A comparison of production parameters and bacterial colonization patterns in pigs raised from birth to nursery phase with two production models

A THESIS
SUBMITTED TO THE FACULTY OF
THE UNIVERSITY OF MINNESOTA
BY

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IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF
MASTER OF SCIENCE

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February 2023

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Acknowledgements

It is with the utmost gratitude that I offer my words of thanksgiving to my advisor Dr. Maria Pieters and co-advisor Dr. Randall Singer for the guidance and support over the past three years. I want to thank Dr. Pieters and Dr. Singer for challenging me to always think critically and do my best, both in course work and in research.

Thank you to my committee, Drs. Pieters, Singer, John Deen and Daniel Linhares. Your willingness to offer your time, knowledge and expertise is greatly appreciated and will always be remembered.

My gratitude also goes out to the University of Minnesota College of Veterinary Medicine Department of Veterinary Population Medicine, Swine Group and MycoLab group. This work would not have been possible without the assistance of so many. From study design, sample collection and laboratory services, a team was willing to offer their assistance and expertise. Thank you to Dr. Marcelo Gottschalk and his team at the University of Montreal for their support of this project with laboratory services.

Thank you to my amazing family and wonderful co-workers. Your patience and support are appreciated beyond words. You have allowed me to pursue this project in a manner that I hope is consistent with the directive of Dr. Bob Morrison, “do work that matters”.

Abstract

Genetic selection for economically important traits has increased growth performance, feed efficiency and litter size, resulting in enhanced sustainability and productivity. An unintended consequence of larger litters has been the increase in preweaning mortality. In this investigation, a production model which allows piglet cross-suckling among multiple sows from one day of age to weaning was evaluated. No difference was observed in preweaning mortality or growth performance from birth to weaning, of piglets raised in the multi-suckle common creep (MSCC) production model, when compared to the conventional (CONV) model. A significant reduction in nursery mortality was observed in the MSCC treatment group ($P < .001$) compared to conventional production. Growth performance from weaning to the end of the nursery phase was observed ($P = 0.01$) with greater average daily weight gain in MSCC pigs. Detection of *Streptococcus suis* serotype 1, *S. suis* species, and *Mycoplasma hyorhinis* with PCR did not differ statistically between the two production models at weaning. There was no difference in the transmission of the bacterial agent *S. suis* serotype 1, with substantial detection in both the MSCC and CONV study groups. The results of this study showed a reduction in nursery mortality and improved growth performance of pigs raised with MSCC. Greater understanding of alternative production models and the impact on pathogen transmission and effect on productivity and sustainability are necessary.

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General Introduction

Commercial swine production in the U.S., as well as throughout much of the world, has evolved from a low capital, high labor industry to a capital-intensive model driven by consistent and efficient throughput. In addition to the structural changes of the pork industry, the transition to multi-site production and segregated early weaning in the 1990s provided the means for effective elimination of several pathogens of economic importance in growing pig populations. While segregated early weaning was invaluable in the elimination of costly diseases such as swine dysentery from herds, application of this methodology for the elimination of other pathogens including *Streptococcus suis* (*S. suis*) and porcine reproductive and respiratory syndrome virus (PRRSV) were not successful.

A review of the literature on changes in litter size, strategies to manage challenges of large litters, the transmission of *Streptococcus suis* and *Mycoplasma hyorhinis* in piglets as well as the application of a new production model to address the issues associated with large litters in presented in Chapter 1.

Litter size has been a focus of genetic selection, resulting in an increase in the average number total born and of pigs born alive per litter. Although production efficiencies have been realized as a result of increased prolificacy, an unintended consequence of larger litter size has been increased preweaning mortality. Management strategies utilized to address the challenge of large litters include split-suckling, cross-fostering, the practice of reducing the variation in both the number and size of pigs within litters at birth, and the use of foster sows to nurse piglets unable to effectively compete with littermates. Novel models seeking to improve piglet survivability and welfare have been introduced to address the challenges of large litter size, including Multi-Suckle Common Creep (MSCC), which allows piglets to nurse from multiple sows and nest in a common area with increased space. The conventional production model (CONV) utilizes the practice of

cross-fostering among sows, with movement a function of piglet birth weight, parity of dam, live born and teat count. Chapter 2 describes the growth performance and survivability of piglets raised with the two production models, MSCC and CONV, from birth to the end of the nursery phase in a field study.

The control of bacterial infections and associated diseases in nursing and nursery phase pigs remains a challenge to efficient and sustainable pig production. *Streptococcus suis*-related diseases are of particular importance and consequence, with prophylactic and therapeutic antibiotic regimens routinely used in the attempt to reduce piglet mortality and morbidity during the nursery phase. The effect of piglet transfer from one or more litters with cross-fostering in the conventional production model and commingling of pigets among multiple litters with MSCC and the frequency of *S. suis* detection during the nursing phase and at weaning is not clearly understood. Analyses of *S. suis* detection and clinical expression of *S. suis*-related disease is complicated by the presence of pathogenic and commensal *S. suis* serotypes in pig populations. Chapter 3 describes the detection frequency of *S. suis* serotype 1 and *S. suis* species in sows and nursing piglets raised under conventionally and with MSCC.

Information on the transmission and colonization pattern of *M. hyorhinis* in nursing and post-weaning pigs is limited, with previous studies identifying prevalence in lactating sows and their piglets at several time points from birth to weaning and during the nursery phase. As litter size has increased, the need to transfer pigs from the birth sow to foster sows, and to implement novel production models which allow piglets to nurse from multiple sows and nest in a common area may alter the ecology of epidemiology of microorganisms including *M. hyorhinis* in the nursing pig. Chapter 4 explores the frequency of detection of *M. hyorhinis* in pigs rasied conventionally in with MSCC.

CHAPTER 1

LITERATURE REVIEW

1.1. Introduction

The U.S. swine production industry, as well as commercial pig-producing countries throughout the world, have evolved from a high labor system to that of a capital-intense model with focus on production efficiencies and consistent throughput. One of the primary means of improving production efficiencies of swine is genetic selection, which has resulted in improved growth performance, feed conversion efficiency, meat quality and reproductive traits for optimal productivity. One of the components of the selection indices utilized for balanced genetic improvement has been litter size. An unintended consequence of large litter size is the increase in piglet mortality from birth to weaning (Rutherford et al., 2013). Efforts have been made to address the challenge of managing large litters with farrowing house management strategies including intervention at birth, cross-fostering of piglets and utilization of nurse sows. Sow and piglet welfare and the effectiveness of cross-fostering as a management strategy in reducing preweaning mortality are areas of ongoing research (Baxter et al., 2013; Vande Pol et al., 2021a). Substantial investment and resources have been allocated in the attempt to modulate piglet survival from birth to weaning through intervention at the time of birth, cross-fostering and nurse sow utilization, with marginal effect on piglet survivability (Baxter et al., 2013). Novel production models with split suckling have been proposed and studied, with the goal of continued improvement in productivity and piglet well-being (Hessel et al., 2006; van Nieuwamerongen et al., 2014; Verdon et al., 2019; Parois et al., 2022). Under these production models, piglets are allowed to nest in a common area and nurse from multiple sows. Development of, and research into, novel production models such as Multi-suckle common creep (MSCC) are necessary in order to improve the pig well-being, sustainability and productivity of pig producers from birth to weaning while providing less complexity, intervention, and labor in the care of piglets at birth.

While pig producers strive to enhance productivity and sustainability through genetic selection and novel production methods, the challenge posed by endemic pathogens hampers efforts for continuous improvement. Pathogens including *Mycoplasma hyopneumoniae, M. hyorhinis, Glaesserella parasuis (G. parasuis), Actinobacillus suis (A. suis), Streptococcus suis* (*S. suis*), Influenza A virus (IAV), porcine circovirus type 2 (PCV-2) and porcine reproductive and respiratory syndrome virus (PRRSV), are endemic in pig populations in multiple phases of production. The elimination of a number of pathogens from weaned pig populations with the implementation of medicated or segregated early weaning led to attempts with similar results with PRRSV and *S. suis*. The application of segregated early weaning as a means of control and elimination of these pathogens proved unachievable. These two pathogens are of particular relevance to the pig producer. Diseases of both *S. suis* and PRRSV result in severe economic losses to pig producers, as well as deprivation of welfare of the pig. Although estimates of the burden of *S. suis* disease are limited, the cost to producers of the control of *S. suis* outbreaks is substantial. *Streptococcus suis* is ubiquitous in swine herds worldwide, with prevalence of carrier animals approaching 100 percent (Brisebois et al., 1990). Additionally, the use of antibiotics to control outbreaks of *S. suis* is reason for concern in light of increasing evidence of antibiotic resistance (Palmieri et al., 2011). Porcine reproductive and respiratory syndrome virus is a pathogen of substantial economic consequence, with the annual cost to U.S. producers estimated to be $664 million (Holtkamp et al., 2013). Control strategies for the elimination of PRRSV from breeding herds, whether endemic or after an outbreak, include the limitation of movement of piglets from the sow of origin to foster sows. Likewise, movement of piglets from sows of origin to foster sows has been implicated in increased mortality in the post-weaning phase. Additional research into the

role of piglet movement in the farrowing room and the transmission of bacterial pathogens including *S. suis* and *M. hyorhinis* as well as viral pathogens including PRRSV and IAV is needed.

1.2 Implications of increased litter size for pig production

Improvements in the efficiency of swine production are the result of decades of intense genetic selection (Dickerson and Grimes, 1947; McLaren and Bovey, 1992; Merks et al., 2011; Knap and Wang, 2012). Swine breeding programs include the use of selection indices rather than single trait selection models (Christians, 1981; Stewart et al., 1991; Amer et al., 2014). An unintended consequence of genetic selection for increased litter size is the difficulty in effectively managing litters with the increased number of high-risk, small birth weight pigs (Quiniou et al., 2002; Baxter et al., 2013; Andersson et al., 2015; Zeng et al., 2019; Gourley et al., 2020a; Kobek-Kjeldager et al., 2020; Ward et al., 2020; Peltoniemi et al., 2021). From 1996 to 2011, the average number of pigs born alive per litter increased from 11.2 to 14.8, while the pre-weaning mortality increased from 18.2% to 23.5% (Rutherford et al., 2013). A 2018 productivity analysis prepared by the National Pork Board reported the U.S. swine herd average of 17.8% mortality from birth to weaning, in addition to the 9.8% of total born that are stillborn and mummified fetus. In total, over 25% of total born piglets are lost before weaning (Stadler, 2018).

1.3 Attempts to reduce pre-weaning mortality

Efforts to reduce piglet losses from birth to weaning include dietary supplementation to the pig (Declerck et al., 2016; Peltoniemi et al., 2021) and the piglet at birth (Schmitt et al., 2019), as well as management strategies including piglet drying at birth (Anderson et al., 2009; Vande Pol et al., 2021), have been investigated. Increased lysine and energy duration on sow and piglet performance, piglet survival and colostrum quality has been evaluated. Colostrum quality and

piglet colostrum intake were not impacted by treatment (Gourley et al., 2020b). Dietary zinc supplementation indicated a reduction in mortality of piglets classified as low (<1 kg) weight. Sows were assigned to one of three dietary zinc sulfate levels. The percentage piglet mortality classified as low birth weight in the high zinc sulfate treatment group was significantly lower than that of low birthweight pigs in the medium and control dietary zinc sulfate levels (Holen et al., 2020). The effect of supplemental dietary fiber fed during the last two weeks of gestation has been evaluated as well. Both the proportion of stillborn piglets and piglet mortality as a proportion of total born was reduced in litters from sows in the dietary fiber treatment group, however, no impact on preweaning mortality was observed (Feyera et al., 2017). A study evaluating the efficacy of energy supplementation at birth was conducted using treatment with coconut oil or a commercial product in reducing preweaning mortality of pigs born less than 1.1 kg. No difference in the proportion of piglet mortality between the energy in either supplemented treatment groups and the control groups at 24 hours or at weaning was observed (Schmitt et al., 2019).

The practice of drying piglets at the time of birth, either as the sole intervention, or in conjunction with individually warming each pig, has been used as a management practice attempting to reduce piglet mortality. A study was conducted to compare the piglet weaning weight and preweaning mortality on a commercial farm. Piglets were allotted into either the treatment group (dried and placed in a warming box for 30 min before being returned to the farrowing pen) or the control group. Difference in piglet mortality or weaning weight between treatments was not observed (Vande Pol et al., 2021). Management interventions including the practice of split suckling have been utilized. A recent study evaluated the effectiveness of the practice of split suckling in reducing piglet mortality before weaning. In the study, litters were allocated to one of three treatment groups, no split suckle, split suckle on the first day of life only, and split suckle for three

days. Colostrum intake did not differ between the treatment groups, however colostrum intake was reduced among the biggest pigs in both the one-day and three-day split suckle treatment. Mortality did not differ among the treatment groups, and piglet growth performance in the one day split suckle group was not affected. Growth performance was negatively impacted in the three days split suckle treatment group (Vandaele et al., 2020).

1.4 Managing large litters: implications of cross-fostering and cross-suckling

Increased litter size has resulted in more variation within litter birth weight, with a greater proportion of small birthweight piglets being born (Quiniou et al., 2002; Baxter et al., 2013). As litter size has increased, the proportion of litters with more pigs born alive than functional glands on the birth sow has also increased (Wensley et al., 2021). For example, the percentage of litters with 17 or more pigs born alive increased from 0.5% to 39.5% from 2005 to 2021 in one production system. Eighty six percent of litters had recorded foster movements (Schwartz, 2021). A management response to increased litter size, with the higher proportion of small birthweight pigs has been the implementation of cross-fostering as a management strategy (Straw et al., 1998a; Straw et al., 1998b; Calderón-Diaz et al., 2018). Cross-fostering is defined as the transfer of piglets at birth to equalize litter size according to weight (Heim et al., 2012; Calderón-Diaz et al., 2018). Vande Pol et al., in 2021a, compared the preweaning mortality and growth performance to weaning as a function of within-litter weight variation after cross-fostering. Piglets were classified into one of three birthweight categories, namely light, medium or heavy, and foster litter categories uniform light, uniform medium, uniform heavy or mixed with two or more birthweight categories within the litter after cross-fostering. In general, within each birth weight category, weaning weight increased and preweaning mortality decreased as littermate weight decreased. Preweaning mortality was lowest for medium weight category pigs in the mixed medium and large litters and

for heavy weight category pigs in the mixed light, medium and heavy litters. Litter composition had no effect on the preweaning mortality of light birth weight pigs. Increasing the average litter weight after cross-fostering decreased weaning weight and increased preweaning mortality across all birth weight categories (Vande Pol et al., 2021a). An observational study conducted in a commercial sow farm measured piglet preweaning mortality, growth performance and welfare (Calderón-Diaz et al., 2018). Piglets were classified into one of three treatment groups non-cross-fostered, cross-fostered within the first week of birth and cross-fostered in the second and third weeks from birth. Welfare was measured by scoring tail, ear and body lesions at weaning and at several points during the nursery and grow-finish phases. Of all cross-fostered piglets, 40.8% and 59.2% were classified as cross-fostered within the first week of birth and cross-fostered in the second and third weeks, respectively. Cross-fostered pigs were born into larger litters than non-cross-fostered pigs. No differences in tail or body lesions or growth rate were observed between cross-fostered and non-cross-fostered treatment groups. Pigs cross-fosterd within the first week of birth were more likely to have ear lesions than non-cross-fostered pigs and pigs cross-fostered in the second and third weeks from birth (Calderón-Diaz et al., 2018). A study which evaluated the association between cross fostering small birthweight piglets onto either large or small litters, with piglets classified as either average or large birthweight littermates was conducted on a commercial farm. Large and small litter size was defined as 14 pigs and 13 pigs after cross-fostering, respectively. The growth rate of the small birthweight piglets that were cross-fostered to make small litters with average birthweight littermates was greater than that of the small birthweight piglets that were cross-fostered to make either large or small litters of large birthweight pigs. The preweaning mortality of small birthweight pigs fostered into large litters of large birthweight pigs was significantly greater than that of the other three treatment groups (Deen et al., 2004). The

effect of repeated fostering events was evaluated in a study in which a subpopulation of piglets were moved to adoptive sows every third day. Adopted piglets were 13% lighter at weaning than non-adopted (control) piglets. Sows nursing adoptive piglets spent between 15% and 30% less time in lateral recumbancy than control sows (Robert and Martineau, 2001). In a study comparing colostrum intake, mortality and growth performance, piglets fostered to primiparous sows consumed less colostrum and were lighter at 20, 28 and 42 days of age than pigs fostered to multi-parity sows. Piglets that died by 42 days was associated with low birth weight and colostrum intake as well as IgG score at 24 hours compared to surviving piglets (Ferrari et al., 2014). While cross-fostering is a predominant and common procedure for managing large litters with the ultimate goal of producing robust piglets (Baxter et al., 2013), research into new models and strategies during the nursing phase to enhance the growth performance post-weaning pig is needed (Wensley et al., 2021).

