Antibody responses to porcine reproductive and respiratory syndrome virus, influenza A virus, and Mycoplasma hyopneumoniae and their association with single-nucleotide variants

by
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ABSTRACT

ANTIBODY RESPONSES TO PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS, INFLUENZA A VIRUS, AND MYCOPLASMA HYOPNEUMONIAE AND THEIR ASSOCIATION WITH SINGLE-NUCLEOTIDE VARIANTS

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Swine respiratory diseases are a significant problem for pork producers. This study analyzed antibody responses to porcine reproductive and respiratory syndrome virus (PRRSV), influenza A virus (IAV), and Mycoplasma hyopneumoniae (M. hyopneumoniae), their association with variants in the porcine genome, and the effects of nursery diet complexity on antibody levels. Fourteen groups of pigs (n=618) from 8 farrowing sources were tested 4 times from weaning to the end of the grower-finisher stage. Blood samples were genotyped, and sera were analyzed for antibodies using commercial ELISAs. Data were analyzed using mixed-effects multi-level logistic regression methods. Pigs were more likely to test seropositive at weaning compared to end of nursery for all three pathogens (p<0.05). Variants in/near RAB38 and BRINP3 for PRRSV; PARP14, ADCY6, and TG for IAV; and ENSSSCG00000051043, DNASE1L3, and TEKT2 for M. hyopneumoniae seropositivity were approaching suggestive associations. These findings may help identify options for improved swine breeding programs.
ACKNOWLEDGEMENTS

I would like to firstly and most sincerely thank my advisors, Dr. Brandon Lillie and Dr. Vahab Farzan, for seeing my potential and granting me the opportunity to work with you. To Brandon, I’m thankful for our occasionally on-topic chats, your willingness to solve any problem I throw at you, and your attention to detail. Your ability to turn mountains into molehills is one I’ve always admired and appreciated. To Vahab, thank you for your patience and dedication when teaching and your passion for your field – I like to think it helped me foster passion for it as well. I would also like to thank Dr. Robert Friendship and Dr. Russell Fraser, members of my advisory committee, without whom I would not have been able to finish this project.

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To my family and friends, the belief you had in me and the support you gave was instrumental, and not a day goes by that I’m not thankful for your unending love.
STATEMENT OF WORK PERFORMED

I have performed all of the work described in this thesis, aside from the exceptions listed below:

Dr. Brandon Lillie and Dr. Vahab Farzan designed the study and supervised on-farm sample collection and laboratory sample processing. Dr. Farzan also supervised the data analysis in Chapter 2.

Margaret Ainslie-Garcia and Heather Reinhardt, now-graduated Master’s students on this project from the Departments of Pathobiology and Animal Biosciences, respectively, performed the majority of sample collection from 2015 to 2016.

Pauldeep Virk and Bailey Fuller, a now-graduated Biomedical Sciences student and a veterinary student, respectively, assisted with laboratory analysis of sera for Chapter 2.

Dr. Russell Fraser programmed and performed the majority of the genome-wide association study analysis in Chapter 3.
## TABLE OF CONTENTS

Abstract .......................................................................................................................................................... ii
Acknowledgements .......................................................................................................................................... iii
Statement of work performed ....................................................................................................................... iv
Table of contents .......................................................................................................................................... v
List of tables ................................................................................................................................................ viii
List of figures .............................................................................................................................................. ix
List of abbreviations .................................................................................................................................... x
List of appendices ........................................................................................................................................ xiv
General introduction ................................................................................................................................. 1
Objectives .................................................................................................................................................. 2
Hypotheses .................................................................................................................................................. 2

Chapter 1: Porcine reproductive and respiratory syndrome, swine influenza, and mycoplasmal pneumonia.................................................................................................................................................. 4

1.1 The porcine immune system ................................................................................................................... 5
1.1.1 Innate immune responses .................................................................................................................. 5
1.1.2 Adaptive immune responses ............................................................................................................. 8

1.2 Porcine reproductive and respiratory syndrome ................................................................................. 11
1.2.1 Taxonomy .......................................................................................................................................... 11
1.2.2 Transmission .................................................................................................................................... 11
1.2.3 Pathogenesis .................................................................................................................................... 12
1.2.4 Clinical signs .................................................................................................................................... 13
1.2.5 Porcine reproductive and respiratory syndrome virus and the host ............................................. 14

1.3 Swine influenza ........................................................................................................................................ 15
1.3.1 Taxonomy .......................................................................................................................................... 15
1.3.2 Pathogenesis .................................................................................................................................... 15
Chapter 1: Prevalence and Control of Porcine Respiratory Diseases

1.3.3 Transmission..................................................................................................................16
1.3.4 Clinical signs ..................................................................................................................16
1.3.5 Influenza A virus and the host ......................................................................................16

1.4 Mycoplasma pneumonia .....................................................................................................17
1.4.1 Taxonomy ........................................................................................................................17
1.4.2 Pathogenesis ....................................................................................................................17
1.4.3 Transmission ....................................................................................................................18
1.4.4 Clinical signs ...................................................................................................................18
1.4.5 Mycoplasma hyopneumoniae and the host .....................................................................19

1.5 Control strategies ..............................................................................................................20
1.5.1 Basic control measures ..................................................................................................20
1.5.2 PRRSV............................................................................................................................21
1.5.3 IAV .................................................................................................................................22
1.5.4 M. hyopneumoniae ..........................................................................................................22

1.6 Breeding for genetically resistant pigs ...............................................................................22

1.7 Summary .............................................................................................................................24

Chapter 2: Antibody responses to porcine reproductive and respiratory syndrome virus, influenza A virus, and Mycoplasma hyopneumoniae from weaning to finisher in fourteen groups of pigs in Ontario .................................................................................................................................26

2.1 Abstract ..............................................................................................................................26

2.2 Introduction .........................................................................................................................27

2.3 Materials and methods ......................................................................................................30
2.3.1 Study design .....................................................................................................................30
2.3.2 Sample collection .............................................................................................................30
2.3.3 Antibody detection ..........................................................................................................31
2.3.4 Multivariable analyses ..................................................................................................32

2.4 Results .................................................................................................................................33
2.4.1 Antibody responses .........................................................................................................33
2.4.2 Multivariable analyses ..................................................................................................34
<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.5 Discussion</td>
<td>35</td>
</tr>
<tr>
<td>2.6 Conclusion</td>
<td>39</td>
</tr>
<tr>
<td>2.7 Acknowledgements</td>
<td>39</td>
</tr>
<tr>
<td>2.8 Tables and figures</td>
<td>40</td>
</tr>
<tr>
<td>Chapter 3: Genetic variants associated with antibody responses to porcine reproductive and respiratory syndrome virus, influenza A virus, and <em>Mycoplasma hyopneumoniae</em> in swine</td>
<td>51</td>
</tr>
<tr>
<td>3.1 Abstract</td>
<td>51</td>
</tr>
<tr>
<td>3.2 Introduction</td>
<td>52</td>
</tr>
<tr>
<td>3.3 Materials and methods</td>
<td>55</td>
</tr>
<tr>
<td>3.3.1 Study design</td>
<td>55</td>
</tr>
<tr>
<td>3.3.2 Sample collection</td>
<td>56</td>
</tr>
<tr>
<td>3.3.3 Antibody detection</td>
<td>56</td>
</tr>
<tr>
<td>3.3.4 Phenotype designation</td>
<td>57</td>
</tr>
<tr>
<td>3.3.5 DNA extraction and genotyping</td>
<td>57</td>
</tr>
<tr>
<td>3.3.6 Quality control (QC) and genome-wide association study</td>
<td>58</td>
</tr>
<tr>
<td>3.4 Results</td>
<td>59</td>
</tr>
<tr>
<td>3.4.1 Genome-wide association study</td>
<td>59</td>
</tr>
<tr>
<td>3.5 Discussion</td>
<td>60</td>
</tr>
<tr>
<td>3.6 Conclusion</td>
<td>65</td>
</tr>
<tr>
<td>3.7 Acknowledgments</td>
<td>65</td>
</tr>
<tr>
<td>3.8 Tables and figures</td>
<td>66</td>
</tr>
<tr>
<td>General discussion and conclusions</td>
<td>71</td>
</tr>
<tr>
<td>Summary</td>
<td>75</td>
</tr>
<tr>
<td>References</td>
<td>76</td>
</tr>
<tr>
<td>Appendices</td>
<td>102</td>
</tr>
</tbody>
</table>
LIST OF TABLES

