and respiratory syndrome virus

Commercially available ELISAs can be used to detect antibodies to both the North American and European strains of the PRRS virus. While a seroconversion from negative to positive status within sera samples is the standard method of diagnosing PRRS serologically, an increase in antibody titres against the virus can also indicate an infection with the virus (Zimmerman et al., 2012). The advantage of using ELISAs to detect PRRSv antibodies is that they are standardized, quick and highly specific and sensitive (Zimmerman et al., 2012). After 9 days post-infection, PRRS virus antibodies can be detected and they peak between 30 and 50 days post-infection before declining between 4 and 12 months after an infection has occurred (Zimmerman et al., 2012).

Serological assays, however, cannot detect the difference between antibodies resulting from a new infection or those resulting from a re-infection or vaccination (Zimmerman et al., 2012). Thus, these assays cannot be used to detect a new PRRSV infection in a herd that has either been previously exposed to the virus or has been vaccinated. Also, if antibodies to the

PRRSV are detected in nursery pigs younger than 5-weeks-old, they could be the result of maternal antibodies passed down from the dams (Zimmerman et al., 2012).

It can be challenging to interpret ELISA PRRSV antibody tests collected at a single point in time for individual animals, since swine can be persistently infected with the virus and the antibody levels vary significantly based on the time since infection (Zimmerman et al., 2012). Instead, taking multiple ELISA tests over time for each animal can give a better indication of the true health status of each animal (Zimmerman et al., 2012).

1.13.2 ELISA tests for Mycoplasma hyopneumoniae

Serology testing is a commonly used tool to determine the status of *M. hyopneumoniae* in a herd (Thacker & Minion, 2012). As outlined by Thacker and Minion (2012), multiple studies have shown that ELISAs have been shown to be more effective at detecting antibodies to the bacterium than the previously used complement fixation assays. This research has resulted in ELISAs being the most commonly used assays for detecting *M. hyopneumoniae* antibodies (Thacker & Minion, 2012). A study conducted in 2005, compared the performance of the three ELISA tests, used in the United States that detect *M. hyopneumoniae* antibodies and determined that each of the ELISA tests had high specificities but that the sensitivities of the assays were much lower, ranging from 35% to 49% (Erlandson et al., 2005). Low sensitivities of *M. hyopneumoniae* ELISA assays indicates that there is a high likelihood of false-negatives, or in other words instances where truly positive pigs are deemed negative. The low sensitivity is likely a result of delayed immune responses in the pigs to *M. hyopneumoniae* exposure, as is characteristic to the bacterium, rather than an inability of the tests to properly detect antibodies. It should also be noted that antibodies to *M. flocculare* have been shown to cross-react with *M. hyopneumoniae* ELISA assays (Bereiter et al., 1990).

As discussed by Thacker and Minion (2012), studies have shown that the type of vaccine administered, and the assay used as well as the infection status of pigs can cause variations to be seen in antibody levels of pigs that have been vaccinated against *M. hyopneumoniae*. Thacker and Minion (2012) also outline how some studies have shown that no correlation exists between vaccine driven antibody levels and protection against *M. hyopneumoniae*. As with PRRSV, using ELISAs to draw conclusions about the health status of individual animals for *M. hyopneumoniae* can be problematic. The *M. hyopneumoniae* ELISAs are better suited for indicating the health status of an entire herd rather than individual animals.

1.13.3 ELISA tests for influenza A virus

As with PRRSV and *M. hyopneumoniae,* serological tests can also be used to detect antibodies to influenza virus (Van Reeth et al., 2012). While the HI test is the most commonly used assay for testing for influenza virus antibodies, ELISA tests are also used (Van Reeth et al., 2012). There are two different types of ELISAs, the first type simply tests for general antibodies to influenza A virus while the other tests for specific antibody subtypes (Van Reeth et al., 2012). General influenza ELISAs usually have good sensitivity values but ELISAs that test for specific subtypes can have a much lower sensitivity than other serological diagnostic tests (Van Reeth et al., 2012). Also, similar to the previously mentioned pathogens, the proper diagnosis of an influenza infection requires testing multiple sera samples taken at different times rather than just testing samples from a single time point (Van Reeth et al., 2012).

While interpreting any serological diagnostic test can be difficult, it can be especially difficult with IAV tests. This is due to the ability of a herd to have multiple strains of the influenza virus circulating at the same time, as these strains can cross-react to diagnostic tests (Van Reeth et al., 2012).

1.14 Purpose and research objectives

The main purpose of this research is to determine how nursery pigs raised in antibiotic-free programs compare to conventionally-raised nursery pigs for growth rates and to provide a summary of the serological status of nursery pigs for PRRS, *M. hyopneumoniae* and IAV. This can be further divided into the following objectives:

1) To summarize key management differences between conventional and antibiotic-free nurseries
2) To determine an estimate of the average growth rate of nursery pigs raised antibiotic-free
3) To compare if growth rates of nursery pigs raised antibiotic-free differ from conventional nursery pigs
4) To provide a serological profile of respiratory pathogens (PRRSV, IAV, or *M. hyopneumoniae*) in the nursery pig population.
5) To evaluate the impact of exposure to PRRSV and IAV on individual nursery pig performance

1.15 References

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Table 1.1: Summary of key management practices of conventional, raised-without-antibiotics (RWA), organic and humane nurseries

Management Practice	Conventional	RWA[a]	Organic[b]	Humane[c]
Weaning Age (days)	21	21	28 +	28+
Individually treat with antibiotics	Yes	No	No	Yes
Mass treat with antibiotics in feed or water	Yes	No	No	No
Bedding	No	No	Yes	Yes
Access to the outdoors	No	No	Yes	Preferred
Type of feed	Conventional	Conventional	Organic	Conventional
Vaccines	Yes	Yes	Restricted	Yes
Animal by-products in feed	Yes	Varies	No	No
Parasiticides	Yes	Yes	Restricted	Yes
Premium paid for product	No	Yes	Yes	Yes

[a] CFIA, 2019a

[b] Canadian General Standards Board, 2015a

[c] HFAC Scientific Committee, 2014

CHAPTER 2: A COMPARISON OF NURSERY PIG PERFORMANCE AND MANAGEMENT PRACTICES ON CONVENTIONAL VS. ANTIBIOTIC-FREE FARMS

2. 1 Introduction

Weaning is an especially challenging time in a pig's life. Most modern Ontario swine farms wean pigs around 21 days of age, before a pig's active immunity has fully developed (Chase & Lunney, 2012). The lag in the complete development of pigs' immune systems, in addition to the stress from the nutritional and environmental changes that occur at weaning, results in nursery pigs being more susceptible to disease. A commonly used practice to reduce disease and optimize growth during the nursery period is to include antimicrobials prophylactically in feed. This practice has proven to be effective as documented by Cromwell (2002) who summarized more than 1,000 studies showing that in-feed antimicrobials increased the daily gain of nursery pigs by an average of 16.4%.

However, concern about antimicrobial resistance has led to increasing public pressure to reduce antibiotic use in livestock production (O'Neill, 2015; Lusk et al., 2006), and therefore different systems that market pork as being raised antibiotic-free, and thus different from conventional pork production, have arisen to capitalize on this niche market.

Raised-without-antibiotics (RWA) is a production system that markets pork from pigs that have never been exposed to antimicrobials. Unlike the organic production system, the RWA program places no other production restrictions on producers (CFIA, 2019a). To be certified as organic, livestock production must follow specific management practices outlined by the Canadian General Standards Board committee on organic agriculture (CFIA, 2019b). Specific organic production practices include feeding only organic feed, allowing pigs access to the

outdoors, providing pen bedding, and having a minimum weaning age of 28 days (Canadian General Standards Board, 2009). The last system to be included in this study is Certified Humane. Although this system does not require feeding organic feed, it follows similar practices to the organic production system (HFAC Scientific Committee, 2014).

In 2018, it was estimated that antibiotic-free swine production made up 9% of the total number of sows in Ontario (Burlatschenko et al., personal communication, 2019). However, the publicly available research and information on nursery pigs raised in these programs is limited. It is unknown how the growth rates of these nursery pigs compare to those grown with access to antimicrobials and whether specific management practices used in antibiotic-free programs can reduce the need for antimicrobial use in the nursery without compromising performance.

The objectives of this study were 1) to summarize key management practice differences between conventional and antibiotic-free systems, 2) to determine an estimate of the growth rates of nursery pigs raised antibiotic-free, and 3) to compare the growth rates of nursery pigs raised antibiotic-free to conventional nursery pigs.

2.2 Materials and methods

This study was approved by the University of Guelph Animal Care Committee and the University of Guelph Research Ethics Board.

Farm selection and sample collection

Nurseries were recruited through a convenience sample using veterinary and industry contacts. Fifty Ontario nurseries, 30 of which were conventional and 20 that were antibiotic-free were sampled between May 2016 and January 2019. Of the 20 antibiotic-free nurseries, 10 were within the RWA system, nine were certified organic and one was certified humane. Each nursery

was visited twice, within a week after weaning and within a week before the pigs left the nursery. At the initial visit, producers answered a questionnaire describing the size and type of operation, facility, pig flow, genetics, health concerns, nutrition, production parameters, biosecurity protocols, and key management practices (Appendix I). At the first visit, 20 pigs of an average size of the weaned batch were selected. These pigs were ear tagged with a unique numerical identification. Pigs were placed in a bucket and then weighed using a tared postal scale at each visit. The scale calibration was verified using check weights of known amounts. Blood samples were collected from the 20 ear tagged pigs at both visits via the infraorbital sinus into 10 mL serum tubes (BD Vacutainer ®, Franklin Lakes, NJ, USA). Pigs were restrained for blood sample collection by using a custom-made V-trough. Blood samples were placed in ice for transportation before processing.

Serology

The serology is explained in further detail in chapter 3. To summarize, blood samples were centrifuged, and serum samples were stored and then tested for antibodies to porcine reproductive and respiratory syndrome virus, influenza A virus and *Mycoplasma hyopneumoniae*, respectively using enzyme-linked immunosorbent assays (ELISAs) (IDEXX Laboratories, Westbrook, Maine, USA). ELISAs were conducted according to kit instructions.

Data analysis

Due to the similarity in production practices, the farm raising pigs in the certified humane system was categorized with those farms raising pigs as certified organic. For this study the nursery performance of 3 types of production (conventional, RWA, and organic) were compared.