Cross-suckling, also referred to as allo-suckling, occurs in many mammalian species that live in groups (Riedman, 1982) and is defined in swine as the presence of alien piglets at the udder during milk ejection (Maletínská and Spinka, 2001), which occurs in open farrowing production models including MSCC. The degree to which piglet cross-suckling occurs and the effect of cross-suckling on growth performance and agonistic behavior was investigated (Olsen et al., 1998; Maletínská and Spinka, 2001; van Nieuwamerongen, 2014). Multiple variations of open farrowing exist with loose housed sows and piglets (van Nieuwamerongen, 2014) allowed to cross-suckle in early lactation (Mesarec et al., 2020) and at a point in mid to late lactation (Olsen et al., 1998; Maletínská and Spinka, 2001; Kanaan et al., 2008; Camerlink et al., 2018). The objectives of utilizing cross-suckling through open farrowing production include enhanced sow welfare (van Nieuwamerongen, 2014), piglet socialization before weaning (van Nieuwamerongen, 2014,

Mesarec et al., 2020), enhanced piglet growth performance (Olsen et al., 1998; van Nieuwamerongen, 2014; Mesarec et al., 2020) improved piglet survivability (van Nieuwamerongen, 2014) and simplified litter management at birth. Approximately one third of piglets cross-suckle (Mesarec et al., 2020) from sows other than the birth dam, consistent with earlier studies (van Nieuwamerongen, 2014). Ear and skin lesions were more prevalent in piglets from cross-suckled litters (Olsen et al., 1998) than in those nursed individually, and increased sow aggression toward cross-suckling and cross-fostered piglets was observed. No difference in piglet growth performance was noted between the cross-suckled and individually reared piglets (Olsen et al., 1998; D'Earth, 2005; Salazar et al., 2018), while others observed improved growth in the nursery phase (Hessel et al., 2005; Mesarec et al., 2020). Although these studies have contributed to an understanding of piglet performance and well-being under alternative production models, additional work is needed. The effect of MSCC on pre-weaning mortality and growth performance has not be thoroughly investigated.

1.5 Bacterial transmission and prevalence prior to weaning: *Streptococcus suis* and *Mycoplasma hyorhinis.*

The progression of the U.S. pig production industry from single-site to the multi-site production model occurred rapidly, beginning in the late 1980s (Fangman and Tubbs, 1997; Harris, 2000). Multi-site production was utilized in combination with segregated early weaning and was adopted as a means for improved health and growth performance with reduced pathogen prevalence at the time of weaning (Harris, 2000).

Medicated and segregated early weaning as a means of controlling or eliminating pathogens that had been endemic in commercial pig populations was described in the 1980s and early 1990s. One of the first field trials was conducted to evaluate the efficacy of the elimination of pathogens

including *M. hyopneumoniae* and *Bordetella bronchiseptica,* as well as colonic treponomas (Alexander et al., 1980). *Mycoplasama hyopneumoniae* and *B. bronchiseptica* were not detected in 10 of 11 herds in which boars and gilts weaned under the medicated early wean protocol and were weaned into herds classified as *M. hyopneumoniae* and *B. bronchiseptica* negative. Another study evaluated the inclusion of tiamulin in late gestation and early lactation diets, as well as daily administration of tiamulin to piglets. Candidate piglets were removed from the source population at day six of age and moved to an isolated farm. Repeated serological, clinical and pathological tests were conducted. No evidence of *M. hyopneumoniae*, *Treponema hyodysenteriae*, Aujeszky's disease virus and *Leptospira* species were detected during the three-year study, including second and third generations from the source farm (Mészáros et al., 1985). The notion that segregated early weaning would result in a pig population free of various pathogens including PRRSV, *S. suis***,** *Pasteurella multocida*, *M. hyopneumoniae* and Aujesky's disease lead to recommendations for a specific maximum weaning age for each pathogen (Harris, 2000). Efforts were made to establish PRRSV negative pig populations weaned from PRRSV positive source farms (Torremorell et al., 2002). The increase in litter size also affected the ecology of infectious diseases of the nursing pig (Declerck et al., 2016a). Strategies to change the viral or bacterial pathogen transmission and thereby reduce the related diseases include limited cross-fostering (McCaw, 2000), limited nurse sow utilization (Garrido Mantilla et al., 2021) and pathogen exposure (Torremorrell et al., 1999).

Multiple viral and bacterial agents affect the welfare of both breeding animals and growing pigs as well as the efficiency of pork production. Two agents in particular, PRRSV and *S. suis*, are of significant consequence to the nursing piglet and have generated substantial research into control and elimination (Amass et al., 1996; Amass et al., 2000; Corzo et al., 2010; Segura et al., 2020;

Magalhães et al., 2021). A third agent, *M. hyorhinis*, is a commensal of swine and pathogenic in nursing and post-weaning piglets (Clavijo-Michelangeli, 2014; Roos et al., 2019). Although recent research into the prevalence of *M. hyorhinis* has been conducted (Clavijo, 2014, Roos et al., 2019), understanding of the epidemiology and ecology of *M. hyorhinis* is limited.

1.5.1. *Streptococcus suis*

Streptococcus suis is a bacterium of significant consequence as the causative agent of *S. suis* diseases and the resulting animal welfare concerns and economic losses for pig producers (Gottschalk and Segura, 2019; Segura, 2020). The ubiquitous nature of *S. suis*, with nearly 100% prevalence of carrier pigs in herds around the world, make it one of the most important bacterial pathogens of swine (Goyette-Desjardins et al., 2014). Additionally, *S. suis* has been identified as a zoonotic agent and a threat to human health as recent outbreaks of *S.suis* infection in humans in multiple regions globally (Perch et al., 1968; Arends et al., 1988; Staats et al., 1997; Gottschalk, 2004; Gottschalk et al., 2010; Goyette-Desjardins, 2014; Segura, 2020). The frequency of prophylactic and therapeutic antibiotic use in the effort to reduce losses from *S. suis* infection in pigs is of concern as information suggests the role of *S. suis* in antibiotic resistance (Palmieri et al., 2011). *Streptococcus. suis* is a facultatively anaerobic, Gram-positive bacterium with a polysaccharide capsule (Elliott, 1966; Kilpper-Bälz and Schleifer, 1987), with strains that are serologically classified by antigenicity of the capsular polysaccharide (Elliott, 1966; Windsor and Elliott, 1975; Perch et al., 1983; Gottschalk et al., 1989; Gottschalk et al, 1991; Higgins et al., 1995), with 35 serotypes identified to date. *Streptococcus suis*, a normal inhabitant of the tonsil, nasal passages and upper respiratory tract of the pig, (Dutkiewicz et al., 2017) has been identified in other mammalian species including cattle (Komatsu et al., 2018), dogs (Muckle et al., 2010),

cats (Wood et al., 2021), humans (Gottschalk et al., 2007; Hughes et al., 2009; Jiang et al., 2020), as well as non-mammalian species (Devriese et al., 1994). Pathologically, the disease caused by *S. suis* includes meningitis, arthritis, endocarditis, peritonitis, myocarditis and polyserositis (Goyette-Desjardins, 2014), affecting pigs from two to 24 weeks of age (Dutkiewicz et al., 2017; Segura, M., 2020). The first recorded serious outbreak of meningitis attributed to *S. suis* in 1973 was in the UK (Alexander, 1995a). It was observed that *S.suis* most probably inhabited pigs long before 1973, but that change in weaning age, population size and stocking density, created an environment for the selection and transmission of virulent strains of streptococci (Alexander, 1995b). Of the 35 *S. suis* serotypes, types 1 to 8 are most commonly identified (Higgins and Gottachalk, 2005), with type 2 frequently isolated from diseased animals. Geographic and temporal difference have been noted, with much greater prevalence of serotype 2 in France than in Canada, as well as a shift to lesser prevalence over time in Canada (Gottschalk and Segura, 2000). Serotype 14 has been identified in clinical outbreaks in the UK (Heath et al., 1996), as well as serotype 5 in Canada (Cloutier et al., 2003). Of submissions to a diagnostic laboratory, serotype 2 was the most commonly detected strain from respiratory, neurologic and septicemic cases (Galina et al., 1992) with serotypes 3, 4 and 7 also found in common among the categories of clinical disease. Mortality and morbidity from *S. suis* diseases vary greatly. Co-infections as well as environmental stressors are implicated with severity of clinical expression (Dee et al., 1993; Galina et al., 1994; Halbur et al., 2000; Feng et al., 2001; Pallarés et al., 2003; Segura et al., 2020; Obradovic et al., 2021). Outbreaks can occur in pigs of all ages, but most commonly in the nursery phase, with mortality from four to 14% (Torremorell et al., 1998). Studies attempting to quantify the economic cost of *S. suis* disease in pigs are limited. Utilizing a questionnaire of swine veterinarians, a study found a range from €0.60 to €1.30 per pig across all phases of production on German, Dutch and Spanish

farms (Neila-Ibáñez et al., 2021). Interventions to reduce the economic losses from *S. suis* diseases include prophylactic and therapeutic antibiotic treatment and as well as vaccination of both, sows and piglets, using autogenous vaccines.

Research into the colonization, transmission and pathogenicity of *S. suis* began with the earliest observations of clinical disease from streptococcal infection. *Streptococcus suis* disease was described as outbreaks of meningitis and arthritis typically within an individual litter (Field et al., 1954), and concluded that the sow was the most probable source of infection. A study was conducted to determine the source and route of *S. suis* infection in the pig, in which experimental exposure to *S. suis* occurred by spraying culture of the bacterium obtained from non-clinical adult gilts into the noses and throats of piglets (Elliott et al., 1966). The organism was isolated from the throats of both infected and from healthy littermates and the observations of meningitis and arthritis in the affected pigs followed bacteremia. Additionally, the authors found that serum from previously infected pigs, when injected into susceptible piglets, was protective of subsequent exposure to *S. suis* culture. An *S. suis* outbreak was described (Windsor and Elliott, 1975) in growing pigs. It was hypothesized that continuous introduction of susceptible pigs from the farrowing unit to the continuous flow growing site at a time of diminishing maternal immunity and limited resistance resulted in *S. suis* infection post-weaning. The movement to medicated, modified medicated, and segregated early weaning production models was predicated upon the theory of pathogen reduction or elimination of piglets at weaning with movement to locations separated from the breeding herd. The findings from research conducted in the late 1980s promoted the concept of pathogen reduction or elimination in weaned pig populations with the use of medicated early weaning (Alexander et al., 1980). The implementation of medicated early weaning was successful in the elimination of pathogens including *Brachyspira hyodysenteriae* and *A.*

pleuropneumoniae from populations of weaned pigs raised in multi-site production. The possibility of eliminating pathogens including *S. suis* from weaned pig populations with medicated or segregated early weaning was found to be unattainable. In a literature review of medicated and segregated early weaning the authors concluded that the technologies at times had been undertaken without sufficient understanding of the underlining physiology of the nursing pig (Fangman and Tubbs, 1997). In a review and compendium, describing the history and evolution of multi-site production the author states that emerging infectious diseases including *S. suis* may be exacerbated with younger weaning age (Harris, 2000c). The dynamics of early but low prevalence colonization of *S. suis*, depletion of maternal antibodies in the population of early weaned pigs and the subsequent acute outbreaks of disease from *S. suis* was observed. It has been hypothesized that if an ideal weaning age exists, it would be the age at which a high proportion of the population has been colonized, while still having sufficient maternal antibody protection (Pijoan, 1995). The results of research into the source and timing of *S. suis* infection in the nursing piglet and the utilization of antibiotic therapy in conjunction with early weaning (Clark et al., 1994; Amass et al., 1995) indicated the *S. suis* elimination with medicated early weaning to be unlikely.

The age at which piglets become infected and the source of infection with *S. suis* has been an on-going focus of research. While early work indicated the importance of spread among piglets at the time of weaning (Clifton-Hadley, 1984), the role of the carrier animal, and the sow as a carrier of *S. suis* and the subsequent passage of the organism to the piglet at birth became apparent after additional research (Amass et al., 1996). In one study of 19 sow herds not experiencing clinical *S. suis* disease, 1.5% of nasal swab samples were positive for serotype 2. Overall, 75% pigs harbored one of several *S. suis* serotypes (Gottschalk et al, 1995). Post-mortem tonsil surveillance at a slaughter plant found *S. suis* PCR positive at the greatest frequency (53.7%) compared to other

pathogens including *S. equisimilis* (29.4%), *P. multocida* (27.3%) and *Staphylococcus aureus* (3.5%), (O'Sullivan et al., 2011). In a two-part study of a clinically affected PRRSV-negative, farrow to finish farm, serotype 5 was detected in 43% and 28% of vaginal and nasal swabs, respectively, from sows prior to farrowing. Fifteen percent of piglet nasal swabs were positive at birth and 35% at 5 to 8 weeks of age. Of the 20 piglet mortalities (21%), *S. suis* serotype 5 was isolated in predominant or pure culture in 55%. Twenty four percent of tonsillar samples obtained at the slaughterhouse were positive for *S. suis* serotype 5. One year later, in the absence of clinical signs, described as the spontaneous disappearance of *S. suis* disease, 53% of sows were *S. suis* serotype 5 positive, while no piglets were positive at 4, 6 and 8 weeks of age. Thirty two percent of tonsillar samples obtained at the slaughterhouse were *S. suis* serotype positive (Cloultier et al, 2000). In a study of the prevalence of *S. suis* in healthy populations, nasal swabs were obtained from four-to-eight week old healthy pigs on farms in Quebec. Ninety four percent of 388 piglet samples from 49 different farms were detected positive to multiple serotypes of *S. suis*. Almost one third of the pigs were positive for two different serotypes and one percent were positive with three serotypes (Brisebois et al., 1990).