Table 2.1. Seropositivity to PRRSV, IAV, and M. hyopneumoniae in 14 groups of pigs........ 46
Table 2.2. Seropositivity profiles in pigs tested for PRRSV, IAV, and M. hyopneumoniae....... 47
Table 2.3. Mixed-effects multi-level logistic regression analysis for porcine reproductive and respiratory syndrome virus (PRRSV) seropositivity ................................................................. 48
Table 2.4. Mixed-effects multi-level logistic regression analysis for influenza A virus (IAV) seropositivity ................................................................................................................................. 49
Table 2.5. Mixed-effects multi-level logistic regression analysis for Mycoplasma hyopneumoniae seropositivity ................................................................................................................................. 50
Table 3.1. Top 15 SNVs ranked by significance for the GWAS analysis of PRRSV seropositivity from end of nursery to end of finisher ................................................................. 68
Table 3.2. Top 15 SNVs ranked by significance for the GWAS analysis of IAV seropositivity from end of nursery to end of finisher ................................................................. 69
Table 3.3. Top 15 SNVs ranked by significance for the GWAS analysis of M. hyopneumoniae seropositivity from end of nursery to end of finisher ................................................................. 70
LIST OF FIGURES

Figure 2.1. Percentage of pigs testing seropositive for PRRSV at least once from weaning to end of finisher ................................................................. 40
Figure 2.2. Percentage of pigs testing seropositive for IAV at least once from weaning to end of finisher ................................................................................................................................. 41
Figure 2.3. Percentage of pigs testing seropositive for *M. hyopneumoniae* at least once from weaning to end of finisher ................................................................. 42
Figure 2.4. Percentage of pigs testing seropositive for PRRSV, IAV, and *M. hyopneumoniae* at each production stage from weaning to the end of finisher ................................................................. 43
Figure 2.5. Number of times pigs tested seropositive for PRRSV, IAV, and *M. hyopneumoniae* ................................................................................................................................. 44
Figure 2.6. PRRSV, IAV, and *M. hyopneumoniae* seropositivity in pigs fed conventional or low complexity nursery diets ................................................................................................................................. 45
Figure 3.1. Manhattan plots of the GWAS analyses for seropositivity from end of nursery to end of finisher for PRRSV (A), IAV (B), and *M. hyopneumoniae* (C) ................................................................................................................................. 66
Figure 3.2. Quantile-quantile plots for PRRSV (A), IAV (B), and *M. hyopneumoniae* (C) seropositivity from end of nursery to end of finisher ................................................................................................................................. 67
# LIST OF ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADCY6</td>
<td>Adenylate cyclase 6</td>
</tr>
<tr>
<td>AMIR</td>
<td>Antibody-mediated immune response</td>
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<td>APC</td>
<td>Antigen-presenting cell</td>
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<tr>
<td>BCR</td>
<td>B-cell receptor</td>
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<tr>
<td>BRINP3</td>
<td>BMP/retinoic acid-inducible neural-specific protein 3</td>
</tr>
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<td>C</td>
<td>Cytosine</td>
</tr>
<tr>
<td>CMIR</td>
<td>Cell-mediated immune response</td>
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<td>COL8A2</td>
<td>Collagen type VIII, alpha 2</td>
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<tr>
<td>DC</td>
<td>Dendritic cell</td>
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<tr>
<td>DNASE1L3</td>
<td>Deoxyribonuclease 1 like 3</td>
</tr>
<tr>
<td>dpi</td>
<td>Days post-infection</td>
</tr>
<tr>
<td>EAV</td>
<td>Equine arteritis virus</td>
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<td>ELISA</td>
<td>Enzyme-linked immunosorbent assay</td>
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<td>ETEC</td>
<td>Enterotoxigenic <em>Escherichia coli</em></td>
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<td>G</td>
<td>Guanine</td>
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<td>GRM</td>
<td>Genomic relatedness matrix</td>
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<td>GWAS</td>
<td>Genome-wide association study</td>
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<tr>
<td>HA</td>
<td>Hemagglutinin</td>
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<td>HC</td>
<td>High complexity</td>
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<td>HPS</td>
<td>Hermansky-Pudlak syndrome</td>
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<td>IAV</td>
<td>Influenza A virus</td>
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<td>IFN</td>
<td>Interferon</td>
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<td>Description</td>
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<td>Ig</td>
<td>Immunoglobulin</td>
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<td>Immunoglobulin G</td>
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<td>IL</td>
<td>Interleukin</td>
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<td>IR</td>
<td>Immune response</td>
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<td>LDV</td>
<td>Lactase-dehydrogenase-elevating virus</td>
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<td>IncRNA</td>
<td>Long non-coding RNA</td>
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<td>LV</td>
<td>Lelystad virus</td>
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<td>M</td>
<td>Matrix</td>
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<td>M. hyopneumoniae</td>
<td><em>Mycoplasma hyopneumoniae</em></td>
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<td>mAbs</td>
<td>Maternal antibodies</td>
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<tr>
<td>MHC</td>
<td>Major histocompatibility complex</td>
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<td>MLV</td>
<td>Modified live vaccine</td>
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<td>MPP</td>
<td><em>Mycoplasma pneumoniae</em> pneumonia</td>
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<tr>
<td>NA</td>
<td>Neuraminidase</td>
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<tr>
<td>NK</td>
<td>Natural killer</td>
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<tr>
<td>NLR</td>
<td>NOD-like receptor</td>
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<tr>
<td>NP</td>
<td>Nucleoprotein</td>
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<tr>
<td>NRAMP1</td>
<td>Natural resistance-associated macrophage protein 1</td>
</tr>
<tr>
<td>PAM</td>
<td>Porcine alveolar macrophage</td>
</tr>
<tr>
<td>PAMP</td>
<td>Pathogen-associated molecular patterns</td>
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<td>PARP</td>
<td>Poly(ADP-ribose) polymerase</td>
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<tr>
<td>PCR</td>
<td>Polymerase chain reaction</td>
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<td>PEARs</td>
<td>Porcine endemic abortion and respiratory syndrome</td>
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<td>PRR</td>
<td>Pattern recognition receptor</td>
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<td>PRRS</td>
<td>Porcine reproductive and respiratory syndrome</td>
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<td>PRRSV</td>
<td>Porcine reproductive and respiratory syndrome virus</td>
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<td>QC</td>
<td>Quality control</td>
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<td>qq</td>
<td>Quantile-quantile</td>
</tr>
<tr>
<td>RAB38</td>
<td>Ras-related protein Rab-38</td>
</tr>
<tr>
<td>rs</td>
<td>Reference SNV</td>
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<tr>
<td>SC</td>
<td><em>Salmonella enterica</em> serovar Cholerasuis</td>
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<tr>
<td>SHFV</td>
<td>Simian hemorrhagic fever virus</td>
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<tr>
<td>SIRS</td>
<td>Swine infertility and respiratory syndrome</td>
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<tr>
<td>SLA</td>
<td>Swine leukocyte antigen</td>
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<tr>
<td>S/N</td>
<td>Sample-to-negative</td>
</tr>
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<td>SNV</td>
<td>Single-nucleotide variant</td>
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<tr>
<td>S/P</td>
<td>Sample-to-positive</td>
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<tr>
<td>SSC</td>
<td><em>Sus scrofa</em> chromosome</td>
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<td>TCR</td>
<td>T-cell receptor</td>
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<tr>
<td>TEKT2</td>
<td>Tektin 2</td>
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<tr>
<td>TG</td>
<td>Thyroglobulin</td>
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<tr>
<td>Th</td>
<td>T-helper</td>
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<tr>
<td>TLR</td>
<td>Toll-like receptor</td>
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<tr>
<td>TMB</td>
<td>3,3’,5,5´-tetramethylbenzidine</td>
</tr>
<tr>
<td>Acronym</td>
<td>Description</td>
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</tr>
<tr>
<td>TNF</td>
<td>Tumour necrosis factor</td>
</tr>
<tr>
<td>VL</td>
<td>Viral load</td>
</tr>
<tr>
<td>WG</td>
<td>Weight gain</td>
</tr>
<tr>
<td>$\lambda$</td>
<td>Average genomic inflation factor</td>
</tr>
</tbody>
</table>
LIST OF APPENDICES

Appendix I: ELISA principles ........................................................................................................... 102
Appendix II: Combination frequency of pigs that were seropositive for porcine reproductive and respiratory syndrome virus (PRRSV) at different stages of production ........................................... 103
Appendix III: Combination frequency of pigs that were seropositive for influenza A virus (IAV) at different stages of production .......................................................................................... 104
Appendix IV: Combination frequency of pigs that were seropositive for *Mycoplasma hyopneumoniae* at different stages of production .................................................................................... 105
Appendix V: Mixed-effects multi-level logistic regression analysis for porcine reproductive and respiratory syndrome virus (PRRSV) seropositivity ......................................................... 106
Appendix VI: Mixed-effects multi-level logistic regression analysis for influenza A virus (IAV) seropositivity .................................................................................................................... 107
Appendix VII: Mixed-effects multi-level logistic regression analysis for *Mycoplasma hyopneumoniae* seropositivity ........................................................................................................... 108
GENERAL INTRODUCTION

Swine respiratory diseases are among the most important health concerns for pork producers in Canada, and three of the most significant respiratory disease-causing pathogens affecting swine farm productivity are porcine reproductive and respiratory syndrome (PRRS) virus, influenza A virus (IAV), and *Mycoplasma hyopneumoniae* (*M. hyopneumoniae*) (National Animal Health Monitoring System, 2012). Infectious diseases increase production costs and decrease profits due to reduced growth rate or loss of animals, and they also negatively impact animal health and welfare. Additionally, the pressure to reduce or eliminate the use of antimicrobials on farm is ever rising, as the risk of antimicrobial resistance is a growing concern. Pigs are particularly vulnerable to infectious disease after weaning, when maternal antibodies begin to decline and the adaptive immune system is still in the early stages of development (Chase & Lunney, 2012). As such, novel methods aimed at controlling for and preventing the spread of disease are constantly being sought.