Data were entered into Excel Office 365 (Microsoft Corporation, Redmond, Washington, USA) and the average daily gain (ADG) of individual pigs was calculated using the formula: (weight at visit 2-weight at visit 1)/ (number of days between visits). The data were then imported into STATA 15 for statistical analysis (Stata Corp LLC, College Station, Texas, USA).

Descriptive statistics including mean, minimum and maximum values and standard deviation were sorted by production type and determined for continuous variables at the farm level. Mean ADG values for each production type were calculated by averaging all the ADG values of individual pigs within each system. Summary statistics describing the number of observations and their frequencies were also determined for categorical variables at the farm level.

A mixed-effects linear regression method was used with ADG as the outcome and production type as the explanatory variable and farm included as the random effect in the model. Weight at visit 1 was controlled for by being included as a covariate in the model. Since time in the nursery and weight at visit 2 were part of the calculation for the outcome, ADG, they were not included as covariates in the model. Other variables that were collected through the survey, were screened for inclusion in the model according to a causal diagram (Appendix II). Screening was conducted using descriptive statistics and by testing for significance using a p-value of 0.2 in univariable analysis. Criteria for excluding variables from the model included high numbers of missing observations or a p-value of above 0.2 in the univariable analysis. Pairwise correlations were also tested using the Pearson correlation coefficient. Continuous variables were tested for a linear relationship with the outcome using Lowess smoother curves. Residuals were checked for a normal distribution using histograms. The final model was built by manual forward selection and variables with a $P\text{-}value < 0.05$ were included in the final model. Possible interaction terms

were also tested for significance in the model according to the causal diagram (Appendix II). Possible confounders were tested in the final model and were kept if their exclusion from the model resulted in a 20% change in either the coefficients or p-values in the model. Linear model assumptions, including constant variance and normality of residuals were tested for in the final model.

2.3 Results

One of the conventional nurseries withdrew from the study for an unspecified reason, thus the final sample size was 49 nurseries, 29 of which were conventional, 10 that were RWA and 10 that were organic in the study. From these 49 nurseries, there were 977 pigs sampled, 579 of these were from conventional nurseries, 199 from RWA nurseries and 199 from organic nurseries.

Descriptive statistics summarizing key management practices across systems are highlighted in Table 2.1. Conventional nurseries had the highest mean inventory, followed by RWA and then organic nurseries. Spacing and weaning age were similar in conventional and RWA nurseries but differed in organic nurseries. Other management practices are summarized descriptively across systems in Tables 2.2-2.5. PRRS seropositivity at visit 2 is shown at the pig level in table 2.6.

Table 2.7 provides a summary of the growth rates and associated parameters across the 3 production types. Descriptively, conventional and RWA nurseries were again shown to be similar for the measured parameters while the values for organic nurseries differed for these parameters.

Table 2.8 shows the results of the mixed effects linear regression studying the effect of system on ADG values. System type, weight at visit 1 and PRRS titer status at visit 2 were significant in the model. Variables describing other management information collected were not significant in forward selection and thus were not included in the model. No interactions or confounders were significant in the model. There was no significant difference in growth between RWA and conventional pigs ($P = 0.59$). Organic pigs were found to have a growth rate of 116 g/day less than conventional pigs ($P < 0.001$) Weight at visit 1 had a positive association with ADG ($P<0.001$) while seropositivity for PRRS virus at visit 2, had a negative association with the outcome ($P=0.013$).

RWA pigs were found to have a growth rate of 102 g/day more than organic pigs ($P = 0.001$), (see Table 2.9).

2.4 Discussion

Management practices

The only management difference observed between RWA and conventional nurseries besides antibiotic use, was the barn inventory, with RWA nurseries being smaller. RWA nurseries likely had a lower pig inventory than the conventional farms because smaller swine operations depend on the premium provided by the RWA market to remain profitable. Thus, there is a bias in this system towards smaller farms. The mean spacing per pig and the mean weaning age values of the conventional and RWA production types were the same. Since the RWA restrictions only pertain to antibiotic use and RWA nurseries can follow conventional management practices, the similarity between these systems was expected (CFIA, 2019).

Organic nurseries however, provided more than double the amount of space per pig and had a mean nursery inventory that was less than half that of the conventional and RWA nurseries. The mean weaning age of pigs in organic nurseries was 10.8 days older at 32.5 days than that of the conventional and RWA nursery pigs. This is in accordance with the organic pig production rules that require larger space requirements per pig, a minimum weaning age of 28 days, and labour intensive practices that are more suitable to smaller operations (Canadian General Standards Board, 2015b).

Growth performance

The descriptive statistics showed that RWA nursery pigs outperformed organic pigs for growth rate and feed conversion. While there is limited literature that provides benchmarking data for nursery pigs, the existing literature reports growth rates that are at least 27-73 g/day less than the organic and RWA pigs in this study. A 2005 dataset that included 8.37 million nursery pigs in Canada within 3,527 batches reported a mean average daily gain of 370 ± 50 g/day (Cottrell, 2005). A 2013 report from a Minnesota system containing data from over 7,000 nursery closeouts and more than 13 million pigs, reported a similar mean ADG of 372 g/day (Stein, 2015).

When comparing the ADG values of all three systems, conventional nurseries were shown to have the highest values with a mean ADG of 461.8 g/day. This is nearly 100 g/day higher than the average daily gain values reported by previous studies (Cottrell, 2005; Stein, 2015). The large difference in ADG values reported in this study compared to previous work may be due to industry improvements in efficiency since the previous studies were published. However, the difference in ADG values may also indicate a limitation of this study. Of the 49 nurseries sampled, 12 were sampled more than 3 d after pigs entered and 7 of these nurseries

were sampled more than 5 d after pigs entered with the one nursery being sampled 12 d after weaning. The exclusion of the first week, when pigs tend to acclimatize and grow very little, might have resulted in higher average daily growth rates than if the first week had been included.

In the present study there was no significant difference in the ADG between the conventional and RWA systems. Antibiotic usage was the only management difference between the 2 systems as producers in the RWA system can follow other conventional management practices such as raising pigs indoors, using slatted flooring and feeding conventionally grown feed ingredients. Since only antibiotic use differed between these systems, these findings may indicate that it is possible to raise pigs without antibiotics and obtain similar growth rates to conventional pigs.

In Denmark however, where growth promoting antibiotics have been banned since the 1990s and therapeutic antibiotic use has been restricted since 2010, there have been challenges raising nursery pigs without antibiotics (Jensen & Hayes, 2014). Gastrointestinal and respiratory diseases have been difficult for swine producers to combat without using antibiotics. These diseases have resulted in reduced performance and increased mortality amongst nursery pigs (Jensen & Hayes, 2014).

The nurseries in the organic system recorded poorer growth rate relative to both the conventional and RWA systems and this is likely due to the management practices that are unique to organic production. Outdoor access, feeding only organic feed ingredients and having floors that are only partially slatted (Canadian General Standards Board, 2015a), are all mandatory practices in organic production that can result in issues, such as; possible parasite exposure, lower biosecurity standards, poor quality feed ingredients, and reduced cleanliness compared to other production systems.

Management has long been known to play a critical role in the performance and health of pigs. A review from 1977 summarized hundreds of studies showing that antibiotics are most effective in barns with poor sanitation protocols (Hays, 1977). A 2002 study conducted 9 trials to compare the growth performance of nursery and finishing pigs fed antibiotics to those that were fed a control diet (Dritz et al., 2002). Nursery pigs fed antibiotics had a 5 % increase in growth rate relative to the control, but this difference was more than 10% lower than the improvements found in previous studies. The discrepancy between previous trials and the 2002 trial was explained by industry-wide improvements in hygiene and cleaning practices, and the movement to multi-site production facilities (Dritz et al., 2002).

The experience of Danish producers has shown that antibiotics are still an important tool for treating sick pigs and that the complete elimination of antibiotics, particularly in the nursery stage, can result in poor pig welfare (Jensen & Hayes, 2014). The present study demonstrates however, that growth performance on farms that do not use prophylactic antimicrobials routinely can match the growth rate of pigs receiving antimicrobials. The best option may be found somewhere in the middle of the two extremes, no longer administering antibiotics prophylactically, but still using them when necessary to treat sick animals. This is supported by a 2017 study that implemented farm-specific interventions to reduce antibiotic use in 70 farrow-to-finish farms in Belgium, France, Germany and Sweden. The median value for the reduction on antibiotic use was 47% across all the farms, and this was achieved without negatively impacting the overall measured farm performance parameters, which included nursery pig mortality (Collineau et al., 2017).

2.5 Conclusion

Nurseries in the RWA system were able to maintain growth rates that were not different than nursery pigs raised on farms with access to antimicrobials. Pigs raised in the certified organic system however, had reduced growth rates compared to conventional nursery pigs. This study demonstrates that management plays a critical role in nursery pig performance, and that producers may be able to raise nursery pigs with no or limited antimicrobials without negatively impacting production by using effective and proven management protocols.

2.6 References

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Table 2.1: Summary of management practices in 49 sampled conventional, RWA and organic nurseries in Ontario

	Conventional					Raised without antibiotics (RWA)					Organic				
Management Factor	Mean	N[a]	Min.	Max.	Standard Deviation	Mean	N[a]	Min.	Max.	Standard Deviation	Mean	N[a]	Min.	Max.	Standard Deviation
Nursery inventory [b]	2424	29	220	5000	1414.1	1574	10	550	4200	1020.7	409	10	120.0	600	180.3
Space per pig (m^2) [c]	0.3	27	0.2	0.5	0.1	0.3	10	0.2	0.4	0.1	0.7	10	0.3	1.1	0.2
Weaning age (days)	21.7	29	18	28	2.9	21.7	10	18.0	25.0	1.9	32.5	10	28.0	38.5	3.7
Batch Mortality (%) [d]	2.9	14	0.3	6.9	1.6	2.3	8	1.0	4.3	1.0	2.4	10	0.0	7.5	2.5

[a] N = number of farms with data

[b] Total pig inventory in sampled barns

[c] Determined by dividing the average pen size in the nursery by the number of pigs per pen. Since pen sizes and number of pigs per pen were sometimes different in a barn, this is approximate. Spacing data was only available for 28 of the 29 farms.

[d] Determined by the total number of pigs that died in the batch followed per barn

Table 2.2: Summary of the frequency of production types of 49 sampled nurseries in Ontario

Management Factor	Conventional (%)	RWA (%)	Organic (%)
Part of a larger production system [a]	86.0	40.0	30.0
Farrow-Finish	21.0	60.0	40.0
Farrow-Feeder	10.0	10.0	10.0
Wean-Finisher	10.0	0.0	30.0
Nursery Only	59.0	30.0	20.0
Total number of farms	29	10	10

[a] Defined as a either a contract producer or a nursery that is part of a multi-site operation that is not solely owned by the producer.