Control of *S. suis*-associated diseases in efforts to reduce losses have focused on antibiotic therapy, vaccination of the sow or piglets with autogenous vaccine, with limited work on exposure of either live or attenuated *S. suis* organism to the nursing piglet. Prophylactic and therapeutic antibiotic use in both, the nursing and post-weaning phases, is the primary control method of *S. suis*-associated diseases, even as the use of antibiotics in this manner continues to draw increased scrutiny to the practice (Barton, 2000; Lekagul et al., 2019). A study evaluating the efficacy of treatment options of pigs inoculated with PRRSV and live culture *S. suis* serotype 2 found that ceftiofur injection for three days following *S. suis* challenge was the most effective treatment regimen for minimizing *S.*

suis disease complicated by PRRSV infection (Halbur et al., 2000). Prophylactic administration of water-delivered potassium penicillin G to a nursery population of *S. suis* serotype 2 was evaluated (Byra et al., 2011). There was not significant difference between the treatment (potassium penicillin G at placement and 21 days later) and the control group (no water-delivered potassium penicillin G) in the number of piglets treated with injectable antibiotic (ceftiofur). A significant difference in mortality was observed, with 14% and 7.1% in the control and treatment groups, respectively. The risk of mortality was 1.9 greater in the control group compared to the treatment group. The efficacy of prophylactically administered antibiotic in reducing the colonization and transmission of *S. suis* to piglets at birth was evaluated. Treated and untreated pigs did not differ in growth performance to weaning, *S. suis* isolation frequency or level of opsonizing antibodies (Unterweger et al., 2018). Efficacy of an autogenous bacterin vaccine administered to preparturient sows homologous to the challenge strain used found no difference in mortality, lameness, microscopic lesions of septicemia or frequency of *S. suis* isolation serotype 14 from blood or cerebrospinal fluid of piglets from vaccinated or control sows (Amass et al., 2000). Bacterin vaccination of caesarian-delivered, colostrum-deprived pigs with *S. suis* serotype 9 did not reduce transmission or colonization, nor were there indications that protection against clinical disease signs was induced (Dekker et al., 2012). Maternal immunity transfer, as measured by Ig levels, was high at 7 days of age but deteriorated by 18 days of age resulting in lack of protection against *S. suis* infection post-weaning (Corsaut et al., 2021). A comparative field study of the efficacy of a licensed autogenous *S. suis* bacterin was conducted, (Corsaut et al., 2020), in it both preparturient sow and piglet vaccination protocols were evaluated. Neither preparturient sow vaccination with two doses of *S. suis* autogenous bacterin, nor piglet vaccination provided protection from clinical *S suis* disease as measured by piglet mortality and injectable treatment

frequency by treatment (Corsaut et al., 2020). A field trial was conducted and one group of nursery pigs was allocated into one of four groups, using an *S. suis* serotype 2 isolated from affected pigs on the study farm (experimental intraperitoneal autogenous, standard autogenous, and a commercial bacterin) and a control group. At the initiation of the study, nursery phase mortality approached 17%. Porcine reproductive and respiratory syndrome virus serology indicated seropositive pigs 8 months prior to the trial in the population under study. Mortality in the experimental autogenous vaccine treatment groups trended lower than the other groups. Mortality and morbidity in the control group were 5.5% and 13%, respectively compared to 10% mortality in the commercial vaccine treatment group (Torremorell et al., 1997). Live avirulent *S. suis* inoculum to convey protection of piglets against *S. suis* infection with natural exposure was evaluated. A live, avirulent *S. suis* serotype 2 isolate used in a challenge study provided protection from clinical *S. suis* disease to the pigs in both treatment groups compared to the control (Busque et al., 1997). An *S. suis* serotype 2 pathogenic isolate was used to experimentally expose piglets at 5 days of age in a study evaluating this novel control *S. suis* method in the nursery and finishing phases. Morbidity (lameness) was significantly less in the exposed group. However, no difference in morbidity (central nervous signs) or mortality was noted. More individual pig treatments were administered to pigs in the un-exposed group compared to the exposed group. The novel method of early, uniform exposure of *S. suis* to nursing piglets to reduce *S. suis* disease in nursery and growing pigs deserves additional research (Torremorell et al., 1999). Published research on the transmission of *S. suis*, as well as other pathogens of interest, within the MSCC production model highlights the need to generate information that can contribute to a better understanding of *S.suis* epidemiology in piglets and will enable the development of disease control strategies.

1.5.2 *Mycoplasma hyorhinis*

A commensal of swine, *M. hyorhinis* is the causative agent of *M. hyorhinis*-associated disease with multiple clinical signs in pigs including arthritis, polyserositis, otitis, eustachitis and conjunctivitis (Pieters and Maes, 2019). Of the class *Mollicutes*, *M. hyorhinis* is a facultative anaerobe, lacks a cell wall and is among over 100 mycoplasma species capable of infecting multiple species (Razin, 1998, Citte and Blanchard, 2013). Similar to other mycoplasma species that infect swine, including *M. hyopneumoniae* and *M. hyosynoviae*, *M. hyorhinis* colonizes the airways and upper respiratory tract and is commonly isolated from the nasal passages and tonsils of the pig (Friis and Fenstra, 1994, Roos et al, 2019). Early research described *M. hyorhinis* as the causative agent of polyserositis and arthritis in pigs (Roberts et al., 1963), later work elucidated the possible role of *M. hyorhinis*-associated pneumonia in piglets challenged with a cultured strains of *M. hyorhinis* (Goiš et al., 1971; Poland et al., 1971). In a study utilizing lung lesion evaluation at the time of slaughter and a cough score index of fattening pig groups on *M. hyopneumoniae* negative and positive farms in Switzerland and Germany, respectively, no evidence was reported of *M. hyorhinis* as the uncomplicated causative agent of enzootic pneumonia (Luehrs et al, 2017). The polyserositis is manifested as pericarditis, pleuritis and peritonitis, with progression to chronic serositic adhesions (Goiš et al., 1971; Goiš et al, 1977; Ross, 1992; Friis and Feenstra, 1994; Rovira, 2009). Arthritis associated with *M. hyorhinis* infection results in hypertrophy and hyperemia of the synovial membrane (Goiš et al., 1977; Rovira, 2009; Gomes Neto et al., 2012). Susceptibility to *M. hyorhinis*-associated disease is greatest in pigs three to 10 weeks of age (Kobisch and Friis, 1996; Rovira, 2009). The transition from single-site to multi-site pig production complicates interpretation of earlier work on the transmission and epidemiology of *M. hyorhinis* (Clavijo Michelangeli, 2014). Limited understanding of the ecology and epidemiology,

as well as the timing and extent of transmission to piglets, hampers efforts to control disease associated with *M. hyorhinis*. Prevalence of *M. hyorhinis* was 10% and 30-40% in sows and piglets at weaning, respectively (Ross, 1992), while infection was described as occurring shortly after birth (Kobisch and Friis, 1996) with the source being sows or older pigs. In a comparative study investigating *M. hyorhinis* prevalence from nasal swabs in sows and piglets two breeding herds (Clavijo et al., 2019), a small percentage (0.93%, 3.26%) of piglets were PCR positive at weaning on each of the two farms. Ten days after placement in the nursery, 22% and 100% of the pigs were PCR-positive. Later, at 47 and 64 days post-weaning the PCR-positive rate was 94% and 100%, respectively. Nasal swabs collected at the time of farrowing and enrollment found *M. hyorhinis* PCR-positive of sows 4.7% and 1.8 percent. The possible role of *M. hyorhinis* in porcine respiratory disease complex and the implications for control strategies have been investigated (Kobayashi et al., 1996; Lee et al., 2016; Palzer et al., 2015; Pereira et al., 2017). Control strategies to reduce the incidence and clinical disease from *M. hyorhinis* infection are limited. Acclimation of replacement gilts and boars, minimizing temperature fluctuations and reducing infectious agents including swine influenza virus, and PRRSV are management considerations for prevention of *M. hyorhinis*-associated disease (Leuwerke, 2009; Scheiber and Thacker, 2012). The efficacy of an inactivated, commercial *M. hyorhinis* vaccine was investigated in a challenge study using three antigenic doses and a negative control group. A reduction in pericarditis was demonstrated in pigs in all three antigenic levels compared to the control group, as well as a reduction in lameness, in pigs in the medium and high antigenic levels compared to the control group (Martinson et al., 2018).

Cross-fostering methods vary among farms, with early and late movement methods adopted or restricted depending on health status and management philosophy regarding piglet movement

among litters. Research into the dynamics of pathogen transmission among piglets as a result of cross-fostering and nurse sow movement to fostered litters has focused on PRRSV and swine influenza virus (McCaw, 1996; McCaw, 2000; Garrido Mantilla et al., 2020). Little information regarding the dynamics of bacterial agent transmission among cross-fostered or cross-suckled pigs is available. While cross-fostering is a predominant and common procedure for managing large litters with the ultimate goal of producing robust piglets (Baxter et al., 2013), research into new models and strategies during the nursing phase to reduce piglet mortality and enhance piglet growth performance post-weaning (Wensley et al., 2021) is needed.

Although recent research has shown the changes in *M. hyorhinis* prevalence from birth to weaning and into the nursery phase in conventionally raised pigs (Clavijo et al., 2019; Roos et al., 2019), information regarding the epidemiology of *M. hyorhinis* in nursing piglets in production systems utilizing cross-suckling is necessary to design protocols for disease control at the field level.

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CHAPTER 2

GROWTH AND SURVIVABILITY OF PIGS FROM BIRTH TO THE END OF NURSERY PHASE RAISED UNDER TWO PRODUCTION MODELS

2.1 Abstract

This study was conducted to evaluate the effect of two different nursing piglet production models on piglet growth performance, survival and antibiotic treatment frequency from birth to the end of the nursery phase. Multi-suckle common creep (MSCC), in which piglets are able to nurse from multiple sows and nest in a common area, was compared to the conventional production model (CONV), in which piglets nurse from a single sow, determined after cross-fostering. Ninety-six sows and litters were allocated into each of the two production models (study groups). All piglets were individually weighed and identified at birth, prior to comingling with other litters in MSCC or cross-fostering in CONV. Individual piglet antibiotic treatments and mortalities were recorded. At weaning, individual weights were recorded upon transport to the nursery, and pigs were randomly allocated into mixed-sex pens by study group. No difference in preweaning mortality ($p>0.05$) between the piglets in MSCC or CONV was observed. Significantly fewer MSCC piglets were treated individually with antibiotic from birth to weaning compared to CONV pigs ($P < .001$). A significant difference in growth performance from weaning to the end of the nursery phase was observed ($P = 0.01$) with greater average daily weight gain in MSCC pigs. Estimation of the probability of survival from weaning to the end of nursery was significantly different between study groups ($P < .001$) with lower mortality in MSCC group compared to CONV. Results of this study provide evidence of improved growth performance of pigs raised under the MSCC production model compared to CONV. However, the underlying factors contributing to the difference in survivability between pigs raised in MSCC and CONV remain unidentified.

2.2 Introduction

The U.S. swine industry and most of the commercial pig production throughout the world have evolved from a high labor system to that of a capital-intense model with focus on production

efficiencies and consistent throughput. One of the primary means of improving swine productivity has been genetic selection, resulting in enhanced growth performance, feed conversion, meat quality and reproductive efficiency. One of the components of the genetic selection indices utilized for balanced improvement has been litter size. An unintended consequence of large litter size has been the increase in piglet mortality from birth to weaning. Efforts have been made to address the challenges of managing large litters, such as farrowing house management strategies including intervention at birth, cross-fostering of piglets and utilization of nurse sows. Sow and piglet welfare and the effectiveness of cross-fostering as a management strategy are areas of ongoing research, as genetic selection has resulted in larger litters with a greater number of high-risk, small birth weight pigs (Quiniou et al., 2002; Baxter et al., 2013; Andersson et al., 2015; Zeng et al., 2019; Gourley et al., 2020a; Kobek-Kjeldager et al., 2020; Ward et al., 2020; Peltoniemi et al., 2021). In addition, the increased litter size has resulted in more variation in birth weight, with a greater proportion of small birthweight piglets being born (Quiniou et al., 2002). While cross-fostering is a predominant and common procedure for managing large litters with the ultimate goal of producing robust piglets (Baxter et al., 2013) research into new models and strategies to enhance piglet growth performance during the nursing phase is warranted (Wensley et al., 2021).

Consideration for the welfare of pigs, as well as increased ease of management has resulted in a practice known as community rearing, also referred to cross-suckling, open creep area, open farrowing or multi-suckle common creep (MSCC) model (van Nieuwamerongen et al., 2014; Verdon et al., 2020). In this production model, piglets are allowed to nest in a common area and nurse from multiple sows including the birth sow and others. Cross-suckling with open farrowing has been reported to generate enhanced sow welfare (van Nieuwamerongen et al., 2014), piglet socialization before weaning (van Nieuwamerongen et al., 2014, Mesarec et al., 2020), enhanced

piglet growth performance (Olsen et al., 1998; van Nieuwamerongen et al., 2014; Mesarec et al., 2020) and improved piglet survivability (van Nieuwamerongen et al., 2014).

The novel production model MSCC was initiated in 2018 on a breed-to-wean farm in the Midwest U.S. in order to evaluate its feasibility in commercial production and potential for reduced preweaning mortality, while allowing for simplified protocols for piglet caretakers. Pilot studies have indicated reduced preweaning mortality and increased weight at weaning and at the end of the nursery phase (Schwartz, 2019). However, a comparative evaluation of MSCC in the conditions of the U.S. swine production has not been performed. Therefore, this study was conducted to evaluate the effect of MSCC on production parameters for pigs from birth to the end of the nursery phase.

2.3 Material and Methods

2.3.1 Ethics Statement

All animal handling and sampling procedures for this study were approved by and compliant with the University of Minnesota Institutional Animal Care and Use Committee. Client consent was obtained from the farm owner prior to initiating the study.

2.3.2 Farm and breeding herd description

The study was conducted on a 5,000-sow breed-to-wean farm which had implemented the MSCC production model. The herd was porcine reproductive and respiratory syndrome virus (PRRSV) and *Mycoplasma hyopneumoniae* negative. Piglets were born and weaned on a continuous basis, with all-in, all-out (AIAO) by farrowing room. At weaning, pigs were transported to a 4,800 head commercial research wean-to-finish nursery facility.

2.3.3 Experimental design

A group of 192 sows due to farrow within the same week of production were randomly selected for the study (stratified by parity). Sows were housed in one of four farrowing rooms at least two days prior to the expected farrowing date. Sows were randomly allotted to one of the following study groups: CONV which consisted of sows nursing individual litters (after cross-fostering by farm protocol to minimize the variation of both the number of pigs per litter and within-litter piglet size), and MSCC, which consisted of groups of eight sows communally nursing piglets, with a common creep area in front of the farrowing stalls for the eight litters.

The MSCC and CONV study groups were housed within each of four rooms of 48 farrowing stalls. Each farrowing room housed 24 individual litters in CONV and three groups of eight litters in MSCC, as illustrated in Figure 2.1.