Understanding periods of vulnerability, how diseases spread on farm, and which pathogens are of concern is crucial when developing site specific methods for disease control. For example, depending on the location of the farm, the time of year, the proximity to other herds, the age of the pigs, and numerous other factors, it may be necessary for producers to introduce more stringent protocols for disease prevention. Herds in certain areas may also be burdened by one infectious agent over another and thus may be able to prioritize developing methods for preventing and treating their specific pathogens of concern. In addition, some animals are more genetically capable of mounting a robust immune response to pathogenic exposure, and therefore certain pigs will likely be more susceptible to pathogenic threat and severe infection than others (Crawley et al., 2005; Flori et al., 2011; Shinkai et al., 2012). As such, investigating the genome
of many animals with differing immune responses to pathogen exposure will aid in identifying animals more genetically capable of mounting favourable responses to infection, introducing the opportunity to breed more immunologically robust livestock (Davies et al., 2009; Mozzi et al., 2018). This study measured antibody responses to PRRSV, IAV, and *M. hyopneumoniae* from weaning to the end of the finisher stage while assessing the effects of a low complexity nursery diet (lower level of animal protein, higher level of fiber such as corn and soybean) on antibody responses to these pathogens. Additionally, this study utilized a genome-wide association study (GWAS) approach to identify variants associated with susceptibility to PRRSV, IAV, and *M. hyopneumoniae*.

**Objectives**

1. Measure antibody responses to PRRSV, IAV, and *M. hyopneumoniae* from weaning to the end of the finisher stage.

2. Investigate the relationship between nursery diet complexity and antibody responses to PRRSV, IAV, and *M. hyopneumoniae*.

3. Determine the association of single-nucleotide variants in the porcine genome with antibody responses to PRRSV, IAV, and *M. hyopneumoniae* using a genome-wide association study approach.

**Hypotheses**

1. Antibody levels to PRRSV, IAV, and *M. hyopneumoniae* in pigs under commercial settings will change from weaning to the end of the finisher stage.
2. Nursery diet complexity will have no effect on antibody responses to PRRSV, IAV, or *M. hyopneumoniae*.

3. Variants in innate immune genes and/or elsewhere in the genome will affect antibody responses to PRRSV, IAV, and *M. hyopneumoniae*. 
Chapter 1: Porcine reproductive and respiratory syndrome, swine influenza, and mycoplasmal pneumonia

In the late 1980s, disease outbreaks caused by the emergence of a novel porcine viral agent that would later be identified as porcine reproductive and respiratory syndrome virus (PRRSV) was reported in the United States (Albina, 1997). PRRS was previously named mystery swine disease, blue ear disease, porcine endemic abortion and respiratory syndrome (PEARS), and swine infertility and respiratory syndrome (SIRS) (Merck Animal Health, 2016). After the initial outbreak, similar outbreaks were then reported in Germany in November 1990 and the disease spread quickly throughout most of Europe (Albina, 1997). Today, PRRS is found in most pig producing countries in the world and is considered to be one of the most economically significant diseases affecting swine farm productivity. Losses due to PRRS outbreaks in Canada are estimated to be between $116 - $219 million per year (Morin et al., 2014).

Influenza in swine was first described clinically in 1918, simultaneously with the 1918 Spanish influenza pandemic, reporting clinical signs of respiratory illness closely resembling symptoms of human influenza illnesses (Zimmer & Burke, 2009). The human pandemic strain of influenza A and the swine influenza strain were thought to be closely related (Zimmer & Burke, 2009). Today, influenza in swine causes economic losses to the swine industry and also represents a public health threat as swine are thought to play a role as influenza A virus (IAV) “mixing vessels” because pigs can be infected by influenza viruses of swine, avian, and human origin which can reassort in the pig into novel pandemic strains (Ma et al., 2009).

Mycoplasmal pneumonia is a mild respiratory disease caused by M. hyopneumoniae (Kobisch & Friis, 1996). There can be a significant amount of heterogeneity in isolates of M. hyopneumoniae, both within and between herds, making effective control complicated
(Stakenborg et al., 2005). Additionally, *M. hyopneumoniae* infections predispose pigs to becoming infected with other respiratory pathogens, which contributes to a more severe form of respiratory disease known as enzootic pneumonia (Sibila et al., 2009). Enzootic pneumonia is characterized by a high morbidity and low mortality and is considered one of the most prevalent and economically impactful respiratory diseases in the swine industry worldwide (Kobisch & Friis, 1996; Nathues et al., 2014).

Infectious diseases, and respiratory diseases in particular, are a threat to the commercial swine farm industry in Canada and worldwide as they can have adverse effects on overall herd health, decrease producer profits, and result in poorer quality meat products. Monitoring for the presence of disease is a crucial step in identifying vulnerable populations, prevalent pathogens, and methods to improve control of the spread of disease on farm. In addition, investigating differences in susceptibility to PRRSV, IAV, and *M. hyopneumoniae* has the potential to allow genetically robust individuals to be bred in order to produce immunologically superior lines of pigs capable of mounting more effective immune responses to pathogens.

**1.1 The porcine immune system**

The porcine immune system works to reduce illness caused by infectious organisms. Physical barriers such as the skin and mucous membranes are the first line of defense against infection and work to prevent the invasion of pathogens into the body (Chase & Lunney, 2012). The remainder of the innate response, followed by the adaptive immune response, is engaged in the event that pathogens are able to circumvent these physical barriers.

**1.1.1 Innate immune responses**

The porcine innate immune system is present, although not fully developed, at birth and requires no exposure to pathogenic components to function; however, it lacks the ability to
remember previously encountered pathogens (Murphy, 2012). Instead, the innate immune system responds to common patterns associated with infectious agents, called pathogen-associated molecular patterns (PAMPs) (Kumar, Kawai, & Akira, 2011). A function of particular importance in the innate immune system is the phagocytosis of invading infectious agents by phagocytic cells. These include granulocytes or polymorphonuclear leukocytes, which can be further divided into neutrophils, basophils, mast cells, and eosinophils; and mononuclear phagocytes, which include circulating blood monocytes and tissue macrophages (Murphy, 2012). Phagocytic cells engulf, kill, and digest invading organisms, helping to control bacterial, viral, and fungal infections (Murphy, 2012). Additionally, macrophages are important in processing and presenting antigens to stimulate adaptive immune responses. A unique set of innate immune cells are the natural killer (NK) cells, which are able to kill a variety of virus-infected nucleated cells without previous antigenic stimulation (Murphy, 2012). They also help to drive the adaptive immune response and are activated quickly after infection.

In addition to the cellular components of the innate immune system, many non-cellular entities are involved to coordinate a dynamic immune response. Pattern recognition receptors (PRRs) play a crucial role in the recognition of pathogens and the propagation of the innate immune response. They are expressed on and in cells of the innate immune system, such as dendritic cells (DCs), macrophages, and neutrophils, and are able to recognize PAMPs (Mair et al., 2014). Perhaps the most commonly recognized type of PRRs are the toll-like receptors (TLRs), which are membrane-bound receptors that may be expressed at the cell surface or intracellularly on the surfaces of endosomes (Murphy, 2012). The cell surface TLRs detect extracellular pathogenic components, while the intracellular TLRs recognize pathogenic components such as DNA after the pathogen has been internalized and broken down in the cell
At least 11 mammalian TLR genes have been identified, and each is responsible for responding to a particular ligand. For example, certain TLRs will bind to double-stranded RNA, while others bind to flagellin, but physiological responses after binding to their respective ligands are relatively the same (Murphy, 2012). After recognizing a pathogenic component, TLRs are important in promoting inflammatory responses that in turn help activate the innate and adaptive immune responses (Uenishi & Shinkai, 2009). Similarly, NOD-like receptors (NLRs) are PRRs that behave like TLRs but are located in the cytoplasm (Murphy, 2012). Activation of TLRs and/or NLRs promotes changes in gene expression in macrophages and DCs, which in turn has effects both on the innate and adaptive immune responses (Murphy, 2012). As such, PRRs are crucial components of the immune system.