Table 2.3: Summary of the frequencies of barn flow in 49 sampled nurseries in Ontario

Management Factor	Conventional	RWA	Organic
All-in-all-out by barn (%) [a]	35	80.0	50.0
All-in-all-out by room (%) [b]	38	20.0	40.0
Continuous flow (%) [c]	28	0.0	10.0
Total number of farms with data	29	10	10

[a] The entire barn is emptied before new pigs enter

[b] Rooms are emptied completely before new pigs enter while older pigs remain in other rooms.

[c] New pigs are constantly entering a barn where older pigs are present

Table 2.4: Summary of the frequencies of cleaning practices in 49 sampled nurseries in Ontario

Management Factor	**Conventional**	**RWA**	**Organic**
Clean between batches [a] (%)	96.3	100.0	100.0
Pre-soak pens [b] (%)	74.0	55.3	39.7
Use detergent [c] (%)	55.7	33.5	29.7
Pressure wash with cold water [d] (%)	22.3	44.7	49.8
Pressure wash with hot water [e] (%)	77.7	55.3	40.2
Use disinfectant [f] (%)	88.9	88.8	69.9
Allow dry time [g] (%)	92.6	88.8	90.0
Total number of farms with data	27	9	10

[a] Defined as cleaning out a barn or room between batches of pigs

[b] Defined as watering down pens prior to cleaning them

[c] Defined as using a cleaning detergent when cleaning pens

[d] Defined as washing pens with a high-pressure washer with cold water

[e] Defined as washing pens with a high-pressure washer with hot water

[f] Defined as disinfecting pens after washing

[g] Defined as having a set time for pens to dry before filling after washing and disinfecting

Table 2.5: Summary of the frequencies of biosecurity practices on 49 sampled nurseries in Ontario

Management Factor	Conventional (%)	RWA (%)	Organic (%)
Shower-in [a]	39.2	59.8	0
Danish Entry [b]	75.1	79.9	39.7
Downtime Rules [c]	No data	43.17	40.2
Total numbers of farms with data	27	9	10

[a] Defined as requiring any personnel to shower before entering the barn

[b] Defined as having a Danish entry, upon which outdoor clothing is left on a dirty side and only barn clothing is worn on the clean side

[c] Defined as having a set number of hours that visitors must be free of other pig exposure before entering the barn

Table 2.6: Summary of PRRS seropositivity at visit 2

PRRS antibody titre result at visit 2 [a]	Number of pigs (%)
Positive [b]	175 (18.4)
Negative	776 (81.6)
Total	951

[a] Conducted within a week before pigs exited the nursery

[b] Defined by having an S/P ratio ≥ 0.4 using an ELISA antibody test.

Table 2.7: Summary of growth parameters of pigs in 49 sampled conventional, RWA and organic nurseries in Ontario

	Conventional				RWA				Organic			
Total number of pigs with data	563				195				195			
Management Factor	**Mean**	**Min.**	**Max.**	**Standard Deviation**	**Mean**	**Min.**	**Max.**	**Standard Deviation**	**Mean**	**Min.**	**Max.**	**Standard Deviation**
Weaning age (days)	21.7	18.0	28.0	2.9	21.7	25.0	18.0	1.9	32.5	28.0	38.5	3.7
Duration in nursery (days)	44.6	30.0	56.0	6.8	48.2	34.0	60.0	8.4	38.7	31.0	57.0	7.5
Weight at visit 1 (kg) [a]	7.2	3.6	17.4	2.0	6.8	3.7	12.0	1.6	9.2	4.0	14.6	2.1
Weight at visit 2 (kg) [b]	25.3	12.0	45.5	5.5	25.9	11.2	46.0	6.9	22.5	5.8	40.6	6.6
Average daily gain (g/day) [c]	461.8	114.3	850.0	110.4	445.6	175.8	734.0	108.3	399.1	75.0	763.6	145.8

[a] Visit 1 was conducted within a week after entry

[b] Visit 2 was conducted within a week prior to leaving the barn

[c] Determined using the formula (weight at visit 2-weight at visit 1)/ (number of days between visits). The mean value was generated at the pig level by averaging the ADG values of all individual pigs for each production system type.

Table 2.8: Mixed effects linear regression comparing ADG values of pigs in RWA and organic systems to conventional pigs with farm as the random intercept

Variable		**Coefficient**	**Standard Error**	**95% Confidence Interval**	**P-Value**
System	Conventional	Referent			
	RWA	-13.345	24.775	-61.903, 35.213	0.590
	Organic	-115.539	25.004	-164.547, -66.531	0.000
Weight at visit 1		25.035	1.984	21.145, 28.924	0.000
Seropositivity for PRRS at visit 2 [a]		-34.065	13.784	-61.081, -7.048	0.013

[a] S/P ratio ≥0.4 in an ELISA test

Table 2.9**:** Mixed effects linear regression comparing ADG values of conventional and RWA pigs to organic pigs with farm as the random intercept

Variable		**Coefficient**	**Standard Error**	**95% Confidence Interval**	**P-Value**
System	Organic	Referent			
	Conventional	115.539	25.004	66.531, 164.547	0.000
	RWA	102.194	30.363	42.683, 161.704	0.001
Weight at visit 1 (kg)		25.035	1.984	21.145, 28.924	0.000
Seropositivity for PRRS at visit 2[a]		-34.065	13.784	-61.081, -7.048	0.013

[a] S/P ratio ≥ 0.4 in an ELISA test, visit 2 conducted within a week before pigs exited the nursery

CHAPTER 3: ANTIBODY RESPONSES TO RESPIRATORY PATHOGENS IN SWINE NURSERIES

Introduction

The nursery stage is a challenging period in a pig`s life and impacts the overall health of the entire swine operation. Newly weaned pigs are susceptible to disease because they are exposed to many environmental and nutritional changes at a time when their passive immunity is waning, and their active immunity has not fully developed (Chase & Lunney, 2012). Among the most economically significant diseases affecting weaned pigs are respiratory pathogens such as porcine reproductive and respiratory syndrome virus (PRRSv), influenza A virus (IAV) and *Mycoplasma hyopneumoniae*. Depending on the nursery design and biosecurity, the nursery can act as a reservoir for these pathogens with disease being passed from older pigs to the newly weaned pigs and therefore making eradication of diseases such as PRRS difficult (Chung, Lin, Chang, Hsu, & Yang, 1997). Sometimes, the true impact of these diseases is not realized until after the pigs leave the nursery. For example, on many farms, pigs become infected with *M. hyopneumoniae* in the nursery, but the effects of enzootic pneumonia are most pronounced in the form of reduced growth rates during the grower-finisher stage (Meyns et al., 2004).

Strategies to improve pig health and subsequently reduce the economic impact of disease in the nursery include: regulating pig flow so that newly weaned pigs are housed in a separate room or a separate barn from older pigs, vaccinating either the sow or the piglets directly, and providing antimicrobials prophylactically in nursery pig diets and occasionally as a mass treatment in water (Cromwell, 2002; Maes et al., 1996). However, growing concern about the development of antimicrobial resistance and the presence of antimicrobial residues in meat has resulted in public pressure to reduce or eliminate antimicrobial use in livestock production (Lusk

et al., 2006; O 'Neill, 2015). This has created a niche market for meat produced without the use of antimicrobials. Production systems in Ontario that raise pigs without the use of antimicrobials are outlined in the previous chapter. These include the Raised without antibiotics (RWA) program that prohibits antibiotic usage but otherwise follows conventional management practices and organic production which requires specific management practices to be followed in addition to prohibiting antibiotic use (Canadian General Standards Board, 2015; CFIA, 2019).

Regardless of the production system type, frequent monitoring is a key component to preventing respiratory disease as early detection of disease can restrict or prevent the transmission of respiratory pathogens and reduce the severity of clinical signs (VanAlstine, 2012). Serological tests, such as enzyme-linked immunosorbent assays (ELISAs) are commonly used to monitor health status on pig farms (Christopher-Hennings et al., 2012). There are ELISAs available that can test for antibodies for common pathogens causing disease in swine, including PRRSv, IAV and *M. hyopneumoniae*. The advantages of ELISA tests are that they are quick, generally have a high sensitivity and specificity and allow the testing of many samples at a time (Christopher-Hennings et al., 2012). It should be noted that caution must be taken when using ELISA tests to interpret results for a single animal at one point in time, as ELISAs only report serological responses to pathogens rather than the presence of pathogens themselves. Thus, it is advised to either perform multiple ELISA tests on one pig or to confirm with other types of testing to verify results (Christopher-Hennings et al., 2012).

Sampling the same animal repeatedly over time and measuring antibodies for a specific pathogen using an ELISA can be used with knowledge of herd history, potential exposures and vaccination status, to show whether the health status of an animal is stable or dynamic for pathogens of interest (Zimmerman et al., 2012). The production of antibodies due to vaccination

can also be detected by an ELISA test and help confirm that a group of animals have been vaccinated and that the vaccine has stimulated a humoral and systemic immunological response. Evaluating titre changes during the nursery period can also indicate whether exposure to pathogens is occurring within the nursery and thus affecting the health of an entire herd. Comparing these titre changes across many herds in a specific area could show regional health trends and provide valuable insight regarding disease prevalence.

The primary objective of this study was to provide a serological profile of respiratory pathogens (PRRSv, IAV, or *M. hyopneumoniae*) in a subset of the Ontario nursery pig population. A secondary objective was to evaluate the impact of exposure to PRRSv and IAV on individual pig growth performance.

Materials and methods

This study was approved by the University of Guelph Animal Care Committee and the University of Guelph Research Ethics Board.

Farm selection and sample collection

Data collection is described in detail in chapter 2. To summarize, fifty Ontario nurseries were selected for the study between 2016 and 2019 using convenience sampling, with an effort made to include some farms raising pigs without antibiotics as well as some farms following organic production practices. On each farm one batch of newly weaned pigs was followed from entry to exit from the nursery. Each nursery was visited within a week after pigs entered and within a week before the group of pigs left the nursery. A questionnaire was used to collect information about farm management (Appendix I). On each farm, 20 pigs of estimated average size were individually identified with an ear tag at the first visit. At each visit pigs were weighed,

and blood samples were collected via the infraorbital sinus into 10 mL vacutainer tubes (BD Vacutainer ®, Franklin Lakes, NJ, USA).