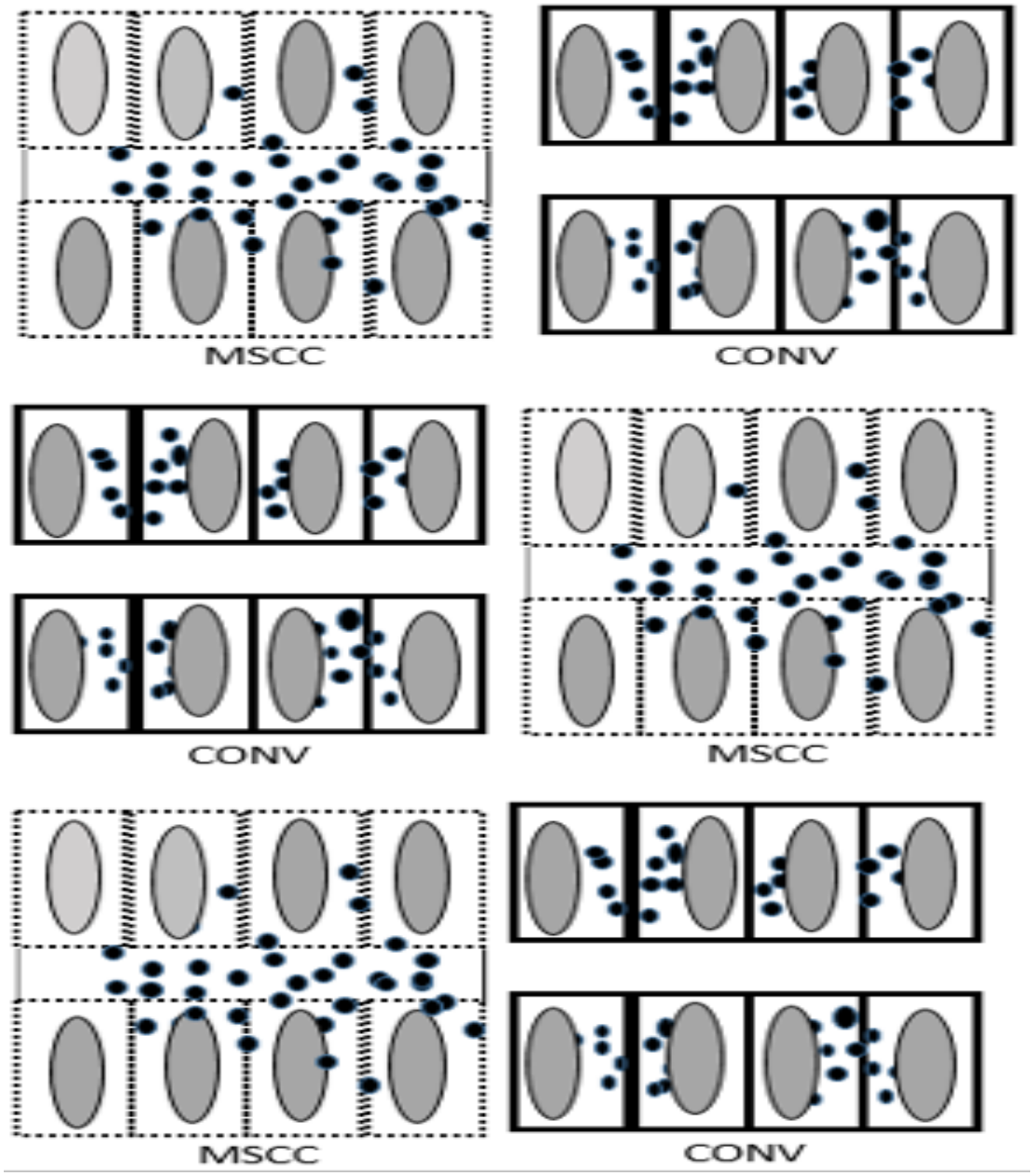

Figure 2.1. Farrowing room diagram showing the location of Multi-suckle Common Creep (MSCC) and Conventional (CONV) groups. Piglets raised in MSCC have additional space in the front alley and are able to nurse from multiple sows. Piglets raised in CONV nurse from only one sow, as determined by birth and cross-fostering. Each farrowing room consisted of 48 farrowing stalls. A total of four farrowing rooms were enrolled in this study. Each oval represents a sow and each dark circle a piglet. Each rectangle of eight sows represents one section of MSCC or CONV. Sows and their corresponding piglets in CONV were separated by solid partitions (dark solid lines). Sows in MSCC were housed in individual stalls (light dashed lines) and piglets were able to move across the eight stalls and the area in front of the stalls of that section.

The study was performed from the selection of sows and allocation into study groups, until the end of the nursery phase of the piglets born to the selected sows. A timeline is shown in Figure 2.2.

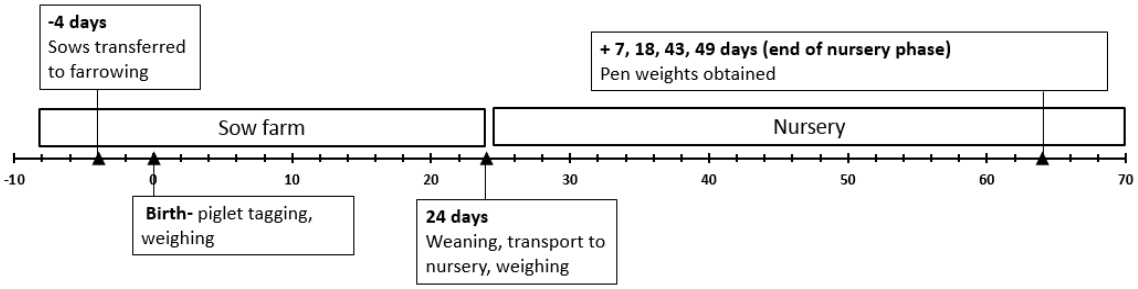

Figure 2.2. Study timeline.

Prior to cross-fostering of CONV litters or opening farrowing pens of MSCC litters, each piglet was weighed and tagged with an ultra-high radio frequency identification tags (LeeO Livestock Precision Farming, Prairie Systems, Spencer, IA, USA). Individual piglet and sow injectable antibiotic treatments and piglet mortalities were recorded daily through to the time of weaning. Sows not having initiated parturition by day 116 of gestation were administered prostaglandin (prostaglandin analog cloprostenol, Estrumate, Merck Animal Health USA, Kenilworth, NJ, USA) to induce farrowing, per farm standard operating procedure. Piglets were provided *ad libitum* a commercial creep feed (Form-A-Feed, Stewart, MN) from approximately day 17 of age to weaning, with individual creep feeders placed in CONV litters (Easton, QC Supply, Columbus, NE, USA) and two feed bowls (Rotecna, Vittetoe, Inc, Keota, IA, USA) provided for each group of eight MSCC litters. At weaning and transfer to the nursery, at approximately 24 days of age, pigs were individually weighed and allocated by treatment into 23 MSCC and 23 CONV pens, balanced by gender into mixed-sex pens of 51 pigs. Pens weights were obtained at seven, 18, 43 and 49 days (end of nursery phase).

2.3.4 Sow and piglet allocation to study group

A total of 192 litters, born to sows which farrowed in four consecutive rooms of 48 farrowing pens each, were enrolled in the study. Based on due-to-farrow date, sows were moved from pen gestation groups to one of the six sections of eight farrowing pens. Each section was allocated randomly by parity, rotationally, while minimizing the range in due date in each MSCC section. First and second parity sows comprised 25% of each study group, with 20.9 and 29.1% parity three sows in MSCC and CONV groups, respectively, and 29.1 and 20.9% of fourth/fifth parity in MSCC and CONV groups, respectively. The number and proportion of sows allocated into each of the two study groups were balanced by parity as shown in Table 2.1.

Table 2.1. Allocation of sows to study groups by parity.

Parity	*MSCC N = 96*	*CONV N =96*
	N (%)	*N(%)*
1	15 (7.8)	18 (9.4)
2	25 (13.0)	27 (14.1)
3	31 (16.1)	27 (14.1)
4/5	25 (13.0)	24 (12.5)

Sows were allocated into study groups MSCC and CONV to balance by parity.

2.3.5. Data collection

Individual birth weight and piglet identification with ear tag was completed within 18 h of birth, prior to cross-fostering of CONV piglets among litters, and opening partitions of MSCC litters. Mortalities and individual piglet antibiotic treatment events were recorded daily. Individual weaning weight was obtained at the time of transport to the nursery. Individual pen weights were obtained at 7, 18, 43 days post-placement and at the end of the nursery phase at 49 days.

2.3.6. Statistical analysis

Significance of the difference among study groups in the proportion of piglet mortality and frequency of individual antibiotic treatment were determined using the two-sample test for proportions with continuity correction. The two-sample t-test was used to determine the significance in the difference in weight gain to weaning. The piglet mortality rate from weaning to the end of the nursery phase was evaluated with Kaplan-Meier survival analysis. All statistical analyses were performed in R Studio (RStudio Team 2021 Boston, MA). The alpha value of .05 was used for statistical significance.

2.4 Results

The mean number of total born, live born, percentage of stillbirth and mummified fetus per litter and the mean piglet birthweight are shown in Table 2.2

Table 2.2. Total born, live born and birth loss (stillbirth and mummified fetuses) by study group.

	MSCC	*CONV*
Total born per litter (n)	15.78	15.63
Born alive per litter (n)	14.54	14.56
Stillbirth per litter (%)	3.48	3.07
Mummified fetus per litter (%)	4.4	3.7
Average piglet birthweight (g)	1360	1420

No significant difference was observed in the proportion of piglet mortality (PWM) from birth to weaning (8.8%, 8.1%) between the MSCC and CONV, respectively. Pigs weaned from the MSCC study group were 6.6 kg compared to 6.8 kg mean weaning weight of pigs in CONV ($P< .001$). The proportion of piglets individually treated with injectable antibiotic from birth to weaning was significantly less in the MSCC study group compared to the CONV group. Sixty MSCC piglets (2.14%) were treated individually with antibiotics, compared to 108 piglets (3.86%) in the CONV group ($Chi^2 = 18.96$, $P < .001$) (Table 2.3).

Table 2.3. Mortality, treatment frequency and growth performance in pigs from birth to end of nursery by study group.

	MSCC	*CONV*	*P-value*
PWM (%)	8.8	8.1	0.53
Wean weight (kg)	6.6	6.8	<0.001
Treatment frequency *(%)*	2.14	3.86	<0.001
Nursery ADG (kg/d)	0.326	0.308	0.01
Nursery survivability (%)	76.1	68.0	<0.001

Pre-weaning mortality (PWM), nursery average daily gain (ADG), Multi-suckle common creep (MSCC) and conventional (CONV).

The growth rate of MSCC pigs was a significantly greater than CONV (P = 0.01) from weaning to the end of the nursery phase. The probability of survival to the end of nursery phase by study group was compared by Kaplan-Meier estimation (Figure 2.3). The probability of survival to the end of nursery phase for the MSCC group was 76.1% compared to 68% in the CONV study group (Chi^2 = 19.6, P < .001).

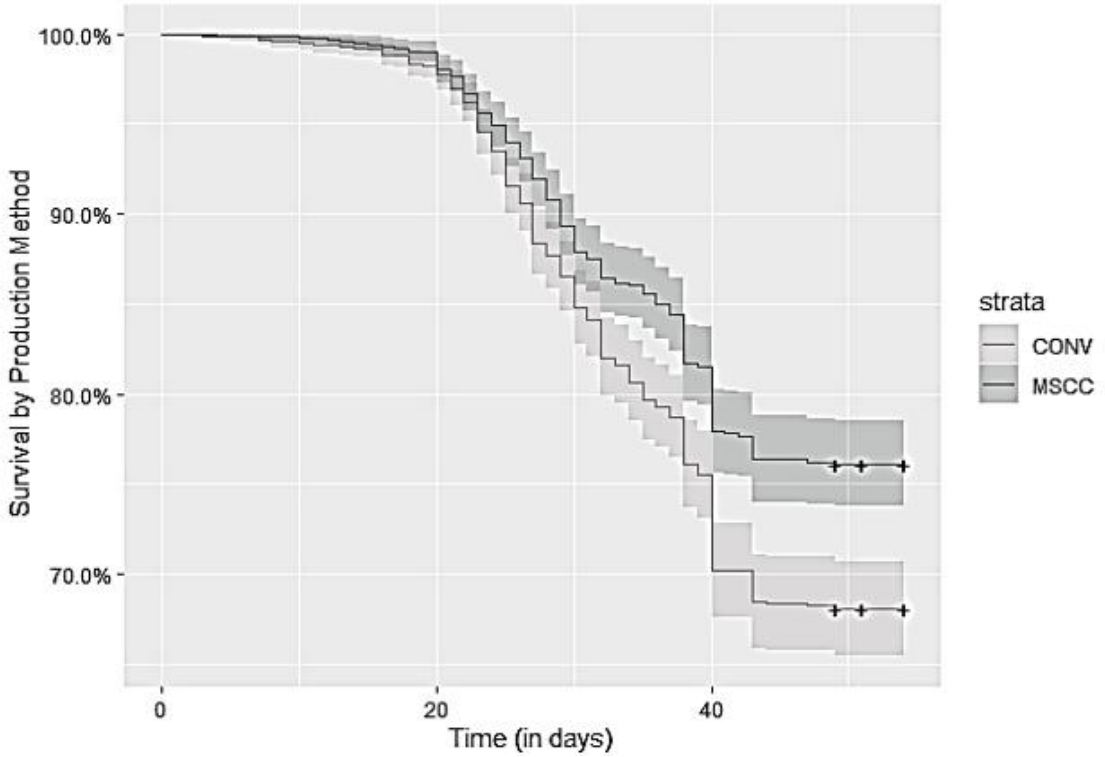

Figure 2.3 Survival curve of pigs from weaning to the end of the nursery phase. The survival rate of pigs

raised with Multi-sucke common creep (MSCC) was 76.11% compared to 68.01% for pigs raised conventionally (CONV) ($Chi^2 = 19.6$, $P < .001$).

2.5 Discussion and conclusions

While larger litters, as an outcome of genetic selection, has resulted in improved productivity and sustainability for pig producers, an unintended consequence has been the subsequent increase in piglet mortality from birth to weaning (Calderón-Diaz et al., 2018). Efforts to effectively manage large litters to maximize pigs weaned include intense interventions at birth as well as cross-fostering (Straw et al., 1998, Calderón-Diaz et al., 2018). This study evaluated the effect of a novel production model, namely MSCC on piglet growth performance and survivability by comparing it to current conventional production.

An outbreak of porcine reproductive and respiratory syndrome virus (PRRSV) was detected by PCR from sera of sows housed in connected buildings adjacent to the farrowing rooms enrolled in the study 14 days before weaning the study pigs to the off-site nursery. Clinical signs of PRRSV infection were observed in the pigs beginning approximately 14 days post-weaning with severe mortality and morbidity.

No difference in preweaning mortality was observed between the MSCC and CONV groups. The mean wean weight of pigs raised in the CONV group was greater than that of MSCC. Outcomes for both preweaning mortality and growth performance to weaning were contrary to findings of pilot studies (Schwartz, 2019). A potential explanation for the lack of difference in this study is the fact that all multi-parity sows enrolled into the study had nursed piglets in the previous lactation(s) under conditions of the MSCC model. Therefore, sows allocated to each study group would have had similar gland utilization and stimulation in the previous lactation period having lactated and nursed piglets under MSCC. Research indicates that mammary glands not suckled

during a lactation period have lower milk yield in the subsequent lactation (Farmer, 2019). With conventional production, gland utilization is a function of the number of piglets born to, and fostered to or from, each individual sow. Piglet mortality and transfers by cross-fostering result in variation among sows in gland utilization and milk production in subsequent lactation periods.

Pilot studies have been conducted to evaluate MSCC compared to CONV production, indicating an improved growth performance in MSCC of pigs raised from birth to weaning as well as from weaning to the end of the nursery phase. Although no difference in growth rate from birth to weaning was observed, the growth rate during the nursery phase was significantly greater in MSCC pigs compared to CONV. The difference in growth rate of MSCC pigs compared to CONV was consistent with pilot studies demonstrating improved growth performance of MSCC pigs. Comingling of litters prior to weaning and the association with a reduction in agonistic behavior and improved growth performance post-weaning has been demonstrated (Hessel et al., 2006).

A difference in the number of piglets individually treated with antibiotic from birth to weaning was observed with significantly fewer piglets in MSCC requiring antibiotic treatment compared to those in CONV.

During the nursery phase, there was no difference in the frequency of individual pig treatment with antibiotic between MSCC and CONV, for both days 1 to 14 post-placement and from placement to the end of the nursery phase.

Mortality during the nursery phase was elevated in both MSCC and CONV. Kaplan-Meier survival analysis indicated significantly lower mortality in the pigs raised from weaning to the end of the nursery phase in MSCC compared to CONV. Extreme mortality events in both MSCC and CONV occurred from approximately day 20 to day 40 post-placement.