The activation of PRRs stimulates the production of cytokines to further mature the immune response. All cells of the immune system can secrete and be affected by cytokines, which are small proteins or glycoproteins that act as intercellular signalling molecules (Murphy, 2012). These include interleukins (ILs), type I interferons (IFNs), and tumour necrosis factors (TNFs) (Murtaugh, 1994). Interleukins have many different functionalities and may be pro- or anti-inflammatory, or they may promote the induction of other cells of the immune system (Murtaugh, 1994). The pro-inflammatory cytokines, IL-1β, IL-6, and TNF-α, are of particular importance to the innate immune response. They are produced mainly by macrophages in response to bacterial infection, although they may also be produced in response to tissue damage as well as viral, protozoal, and fungal infections, and they are required for activation of the adaptive immune response (Murphy, 2012; Murtaugh, 1994). Type I IFNs, which include both IFN-α and IFN-β, are antiviral cytokines that are produced following exposure to viruses and help healthy cells resist viral infection (Chase & Lunney, 2012). They also increase NK cell
activity and swine leukocyte antigen (SLA) expression at the cell surfaces, thereby increasing the amount of antigen presentation to T cells (Lunney et al., 2009).

1.1.2 Adaptive immune responses

The adaptive immune response is triggered when innate defense mechanisms are overwhelmed. The innate immune response alters the cellular environment in response to pathogenic recognition, producing an inflammatory response through the recruitment of effector cells, and this response, along with increased levels of antigen, activates the adaptive immune response (Murphy, 2012). The adaptive immune system has four key features: specificity, in which mature T- and B-cells respond to only one specific antigen; diversity, in which there are a large number of T- and B-cells that are all specific to different antigens; memory, in which adaptive immune cells can remember and respond more quickly to previously encountered antigens; and self/non self-discrimination, or the ability to distinguish between self and non-self so as not to cause autoimmune diseases (Murphy, 2012).

1.1.2.1 Passive immunity

Newborn pigs have a weak functional adaptive immune system compared to that of older pigs (Chase & Lunney, 2012). Serum antibodies are virtually nonexistent in the neonate due to the epitheliochorial placentation of the sow which prevents the transfer of antibodies across the placenta during gestation (Šterzl et al., 1966). As such, the transfer of maternal antibodies (mAbs) from sow to piglet via colostrum and milk is crucial to protect the young pig from infectious agents during this period of increased vulnerability and disease susceptibility (Chase & Lunney, 2012). The maternal immunoglobulins G and A (IgG and IgA) in colostrum are absorbed by the open gut of the neonate before the gut “closes,” typically 12 hours after birth (Walker, 1979). Gut closure refers to a stage of intestinal maturation and the development of the
mucosal barrier in order to protect against exposure to harmful infectious agents (Sangild, 2003; Walker, 1979). As colostrum transitions to milk, the levels of IgA increase and are no longer absorbed by the piglet but instead help develop the mucus coat barrier and support further development of mucosal immunity (Butler et al., 2006; Johansson et al., 2011). Maternal antibodies help the immunologically naïve piglet remain protected against pathogens but are limited to being specific only for pathogens to which the sow has been previously exposed, either through natural infection or vaccination (Chase & Lunney, 2012). Once passive immunity from the sow begins to decline and the piglets begin to be exposed to infectious agents themselves, the active cell-mediated and humoral arms of the adaptive immune response can begin to develop – however, in the period of passive decline before active increase, the weanling is extremely vulnerable to infection (Butler et al., 2006).

1.1.2.2 Active adaptive immunity

The adaptive immune system consists primarily of T- and B-lymphocytes, or T- and B-cells, and their products, which make up the cell-mediated and humoral arms of the adaptive immune system, respectively (Mair et al., 2014). In general, the cell-mediated immune response (CMIR) destroys infected cells while the humoral, or antibody-mediated immune response (AMIR) produces antibodies (Chase & Lunney, 2012). However, they also increase the efficiency of innate immune responses such as phagocytic and NK cells. Once exposed to an antigen, the adaptive immune system takes about 2-3 weeks before it is considered optimally functional (Chase & Lunney, 2012). After exposure to the same antigen, the response is much quicker and of higher magnitude due to the ability of the immune system to “remember” previously encountered antigens.
The CMIR consists of T-cells, which are unable to respond to free soluble antigen or whole bacteria or viruses in order to effectively react to and kill invading pathogens (Murphy, 2012). As such, they depend on antigen processing and presentation by antigen-presenting cells (APCs) through SLA molecules, the porcine equivalent to the human major histocompatibility complex (MHC) (Chase & Lunney, 2012). As in other mammals, there are a few different types of porcine T-cells. CD4+ T-helper (Th) cells recognize antigens only if they are bound to SLA class II molecules and aid B-cells in antibody production. CD8+ cytotoxic T-cells recognize antigens that are bound to SLA class I and are involved in killing virus-infected cells and cancer cells (Chase & Lunney, 2012).

While all nucleated cells express SLA class I and can therefore present antigen to cytotoxic T-cells, it is the professional APCs such as DCs and macrophages that express SLA class II and present antigen to CD4+ Th cells, which is crucial for overall immune function (Murphy, 2012). In the case of infection, lymphocytes are exposed to antigens on cells bearing either SLA class I or II, and T-cells with corresponding T-cell receptors (TCRs) will bind to them. Clonal expansion then occurs, in which multiple copies, or clones, of that T-cell will be produced in order to amplify the response to that antigen (Murphy, 2012).

The humoral immune response refers to the production of antibodies that help destroy extracellular pathogens and prevent the spread of intracellular pathogens. This begins with B-cell development which involves immunoglobulin (Ig) gene rearrangement and alteration. Naïve B-cells express monomeric IgM as their B-cell receptor (BCR) and are stimulated by cognate antigen and cytokines produced by CD4+ Th cells (Murphy, 2012). This causes mitosis of the B-cell to produce many more B-cells with the same antigenic specificity. Increases in antigen-specific IgM causes Th cells to produce cytokines that in turn signal class switching in B-cells
from IgM to IgG, IgA, IgD, or IgE depending on the antigen itself and the location in the body (Butler et al., 2006). B-cells expressing antibodies that are specific to a single antigen and have a high affinity will be positively selected while others are destroyed (Gotoff, 1974). These are known as memory B-cells and are activated following a second exposure to the same antigen (Butler et al., 2006). Antibodies produced by B-cells cannot directly kill invading pathogens but can neutralize them by binding to specific molecules on the target cell, opsonize them by coating the pathogen to enhance phagocytosis, and activate the complement system, which promotes inflammation and enables antibodies and phagocytic cells to clear damaged or infected cells from the body (Murphy, 2012).

1.2 Porcine reproductive and respiratory syndrome

1.2.1 Taxonomy

The PRRS virus is an enveloped, positive-sense linear RNA genome virus from the order Nidovirales, family Arteriviridae, genus Arterivirus, along with equine arteritis virus (EAV), lactase-dehydrogenase-elevating virus (LDV) in mice, and simian hemorrhagic fever virus (SHFV) (Snijder & Meulenberg, 1998). There are two identified genotypes of the virus believed to have evolved from a common ancestor before diverging: type 1, the European genotype, also called Lelystad virus (LV), and type 2, the North American genotype (VR-2332) (Albina, 1997).

1.2.2 Transmission

Transmission of PRRSV can occur horizontally, i.e., pig-to pig or vertically, i.e. transplacentally to fetuses during mid-to-late gestation (Wills et al., 2002). The virus spreads rapidly within naïve herds and can infect the majority of pigs on a farm within 2-3 months (Kristensen et al., 2004)
The survivability of PRRSV in the environment is influenced by temperature, pH, and exposure to detergents; however, it has been known to survive at extended intervals at lower temperatures (Bloemraad et al., 1994; Music & Gagnon, 2010). The virus can also remain stable at pH values between 6.5 to 7.5; however, infectivity is reduced below pH 6.0 and above pH 7.65 (Bloemraad et al., 1994).