Serology

Within 24 hours after each collection, blood tubes were centrifuged for 20 min at 1400 RPM with the Thermo IEC Centra CL3R centrifuge (Thermo Fisher Scientific, Waltham, Massachusetts, USA). Sera was placed in 2 mL tubes and stored at -20°C.

Samples were tested for antibodies to PRRSv, using the IDEXX PRRS X3 enzyme-linked immunosorbent assay (ELISA) test kit (IDEXX Laboratories, Westbrook, Maine, USA) according to the kit instructions. A pig was classified as seropositive if the S/P ratio value was equal to or more than 0.4.

Samples were also tested for antibodies to any subtype of IAV using the IDEXX Swine Influenza general ELISA antibody test kit (IDEXX Laboratories, Westbrook, Maine, USA) according to the kit instructions. A pig was classified as seropositive if the S/N ratio value was less than or equal to 0.6.

Samples were tested for antibodies against *M. hyopneumoniae* using the IDEXX M hyo ELISA test kit (IDEXX Laboratories, Westbrook, Maine, USA) according to the kit instructions. A pig was classified as seropositive if the S/P ratio value was equal to or over 0.4.

Data analysis

The nurseries were categorized into three types of production: conventional (n=30), raised-without-antibiotics (RWA) (n=10) and organic (n=10).

Data were entered into Excel Office 365 (Microsoft Corporation, Redmond, Washington, USA) and the average daily gain (ADG) of individual pigs was calculated using the formula: (weight at visit 2-weight at visit 1)/ (number of days between visits). The data were then imported into STATA 15 for statistical analysis (Stata Corp LLC, College Station, Texas, USA).

Nurseries were classified as seropositive to each pathogen at a visit if 2 or more of the 20 pigs sampled were seropositive for the pathogen of interest (Appendix V). Summary statistics describing the number of observations and their frequencies were sorted by farm and determined for categorical variables. Mean, minimum and maximum values were also determined using individual observations sorted by farm within categorical variables.

Apparent prevalence and exact 95 % confidence intervals were calculated at the herd level for seropositivity to PRRSv, IAV and *M. hyopneumoniae* at both visits using the binomial distribution. Herd-level true prevalence estimates for seropositivity to all three pathogens were calculated for both visits using the Rogan-Gladen estimator and sensitivity and specificity values from the literature (Erlandson et al., 2005; Greiner & Gardner, 2000; Iowa State University, 2010; Maying et al., 2012). The 95 % confidence intervals for the true prevalence estimates were calculated using the normal distribution.

A mixed-effects logistic regression was conducted with *M. hyopneumoniae* seropositivity as the outcome, *M. hyopneumoniae* vaccination status as the fixed explanatory variable and farm as a random effect. Variables were considered for screening according to the causal diagram (Appendix III). Screening was conducted by testing for significance in univariable analysis using a *P-value* of 0.2. Continuous variables were tested for a linear relationship with the logit transformation of the outcome using Lowess smoother curves. The final model was built by manual forward selection and variables with a $P\text{-value} < 0.05$ were included in the final model.

Biologically relevant interaction terms according to the causal diagram (Appendix III) were also tested for significance in the model. Possible confounders, according to the causal diagram, were tested in the final model and were kept if their exclusion from the model resulted in a 20% change in either the coefficients or *P-values.* Logistic model assumptions were tested in the final model.

A mixed-effects linear regression was conducted at the pig level with ADG as the outcome, PRRS seropositivity at visit 2 as the fixed explanatory variable and farm included as a random effect in the model. IAV seropositivity at each visit were screened for inclusion in the model along with other variables according to a causal diagram (Appendix IV). Screening was conducted for significance in univariable analysis using a *P-value* of 0.2. Criteria for excluding variables from the model were high numbers of missing observations, or a *P-value* of above 0.2 in the univariable analysis. Pairwise correlations were also tested using the Pearson correlation coefficient. Continuous variables were tested for a linear relationship with the outcome using Lowess smoother curves and for a normal distribution using histograms. The final model was built by manual forward selection and variables with a $P\text{-value} < 0.05$ were included. Biologically relevant interaction terms according to the causal diagram (Appendix IV), were also tested for significance in the model. Possible confounders, according to the causal diagram, were tested in the final model and were kept if their exclusion from the model resulted in a 20% change in either the coefficients or *P-values*. Linear model assumptions, including constant variance and normality of residuals were tested for in the final model.

Results

One of the conventional nurseries withdrew from the study, leaving a total of 49 nurseries. From these 49 nurseries, there were 951 pigs that were sampled at both visits.

The change in status for the seroprevalence of three pathogens is shown in table 3.1. Two nurseries (4.1 %) had a change in PRRSv status, seven nurseries (14.3 %) had a change in IAV status and 15 nurseries (30.6 %) had a change in *M. hyopneumoniae* status.

Table 3.2 summarizes the herd-level apparent and true prevalence of PRRSv, IAV and *M. hyopneumoniae* seropositivity in the 49 sampled nurseries. *M. hyopneumoniae* estimates were grouped by vaccination status. The herd-level apparent prevalence at visit 2 was 30.6 % (*95 % CI = 18.3-45.4*) for PRRSv and 75.5 % (*95 % CI = 61.1-86.7*) for IAV. For nurseries that vaccinated, the herd-level apparent prevalence of *M. hyopneumoniae* seropositivity at visit 2 was 73.2 % (*95 % CI = 57.1-85.8*). The herd-level true prevalence could not be estimated for *M. hyopneumoniae* seropositivity due to a limitation of the Rogan-Gladen estimator with the specificity and sensitivity values of the ELISA test.

The results of a mixed effects logistic regression with *M. hyopneumoniae* seropositivity at visit 2 as the outcome, vaccination status for *M. hyopneumoniae* as the main explanatory variable and farm as a random effect are shown in table 3.3. Pigs that were vaccinated against *M. hyopneumoniae* had 108 times the odds of being seropositive for the organism at visit 2 compared to pigs that were not vaccinated (*P-value = 0.035*). In addition, pigs that were seropositive for PRRSv at visit 2 had 4 times the odds of being seropositive for *M. hyopneumoniae* compared to pigs that were seronegative for PRRSv, with all other variables being held constant (*P-value = 0.023*). RWA pigs had 389 times the odds of being seropositive

for *M. hyopneumoniae* compared to conventional pigs (*P-value < 0.000*), while organic pigs had 91 times the odds of being seropositive for *M. hyopneumoniae* compared to conventional pigs (*P-value = 0.001*). No interaction terms, including between production system type and *M. hyopneumoniae* vaccination status were significant. The model assumptions were met.

The results of the mixed effects linear regression with pig ADG in g/day as the outcome, PRRS status at visit 2 as the explanatory variable and farm as the random effect, are shown in Table 3.4. Weight at visit 1 and system type were found to be significant in the model during the building process and thus were included. Seropositivity for IAV was not significant in the model for either visit. Barn flow, which was categorized by all-in/all-out by room or site or continuous flow, was found to be a confounder and was included even though it was not a significant variable in the model. A summary of the frequency of barn flow types is shown in table 3.5. No interactions, including between system type and PRRS status at visit 2, that were tested were found to be significant within the model. Model assumptions of homoskedasticity, linearity and normality of residuals were met. Two individual pigs were found to be outliers in the model but since no recording error or other sound reason for removal was found, they were kept in the model.

Discussion

Prevalence of PRRSv, IAV and M. hyopneumoniae seropositivity

The PRRSv estimated herd level prevalence for seropositivity to PRRS of 34.7 % at visit 1 and 30.6 % at visit 2 determined in this study are similar to those found in the literature. A 2015 study used a producer health database to sample 370 production sites from 3 different regions across Ontario (Arruda et al., 2015). ELISAs and polymerase chain reactions (PCRs)

were used to determine disease status and one positive result for either test classified a site as a positive for PRRSv. The study determined the prevalence of PRRS virus to be 16.9%, 48.2 % and 20.6 % at the herd-level on swine production sites in the Niagara, Watford and Perth regions, respectively. The combined herd-level prevalence of PRRSv for all sites in the study was 37.1 %.

Since all the farms in the current study that were seropositive for PRRSv at visit 2 were also seropositive at visit 1, pigs were already entering the nursery with titres against PRRSv on seropositive farms. This indicates that the pigs had either been previously exposed to the virus and built an immune response or that they had acquired passive immunity from their dams (Chung et al., 1997; Zimmerman et al., 2012). Maternal antibodies to PRRSv have been shown to protect piglets until 6-9 weeks of age (Chung et al., 1997). Thus, antibody titres for PRRSv at the end of the nursery period are not maternally derived and indicate that the virus is circulating in the barn. Alternatively, a change from a seropositive to seronegative for PRRS between visit 1 and 2, which occurred in 2 nurseries, indicates that pigs entered the nursery with passive immunity and were not exposed to the virus in the nursery. Lastly, pigs on the 32 nurseries that remained seronegative for PRRSv were likely not exposed to the virus.

The herd-level prevalence for seropositivity to IAV was found to be 85.7 % at visit 1 and 75.5 % at visit 2 in this study. Other estimates in the literature of IAV prevalence differ. In 2004 and 2005 a study was conducted to estimate the prevalence of H1N1 and H3N2 in Ontario finishing pigs (Poljak et al., 2008). The apparent herd-level prevalence was determined to be 19.5 % and 30.6 % for H1N1 and 6.5 % and 40.8 % for H3N2 in 2004 and 2005 respectively. A more recent study in Mexico sampled 150 nursery and finishing pigs from 15 farms (López-Robles et al., 2014). ELISA testing was used to determine that 55 % of the sampled pigs were positive for H1N1, 59 % were positive for H3N2 and 38 % were positive for both subtypes of

IAV. A study conducted in Poland sampled nearly 6000 pigs of all ages from 145 farrow-to-finish farms between 2011 and 2015 and found the herd-level prevalence of antibodies to at least one IAV subtype to be 89.0 % (Czyżewska-Dors et al., 2017). The Polish study determined seroprevalence using hemagglutination inhibition tests that targeted antibodies to specific subtypes of IAV. While the universal ELISA test used in this study cannot identify specific subtypes of the virus, it detects all antibodies to IAV, including subtypes that are not identified by specific HI tests.