Explanatory hypotheses of the observed difference in nursery mortality may be proposed. The hygiene hypothesis (Strachan, 1989) postulated that in humans, certain diseases may be prevented by exposure to infectious agents early in life with household size a factor of exposure. Exposure to pathogens during the nursing phase and subsequent homogenization of colonization may be hypothesized (Oliveira et al., 2004). Additional research into the MSCC production model is necessary. Research into the effect of piglet socialization prior to weaning, although limited, indicates a positive response in nursery phase performance and well-being (Hessel et al., 2006; Parois et al., 2022). Understanding optimal multi-litter group size and weaning age and prevalence of potential pathogens including *M. hyorhinis* and *S. suis*, and the interactions of these factors is lacking.

Limitations of this study include the inability to have raised MSCC pigs within a room, rather than having pigs from both study groups within the same room, which limited the number of litters in each MSCC group compared to group size under commercial production. In addition, pigs raised in the conventional study group nursed from sows that had lactated under MSCC in the previous lactation, altering the gland utilization compared to sows lactating individual litters with conventional production.

As genetic selection continues to provide opportunity for enhanced productivity and sustainability, it also creates additional challenges, requiring the exploration into novel production models to improve the pig welfare and production efficiency. Such proposed models must be applicable to a wide range of commercial production settings and implementable by farm staff at various skill levels. The MSCC production model provides a means of simplifying the processes involved in raising piglets from birth to weaning without compromising the level of care for the sow or the piglets. As pig producers strive to continuously improve productivity, this goal must be balanced

with realistic expectations for the implementation of interventions and practical farrowing house protocols for caregivers.

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CHAPTER 3

EFFECT OF PIGLET PRODUCTION MODEL ON *STREPTOCOCCUS SUIS* COLONIZATION AT WEANING

3.1 Abstract

Piglet movement from the birth to foster sows during the nursing phase has been associated with increased transmission of pathogens like *Streptococcus suis* (*S. suis*), porcine reproductive and respiratory syndrome virus and Influenza A virus. This study was conducted to determine the colonization pattern of *S. suis* in dams and piglets from birth to weaning under two production models, namely Multi-suckle common creep (MSCC) and conventional (CONV) production. Pre-farrow vaginal swabs and oropharyngeal pre-farrowing, mid-lactation and end of lactation swabs were collected from 24 sows in each study group, MSCC and CONV. Tonsillar and nasal swabs were collected at birth, mid-lactation and weaning from 96 piglets born to the sows in the study (two piglets per sow). Multiplex polymerase chain reaction (PCR) targeted *S. suis* serotype 1 and species. A significant increase in the proportion of PCR positive piglets from birth to mid-lactation was observed in nasal and tonsillar samples in both study groups. There were no significant differences in the prevalence of *S.suis* serotype 1 between MSCC and CONV at any collection event or sample type. *Streptococcus suis* serotype 1 detection at birth was significantly greater in nasal samples compared to tonsillar samples ($P < .001$). The detection of *S. suis* serotype 1 was significantly higher in tonsillar samples compared to nasal samples at mid-lactation and weaning. Although piglet movement during the nursing phase has been implicated in increased viral and bacterial transmission among piglets, the data generated in this study indicated no significant difference in the detection of *S. suis* serotype 1 between MSCC and CONV piglets. Differences in the detection of *S. suis* serotype 1 in nasal and tonsillar samples at birth, mid-lactation and weaning merit further investigation.

3.2 Introduction

Streptococcus suis (*S. suis*) is a bacterium of significant consequence as the causative agent of diseases in growing pigs, the resulting animal welfare concerns and economic losses for pig producers, and the potential for zoonotic infections (Goyette-Desjardins et al, 2014). The ubiquitous nature of *S. suis*, with nearly 100% prevalence of carrier pigs in herds around the world make it one of the most important bacterial pathogens of swine (Goyette-Desjardins et al., 2014). The frequency of prophylactic and therapeutic antibiotic use in the effort to reduce losses from *S. suis* infection in pigs is of concern, as information suggests the role of the bacterium in antibiotic resistance (Palmieri et al., 2011). *Streptococcus suis* strains are serologically classified by antigenicity of the capsular polysaccharide (Elliot, 1966; Windsor and Elliot, 1975; Perch et al., 1983; Gottschalk et al., 1989; Gottschalk et al, 1991; Higgins et al., 1995), with 35 serotypes identified to date. *Streptococcus suis* is a normal inhabitant of the tonsil, nasal passages and upper respiratory tract of the pig (Dutkiewicz et al., 2017), has been identified in other species including cattle, dogs, cats, humans, and non-mammalian species (Devriese et al., 1994; Gottschalk et al., 2007; Hughes et al., 2009; Muckle et al., 2010; Komatsu et al., 2018; Jiang et al., 2020; Wood et al., 2021). Pathologically, the disease caused by *S. suis* includes meningitis, arthritis, endocarditis, peritonitis, myocarditis and polyserositis (Goyette-Desjardins, 2014), affecting pigs from two to 24 weeks of age (Dutkiewicz et al., 2017; Segura et al., 2020).

Streptococcus suis disease was described as outbreaks of meningitis and arthritis typically within an individual litter, with the sow as the most probable source of infection (Field et al., 1954**).** Research conducted in the late 1980s promoted the concept of pathogen reduction or elimination in weaned pig populations with the use of medicated early weaning (Alexander et al., 1980), separating piglets from sows at an early age. The implementation of medicated early weaning was

successful in the elimination of pathogens like *Brachyspira hyodysenteriae* and *Actinobacillus pleuropneumoniae* from populations of weaned pigs raised in multi-site production (Alexander et al., 1980). The possibiltiy of eliminating pathogens including *S. suis* from weaned pig populations with medicated (or segregated) early weaning was found to be unattainable (Clark et al., 1994). The age at which piglets become infected and the source of infection with *S. suis* has been an on-going focus of research. While early work indicated the importance of spread among piglets at the time of weaning (Clifton-Hadley, 1984), the role of the pig and sow as a carrier of *S. suis* and the subsequent passage of the organism to the piglet at birth became apparent after additional research (Amass et al., 1996). However, piglet *S. suis* colonization has not been completely elucidated.

Control of *S. suis*-associated diseases in efforts to reduce losses have focused on antibiotic therapy, vaccination of the sow or piglets, with limited work on exposure of either live or attenuated *S. suis* to the nursing piglet. Prophylactic and therapeutic antibiotic use in both, the nursing and post-weaning phases, is the primary control method of *S. suis*-associated diseases (Barton, 2000; Lekagul et al., 2019).

The movement of piglets from the birth to foster sows during the nursing phase has been implicated with increased prevalence of bacterial and viral agents, including *S. suis.* Piglets nursing from multiple sows and nesting in large groups characterize a novel production model, Multi-suckle common creep (MSCC). The objective of this study was to determine to characterize the colonization of both *S. suis* serotype 1 and *S. suis* species at several time points and sample types in dams and their offspring raised in the MSCC and CONV production models from birth to weaning.

3.3 Material and Methods

3.3.1 Ethics statement

All animal handling and sampling procedures were approved by and compliant with the University of Minnesota Institutional Animal Care and Use Committee. Client consent was obtained from the farm owner prior to initiating the study.

3.3.2 Farm and breeding herd description

The study was conducted on a 5,000-sow, wean-to-breed farm in the Midwest of the U.S. The farm was *Mycoplasma hyopneumoniae* (*M. hyopneumoniae*) negative with a history of *S. suis* diagnosis in nursing and post-weaning pigs, with clinical *S. suis*-associated disease during times of acute porcine reproductive and respiratory virus (PRRSV) outbreaks. Piglets were born and weaned on a continuous basis, with four of 16 rooms of 48 farrowing stalls weaned weekly, with all-in all-out (AIAO) by room maintained.

3.3.3. Study design

From 192 sows due to farrow within the same week of production, 48 were randomly selected for the study (stratified by parity). Sows were housed in one of four farrowing rooms, at least two days prior to the expected farrowing date. Sows were randomly allotted to one of the following study groups: MSCC, which consisted of groups of eight sows communally nursing piglets, with a common creep area in front of the farrowing stalls for the eight litters, and CONV, which consisted of sows nursing individual litters (after cross-fostering by farm protocol to minimize the variation of both the number of pigs per litter and within-litter piglet size). The MSCC and CONV study

groups were housed within each of the four rooms in the study. Each farrowing room housed 24 individual litters in CONV and three groups of eight litters MSCC, as illustrated in Figure 3.1.

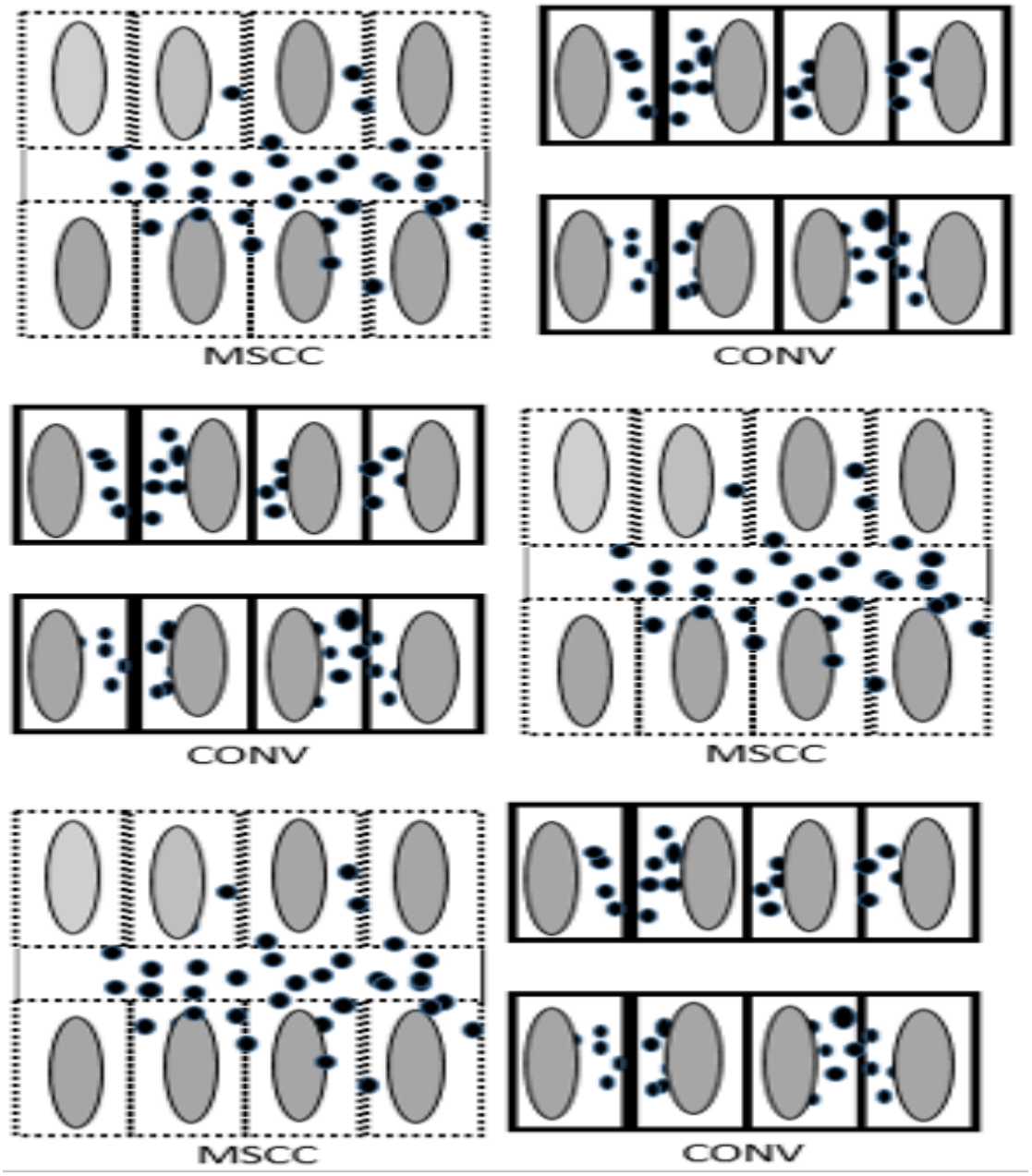

Figure 3.1 Diagram of the Multi-suckle common creep (MSCC) and conventional (CONV) study groups within rooms. Each oval represents a sow, and each dark circle a piglet. Sows were housed individually, represented by the rectangle areas. MSCC piglets were able to move throughout the area shaded in grey. Piglets raised in the MSCC model have additional space and are able to nurse from multiple sows. CONV litters remained within the farrowing stall and nursed from a sow as determined by birth and cross-fostering.

Swab samples were obtained at multiple time points from pre-farrowing to the end of lactation. Pre-farrowing vaginal and sow oropharyngeal swabs at mid-lactation and end of lactation, and piglet tonsillar and nasal swabs were collected at birth, mid-lactation and within 24 hours of weaning, as illustrated in Figure 3.2.

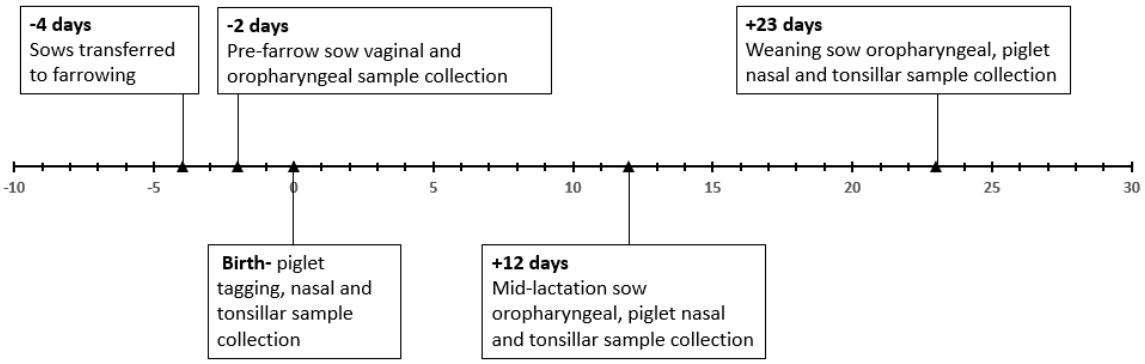

Figure 3.2. Timeline of sample collection.

3.3.4 Sow and piglet allocation to study group

From a total of 192 sows in four consecutive rooms of 48 farrowing stalls, 12 sows from each room were randomly selected for sample collection, balancing by parity, location and by study group (6 MSCC and 6 CONV) for a total of 24 MSCC sows and 24 CONV sows (Table 3.1). Two piglets (48 MSCC and 48 CONV) from each of the selected sows were randomly selected within 18 hours of birth and ear tagged for sample collection.

Table 3.1. Number of sows by parity in MSCC and CONV study groups.

Study group	*Parity 1*	*Parity 2*	*Parity 3*	*Parity 4/5*	*Total*
MSCC	6	6	5	7	24
CONV	6	6	7	5	24

Multi-suckle common creep (MSCC) and conventional (CONV).

3.3.5. Sample collection

Four samples were collected from each of the 48 sows and 6 samples from each of the 96 piglets. Pre-farrow vaginal and oropharyngeal swabs, were collected within 48 hours of farrowing using a sterile swab (BBL™ Culture Swab™, Sparks, MD, USA). Oropharyngeal swabs were collected at mid-lactation (10-12 days post-farrowing) and within 24 hours of weaning. Tonsillar and bilateral nasal swabs (BBL™ Culture Swab™, Sparks, MD, USA) were collected from each randomly selected piglets within 16 hours of birth, at 10 to 12 days of age (mid-lactation), and within 24 hours of weaning. Samples were stored at -20° C until transported to the laboratory for processing.