1.2.3 Pathogenesis

The PRRS virion has a restricted tropism for differentiated cells of the monocytic lineage, specifically porcine alveolar macrophages (PAMs) and differentiated blood monocytes (Nauwynck et al., 1999), though DCs have also been shown to support PRRSV replication (Loving et al., 2007). The virus enters host cells via clathrin-mediated endocytosis (Geldhof et al., 2013; Nauwynck et al., 1999). Heparan sulfate and sialoadhesin have been identified on the surface of macrophages as two receptors for PRRSV (Delputte et al., 2002; Vanderheijden et al., 2001). Low-affinity attachment of virions to heparan sulfate on the surface of susceptible cells initiates infection, followed by a higher affinity binding dependent on the macrophage-specific protein sialoadhesin (Van Gorp et al., 2008). This incites internalization via endocytosis. Additionally, two minor structural proteins, GP2a and GP4, and the scavenger receptor CD163 mediate release of the virus into the cytoplasm (Das et al., 2010; Van Gorp et al., 2008). The presence of sialoadhesin on the cell surface does not ensure PRRS infection will occur, but greatly increases the probability of viral attachment. Conversely, CD163 is crucial for uncoating and releasing the viral genome, and thus cells lacking CD163 are able to internalize the virus but unable to uncoat the genome, making them incapable of supporting a productive infection (Van Gorp et al., 2008).
1.2.4 Clinical signs

There are two distinct types of PRRS infections in the host: reproductive infections, which affect the breeding herd, and respiratory infections, which primarily affect the grower-finisher herd (Klinge et al., 2009). Reproductive complications increase the incidence of premature farrowing, abortion, stillborn or mummified piglets, delayed return to service, and the birth of weak, PRRS-positive piglets (Done & Paton, 1995; Young et al., 2010). Additionally, PRRS positive sows may exhibit signs of anorexia, fever, lethargy, pneumonia, red/blue discolouration of the ears and vulva, subcutaneous hind limb edema, and, in rare cases, death (Young et al., 2010). However, PRRS infections are often subclinical and proceed unnoticed until reproductive failure occurs (Done & Paton, 1995). In neonatal piglets, common clinical signs include dyspnea, tachypnea, and death, with mortality rates reaching up to 100% (Allende et al., 2000).

In grower-finisher pigs, PRRS is also often subclinical and causes macrophage destruction, the thickening of alveolar septa, occasional lesions in blood and lymphatic vessels, and minor heart lesions (Done & Paton, 1995; Klinge et al., 2009). Clinical signs, if present, include hyperpnea, dyspnea, coughing, pneumonia, and oculo-nasal discharge (Lunney et al., 2016).

PRRS virus infections can be further subdivided into three distinct stages following uptake into the cell: acute infection, persistence, and extinction.

In acute PRRS, the virus targets the lungs, with viral replication occurring mainly in the macrophages and DCs of the upper respiratory tract (Gomez-Laguna et al., 2013; Huang et al., 2015; Molina et al., 2008). Systemic distribution to mononuclear cells and tissue macrophages then occurs following acute infection (Merck Animal Health, 2016).
The persistence of PRRS virus is well known and infections can last for weeks to months (Albina et al., 1994; Rossow, 1998; Wills et al., 1997). However, it has been suggested that PRRS is not a true steady-state persistent infection, and instead involves continuous viral replication at a low level (Allende et al., 2000). In persistent infections, viral replication subsides to undetectable levels in the blood and lungs and animals stop displaying overt signs of disease.

Viral replication in the host gradually declines until viral extinction occurs, typically around 150 days post-infection (dpi) (Allende et al., 2000).

1.2.5 Porcine reproductive and respiratory syndrome virus and the host

The PRRS virus has the ability to interfere with or alter numerous processes throughout the development of the innate and adaptive immune responses, mainly type I IFN pathways, delaying the immune response and increasing the duration for the virus to be transmitted to other naïve individuals (Dotti et al., 2013; Huang et al., 2015). The PRRS virus has developed several ways to antagonize type I IFN responses to delay PRR detection (Huang et al., 2014), inhibit IFN production (Huang et al., 2015), and interfere with downstream signal transduction of IFN (Patel et al., 2010). The PRRS virus can also impair antigen presentation by DCs by regulating the expression of SLA II, delaying the onset of an efficient adaptive immune response (IR) (Rodríguez-Gómez et al., 2013). In addition, PRRSV has also been shown to induce apoptosis of DCs, further impairing the ability of the host to mount a sufficient adaptive IR (Rodríguez-Gómez et al., 2013), and can also activate immunosuppressive regulatory T-cells (Tregs) in order to weaken immune responses (Silva-Campa et al., 2012).

Protective immunity is induced following infection but is slow to develop. Additionally, vaccination with modified live vaccines (MLVs) appear to exhibit protection via CMIR and not
AMIR, and the use of killed vaccines does not appear to elicit sufficient protection (Zuckermann et al., 2007).

1.3 Swine influenza

1.3.1 Taxonomy

Influenza A viruses are negative-sense RNA viruses belonging to the family Orthomyxoviridae and are capable of infecting many species, although the natural hosts for all influenza A subtypes are wild birds (Spickler, 2016). Influenza viruses contain two surface antigens: the hemagglutinin (HA) and neuraminidase (NA) proteins, which are used to further classify influenza A viruses into subtypes (Shtyrya et al., 2009; Skehel & Wiley, 2000). There are currently 18 known HA subtypes and 11 known NA subtypes, with combinations of the HA and NA proteins defining different viral subtypes (e.g. H1N1). Influenza can infect a variety of species and each subtype is usually restricted to either birds or mammals. However, cross-infection, while rare, can have high consequences, such as avian H5N1 infections in humans (Spickler, 2016). Pigs are mainly infected by H1N1, H1N2, and H3N2, although they are susceptible to infection with both human and avian influenza viruses as well, and are therefore thought to play a role as “mixing vessels” involved in influenza viral reassortment and the production of novel pandemic strains (Castrucci et al., 1993; Ma et al., 2009).

1.3.2 Pathogenesis

Viral replication takes place in the epithelial cells of the respiratory tract, with a preference for the lower respiratory tract (De Vleeschauwer et al., 2009). In swine, it is unlikely for systemic spread to occur beyond the respiratory tract. Hemagglutinin is responsible for viral binding to the host cell and adheres to sialic acid sugars on the surface of respiratory epithelial cells (Skehel & Wiley, 2000). Sialic acid-α-2,3-galactose is the main sialic acid in birds, while in
mammals, sialic acid-α-2,6-galactose is the most prevalent; however, swine possess both receptor types, furthering their role as IAV mixing vessels for the production of novel influenza viruses. (Ma et al., 2009).

The NA viral glycoprotein is required for virus transmission and breaks the bond between sialic acid and adjacent sugar residues, enabling the release of the virus from the host cell (McAuley et al., 2019).

### 1.3.3 Transmission

Direct transmission, usually through nose-to-nose contact, is the main source of IAV transmission (Strelioff et al., 2013). Aerosolized droplets produced by coughing or sneezing can also be a source of transmission and may be capable of infecting nearby herds (Torremorell et al., 2012). Viral spread through a naïve herd can occur within 2-3 days (Van Reeth et al., 2012).

### 1.3.4 Clinical signs

Influenza infections typically include fever (40.5 – 41.5 °C), anorexia, inactivity, sneezing, nasal discharge, coughing, and laboured breathing, and can lead to pneumonia and reduced growth performance (Janke, 2014; Rajão et al., 2013). Uncomplicated infections elicit high morbidity and low mortality. Influenza infections are often subclinical but can be exacerbated by secondary infections with other bacteria or viruses (Kothalawala et al., 2006). Persistent IAV infections at the herd level tend to be common on farrow-to-finish farms due to the abundance of young, immunologically naïve piglets with declining maternal immunity (Loeffen et al., 2009).

### 1.3.5 Influenza A virus and the host

In experimental conditions, IAV infection promotes the upregulation of type I IFNs, TNF-α, IL-1, and IL-6 in bronchoalveolar secretions (Hemmink et al., 2016). Antibody
production mainly targets HA, NA, matrix (M), and nucleoprotein (NP) proteins, but only antibodies specific to the globular head portion of HA can block attachment of the virus to host cells, neutralizing viral infection (Van Reeth et al., 2012). Influenza A virus can be completely cleared from the respiratory tract within one week, but young piglets and pigs with little or no clinical signs may act as a reservoir for persistent infections (De Vleeschauwer & Van Reeth, 2010).

1.4 Mycoplasmal pneumonia

1.4.1 Taxonomy

*Mycoplasma hyopneumoniae* is a member of the class *Mollicutes*, consisting of eubacteria with a low guanine (G) and cytosine (C) content (Shimkets & Woese, 1992). Mollicutes can infect many hosts, including plants, animals, and humans (Kobisch & Friis, 1996). They are phylogenetically related to the Gram-positive eubacteria bacilli, clostridia, enterococci, lactobacilli, staphylococci, and streptococci (Thacker & Minion, 2012).

The genome of *M. hyopneumoniae* is small, contributing to a lack of biosynthetic pathways in the organism, thus requiring the growth environment to provide many essential amino acids, purines, pyrimidines, and membrane components, which are typically found on mucosal surfaces in animal hosts (Pollack, 1997).