Since IAV is commonly found in swine herds, the primary trend of no change in the seropositive status for IAV between the two visits was expected. On 85.7 % of farms, pigs entered the nursery already having antibodies against IAV. These piglets were either exposed to IAV in the farrowing room or had passive immunity from the colostrum of their dams (Loeffen, Heinen, & Bianchi, 2003). On the second visit, 75.5 % of the nurseries were seropositive for IAV, with all but one of these nurseries also having a positive status at visit 1. As maternally derived antibodies have been shown to persist in pigs until over 10 weeks of age (Loeffen, Nodelijk, et al., 2003), it is possible that the positive titre status at the second visit was a result of lingering maternal antibodies. These maternally derived antibodies however, have also been shown to hinder the development of active immunity (Loeffen, Heinen, et al., 2003). Furthermore, due to the heterogeneity of IAV, antibodies against the virus are strain specific, thus even with antibodies against one strain of IAV, pigs are susceptible to other strains of the virus (Loeffen, Heinen, et al., 2003). A recent Danish study found that piglets could be infected with IAV at less than a week old, despite their dams having maternal antibodies (Ryt-Hansen et al., 2019). Thus, pigs could have entered the nursery with strain specific or maternally derived antibodies and then later been infected with another strain of the virus which they then developed

immunity against. However, clinical signs were not measured in this study, so this theory cannot be verified.

The six nurseries seroconverting from positive to negative for IAV, was likely a result of the pigs not being exposed to IAV after their passive immunity diminished. In the one nursery that seroconverted from negative to positive for IAV, pigs likely did not have passive immunity entering the nursery and were exposed to the virus during the nursery period. Lastly, a consistently seronegative status for IAV, which occurred on 6 farms, indicated that the virus was not present on these farms.

Since most of the nurseries in this study vaccinated against *M. hyopneumoniae*, a limitation of this study was that it was not possible to use the prevalence estimates as an indication of disease status in the nursery, as vaccination against *M. hyopneumoniae* is expected to elicit an immune response that can be detected through serological testing by increased antibody titres (Hodgins, et al., 2004; Seip, 2018; Thacker et al., 1998). In this study however, only 73.2 % of the nurseries that vaccinated had a positive titre status against *M. hyopneumoniae* at the second visit. 11 or 26.8 % of the 41 nurseries that vaccinated did not have more than 1 pig with a positive titre against the organism at the second visit.

Given that *M. hyopneumoniae* is believed to be widespread in Ontario, and with weaned pigs likely encountering the organism as their passive protection wanes and that most of the pigs in this study were vaccinated against the organism, a higher herd-level prevalence was expected for *M. hyopneumoniae* at the end of the nursery period.

Since the ELISA used in this test only has a sensitivity of 37.3 %, the lower than expected seropositivity for *M. hyopneumoniae* may partially be due to false negatives (Erlandson

et al., 2005). ELISA tests also use a cut-off value to differentiate between positive and negative samples. Having a binary outcome like a cut-off value, may miss some information that would be evident using a continuous outcome. For example, a pig may have a S/P value at the second visit just under the cut-off and be considered seronegative when the titre values are showing an upward trend relative to the first sampling. Thus, looking at the trend in S/P values for individual pigs may provide more information than just using a set cut-off S/P value. Alternatively, some studies have shown that a lack of maternal antibodies can result in a reduced immune response in vaccinated piglets (Hodgins et al., 2004), however the seroprevalence of the *M. hyopneumoniae* in the dams in this study was unknown and as reviewed by Thacker et al. (2012), this is still a point of controversy.

Another possible explanation for the lower than expected antibody titres to *M. hyopneumoniae* in vaccinated nurseries may be that producers are not following proper vaccine storage and handling procedures. A Dutch study surveyed swine producers on their knowledge of vaccine storage requirements and then inspected and measured the temperatures of vaccine fridges in 126 farms in Belgium and the Netherlands (Vangroenweghe, 2017). The study determined that only 80 % of surveyed producers knew the proper temperature to store vaccines at and only 10 % of inspected vaccine fridges had a thermometer. In addition, 29 % of the fridges inspected had temperatures above or below the specified range for the proper storage of vaccines.

The IDEXX ELISA test used in this study does not measure IgM and therefore early response to natural antigen exposure or vaccination was not detected. It is common practice on many farms to use a single injection of *M. hyopneumoniae* vaccine rather than following the initial vaccination with a booster and this practice may not elicit an antibody response that would

register as positive. Interestingly, Thacker & Minion (2012) reviewed the literature and concluded that there is no evidence of a correlation between high vaccine-derived antibody levels and disease protection for *M. hyopneumoniae*. Even so, there might be merit in exploring why pigs at the end of the nursery period on some farms have low antibody titres despite vaccination.

Despite there being a lower prevalence for seropositivity to *M. hyopneumoniae* at visit 2 than expected, vaccinating against the bacteria was still shown to increase the odds of seropositivity at visit 2 compared to pigs that were unvaccinated. Seropositivity to PRRS at visit 2 was also shown to increase the odds of being seropositive for *M. hyopneumoniae* at the end of the nursery. This is thoroughly supported in the literature as respiratory diseases often occur in conjunction (Done & White, 2003). Pigs raised in the RWA and organic systems were also shown to have increased odds of *M. hyopneumoniae* seropositivity at visit 2 relative to conventionally raised pigs. Thus, in this study, RWA and organic pigs appeared to be more likely to have been exposed to the bacteria, either through vaccination or infection, by the end of the nursery period than conventional pigs. Since there was no significant interaction between production system type and vaccination status for *M. hyopneumoniae*, this may indicate that the pigs in the RWA and organic nurseries were more likely to be exposed to the bacterium in the nursery than conventional raised pigs. However, this increased likelihood of RWA and organic pigs being seropositive to *M. hyopneumoniae* at visit 2 could also be due to these producers following better vaccination protocols than conventional producers.

Impact of PRRSv, IAV seropositivity on individual pig growth rates

The impact of PRRSv seropositivity on the growth of individual pigs was consistent with the literature. A common clinical sign of PRRS virus in the nursery is a reduced growth rate in

infected animals (Harper, 1991). Even though organic pigs were shown to have a reduced growth rate compared to conventional, there was no interaction between PRRS status at visit 2 and system type. This may be explained by the lack of variation in the PRRS seropositivity status of organic nurseries with only one nursery having a positive status at visit 2.

The lack of relationship between seropositivity to IAV at visit 2 and the growth rate of individual pigs may be explained by the sample size. Since 75.5 % of nurseries had a positive status for IAV at visit 2, the sample size may not have allowed enough variation to properly show an impact of an influenza infection on growth.

A limitation of this study was that the immune status of sows against the pathogens of interest was unknown. The use of an ELISA to evaluate titres without the assistance PCR testing or the evaluation of clinical signs was another limitation, as disease statuses could only be inferred. Future studies to summarize the serological profile of nurseries for respiratory pathogens could determine sow and piglet disease status and seropositivity using PCR testing in addition to ELISA tests and observing clinical signs of disease. This would allow a more complete understanding of the patterns of respiratory diseases in nursery pig populations.

Conclusion

Respiratory pathogens including PRRSv, IAV and *M. hyopneumoniae* are widespread in nursery pig populations. In the nurseries in this study there was serological evidence that influenza was present in the nursery pig population on most farms. Porcine reproductive and respiratory syndrome virus was found to be present in about a third of the nurseries in this study and there was evidence that PRRSv was associated with reduced growth rate. There were herds in this group of nurseries that had no serological evidence of *M. hyopneumoniae*, however most

nurseries were positive based on antibody titres of pigs entering the nursery, reflecting passive immunity. Most farms in the study vaccinated nursery pigs against *M. hyopneumoniae* and therefore it was not possible to differentiate between naturally derived and vaccine-induced antibody titres at the end of the nursery period. Only 73 % of the vaccinated nurseries had a positive antibody status to *M. hyopneumoniae* at the end of the nursery period. This lower than expected prevalence for *M. hyopneumoniae* at the herd level should be studied further using clinical signs and PCR testing as indications of disease status, because it may indicate a lack of protection for pigs entering the grower-finisher stage.

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Table 3.1: Summary of 49 nurseries and their status for 3 respiratory pathogens at the beginning and end of the nursery period

Pathogen	**Number of nurseries that seroconverted from negative to positive (%)**	**Number of nurseries that seroconverted from positive to negative (%)**	**Total number of nurseries that seroconverted (%)**	**Number of nurseries that consistently remained seropositive (%)**	**Number of nurseries that consistently remained seronegative (%)**
PRRSv*	0 (0.0)	2 (4.1)	2 (4.1)	15 (30.6)	32 (65.3)
IAV**	1 (2.0)	6 (12.2)	7 (14.3)	36 (73.5)	6 (12.2)
M. hyo***	6 (12.2)	9 (18.4)	15 (30.6)	26 (53.1)	8 (16.3)

Nurseries were sampled within a week of entry and exit for one batch of pigs. 20 pigs were sampled per nursery. A nursery was classified as seropositive for PRRSv and *M. hyopneumoniae* with an ELISA S/P ratio ≥ 0.4 and for IAV with an ELISA S/P ratio of ≤ 0.6.

*PRRSv –porcine reproductive and respiratory syndrome virus

**IAV- Influenza A virus

****M. hyo- Mycoplasma hyopneumoniae*

Table 3.2: Summary of the seroprevalence of porcine reproductive and respiratory syndrome virus (PRRSv), influenza A virus (IAV), and *Mycoplasma hyopneumoniae* (M hyo) at beginning and the end of a batch for 49 nurseries

Pathogen	Visit [a]	Vaccination Status [b]	Number of seropositive nurseries [c]	Herd-level apparent prevalence, % (95 % confidence interval) [d]	Herd-level true prevalence, % (95 % confidence interval) [e]	Mean number of seropositive pigs per nursery (range) [f]
PRRSv	1	0	17	34.7 (21.7, 49.6)	35.1 (21.2, 48.2)	7 (2-17)
	2	0	15	30.6 (18.3, 45.4)	30.9 (17.5, 43.7)	12 (2-20)
IAV	1	0	42	85. 7 (72.8, 94.1)	99.6 (72.6, 98.8)	15 (4-20)
	2	0	37	75.5 (61.1, 86.7)	86.0 (59.4, 91.6)	10 (2-20)
M hyo	1	1	31	75.6 (59.7, 87.6)		7 (2-20)
		0	2	40.0 (5.0, 85.3)		7 (4-10)
		Unknown	2	66.7 (9.4, 99.2)		5 (2-8)
	2	1	30	73.2 (57.1, 85.8)		12 (2-20)
		0	1	20.0 (0.5, 71.6)		3
		Unknown	1	33.3 (0.8, 90.6)		2

[a] Visits were conducted within a week of entry and exit from the nursery.