3.3.6. Sample processing and testing

DNA extraction was performed using MagMAX-96 isolation kits (Life Technologies, Grand Island, NY, USA). PCR testing was completed using EXOone *Streptococcus suis* oneMIX qPCR kit (Exopol, San Mateo de Gallego, Spain) according to manufacturer instructions. The cycle threshold (Ct) value of less than 40 was used for positive *S. suis* serotype 1 and *S. suis* species PCR.

3.3.7. Statistical Analysis

Differences in the proportions of PCR test positive and cycle threshold (Ct) value among the study groups and sampling events was determined with the two-sample test for proportions with continuity correction, pairwise proportion test and two sample t-test. All statistical analysis was performed in R Studio (RStudio Team 2021 Boston, MA). The alpha value of .05 was used for statistical significance.

3.4 Results

Streptococcus suis serotype 1 was detected in 4.2% (1/24) of the pre-farrow vaginal samples in both study groups and ranged from 79.2% to 95.8% of oropharyngeal samples obtained from sows, across sampling events and study group, with no statistical difference among events or groups (Table 3.2).

Streptococcus suis species was detected in 95.8% of CONV vaginal samples and 100% of MSCC vaginal, and all pre-farrow, mid-lactation and weaning oropharyngeal samples from both study groups, with no statistical difference among sampling events or study groups (data not shown).

Table 3.2. Sow *Streptococcus suis* serotype 1 testing by PCR, by sampling event and study group.

	Sample type			
Study group	Vaginal	Pre-OR	Mid-OR	Wean-OR
	# pos/#tested (%)	# pos/#tested (%)	# pos/#tested (%)	# pos/#tested (%)
MSCC	1/24 (4.2%)	20/24 (83.3%)	20/22 (90.9%)	19/24 (79.2%)
CONV	1/24 (4.2%)	19/24 (79.2%)	22/24 (91.7%)	23/24 (95.8%)

Vaginal and Pre- OR: pre-farrow vaginal and oropharyngeal samples, Mid-OR: mid-lactation oropharyngeal sample, Wean-OR: weaning oropharyngeal sample.

Thirty one and 39% of birth nasal samples were *S.suis* PCR positive from the MSCC and CONV groups, respectively. No statistical differences in PCR positive detection for the three sampling events between the study groups were observed. A significant increase in the proportion of *S. suis* serotype 1 PCR positive nasal samples from birth to mid-lactation was observed both study groups. For the MSCC group the proportion of PCR positive increased from 31% at birth to 86% at the mid-lactation collection event (95% CI .368, .740, $Chi^2 = 27.06$). Likewise, the proportion PCR positive in the CONV group increased from 39.6% at birth to 71.1% at mid-lactation (95% CI

.102, .528, Chi^2 = 8.09). There were no statistical differences in the proportion of *S. suis* PCR positive, with a numerical decrease from mid-lactation to weaning in both groups (Table 3.3).

Table 3.3. Piglet *Streptococcus suis* serotype 1by PCR, by sampling event and study group in nasal samples.

	Nasal sample collection event		
Study group	Birth #pos/#tested (%)	Mid-lactation #pos/#tested (%)	Wean #pos/#tested (%)
MSCC	15/48 (31.2%)	39/45 (86.7%)	32/44 (72.7%)
CONV	19/48 (39.6%)	32/45 (71.1%)	30/45 (66.7%)
Total	34/96 (35.4%)	71/90 (78.9%)	62/89 (69.7%)

Tonsillar samples from pigs at birth were PCR positive in 12.8% of MSCC and 10.9% of CONV, with no statistical difference in prevalence of *S. suis* detection between the study groups. A significant increase in the proportion of PCR positive *S. suis* serotype 1 was observed in both study groups with age. The percentage PCR positive increased from 12.8% to 95.5% and 10.9% to 86.1% in the MSCC (95% CI .693, .962, Chi^2 = 60.01) and CONV (95% CI .602, .914, Chi^2 = 49.34) groups, respectively. While a numerical increase in the proportion of PCR positive tonsillar samples was observed from mid-lactation to weaning for both groups, this difference was not statistically significant (Table 3.4).

Table 3.4. Piglet *Streptococcus suis* serotype 1 PCR by study group and sampling event, in tonsillar samples.

	Tonsillar sample collection event		
Study group	*Birth #pos/#tested (%)*	*Mid-lactation #pos/#tested (%)*	*Wean #pos/#tested (%)*
MSCC	6/47 (12.8%)	43/45 (95.5%)	44/44 (100%)
CONV	5/46 (10.9%)	39/45 (86.7%)	45/45 (100%)
Total	11/93 (11.8%)	82/90 (91.1%)	89/89 (100%)

Significant differences in *S. suis* serotype 1 detection were observed between nasal and tonsillar samples at each sampling event (Table 3.5). Thirty five percent (34/96) of the birth nasal samples were positive for *S. suis* serotype 1, compared to 11.8% (11/93) of the birth tonsillar samples (95% CI for difference in proportion .109, .362, Chi^2 = 13.22). Nasal mid-lactation samples were PCR positive at a lesser percentage (78.9%) than the mid-lactation tonsillar samples (91.1%) which was also statistically significant (95% CI for the difference in proportions .008, .236, Chi^2 = 4.35). Likewise, *S. suis* serotype 1 detection was greater in the weaning tonsillar samples (100%) than in the weaning nasal samples (69.2%), which was also statistically significant (95% CI for difference in proportion .196, .410, Chi^2 = 29.0).

Table 3.5. *Streptococcus suis* serotype 1 detection by PCR, by sampling type and event.

Sampling event	*Nasal swab*	*Tonsillar swab*
Birth	35.4%[a]	11.8%[b]
Mid-lactation	78.9%[a]	91.1%[b]
Weaning	69.2%[a]	100%[b]

Streptococcus suis species was detected in 95.7% to 100% of nasal and tonsillar samples at all sampling events, with no statistical differences among sampling events or study groups (data not shown).

The mean Ct value of the sow vaginal and pre-farrow, mid-lactation and weaning oropharyngeal *S. suis* serotype 1 and *S. suis* species samples are shown in Figure 3.3.

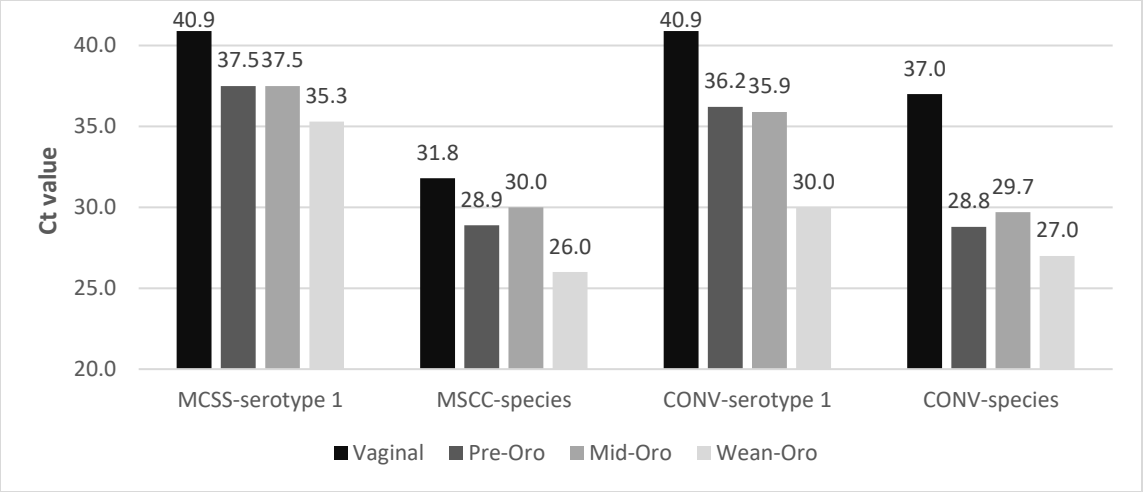

Figure 3.3. Mean cycle threshold (Ct) values of sow *Streptococcus suis* serotype 1 and *Streptococcus suis* species at each sampling event. Oro: Oropharyngeal samples.

There were no significant statistical differences in Ct values among oropharyngeal sampling events of both *S. suis* serotype 1 and *S. suis* species in sows. Analysis of variance (ANOVA) showed no differences in Ct values from pre-farrow to mid-lactation, pre-farrow to weaning and mid-lactation to weaning oropharyngeal samples among parities (data not shown).

Numerical decreases in Ct values were observed from birth to weaning for both *S. suis* serotype 1 and *S. suis* species. No statistical differences were observed between study groups MSCC and CONV, at any sampling event within sample type.

The mean Ct value of *S. suis* serotype 1 and *S. suis* species of piglet nasal and tonsillar samples are illustrated in Figures 3.4 and 3.5.

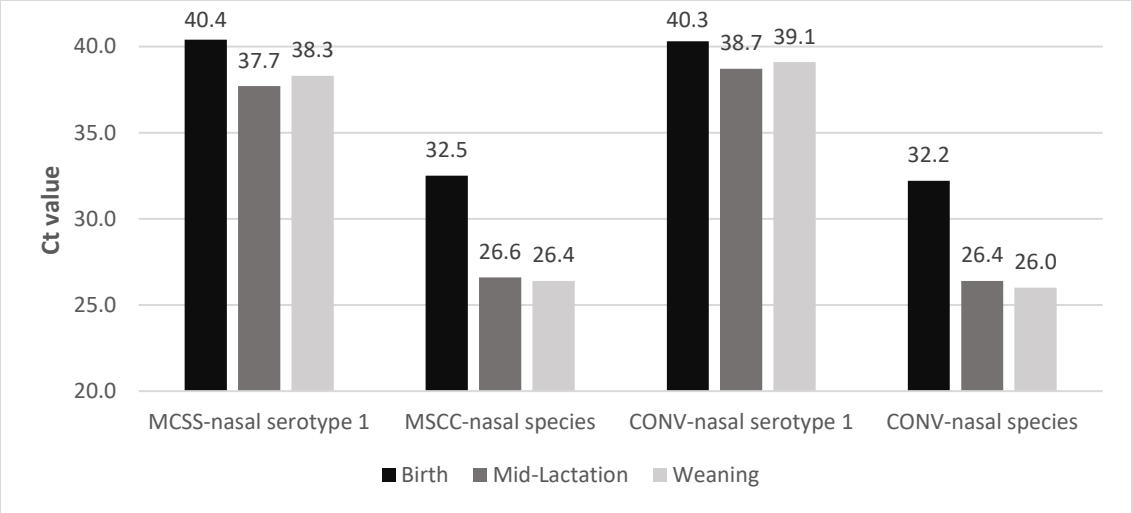

Figure 3.4. Mean cycle threshold (Ct) values of piglet nasal samples at birth, mid-lactation and weaning.

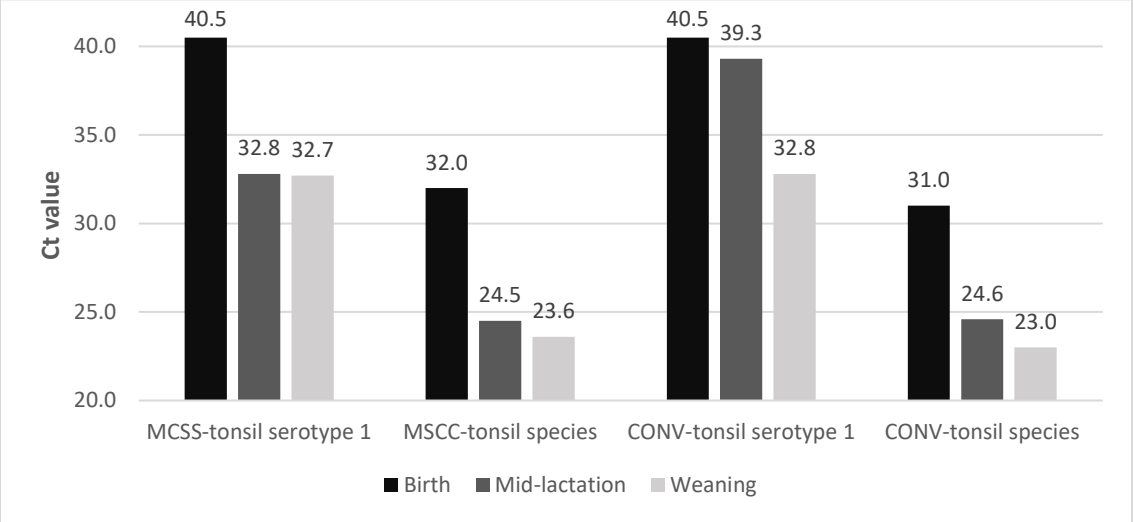

Figure 3.5. Mean cycle threshold (Ct) values of piglet tonsillar samples at birth, mid-lactations and weaning.

Cycle threshold values were significantly different between nasal and tonsillar samples at each sampling event for both *S. suis* serotype 1 and *S. suis* species with the exception of the *S. suis* serotype 1 birth sample. The absolute value of the difference in Ct value was greater for *S. suis* serotype 1 mid-lactation and weaning samples and the least disparate Ct values were from the samples at birth for both the *S. suis* serotype 1 and *S. suis* species (Table 3.6).

Table 3.6. Tonsillar to nasal Ct value difference by sampling event and *Streptococcus suis* serotype 1 vs *Streptococcus suis* species

Age group	***S. suis serotype 1***		***S. suis species***	
	Ct value diff	***95% CI***	***Ct value diff***	***95% CI***
Birth	0.16	(-0.15, 0.49)	-0.87	(-1.67, -0.63)*
Mid	-4.60	(-5.94, -3.91)*	-2.07	(-2.75, -1.40)*
Wean	-5.96	(-6.70, -5.22)*	-2.56	(-3.27, -1.66)*

* denotes a statistical difference (95% C.I.) in the mean difference in Ct value.

3.5 Discussion and conclusions

The objective of this study was to characterize the colonization pattern of *S. suis* serotype 1 and *S. suis* species of nursing piglets and their dams raised under two different production models, namely MSCC and CONV at various time points from pre-farrow and birth to weaning. Under the conventional production model, the management strategy of cross-fostering is utilized to reduce the variation of the number of pigs nursing each sow and in the size of individual pigs within each litter. With MSCC, piglets nurse from multiple sows and nest in a common area with up to 24 litters in a group after having nursed colostrum from the birth dam. Piglet interaction and socialization from multiple litters and the association with differences in prevalence and colonization patterns of organisms including *S. suis* under the MSCC production model have not been described. *Streptococcus suis* transmission from sow to piglet and among piglets, and interventions to reduce the transmission, colonization and *S. suis* related disease has been and continues to be an area of investigation (Field et al., 1954; Windsor and Elliot, 1975; Clifton-Hadley, 1984; Dee et al., 1993; Clark et al., 1994; Galina et al., 1994; Amass et al., 1995; Halbur et al., 2000; Feng et al., 2001; Pallarés et al., 2003; Segura, 2020; Segura et al., 2020; Obradovic et al., 2021). The potential for increased pathogen transmission with movement of sows and

piglets, with both cross-fostering and nurse sow utilization, has been hypothesized (McCaw, 1996; McCaw, 2000; Garrido-Mantilla et al., 2021). Since *S. suis* remains a bacterial agent of economic consequence for the producer (Goyette-Desjardins et al., 2014), alternative methods of prevention and control of *S. suis*-related diseases in nursing and growing pigs are sought, as the prophylactic and therapeutic use of antibiotics to prevent and treat *S. suis*-related and other diseases of the growing pig continues to gain scrutiny (Palmieri et al., 2011; Segura et al, 2020).