1.4.2 Pathogenesis

*Mycoplasma hyopneumoniae* binds to porcine epithelial cells of the respiratory tract, specifically tracheal and bronchial epithelial cells, after inhalation, adhering directly to the ciliary membrane in order to colonize the respiratory tract (Blanchard et al., 1992). Adherence to the cilia of the respiratory epithelium causes ciliary clumping, ciliostasis (wherein ciliary beating is minimized), loss of cilia, and exfoliation of ciliated cells (Bin et al., 2014; Blanchard et al., 1992;
DeBey & Ross, 1994). In order to facilitate further development of mycoplasmal pneumonia, *M. hyopneumoniae* must adhere to the distal portion of the respiratory tract in order to prevent the removal of secretory and cellular debris, allowing it to gravitate deeper into the lungs (Kwon, et al., 2002). The inhibition of ciliary clearance of cellular debris increases the susceptibility of the host to secondary infections, both bacterial and viral (Livingston et al., 1972).

Adherence of *M. hyopneumoniae* to the cilia of respiratory epithelia depends mainly on a group of adhesins belonging to the P97/P102 family (Hsu & Minion, 1998; Zhang et al., 1995). The P97/P102 proteins create a “fuzzy layer” of fibrils starting from the plasma membrane of *M. hyopneumoniae* cells and extending to susceptible host cells (Tajima & Yagihashi, 1982).

### 1.4.3 Transmission

The primary forms of transmission for *M. hyopneumoniae* are direct contact between sow and piglet in gestation crates or nose-to-nose contact between carrier and naïve swine in grower-finisher stages (Morris et al., 1995). Transmission can also occur via aerosolized droplets expelled from infected pigs, which may be able to travel up to 9 km (Otake et al., 2010).

### 1.4.4 Clinical signs

Clinical signs of mycoplasmal or enzootic pneumonia are rarely seen in lactating sows and piglets unless *M. hyopneumoniae* has been introduced into a naïve herd (Batista et al., 2004; Nathues et al., 2014). Clinical signs are more often seen in grower and finisher pigs over 12 weeks of age and include a dry, non-productive cough, mild chronic pneumonia, and decreased growth rate, and they can be exacerbated by co-infection with secondary viral and/or bacterial agents such as IAV and PRRSV, *Pasteurella multocida*, and *Actinobacillus pleuropneumoniae* (Kobisch & Friis, 1996).
1.4.5 *Mycoplasma hyopneumoniae* and the host

The survival and proliferation of *M. hyopneumoniae* within host tissues depends on its ability to alter the porcine innate and adaptive immune responses (Messier et al., 1990). This altering of the immune response prevents systemic spread but ensures the host will not be able to clear the infection, leading to chronic colonization of airways and a prolonged pulmonary inflammatory response (Huang et al., 2014; Thacker et al., 2000).

*Mycoplasma hyopneumoniae* adheres to cilia of the respiratory tract via adhesin proteins, resulting in ciliary clumping, damage, and loss (Bin et al., 2014; Blanchard et al., 1992). Cilia loss contributes to the buildup of cellular debris in the respiratory tract, allowing it to migrate deeper into the lungs and increasing the opportunity for opportunistic pathogens to colonize the respiratory tract (Kwon et al., 2002).

Infection with *M. hyopneumoniae* induces a general immunosuppressive effect on the CMIR in a number of ways. It suppresses phagocytic capabilities of pulmonary alveolar macrophages, preventing the uptake and presentation of antigens thereby interrupting an important first line of adaptive immune defense in the host (Caruso & Ross, 1990). This, in turn, reduces the clearance of *M. hyopneumoniae* and other bacterial pathogens, further promoting the development of disease (Caruso & Ross, 1990). In addition, *M. hyopneumoniae* induces macrophages and monocytes to produce the pro-inflammatory cytokines IL-1, 6, 8, and TNF-α in excess, leading to host-mediated tissue injury (Ahn et al., 2009; Asai et al., 1994; Choi et al., 2006). A potent inducer of the CMIR, IFN-γ, is inhibited following *M. hyopneumoniae* infection, and the combination of excess pro-inflammatory cytokines and immunosuppressive events caused by *M. hyopneumoniae* encourages inflammation, causing lung injury (Thanawongnuwech et al., 2004; Thanawongnuwech & Thacker, 2003).
Pigs typically seroconvert between 10 and 21 dpi after experimental infection with *M. hyopneumoniae* (Sheldrake et al., 1990). Elevated levels of immunoglobulins can be found two weeks after infection in experimental conditions, which peak 3-4 weeks after infection (Thacker et al., 2000) and are associated with decreased bacterial load and lung lesion severity (Messier et al., 1990). IgA secretion at the site of bacterial infection may prevent adherence of *M. hyopneumoniae* to cilia, and IgG could enhance phagocytosis of mycoplasma cells by alveolar macrophages (Messier et al., 1990; Suter et al., 1985). However, due to the immunosuppressive effects of *M. hyopneumoniae* infection, the protective effects of immunoglobulins are significantly decreased.

Animals that have successfully cleared *M. hyopneumoniae* are highly resistant to reinfection with the same strain, although susceptibility can increase during late gestation when antibodies are transferred from the maternal blood to colostrum (Bandrick et al., 2008).

### 1.5 Control strategies

#### 1.5.1 Basic control measures

Enhancing biosecurity measures can greatly decrease the incidence of infectious disease on swine farms. The main goals are to prevent infectious agents from entering the barn and preventing the spread of illness if these barriers are breached. Isolating pigs with signs of illness, separating groups of pigs by production stage (newborn, weaned, grower, finisher, breeding), moving groups in an all-in/all-out manner, and ensuring sufficient air quality and flow are some of the methods that can help reduce the spread of disease (Levis et al., 2015). Depending on a number of variables such as the size and density of the farm, the source of replacement breeding stock, and the proximity to other farms, biosecurity measures may need to be more stringent in order to successfully limit the incidence of disease. While antimicrobial use is implemented
widely on farms to prevent the incidence of bacterial infection, there has been significant public pressure to limit the use of antimicrobials in order to decrease the potential for antimicrobial resistance.

The dynamics of infection are complex and multifactorial, further increasing the difficulty involved in keeping herds disease-free. Certain methods have been adopted to combat the incidence of certain diseases, discussed below. In addition to these methods for controlling infections, frequent monitoring for the emergence of new bouts of disease should be performed. Using techniques such as ELISA and/or polymerase chain reaction (PCR) to detect antibodies or virus/bacteria, respectively, would help keep producers informed regarding the disease status for their farm (Bertrand et al., 2013).

1.5.2 PRRSV

Controlling for PRRSV-related disease on farm is difficult. The virus is complex and has a high genetic diversity, making development of a widely efficacious vaccine complicated (Murtaugh et al., 2010). In addition to improving biosecurity measures as mentioned above, there are a number of other methods that are used specifically to control PRRS.

In the breeding herd, stabilization through mass exposure or mass vaccination is crucial to ensure piglets aren’t infected before weaning. This involves exposing gilts to PRRS-infected pigs or vaccinating them before introducing them to the herd to help acclimatize them and make them less likely to transmit the virus (Chae ChanHee, 2013). However, due to the number of PRRSV variants, vaccination may help to lower the number of susceptible individuals but may not offer complete protection (Zuckermann et al., 2007).

Herd closure of PRRS-positive farms may also help to clear the virus from a herd (Rathkjen & Dall, 2017). This will lower the number of viremic pigs that can infect naïve gilts.
and prevent them from acting as reservoirs to in turn infect other pigs (Rathkjen & Dall, 2017). If controlled for in the breeding herd, only PRRSV-negative piglets should exist at weaning. Vaccinating piglets before introducing them to the growing population can help ensure pigs remain negative and are not a threat to the health of the rest of the pig population. However, in instances of PRRSV-positive nursery pigs, nursery depopulation can help to prevent transmission to older pigs, and also improve growth rate and decrease incidence of mortality (Corzo et al., 2010; Dee et al., 1996).

1.5.3 IAV

Vaccinating for IAV is the most widely used method for controlling incidence on farm. Producers are recommended to administer two vaccinations to piglets 2 – 4 weeks apart beginning after 3 weeks of age, and boosters to sows on a biannual basis (Van Reeth et al., 2012). Due to the public health threat of viral reassortment in swine and their role as influenza “mixing vessels”, reducing the amount of interaction between human traffic on farm, as well as restricting access to anyone with flu-like symptoms is also advised (Detmer, 1987).

1.5.4 M. hyopneumoniae

In the case of M. hyopneumoniae, aside from the methods mentioned previously, stocking density can play an important role in disease transmission, and therefore maintaining optimal stocking densities can help to reduce the risk of transmission (Maes et al., 2008). Vaccination is widely practiced, and prophylactic antibiotic use is used by the swine industry to control mycoplasmal pneumonia.