[b] Describes the vaccination status against the pathogens of interest, with 0 meaning not vaccinated, 1 meaning vaccinated and unknown meaning that the vaccination status was not known.

[c] 20 pigs were sampled per nursery. Seropositive classification of a nursery was determined as having 2 or more pigs with S/P ratio $\geq$ 0.4 for PRRSv and *M. hyopneumoniae* and S/N ratio $\leq$ 0.6 for IAV.

[d] Herd-level apparent prevalence was calculated by dividing the number of seropositive nurseries by the total number of nurseries or in the case of M. hyo, the total number of nurseries in the vaccination category. There were 49 nurseries in total, 41 nurseries that vaccinated for M. hyo, 5 nurseries that didn't vaccinate for M. hyo and 3 nurseries that had an unknown vaccination status for M. hyo. 95 % confidence intervals were calculated using the binomial distribution

[e] True prevalence was calculated using the Rogan-Gladen estimator to account for the sensitivity and specificity of the ELISA tests used. The normal distribution was used to calculate the 95 % confidence intervals. True prevalence could not be estimated for *M. hyopneumoniae* due to a limitation of the estimator with the low test sensitivity and high specificity values of the ELISA test used.

[f] Determined by taking the average number of seropositive pigs in all nurseries that were classified as seropositive (having 2 or more pigs with a positive titre status).

Table 3.3: Mixed effects logistic regression at the pig level with *M. hyopneumoniae* seropositivity at visit 2 as the outcome and farm as a random effect

Variable		**Odds Ratio**	**Standard Error**	**95% Confidence Interval**	**P-value**
Vaccinate for *M. hyopneumoniae*		107.449	238.145	1.395, 8274.824	0.035
Production system type	Conventional	Referent			
	RWA	388.448	530.130	26.771, 5636.388	0.000
	Organic	91.360	120.443	6.896, 1210.383	0.001
PRRS seropositivity at visit 2[a]		3.811	2.242	1.203, 12.073	0.023

[a] Defined by having a S/P ratio ≥ 0.4, visit 2 was conducted within a week before weaning.

Table 3. 4: Mixed effects logistic regression at the pig level with seropositivity for *M. hyopneumoniae* at visit 2 as the outcome and farm as the random effect

Variable		Coefficient	Standard Error	95 % Confidence Interval	P-Value
PRRS seropositivity at visit 2[a]		-35.075	13.695	-61.917, -8.232	0.010
Weight at visit 1[b]		25.043	1.984	21.155, 28.931	0.000
Production system type	RWA	-23.077	25.636	-73.323, 27.169	0.368
	Organic	-120.207	24.674	-168.568, -71.846	0.000
Flow	Continuous	Referent			
	All-in-all-out by room	41.707	24.402	-6.120, 89.534	0.087
	All-in-all-out by barn	23.258	26.737	-29.145, 75.661	0.384

[a] Defined as having an S/P ≥ 0.4 at second visit, which is conducted within a week prior to exit from the nursery

[b] Visit 1 is conducted within a week upon entry to the nursery

Table 3.5: Summary of the frequency of barn flow types in 49 sampled nurseries

Management Factor	Conventional	RWA*	Organic
All-in-all-out by barn (%) [a]	35.0	80.0	50.0
All-in-all-out by room (%) [b]	38.0	20.0	40.0
Continuous flow (%) [c]	28.0	0.0	10.0
Total number of farms with data	29	10	10

[a] The entire barn is emptied before being filled again

[b] Rooms are emptied completely before being filled again

[c] New pigs are constantly entering a barn where older pigs are present

*RWA-raised-without-antibiotics

CHAPTER 4: CONCLUSIONS

4.1 Research Summary and Conclusions

The nursery stage is a critical time in the health and performance of individual pigs and entire swine operations. The susceptibility of newly weaned pigs to disease causes the nursery to be a reservoir for pathogens within a herd. Respiratory pathogens including porcine reproduction and respiratory syndrome virus (PRRSv), *Mycoplasma hyopneumoniae* and influenza A virus (IAV) commonly infect nursery pigs and may impact their health and performance. In addition, these diseases can remain endemic in the nursery, with the nursery acting as a source of infection for subsequent weaned pigs and the rest of the herd (Chung, et al., 1997). Management plays a key role in preventing disease and maintaining high health and performance in nursery pigs. One of the key strategies for controlling respiratory disease is the feeding of antimicrobials in starter diets to reduce clinical signs of disease and optimize growth (Cromwell, 2002). In the case of viral diseases, antimicrobials are often justified as a control of secondary bacterial infections because both PRRSv and IAV are known to suppress immunity and act synergistically with bacterial pathogens like *M. hyopneumoniae* (Done & White, 2003).

Due to increased public concern over the role of food animal antimicrobial use in the emergence of antimicrobial resistance, niche markets have formed for pork originating from farms that do not use antimicrobials. In general, retailers have been able to charge a premium for pork that is identified as having been raised “antibiotic-free”. Three systems reviewed in this study were “raised-without-antibiotics” (RWA), “certified organic” and “certified humane”. The RWA system allows producers to use traditional management practices except for the ban on antimicrobial use (CFIA, 2019). Organic and humane production systems require other

management practices to be followed in addition to the prohibition of antimicrobial use. Organic production insists on the exclusive feeding of organic feed ingredients (Canadian General Standards Board, 2015), while both the organic and humane production systems require specific spacing per animal, use of solid flooring and access to bedding material and the outdoors (Canadian General Standards Board, 2015; HFAC Scientific Committee, 2014). No previous studies comparing the growth of pigs raised in these speciality systems to conventional pigs have been conducted in Ontario.

In chapter 2 of this thesis, the growth of conventional nursery pigs in Ontario was compared to antibiotic-free nursery pigs. Fifty Ontario nurseries were sampled, 30 of which were conventional, 10 that were RWA and 10 that were organic. A subset of twenty pigs were selected at each nursery to represent a batch of weaned piglets. Nurseries were visited twice, within a week after weaning and then a week prior to shipping from the nursery. Pigs were weighed at each visit and the weights were used to calculate individual average daily gain (ADG) values. Using linear regression modeling and controlling for the effect of individual nursery variation, it was determined that the production system type affected individual pig growth rates. There was no difference found between the growth rates of nursery pigs from RWA and conventional nurseries while organic pigs grew significantly slower than pigs in the other two production programs, even though pigs on organic farms are weaned at an older age.

Since conventional and RWA farms follow the same management protocols other than antimicrobial usage, the difference found in growth between organic and conventional pigs was attributed to the management practices that are unique to the organic system, such as pigs having access to the outdoors and pens being bedded with straw. Some of the sanitation practices that are available to conventional producers are not possible with the constraints imposed by the

organic requirements. The need to utilize only organic feed ingredients may also be major contributing factor of the reduced growth rates, however this was not studied in this research.

The results in chapter 2 indicate that growth rate appears to be similar for nursery pigs on RWA farms and on conventional farms that typically feed antimicrobials at least for the first few weeks after weaning. This suggests that routine antimicrobial use in starter diets may not be essential as far as maximizing nursery performance. It has been suggested in the literature that with increased sanitation, all-in/all-out production, and all the other advances in housing and management the need for antimicrobials is greatly decreased (Dritz, et al., 2002). From this research, it appears possible that swine producers may remove antimicrobials from their starter diets without a noticeable decrease in growth rate.

In Chapter 3, the situation of endemic diseases circulating in nurseries was explored. The status of three common respiratory diseases was used in this study of the same 50 nurseries as the previous chapter. Blood samples were collected from 20 pigs sampled at the beginning and end of the nursery period. ELISA tests were conducted to determine titer status to PRRSv, IAV and *M. hyopneumoniae*, according to kit instructions. The criteria for a nursery to be considered seropositive for PRRSv, IAV or *M. hyopneumoniae* was to have 2 or more of the 20 pigs that were seropositive. Herd-level prevalence estimates were calculated at the herd level for seropositivity to the three pathogens. Using a mixed-effects logistic regression and controlling for individual nursery variation, the relationship between vaccination status and *M. hyopneumoniae* seropositivity was explored at the pig level. A mixed effects linear regression model was also used to explore the relationship between seropositivity to PRRSv and IAV and growth rate at the pig level, controlling for individual nursery variation.

The apparent herd-level prevalence of PRRSv in the 49 nurseries was determined to be 34.7 % and 32.7 % at visit 1 and visit 2, respectively. The apparent herd-level prevalence of IAV in 50 nurseries was determined to be 85.7 % and 79.6 % at visit 1 and visit 2, respectively. Lastly, the apparent herd-level prevalence of *M. hyopneumoniae* was 63.3 % and 61.2 % for nurseries that vaccinated against the organism.

The lower than expected apparent herd-level prevalence of *M. hyopneumoniae* seropositivity in vaccinated nurseries could have been attributed to the low sensitivity of the ELISA test used as well as the limitation of using a binary outcome, like S/P ratios, in serological testing. Another explanation proposed for the lower than expected prevalence was producers not following proper vaccination strategies or proper vaccine storage and handling, as producer knowledge on vaccines has been shown to be limited (Vangroenweghe, 2017).

Despite the lower than expected herd-level prevalence of *M. hyopneumoniae* seropositivity, vaccinating against the organism was shown to increase the odds of a seropositive response at the end of the nursery period at the pig level. In addition, RWA and organic pigs were shown to have higher odds of being seropositive for *M. hyopneumoniae* at the end of the nursery period compared to conventional pigs. Since there was no interaction found between *M. hyopneumoniae* vaccination status and production system type, this could be attributed to a higher prevalence of *M. hyopneumoniae* in RWA and organic nurseries. However, it could also be due to producers being more diligent when vaccinating pigs in these programs compared to conventional producers.

Among the nursery in the study it was found that pig growth rate was impacted by PRRSv. This finding is consistent with other studies that have found decreased growth rates due PRRSv infections. In endemic situations the nursery is commonly the reservoir for the PRRSv

with naive pigs entering the nursery and becoming exposed and developing antibodies but also remaining viremic for several weeks. Without serological monitoring the producer may remain unaware of the presence of the virus even though performance is often affected. The lack of relationship between IAV seropositivity and growth rates noted in this study may be attributed to the sample size not allowing enough variation in IAV seropositivity as the virus was seroprevalent on most farms.

Overall, this research provided insight into the growth rates and health status of nursery pigs in a variety of production systems. Management practices other than antimicrobial use were found to play a critical role in the growth rates of pigs suggesting that producers may be able to raise pigs effectively in the nursery with limited or no antimicrobial usage. PRRSv is present in Ontario nurseries and does have a negative impact on the growth of individual pigs. Influenza A virus, however, while highly prevalent in nurseries in Ontario was not found to affect individual pig growth rates. Lastly, Ontario producers may not be eliciting protective immune response to *M. hyopneumoniae* in vaccinated animals, however future research is needed to support this claim.