The commensal nature of *S. suis*, with near 100% prevalence of both serotype 1 and *S. suis* species in both, sows and piglet populations, with the limited clinical expression in piglets illustrates the difficulty in gaining understanding of the ecology and epidemiology of this microorganism with field studies. This study showed that a large percentage of sows were carriers of *S.suis* species, including serotype 1, prior to farrowing and throughout lactation to the time of weaning. The prevalence of *S. suis* serotype 1 and *S. suis* species in piglets was similar between the MSCC and conventionally raised pigs at each sampling event, with detection approaching 100% in both populations. The results of this study suggest that *S. suis* serotype 1 and *S. suis* species colonization is similar between the MSCC and conventional groups, and therefore not showing evidence to support the hypothesis of increased transmission and prevalence of *S. suis* in pigs raised under the MSCC production model.

This pilot study, although limited by sample size of sows farrowing and piglet born over one week on one breed-to-wean farm, provides prefatory data and insight into colonization of *S. suis* serotype 1 and *S. suis* species in lactating sows and nursing piglets under two different production models. Research has been conducted into the association between piglet movement among litters during the nursing phase and an increase in the transmission of viral and bacterial pathogens among piglets and subsequent diseases (McCaw, 1996; McCaw, 2000; Garrido-Mantilla et al., 2021). The

results of this study provide a basis for interpolation of associations between piglet movement among litters and the transmission and colonization patterns of other pathogens including PRRSV, Influenza A virus and *M. hyorhinis* and the implications for pathogen elimination strategies.

In this study, *S. suis* serotype 1 and *S. suis* species colonization occurred in a large percentage of sows and piglets, with *S. suis*-related clinical disease observed in the population of piglets. It is not understood if this finding would be consistent across breeding herd populations or if prevalence of *S. suis* colonization patterns are variable among herds. Additional research into the colonization patterns of *S. suis* among sows and piglets in multiple breeding herds is important in gaining understanding of the association of *S. suis* prevalence and clinical *S. suis*-related disease across a cross section of breeding herd and piglet populations. Previous research suggests early colonization of *S. suis*, with delayed colonization of pathogenic strains (Torremorell et al., 1998; Segura et al., 2020)

This study was conducted over a short time period, with sample collection completed over one week. Temporal variability correlated with changes in prevalence of other bacterial pathogens, presence or absence of viral agents, vaccination protocol and differences in *S. suis* colonization patterns in sows and piglets, and association with incidence of clinical *S. suis* disease under MSCC and conventional productions models are not clearly understood, with additional work justified.

Multi-suckle common creep is a production model that allows piglets to nurse from multiple sows and nest in large groups, simplifying procedures for the piglet caretakers and potentially decreasing piglet mortality. Due to the novelty of MSCC, research into possible differences in pathogen transmission and colonization patterns of pathogens including *S. suis* is limited. This study provided information on the colonization pattern of *S. suis* in a commercial sow farm. Additional

research into these areas of study are necessary as the MSCC model is implemented in commercial breeding herds.

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CHAPTER 4

EVALUATION OF THE DETECTION OF *MYCOPLASMA HYORHINIS* IN INDIVIDUAL OR COMMINGLED PIG LITTERS

4.1 Abstract

Detection of *Myoplasma hyorhinis* (*M. hyorhinis*) in nursing piglets has been investigated. Differences in colonization and detection of *M. hyorhinis* in nursing piglets, as a function of commingling, is not clearly understood. This study was conducted to determine the detection patterns of *M. hyorhinis* at various time points from birth to the end of lactation in sows and in piglets raised in two different production models, namely Multi-suckle common creep (MSCC) and the conventional production model (CONV). Pre-farrow vaginal and pre-farrow, mid- and end of lactation oropharyngeal swabs were collected from 24 sows in each study group, MSCC and CONV. Tonsillar and nasal swabs were collected at birth, mid-lactation and weaning from 96 piglets born to sows in the study. *Mycoplasma hyorhinis* was detected in 8.2% and 4.1% of pre-farrow oropharyngeal samples in MSCC and CONV sows, respectively. At the end of lactation, *M. hyorhinis* was detected in 4.2% of sow oropharyngeal samples in both study groups. There was not a significant difference in *M. hyorhinis* detection at any sampling event between piglets from MSCC and CONV ($P > 0.05$). A significant difference in cycle threshold (Ct) value was observed in PCR positive tests between MSCC and CONV ($P < .001$). A difference in detection of *M. hyorhinis* was observed among piglet between farrowing rooms in the study. Although piglet movement during the nursing phase has been implicated in increased transmission and colonization of various pathogens among nursing piglets, these data indicated no significant difference in the prevalence of *M. hyorhinis* between MSCC and CONV piglets.

4.2 Introduction

A commensal of swine, *Mycoplasma hyorhinis* (*M. hyorhinis*) is the causative agent of *M. hyorhinis*-related disease with multiple clinical signs in pigs including arthritis, polyserositis, otitis, eustachitis and conjunctivitis (Morita et al., 1995, Resende et al., 2019; Ustulin et al., 2021).

The polyserositis is manifest as pericarditis, pleuritis and peritonitis, with progression to chronic serositic adhesions (Goiš et al., 1971; Goiš et al, 1977; Ross, 1992; Friis and Feenstra, 1994; Rovira, 2009). Of the class *Mollicutes*, *M. hyorhinis* is a facultative anaerobe, lacks a cell wall and is among over 100 mycoplasma species capable of infecting multiple species (Razin, 1998; Citte and Blanchard, 2013). Similar to other mycoplasma species that infect swine, including *M. hyopneumoniae* and *M. hyosynoviae*, *M. hyorhinis* colonizes the airways and upper respiratory tract and is commonly isolated from the nasal passages and tonsils of the pig (Friis and Fenstra, 1994; Roos et al, 2019).

The epidemiology and ecology of *M. hyorhinis* has not been elucidated. Moreover, the transition from single-site to multi-site pig production complicates the interpretation of earlier work on the transmission and epidemiology of *M. hyorhinis* (Clavijo Michelangeli, 2014). Although historically considered as an opportunistic pathogen, recent diagnostics and assay development implicate *M. hyorhinis* as a primary pathogen (Leuwerke, 2009, Clavijo-Michelangeli, 2014). The similar clinical presentation and gross lesions of *Glasserella parasuis* (*G. parasuis*) and *Streptococcus suis* (*S. suis*) to those of *M. hyorhinis*, as well as the frequent concurrent detection of *G. parasuis* and *S. suis* by PCR blurs definitive differential diagnoses.

Transmission of *M. hyorhinis* is likely by aerosolized nasal secretions or direct contact from sow to piglets during the nursing phase (Helke et al., 2015). Differences in frequency of *M. hyorhinis* detection in sows and nursing piglets among farms has been demonstrated (Clavijo et al., 2012, Clavijo et al., 2019, Roos et al., 2019). Recent research has shown differences in *M. hyorhinis* prevalence from birth to weaning and into the nursery phase in conventionally raised pigs (Clavijo et al., 2019; Roos et al., 2019).

A novel production model, Multi-suckle common creep (MSCC), in which piglets are able to nurse from multiple sow and nest in a common area, was implemented on a commercial farm. Evaluation of differences in survivability and growth performance of piglets raised from birth to weaning with MSCC was compared to the conventional production model (CONV) which utilizes cross-fostering to minimize variation among litters of both the number and size of piglets nursing on an individual sow. Implications for transmission and differences in colonization pattern of *M. hyorhinis* of piglets raised under MSCC is lacking.

Therefore, the objective of this study was to investigate the detection of *M. hyorhinis* in piglets raised from birth to weaning with the MSCC and conventional production models, and to determine if a difference in the *M. hyorhinis* colonization pattern between pigs raised under the two production models can be identified.

4.3 Material and Methods

4.3.1 Ethics Statement

All animal handling and sampling procedures were approved by and compliant with the University of Minnesota Institutional Animal Care and Use Committee. Client consent was obtained from the farm owner prior to initiating the study.

4.3.2 Farm and breeding herd description

The study was conducted on a 5,000-sow, wean-to-breed farm in the Midwest of the U.S. The herd was *M. hyopneumoniae* negative with a history of *M. hyorhinis* diagnosis by PCR in post-weaning pigs, with clinical *M. hyorhinis*-associated disease during times of acute porcine reproductive and

respiratory syndrome virus (PRRSV) outbreaks. Piglets were born and weaned on a continuous basis, applying all-in all-out (AIAO) by room.

4.3.3. Study design

A group of 192 sows, due to farrow over one week, were housed in four adjacent farrowing rooms, each with 48 farrowing stalls. From this population, 24 sows were allocated randomly into each of the two study groups (MSCC and CONV), balanced by parity and group within each of the four rooms. In addition, two piglets from each of the 48 sows were randomly selected at birth for sample collection. In each farrowing room, 24 CONV sows nursed individual litters, after cross fostering by farm protocol. MSCC sows nursed in sections of eight litters, with three sections of MSCC sows in each room, as illustrated in Figure 4.1

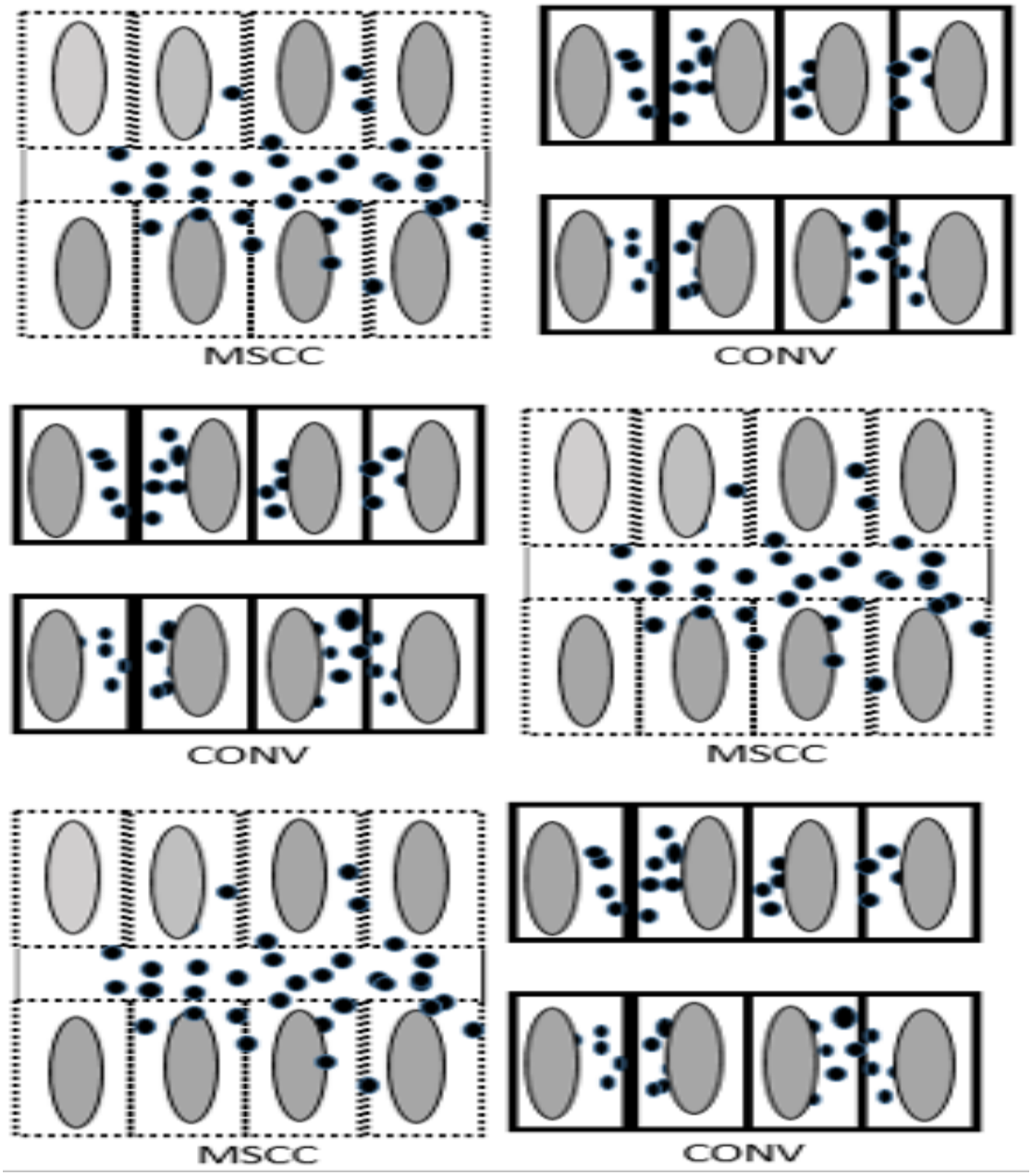

Figure 4.1 Farrowing room layout showing study groups Multi-suckle common creep (MSCC) and conventionally housed (CONV) sows and litters. MSCC sows nursed eight litters in each of the three sections in each room. Twenty-four CONV sows individually nursed litters after cross-fostering. Each grey oval represents a sow in a farrowing stall. Solid black lines represent the solid partitions separating piglets in each CONV litter. Light grey dashed lines represent individually housed MSCC sows with piglets able to nurse and nest throughout the area encompassed by the section of eight MSCC stalls.

Swab samples were collected from sows and piglets at multiple time points from pre-farrowing to weaning. Pre-farrowing vaginal swab samples and pre-farrowing, mid-lactation and end-of-lactation oropharyngeal swabs were obtained, as well as piglet nasal and tonsillar swabs at birth, mid-lactation and at weaning, illustrated in Figure 4.2.

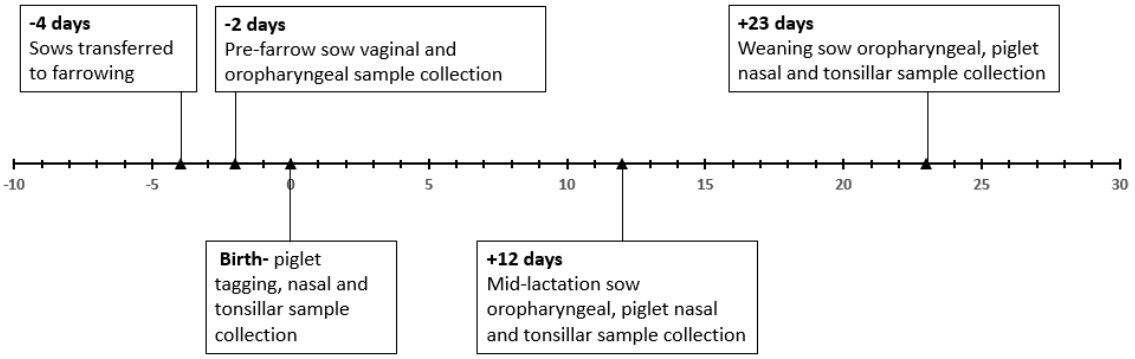

Figure 4.2. Timeline of sample collection.

4.3.4 Sow and piglet allocation to study groups.

From a total of 192 sows in four consecutive rooms of 48 farrowing stalls, 12 sows from each room were randomly selected for sample collection, balancing by parity, location and by study group (6 MSCC and 6 CONV), for a total of 24 MSCC sows and 24 CONV sows. Two piglets (48 MSCC and 48 CONV) from each of the selected sows were randomly selected within 18 hours of birth and ear tagged for sample collection.

To identify possible differences in *M. hyorhinis* detection and timing of colonization, four samples were collected from each of the 48 sows and 6 samples from each of the 96 piglets. Pre-farrow vaginal and oropharyngeal swabs, were collected within 48 hours of farrowing at mid-lactation (10-12 days post-farrowing) and 12 to 24 hours prior to weaning were collected, using sterile swabs (BBL™ Culture Swab™, Sparks, MD, USA),. Tonsillar and bilateral nasal swabs were collected from each randomly selected piglet within 16 hours of birth, at 10 to 12 days of age and

within 24 hours of weaning. Samples were stored at -20 ° C until transport to the laboratory for processing.