1.6 Breeding for genetically resistant pigs

Despite efforts to limit infectious disease on farm through enhanced biosecurity and new and more efficacious vaccines, infectious disease continues to pose a threat to animal health and
welfare and producer profits. As such, breeding for pigs that are more immunologically robust may assist in reducing or negating the impact of infectious disease (Mozzi et al., 2018).

Selecting for pigs that are phenotypically better immune responders results in the production of offspring that are also better immune responders more capable of responding favourably to infectious disease (Crawley et al., 2005; Mallard et al., 2015). Identifying variants in genes that affect immune responses at the genomic level using GWAS techniques is also a promising method for selecting animals that may be more capable of mounting a robust immune response to a wide array of pathogens (Davies et al., 2009). Genome-wide association studies are techniques interested in identifying single-nucleotide variants (SNVs) that are associated with the expression of certain traits. Typically, these studies are interested in breeding and production traits such as teat number and growth rate (Horodyska et al., 2016; Lopes et al., 2014). However, identifying pigs that are more capable of mounting an effective immune response or pigs that are less susceptible to infection may allow for selective breeding of more robust hosts, which has the potential to improve overall animal health, reduce disease-associated production costs, and limit the use of on-farm antimicrobials (Davies et al., 2009; Georges et al., 2019).

Variation in resistance to a number of infectious diseases, including *Salmonella enterica* serovar Cholerasuis (SC) (Hiroki et al., 2011), neonatal diarrhea caused by enterotoxigenic *Escherichia coli* (ETEC) (Fu et al., 2012), and bovine tuberculosis (Acevedo-Whitehouse et al., 2005) have been identified in pigs. Additionally, a SNV on *Sus scrofa* chromosome (SSC) 4 was found to be associated with increased weight gain and decreased viral load following infection with a highly virulent strain of PRRSV, suggesting that this SNV may be associated with favourable immune responses to PRRSV infection (Boddicker et al., 2012). While no studies have identified SNVs associated with increased resistance to IAV or *M. hyopneumoniae*,
previous findings in disease resistance and susceptibility, such as the heritability of *M. hyopneumoniae* resistance (Kadowaki et al., 2012) and the findings mentioned above, suggest that disease resistance is a trait that can be improved through genetic selection. Identifying SNVs associated with resistance to important diseases is a promising field of research, as this would widely improve herd health and also offer substantial cost-saving benefits to producers if animals experience milder symptoms or are infected less frequently.

Due to the specificity of the adaptive immune response, selecting variants that enhance the efficiency of the system to respond to a wide range of pathogens would be difficult as cells of the adaptive immune system are specific to one antigen (Wilkie & Mallard, 1999). However, selecting genetic variants that broadly enhance the efficacy of the innate immune response could confer protection to a wider assortment of pathogens and also in turn increase the adaptive immune response (Kaiser, 2010). Because the immune response is complex and multifactorial, it is unlikely for a single SNV to have a major effect on immune responses; however, it is possible that multiple SNVs may work in conjunction to positively affect the immune response.

### 1.7 Summary

The presence of PRRSV, IAV, and *M. hyopneumoniae* on swine farms leads to decreased producer profits and adversely affects animal health and welfare. These respiratory pathogens are dynamic and difficult to control with traditional means. Methods for reducing the burden of disease while avoiding the use of antimicrobials are therefore highly desirable.

In order to develop effective methods of control, serological monitoring for the presence and cycles of various disease-causing pathogens at different stages of production is necessary. In addition, understanding the relationship between genetic variants and immune responses may help uncover the possibility for selectively breeding animals with a wide array of disease
resistance, which in turn would help to reduce the costs associated with disease management and improve overall animal health.
Chapter 2: Antibody responses to porcine reproductive and respiratory syndrome virus, influenza A virus, and Mycoplasma hyopneumoniae from weaning to finisher in fourteen groups of pigs in Ontario

This chapter corresponds to the following manuscript currently under review:


2.1 Abstract

Porcine reproductive and respiratory syndrome, swine influenza, and mycoplasmal pneumonia are some of the most prevalent respiratory diseases affecting swine farm productivity in Canada. Monitoring for the prevalence of the infectious agents associated with these diseases on farm may help to improve herd-specific control strategies and to minimize the impact of disease on commercial swine farms. The objectives of this study were to investigate antibody responses to porcine reproductive and respiratory syndrome virus (PRRSV), influenza A virus (IAV), and Mycoplasma hyopneumoniae (M. hyopneumoniae) from weaning to the end of the finisher stage on 14 groups of commercial swine farms in Ontario and to examine the effects of nursery diet on antibody responses. Serology found that 8, 61, and 31 % of pigs at weaning, 1, 31, and 22 % at the end of nursery, 8, 38, and 18 % at the end of grower, and 11, 48, and 25 % at the end of the finisher stage tested seropositive for PRRSV, IAV, and M. hyopneumoniae, respectively. Three, 14, and 5 groups had > 20 % of pigs that tested seropositive at least once over the course of production (“high seropositivity”) for PRRSV, IAV, and M. hyopneumoniae, respectively. In general, seropositivity was more likely to be lower at the end of nursery compared to weaning for all three pathogens, and more likely to be higher for PRRSV and IAV at weaning, end of grower, and end of finisher. Pigs that were seropositive for PRRSV were
more likely to be seropositive for *M. hyopneumoniae* (p < 0.001). Overall, pigs fed a low
complexity diet during nursery were more likely to be seropositive for PRRSV (p < 0.001) and
IAV (p = 0.04). This study provides information regarding changes in serum antibody in pigs
across different stages of production and highlights periods of vulnerability. Additionally, these
findings may encourage further research into the effects of nursery diet complexity on disease
susceptibility and immune response.

### 2.2 Introduction

Infectious diseases on swine farms can have detrimental effects on both producer profits
and animal health and welfare. Porcine reproductive and respiratory syndrome virus (PRRSV),
influenza A virus (IAV), and *Mycoplasma hyopneumoniae (M. hyopneumoniae)* are three of the
most significant pathogens affecting swine-farm productivity (USDA, 2012). Despite efforts to
eradicate these pathogens and their associated diseases, they continue to be widespread in the
global swine population and result in huge economic losses for pork producers (Haden et al.,
2002).

One difficult aspect of disease eradication on swine farms is the variation in immunity
throughout the lifecycle of the animals. After weaning, levels of maternal antibodies in the
weaner decline, after which pigs begin to produce their own antibodies as their immune systems
develop (Chase & Lunney, 2012). The initial decrease in antibody levels with waning passive
immunity increases disease susceptibility in young pigs, and as such, it is important to monitor
for the presence of pathogens at multiple stages of production in order to identify herd-specific
patterns of infection so as to better protect immunologically naïve pigs from infection and avoid
the spread of disease on farm.
Further complicating the issue of infection on commercial swine farms is the potential presence of additional pathogens, which may take advantage of the already fragile immune system of an infected individual to co-infect their host (White, 2011). For example, *M. hyopneumoniae* adheres to cilia of the respiratory epithelium preventing clearance of cellular debris, thereby increasing the potential for co-infection (Bin et al., 2014; Blanchard et al., 1992; DeBey & Ross, 1994). Similarly, PRRSV suppresses the innate immune response mainly through its selective tropism and subsequent destruction of porcine alveolar macrophages (PAMs) (Zimmerman et al., 2012), which leads to the suppression or alteration of type I interferon (IFN) pathways (Huang et al., 2015), which may also increase the susceptibility for co-infection.

Not only does infection with certain pathogens increase the potential for further infection, but co-infection is often more severe than infection by either pathogen alone. Previous research determined that *M. hyopneumoniae* infection increased and prolonged PRRSV-induced pneumonia (Thacker et al., 1999), and pigs co-infected with both *M. hyopneumoniae* and IAV have been shown to exhibit more severe clinical disease than pigs infected singly with either agent (Thacker et al., 2001). These interactions may further exacerbate declines in producer profits and animal welfare, and thus avoiding infection with multiple pathogenic agents on farm is crucial in order to minimize the impact of disease.

Another aspect of swine production that may influence animal health and producer profits is feed. Not only is feed the costliest aspect associated with pork production, but nursery diet costs are especially high due to the need for a highly palatable feed that will allow the immature gut of the pig to adapt from an easily digestible milk diet to solid, grain-based feed (Hazzledine & Whittemore, 2006). Common industry practice recommends producers feed a series of starter
feeds in order to slowly transition weanlings from expensive, complex diets containing milk products or fishmeal, for example, to less expensive diets consisting of simpler plant-based ingredients (McManus, n.d.). This allows pigs to develop the necessary enzymes required for digesting the constituents found in adult diets (Hazzledine & Whittemore, 2006; McManus, n.d.). Additionally, the composition of nursery diets, particularly protein content and quality, may affect animal health, carcass quality, and growth rate (Hazzledine & Whittemore, 2006). Nursery diets that substitute animal proteins with plant-based proteins may decrease costs associated with pork production without sacrificing meat quality or body weight (Skinner et al., 2014) but the effects these diets may have on antibody responses, if any, are not yet fully understood (Schut et al., 2019).