There were several weaknesses in this research. Pigs were sampled within a week after entry and week before exit, thus the whole nursery period was not captured on every farm. This might make it difficult to compare growth rate data from this study with "close-out data" which typically is calculated based on the weight of all pigs entering, subtracted from the weight of all pigs leaving the nursery and then divided by the number of pigs in the group. In chapter 2, the exclusion of the first week, when pigs tend to acclimatize and grow very little, might have resulted in higher average daily growth rates than if the first week had been included. Alternatively, not including the last few days when pigs are growing the fastest might mean the

calculated growth rate of this study underestimates nursery performance. While the differences in management practices were summarized across production system types in chapter 2, another limitation was their individual effects on pig growth rates were not studied. A limitation of chapter 3 was the use of an ELISA test to determine seropositivity to *M. hyopneumoniae*, as this type of test cannot differentiate between vaccine-induced and naturally derived antibodies. In addition, it takes weeks for antibodies to develop, so serological testing cannot show recent infections. Thus, some of the animals testing positive at the end of the nursery in chapter 3 may have been recently exposed and were infected with one of the pathogens of interest but were serologically negative. While serological testing is widely used for monitoring respiratory disease in swine due to advantages of cost and speed, the interpretation of results must be done carefully.

Future research from this thesis could include controlled experimental studies comparing the growth rates of nursery pigs raised with and without antibiotics in a commercial farm setting. Specific management practices that differ in organic production compared to other production system types should also be isolated in future studies to study how they impact pig growth rates. Future studies to summarize the serological profile of nurseries for respiratory pathogens could determine sow and piglet disease status and seropositivity using polymerase chain reaction testing and observing clinical signs in addition to ELISA tests. In addition, observational research on vaccine storage and administration on Ontario swine farms could be beneficial.

4.2 References

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Appendix I: The producer questionnaire used for chapter 2 and 3

Nursery barn survey

Date: _____

Farm code: _____

Farm veterinarian:

The Facility

1. How best to describe the barn: -part of farrow-to-finish operation (if loop, name)

-part of farrow-to-feeder operation

-off-site nursery

- other_____

2. How many buildings on site?

3. What is the size of your herd? (Number of sows, or number of pigs at facility)

4. If animals are part of a loop, or bought in, who is your sow source?

5. Are you organic?

6. How long have you been organic/antibiotic-free?

7. The number of rooms_____

8. Number of pens in a room_____

9. Number of pigs in a pen_____

10. Number of water drinkers per pen_____

11. Number of feeding places per pen_____

12. What are the dimensions of the nursery barn pens?

Pig Flow

13. Is the barn operated as: - all-in/all-out by site

- all-in/all-out by barn

- all-in/all-out by room

- continuous flow

14. The average pig inventory of the nursery barn_____

15. How many pigs are in the batch being sampled?

16. What genetic company do you buy your pigs from?

Sow breed?.....

Boar breed?

Management

17. Typical age that pigs enter _____

18. Number of weeks they stay in the nursery_____

19. Are there age or weight limits? Yes/No, and if yes please list _____

20. Are pigs sorted by size or age in the nursery?

21. Is there a hospital pen?

22. Are pigs vaccinated? Yes/No, and if yes please list_____

23. How do you dispose of deadstock? Compost?___Incinerate?___Deadstock? Do you have a CAZ for the compost? Is the deadstock picked up here?

24. Are sows vaccinated for *Mycoplasma Hyopneumoniae*, PRRS, or influenza?

25. If pigs become sick in the nursery are they separated and treated with an injectable antibiotic? Yes/No

Feeding

26. How many different diets are used during the nursery phase? _____

And what are they? _____

Feed supplier: _____
Type of feed:

a. Pellet feed
b. Mash
c. Liquid feed
d. Wet/dry feed
e. Other: _____

2. Do you use creep feed?

Yes (please specify): _____

27. Would it be possible to record the amount of feed this batch of pigs consumes?

Production Records

28. Do you record total weight in and out of the nursery? Yes/No

29. What is your average growth rate during the nursery phase?

30. Do you record mortality? Yes/No

31. What is your average nursery mortality? _____

32. Can you record the mortality for this batch?

Health Concerns

33. What are common disease challenges that you face in your nursery? Please list_____

Biosecurity

34. Do you require visitors to be away from pigs for a certain time? Yes/No and if yes how long? _____

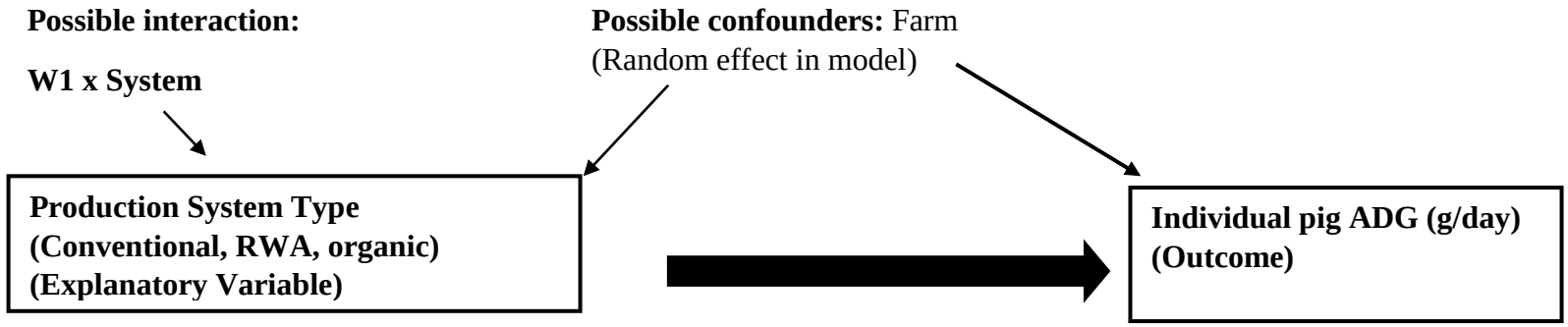
35. What is your washing protocol?_____ Do you wash between batches of pigs? Always or sometimes? _____Pressure wash- hot or cold._____ Do you presoak?

____Do you use detergent?_____ Is there dry time?_____ How long?

36. Do you use any disinfectants in your nursery barn and if so, what kind?

37. How best to describe biosecurity procedures for visitors: Shower-in, Danish entry, change into clean boots and coveralls, or no specific rules (circle one that most closely describes your situation)

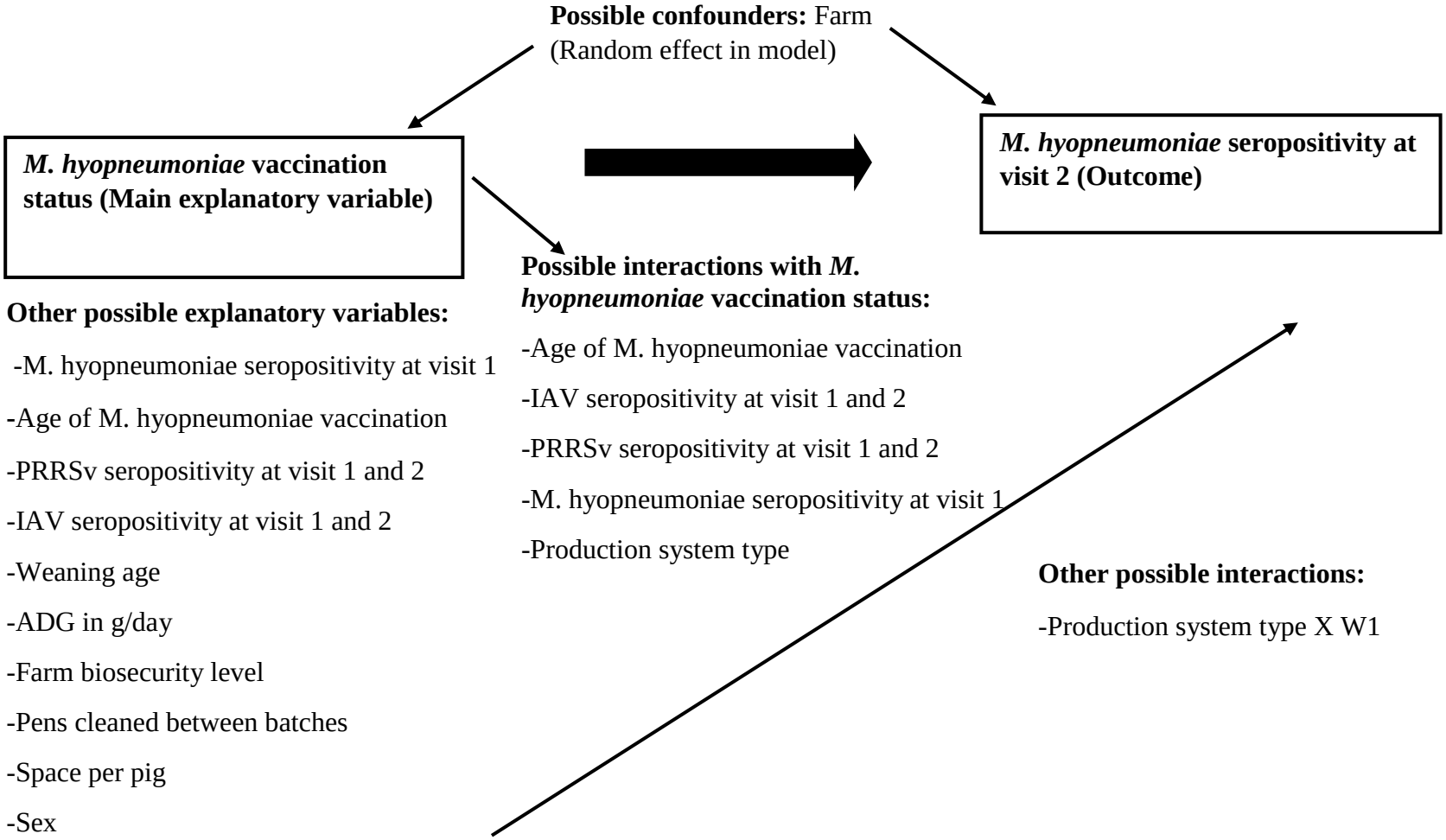
Appendix II: Causal diagram explaining screening of variables for the mixed effects linear regression between pig ADG and system type with farm as a random effect in chapter 2.