4.3.5. Sample collection

Four samples were collected from each of the 48 sows and 6 samples from each of the 96 piglets. Pre-farrow vaginal and oropharyngeal swabs, were collected within 48 hours of farrowing using a sterile swab (BBL™ Culture Swab™, Sparks, MD, USA). Oropharyngeal swabs were collected at mid-lactation (10-12 days post-farrowing) and within 24 hours of weaning. Tonsillar and bilateral nasal swabs (BBL™ Culture Swab™, Sparks, MD, USA) were collected from each randomly selected piglets within 16 hours of birth, at 10 to 12 days of age (mid-lactation), and within 24 hours of weaning. Samples were stored at -20° C until transported to the laboratory for processing.

4.3.6. Sample processing and testing

DNA extraction was performed using MagMAX-96 isolation kits (Life Technologies, Grand Island, NY, USA). Real-time PCR testing was completed using QuantiFast Probe PCR kit (Qiagen Inc, Germantown, MD, USA) by standard operating procedures, utilizing custom primers and probe described by Clavijo et al. (2014a). The cycle threshold (Ct) value <40 was used to determine samples positive for *M. hyorhinis*.

4.3.7. Statistical Analysis

Differences among study groups in the proportions of PCR test positive and cycle threshold values, and odds ratios were determined with Fisher exact and Chi square tests, with Yates continuity correction for small sample size. Mann-Whitney rank sum test was employed to assess the

difference in weaning nasal swab and weaning tonsil swab Ct values between the study groups. Comparison of median Ct values of tonsil and nasal from PCR positive was carried out with the Wilcoxin Rank-Sum test. All statistical analysis was performed in R Studio (RStudio Team 2021 Boston, MA). The alpha value of .05 was used for statistical significance.

4.4 Results

Mycoplasma hyorhinis genetic material was not detected from pre-farrow vaginal swabs and mid-lactation sow oropharyngeal by PCR. Two (8.33%) pre-farrow oropharyngeal swabs from MSCC sows and one (4.16%) from a CONV sow were detected positive for *M. hyorhinis.* One (4.16%) weaning sow oropharyngeal swab from a sow in each study groups was PCR positive. The pre-farrow and weaning oropharyngeal PCR positive Ct values were 31.57, 37.3 and 39.01 and 32.7 and 34.8, respectively.

Mycoplasma hyorhinis genetic material was not detected in the piglet tonsillar swabs collected at the birth. The number and percent of positive PCR tests from each study group and collection event in piglets is shown in Table 4.1.

Table 4.1. *Mycoplasma hyorhinis* PCR positive from piglet nasal and tonsillar swabs at each sampling event.

Study group/N	*Birth*		*Mid-lactation*		*Weaning*	
	tonsillar	*nasal*	*tonsillar*	*nasal*	*tonsillar*	*nasal*
N	48	48	45	46	44	45
MSCC	0	3 (6.25)	5 (11.11)	5 (11.11)	27(61.36)	24(54.55)
CONV	0	1 (2.08)	0 (0.0)	2 (4.35)	24(53.33)	20(44.44)

Multi-suckle common creep (MSCC) and conventional (CONV).

There were no significant differences in the detection of *M. hyorhinis* colonization between the study groups at any sample collection event in piglets. Differences were not observed in number of PCR positive weaning tonsil swab samples (Chi square = 3.32, *P* value = .345), nor in the number of PCR positive weaning nasal swab samples (Chi square = 7.29, *P* value = .063) among the offspring by parity.

There was no significant difference in median Ct values of tonsillar samples between piglets in MSCC and CONV (*P* = .44). However, there was a significant difference between study groups in the median Ct values of nasal weaning samples (*P* = .004). Differences in the detection of *M. hyorhinis* by PCR among the four rooms in the study were observed (Table 4.2). Pairwise comparison using Fisher exact test indicated a greater number of PCR positive tonsil swab samples in the fourth room enrolled compared to the first and second rooms enrolled in the study (*P* = .005, *P* < .001, respectively). There were a significantly greater number of PCR positive nasal swab samples in the first room compared to the second room enrolled in the study (*P* = .015), the first and second rooms compared to the fourth room (*P* < .001) and significantly greater PCR positive nasal samples in the third room compared to the second room (*P* < .001).

Table 4.2. Tonsillar and nasal swab sample positive by PCR by room in piglets.

Sample type	*Room 1*	*Room 2*	*Room 3*	*Room 4*
Tonsillar	11 (45.8)[a]	7 (30.4)[a]	14 (66.6)[ab]	19 (90.5)[b]
Nasal	9 (37.5)[df]	1 (4.3)[e]	14 (66.6)[fd]	20 (95.2)[f]

4.5 Discussion and conclusions

Interaction and socialization of piglets among multiple litters and the association with differences in the colonization pattern of *M. hyorhinis* in the nursing piglet is not clearly understood. The objective of this study was to detect the colonization pattern of *M. hyorhinis* in dams and their piglets raised in MSCC and to compare it to conventional production. Cross-fostering of piglets from the birth sow onto another sow is a common practice under conventional production conditions and is utilized as a management strategy for nursing piglets. Under the MSCC production model, piglets are able to nurse from multiple sows and nest in a common area with up to 24 litters in a group after having nursed colostrum from the birth sow. Movement of piglets among litters during the nursing phase has been associated with increased prevalence of pathogens including Influenza A virus (Garrido-Mantilla et al., 2021) and PRRSV (McCaw, 1996; McCaw, 2000). Multi-suckle common creep is a novel production model on commercial sow farms, as such, research into the effect of piglet movement among litters during the nursing phase on pathogen transmission and colonization patterns is limited.

This pilot study investigated the detection of *M. hyorhinis* in sows and piglets at various time points with several sampling types in piglets raised under MSCC compared to conventional piglet production.

The detection of *M. hyorhinis* in sow samples in this study was low, consistent with result of other studies (Clavijo et al., 2012; Clavijo-Michelangeli, 2014; Clavijo et al., 2019). However, increased detection of *M. hyorhins* in sows was shown in a study, with 40% prevalence at one and three weeks post-farrowing (Roos et al., 2019). Sampling method varied between studies, with the use of nasal swabs (Clavijo et al., 2012; Clavijo-Michelangeli, 2014; Clavijo et al., 2019) and tonsillar swabs (Roos et al., 2019), which could have influenced results in those investigations. In this study,

a statistical difference in *M. hyorhinis* detection in first parity to all other parity sows was observed, similar to Roos et al. (2019), but contrary to another study in which no association of parity and *M. hyorhinis* prevalence was shown (Clavijo et al., 2019).

Low detection frequency of *M. hyorhinis* was observed in piglets at birth and mid-lactation in both sample types. At the third and last sampling event, prior to weaning, detection increased across sample type and study group, with no significant difference in the proportion positive depending on the production model or sample type. The percentage positive *M. hyorhinis* by PCR at birth and weaning was consistent with a recent study (Roos et al., 2019) which showed less than 10% and 50% *M. hyorhinis* positive by PCR at one week of age and weaning, respectively. While Clavijo et al. (2019) showed a similar prevalence of *M. hyorhinis* in both sow samples and piglet birth samples, increased prevalence at weaning in that study was not observed with 2.7% and 3.3% prevalence in two breeding herds. There were differences in litter selection criteria among the studies, with sample collection from intact (non-cross fostered) litters only (Clavijo et al., 2019) and after cross-fostering of pigs among litters (Roos et al., 2019). The proportion of litters with pigs fostered on and the parity from which pigs were fostered from is not apparent in Roos et al., 2019. An association between litter selection criterion and low prevalence of *M. hyorhinis* could be hypothesized.

There was a statistical difference between the median cycle threshold (Ct) value of weaning nasal samples from MSCC and conventionally raised pigs, although no difference in the median Ct value of weaning tonsillar samples was observed. The significance of the observed difference in median Ct value of nasal weaning samples, which can be a relative quantification of bacterial load, is not readily apparent. Although acceptable detection efficiency of *M. hyorhinis* is expected, and most Ct values are repeatable to within 0.5, variation of 1 to 2 Ct values can occur when values approach

mid-30s. Interpretation is limited by sample size and reproducible results over multiple populations (Bouzid et al, 2021). Accurate interpretation of the difference in Ct value between the two production models is limited by the time interval between sampling events, from mid-lactation (12 days) to weaning. Additional sampling events from mid-lactation to weaning may provide additional clarity to the observed difference.

Significant differences in the *M. hyorhinis* detection in piglet weaning samples among the four farrowing rooms in which the study took place were observed. Tonsillar swab sample *M. hyorhinis* detection by PCR ranged from 30% to 90%. It is not clear if piglet swabs were collected in a room or across multiple farrowing rooms in the Clavijo and Roos studies. The significant differences observed among rooms in this study suggests that means of *M. hyorhinis* transmission other than, or in addition to, birth sow to piglet may be considered in farms with continuous farrowing and weaning, potentially including transmission from farm personnel and supplies in contact with pigs at various ages within the farm.

The commensal nature of *M. hyorhinis,* with demonstrated variability of prevalence in piglets of weaning age, and the limited clinical expression of disease, illustrates the difficulty in gaining a better understanding of the ecology and epidemiology in nursing piglet populations (Clavijo-Michelangeli, 2014). In this study, approximately 50% of piglets were *M. hyorhinis* positive by PCR at the time of weaning. A small percentage of the population under study expressed clinical disease, which could be attributed to several pathogenic organisms, including *M. hyorhinis.* Co-infections with *S. suis, G. parasuis*, PRRSV and Influenza A virus further complicate interpretation of *M. hyorhinis* colonization and differences in prevalence in piglet populations (Obradovic et al., 2021). Additional investigations into *M. hyorhinis* colonization patterns in nursing piglets across

multiple sow farms of unique pathogenic bacterial and viral status is necessary to gain understanding of *M. hyorhins* epidemiology in nursing piglets.

This study provided information on the colonization pattern of *M. hyorhinis* of piglets raised under MSCC or conventionally. The results of this pilot study indicate that colonization patterns and detection of *M. hyorhinis* in pigs raised in MSCC at weaning were not significantly different from that of pigs raised conventionally. Although sampling collection was limited to a week of farrowing on a farm, the results of this study provide basis for further investigation into transmission and colonization of *M. hyorhinis* and other swine pathogens including PRRSV, Influenza A virus and *S. suis* of nursing piglets raised under MSCC.

This study provided information on the transmission of *M. hyorhinis* in a commercial farm under MSCC and the conventional production model. While MSCC may provide enhanced piglet welfare and improved productivity, there is limited information on the effect of MSCC on the transmission and colonization pattern of organisms including *M. hyorhinis*. Additional research into the effect of MSCC on *M. hyorhinis* and other swine pathogens of interest is necessary as MSCC is implemented in commercial breeding herds.

4.6 References

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5.1 General discussion and conclusions

The U.S. and world swine production industries have transformed from a seasonal, low-capital, high-labor model to one of continuous output with focus on throughput and production efficiency. In the midst of this major transition, another shift was occurring, from single to multi-site production, which provided the opportunity to eliminate certain pathogens from the growing pig population. Nevertheless, attempts to eliminate a broad range of pathogens, both viral and bacterial, with the use of techniques such as segregated early weaning, for example, has not been successful.

As commercial swine producers continue to strive toward improved productivity and sustainability, genetic selection has been a tool that has proved valuable in improving multiple traits including rate of gain, efficiency of feed conversion and reproductive parameters, including litter size. An unintended consequence of larger litters has been the increase in piglet mortality from birth to weaning. Management strategies to address the increased piglet losses included practices detailing cross-fostering protocol, nurse sow utilization and intensive interventions at the time of birth, with drying and split suckling piglets included in standard operating procedures.

The effects of intensive interventions at birth, including cross-fostering, is a subject of debate and research. Although cross-fostering and piglet sizing have been used to improve piglet survivability, studies to evaluate the efficacy of such practices produce equivocal results. One focus of study of the practice of cross-fostering is the effect of routine movement of piglets and the potential transmission and prevalence of viral and bacterial pathogens amoung piglets, including Influenza A virus (IAV), porcine reproductive and respriratory syndrome virus (PRRSV), *Streptococcus suis* (*S. suis*) and *Mycoplasma* species. Protocols have been implemented in the attempt to reduce the

transmission of infectious agents among litters, with the goal of decreased prevalence and subsequent diseases.

Control and treatment of bacterial pathogens including *S. suis* with prophylactic and therapeutic administration of antibiotics have produced variable results. The prophylactic administration of antibiotics with the goal of prevention of diseases has come under increased scrutiny. Methods of manipulating the immune status of nursing and post-weaning piglets with changes in weaning age and the effect of pathogen exposure homogenization before weaning are necessary areas of research, with the goal of reduced antibiotic usage and improved performance of birth to weaning outcomes of interest.

The growth performance, antibiotic treatment frequency and mortality by each production phase, birth to weaning and weaning to end of nursery, under Multi-suckle common creep (MSCC) and conventional production (CONV) was evaluated. Although pilot studies have indicated a reduction in preweaning mortality and improved growth performance from birth to weaing, the results of this study did not support previous outcomes of preweaning mortality and weaning weight. The improved growth performance from weaning to the end of the nursery phase of pigs in MSCC compared to CONV was consistent with previously conducted pilot studies.

Streptococcus suis species was detected at each sampling event in both sows and piglets with 95% to 100% detection at each sampling event and sample type. Prevalence of *S. suis* serotype 1 varied by sampling event and type, with more than three fourths of sow oropharyngeal *S. suis* serotype 1 detection at each sampling event. Piglet nasal and tonsillar *S. suis* serotype 1 prevalence increased from birth to mid-lactation. There was a significant difference at birth and weaning in the prevalence of *S. suis* serotype 1 between piglet nasal and tonsillar samples.

The interpretation of the prevalence and differences among sampling events and types of *S. suis* serotype 1 is not apparent. Although more than 95% of tonsil, nasal cavity and oropharyngeal fluids are positive *S. suis* species and serotype 1, a small percentage of piglets are identified with *S. suis*-related disease. Understanding the epidemiology and transmission of of *S. suis* among piglets and litters is limited, the commensal nature of *S. suis* creates additional challenges in this area of research. The prevalence of *S. suis* colonization was similar between pigs raised under MSCC and CONV in this study. A clearer understanding of *S. suis* colonization and the effect of MSCC on prevalence is needed.

Myoplasma hyorhinis was not detected in sow pre-farrow vaginal and mid-lactation oropharyngeal samples. The prevalence of *M. hyorhinis* increased from birth and mid-lactation to weaning, with one-half of nasal and tonsillar samples PCR positive at weaning. There was no difference in the prevalence of *M. hyorhinis* between MSCC and CONV. Differences in *M. hyorhinis* prevalence at the room level were observed, from the four farrowing rooms enrolled in the study.

The significance of *M. hyorhinis* colonization is not apparent, as the commensal nature of the organism creates difficulty in gaining understanding of the epidemiology and transmission of *M. hyorhinis* among sows, piglets and litters. Similar to *S. suis*, the implications of piglet movement and any association with differences in prevalence of *M. hyorhinis* between pigs raised under MSCC and CONV are not clearly understood. In this study, there was no difference in the prevalence of *M. hyorhinis* at weaning between pigs raised under MSCC compared to CONV. Additional research is needed to gain understanding in the transmission and colonization of *M. hyorhinis*, co-colonizations and the effect of MSCC on *M. hyorhinis* prevalence at various stages of production.