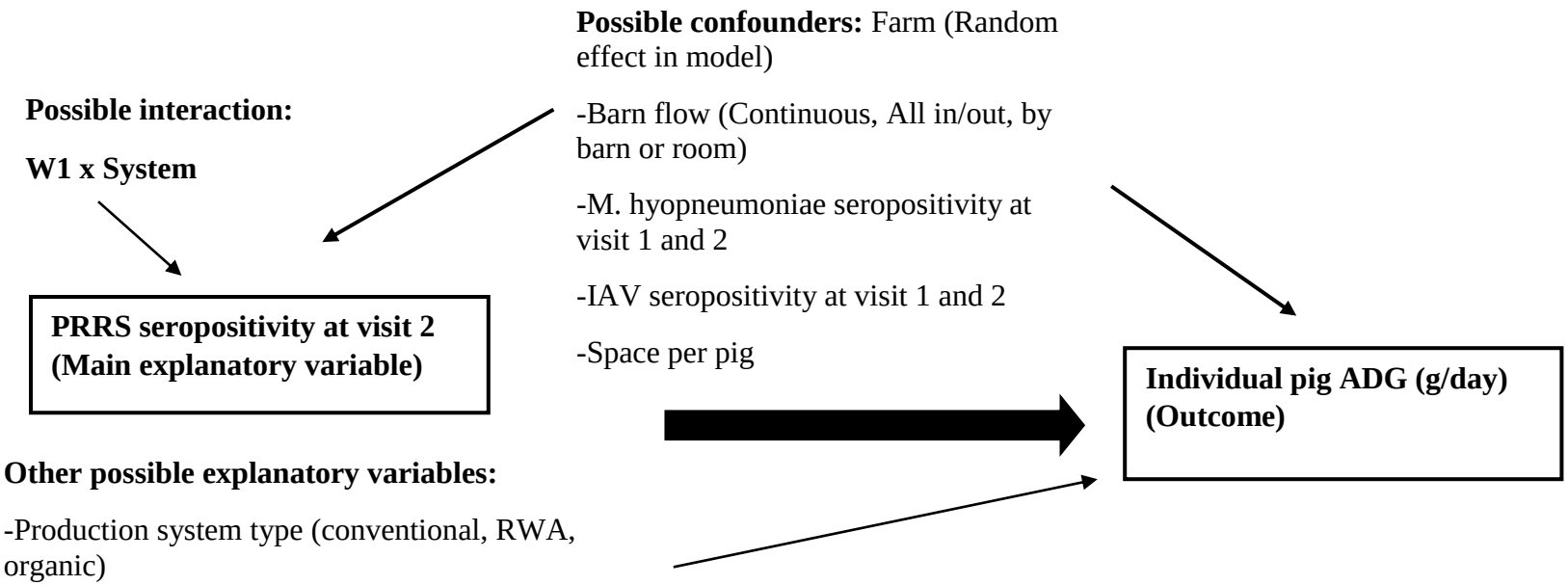
Other possible explanatory variables:

- Farm type (farrow-finish, wean-finish, nursery only)
-*M. hyopneumoniae* seropositivity at visit 1 and 2
-PRRSv seropositivity at visit 1 and 2
-IAV seropositivity at visit 1 and 2
-Weaning age
-Weight at visit 1
-Farm biosecurity level
-*M. hyopneumoniae* vaccination status
-Pens cleaned between batches
-Space per pig
-Sex
-Barn flow (continuous, all/ all out by room or barn)

Appendix III: Causal diagram explaining screening of variables for the mixed effects logistics regression between *M. hyopneumoniae* seropositivity at visit 2 and *M. hyopneumoniae* vaccination status at the pig level with farm as a random effect in chapter 3



Appendix IV: Causal diagram for the mixed effects logistic regression between PRRS seropositivity at visit 2 and ADG at the pig level with farm as a random effect in chapter 3



Appendix V: Summary of the seropositivity of *M. hyopneumoniae*, IAV and PRRSv at visit 1 and 2

Farm ID	# pigs pos for *M. hyo* V1 (%)	# pigs pos for *M. hyo V2*	Vaccinate for *M. hyo*	# pigs pos for influenza V1	# pigs pos for influenza at V2	# pigs pos for PRRSv at V1	# pigs pos for PRRSv at V2
1	11 (55.0)	1 (5.0)	1	18 (90.0)	11 (55.0)	0 (0.0)	0 (0.0)
2	3 (15.0)	7 (35.0)	1	13 (65.0)	3 (15.0)	3 (15.0)	20 (100.0)
3	2 (10.0)	1 (5.0)	1	15 (75.0)	8 (42.11)	6 (30.0)	15 (79.0)
4	6 (30.0)	1 (5.0)	1	18 (90.0)	10 (52.6)	3 (15.0)	14 (70.0)
5	1 (5.0)	20 (100.0)	1	19 (95.0)	13 (65.0)	6 (30.0)	2 (10.0)
6	2 (10.0)	5 (25.0)	1	9 (45.0)	1 (5.0)	3 (15.0)	2 (10.0)
7	0 (0.0)	1 (5.0)	1	17 (85.0)	10 (52.6)	0 (0.0)	19 (100.0)
9	18 (90.0)	13 (65.0)	1	14 (70.0)	4 (22.2)	0 (0.0)	0 (0.0)
10	1 (5.0)	0 (0.0)	1	18 (90.0)	16 (80.0)	0 (0.0)	0 (0.0)
11	4 (20.0)	4 (20.0)	1	15 (75.0)	9 (45.0)	13 (65.0)	20 (100.0)
12	2 (10.0)	1 (5.0)	1	13 (65.0)	3 (15.8)	0 (0.0)	0 (0.0)
13	0 (0.0)	0 (0.0)	1	0 (0.0)	20 (100.0)	0 (0.0)	0 (0.0)
14	2 (10.0)	1 (5.0)	1	17 (85.0)	14 (73.7)	0 (0.0)	0 (0.0)
15	13 (65.0)	2 (10.0)	1	15 (75.0)	4 (20.0)	0 (0.0)	0 (0.0)
16	20 (100.0)	8 (40.0)	1	12 (60.0)	0 (0.0)	5 (25.0)	20 (100.0)
17	8 (40.0)	1 (5.0)	un	15 (75.0)	16 (84.2)	0 (0.0)	0 (0.0)
18	1 (5.3)	1 (5.3)	1	1 (5.3)	0 (0.0)	0 (0.0)	0 (0.0)
19	7 (35.0)	0 (0.0)	1	17 (89.5)	13 (65.0)	0 (0.0)	0 (0.0)
20	0 (0.0)	11 (55.0)	1	16 (80.0)	5 (25.0)	6 (30.0)	6 (30.0)
21	3 (15.0)	2 (10.0)	1	16 (80.0)	13 (72.2)	3 (15.0)	12 (66.7)
22	0 (0.0)	0 (0.0)	0	16 (80.0)	13 (65.0)	0 (0.0)	0 (0.0)
23	10 (50.0)	3 (15.0)	0	13 (65.0)	4 (20.0)	0 (0.0)	0 (0.0)
24	0 (0.0)	1 (5.0)	0	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
25	2 (10.0)	2 (10.0)	un	11 (55.0)	5 (25.0)	0 (0.0)	0 (0.0)
26	8 (40.0)	19 (95.0)	1	19 (95.)	4 (20.0)	17 (85.0)	2 (10.0)
27	4 (20.0)	0 (0.0)	0	18 (90.0)	4 (20.0)	0 (0.0)	0 (0.0)
28	2 (10.0)	2 (10.0)	1	20 (100.0)	8 (44.4)	14 (70.0)	17 (94.4)
29	2 (10.0)	5 (25.0)	1	12 (60.0)	1 (5.0)	0 (0.0)	0 (0.0)
30	20 (100.0)	20 (100.0)	1	20 (100.0)	15 (100.0)	9 (45.0)	15 (100.0)
31	0 (0.0)	0 (0.0)	un	20 (100.0)	14 (70.0)	0 (0.0)	0 (0.0)
32	14 (70.0)	20 (100.0)	1	16 (80.0)	12 (60.0)	0 (0.0)	0 (0.0)
33	0 (0.0)	2 (10.0)	1	15 (75)	2 (10.0)	0 (0.0)	0 (0.0)
101	2 (10.0)	19 (95.0)	1	0 (0)	0 (0.0)	0 (0.0)	0 (0.0)
102	3 (15.0)	13 (65.0)	1	9 (52.9)	19 (95.0)	3 (17.7)	20 (100.0)
103	8 (40.0)	18 (90.0)	1	12 (60)	1 (5.0)	0 (0.0)	0 (0.0)
104	0 (0.0)	9 (45.0)	1	0 (0.0)	20 (100.0)	0 (0.0)	0 (0.0)
105	3 (15.0)	20 (100.0)	1	1 (5.0)	1 (5.0)	0 (0.0)	0 (0.0)

106	1 (5.0)	20 (100.0)	1	18 (90.0)	2 (10.5)	0 (0.0)	0 (0.0)
107	14 (70.0)	6 (30.0)	1	18 (90.0)	16 (80.0)	8 (40.0)	5 (25.0)
108	2 (10.0)	7 (35.0)	1	19 (95.0)	0 (0.0)	0 (0.0)	0 (0.0)
109	3 (15.8)	19 (100.0)	1	13 (68.4)	12 (70.6)	5 (26.3)	0 (0.0)
110	0 (0.0)	20 (100.0)	1	14 (70.0)	6 (31.6)	2 (10,.0)	0 (0.0)
111	2 (10.0)	5 (25.0)	1	4 (20.0)	18 (100.0)	0 (0.0)	0 (0.0)
112	3 (15.8)	19 (100.0)	1	15 (79.0)	6 (31.6)	0 (0.0)	0 (0.0)
113	5 (25.0)	18 (90.0)	1	20 (100.0)	20 (100.0)	11 (55.0)	5 (25.0)
114	3 (15.0)	0 (0.0)	1	0 (0.0)	1 (5.0)	0 (0.0)	0 (0.0)
115	20 (100.0)	18 (90.0)	1	18 (90.0)	2 (10.0)	0 (0.0)	0 (0.0)
116	8 (40.0)	4 (20.0)	1	9 (45.0)	12 (63.2)	0 (0.0)	0 (0.0)
117	0 (0.0)	0 (0.0)	0	8 (40.0)	2 (10.0)	0 (0.0)	0 (0.0)