Infections with PRRSV, IAV, and *M. hyopneumoniae* are frequently chronic and/or subclinical, and due to the many factors affecting the incidence of the associated diseases, monitoring for the presence and distribution of microorganisms on-farm is an important step in identifying herd-specific patterns of illness and susceptible populations in order to implement effective control programs. The use of serological assays such as enzyme-linked immunosorbent assay (ELISA) tests are frequently used to monitor antibody responses to infectious agents rather than testing for the presence of the pathogen itself (Robben, 2017), because they tend to be less expensive and are fast and efficient to perform on large numbers of samples, allowing for a broader picture of pathogenic threats to be painted on a farm-by-farm basis and providing producers with the means to administer tailored preventative medicines and treatments specific to their hazards.

The objectives of this chapter were: to measure antibody responses to PRRSV, IAV, and *M. hyopneumoniae* in pigs from weaning until the end of the finisher stage; to examine the
relationship in antibody responses among those three pathogens; and to determine the impact of a nursery diet that uses mostly plant protein compared to the typical complex animal protein-based diet on seropositivity to PRRSV, IAV, and M. hyopneumoniae.

2.3 Materials and methods

2.3.1 Study design

The farrowing source and pig selection for this study have been previously described (Ainslie-Garcia et al., 2018; Schut et al., 2019). Briefly, fourteen groups of 50 – 60 pigs originating from eight farrowing sources in Southwestern Ontario were selected. Two cohorts (Cohort 1 and 2) were included in the study from six of the eight farrowing sources, while the other two farrowing sources included only one cohort (Cohort 1). Pigs in Cohort 1 were born between May and August, while pigs in Cohort 2 were born between October and January. All sources but one utilized off-site nursery and finishing, while the other was farrow-to-finish for Cohort 1 and off-site finisher for Cohort 2. Pigs received either a standard high-complexity (HC) nursery diet or experimental lower-cost, low-complexity (LC) diet in which the majority of the animal protein was replaced with plant protein throughout the nursery phase (Skinner et al., 2014). The diets for all pigs were the same at all other production stages. Surveys were provided to producers to obtain information regarding farm management practices.

2.3.2 Sample collection

Blood samples were collected from pigs at weaning and at the end of the nursery, grower, and finisher stages in all 14 groups except for one, where samples were not collected at the end of the finisher stage. Blood samples were collected from either the jugular vein or suborbital sinus and centrifuged at 1500 x g for 20 minutes. The serum samples were then stored at -20 °C.
2.3.3 Antibody detection

Sera were analyzed for the presence of PRRSV, IAV, and *M. hyopneumoniae* antibodies using three commercially available ELISA Kits (IDEXX Laboratories, Inc., Westbrook, Maine, USA) as per the manufacturer’s instructions (Appendix I). Groups were classified as high seropositivity for each pathogen if at least 20% of pigs in a group were seropositive for that pathogen at least once over the course of production; otherwise, they were classified as low seropositivity.

Direct ELISAs were used to determine sample-to-positive (S/P) ratios for PRRSV antibodies, calculated as follows:

\[
S/P = \frac{\text{Sample absorbance (650)} - \text{Mean}_{\text{negative control}}}{\text{Mean}_{\text{positive control}} - \text{Mean}_{\text{negative control}}}
\]

A pig was considered seropositive for PRRSV if the S/P ratio was \( \geq 0.4 \).

Blocking ELISAs were used to determine sample-to-negative (S/N) ratios for IAV, calculated as follows:

\[
S/N = \frac{\text{Sample absorbance (650)}}{\text{Mean}_{\text{negative control}}}
\]

A pig was considered seropositive for IAV if the S/N ratio was < 0.6.

Direct ELISAs were used to determine S/P ratios for *M. hyopneumoniae*, calculated as follows:

\[
S/P = \frac{\text{Sample absorbance (650)} - \text{Mean}_{\text{negative control}}}{\text{Mean}_{\text{positive control}} - \text{Mean}_{\text{negative control}}}
\]

A pig was considered seropositive for *M. hyopneumoniae* if the S/P ratio was > 0.4.
2.3.4 Multivariable analyses

Data were cleaned in Excel (Microsoft 2016, Redmond, Washington, USA) and transferred to Stata (Stata/MP-13 StataCorp, College Station, Texas, USA) for analysis. All statistical analyses were conducted only on high seropositivity groups. A mixed-effects multi-level logistic regression method with farrowing source and sow as random effects was used to compare IAV and *M. hyopneumoniae* seropositivity at different stages of production. As only three groups were classified as high PRRSV seropositivity, a mixed-effects multi-level logistic regression method with only sow as a random effect and farrowing source as fixed effect was used to compare PRRSV seropositivity at different production stages. An additional mixed-effects multi-level logistic regression method with farrowing source, sow, and pig (repeated measurement) as random effects was used to determine the association between seropositivity to different pathogens for the IAV and *M. hyopneumoniae* models, as well as the effects of nursery diet complexity on antibody responses. Similar models were prepared for PRRSV with only sow and pig as random effects and farrowing source as fixed effect. While this study was not designed to evaluate risk-factors associated with disease susceptibility, certain parameters were included to control for managerial differences between farms. The independent variables considered in the univariable analyses were Cohort (1/2), nursery diet (HC/LC), production stage (at weaning/end of nursery/end of grower/end of finisher), seropositivity to other pathogens of interest for the present study (yes/no), and farrowing source. These variables were first screened by univariable analysis and considered for inclusion in the final model if \( p < 0.2 \). Models were then built using a manual forward stepwise approach and variables were included in the final model if \( p < 0.05 \).
2.4 Results

2.4.1 Antibody responses

Overall, 6.6 % (144/2182), 44 % (964/2180), and 24 % (502/2078) of samples from all farms were seropositive for PRRSV, IAV, and *M. hyopneumoniae*, respectively. In total, 618, 618, and 590 pigs were tested twice, 537, 536, and 507 were tested three times, and 409, 408, and 391 were tested four times for PRRSV, IAV, and *M. hyopneumoniae*, respectively. There were 336 pigs tested for all three pathogens at four visits. Of the pigs tested for three pathogens at four visits, 24 (7.1 %) were seronegative for all three pathogens throughout production, 165 (49.1 %) were seropositive for one at least once over the course of production, 124 (36.9 %) were seropositive for two, and 23 (6.9 %) were seropositive for all 3. The proportions of pigs in 14 groups that were seropositive over the course of production for PRRSV, IAV, and *M. hyopneumoniae* are shown in Figure 2.1, Figure 2.2, and Figure 2.3, respectively. PRRSV, IAV, and *M. hyopneumoniae* seropositivity for all pigs was 7.9, 61.0, and 31.1 %, respectively, at weaning; 1.1, 30.6, and 21.5 % at the end of the nursery stage; 8.2, 37.7, and 18.3 % at the end of the grower stage; and 10.8, 48.0, and 25.1 % at the end of the finisher stage (Figure 2.4). A group was classified as high seropositivity for any pathogen if at least 20 % of pigs in that group were seropositive at least once for that pathogen over the course of production. Three groups were classified as high seropositivity for PRRSV (40.4 – 88.6 %), all fourteen groups were high seropositivity for IAV (36.7 – 100 %), and five were high seropositivity for *M. hyopneumoniae* (72.9 - 100 %) (Table 2.1). All statistical models were constructed using the high seropositivity groups only. There were two groups in this study that were vaccinated for *M. hyopneumoniae*, as determined by the survey distributed to producers, and the corresponding groups were removed.
from further analyses. The subsequent increases in seropositivity in the remaining groups were therefore assumed to be due to natural infection.

Seropositivity profiles for pigs tested at 4 production stages for all groups are shown in Table 2.2. Alternate figures for displaying seropositivity profiles for PRRSV, IAV, and *M. hyopneumoniae* are presented in Appendix II, Appendix III, and Appendix IV, respectively. The number of times pigs were seropositive if tested at four visits in all 14 groups are shown in Figure 2.5.

2.4.2 Multivariable analyses

Multivariable analyses were performed including only the high seropositivity groups for each pathogen of interest.

2.4.2.1 PRRSV

On the three high seropositive cohorts, pigs were more likely to be seropositive for PRRSV at weaning (*p* < 0.001), the end of grower (*p* < 0.001), and the end of finisher (*p* < 0.001) compared to the end of nursery. Pigs fed a low complexity nursery diet were more likely to be seropositive than those fed a conventional high complexity diet (*p* < 0.001), and farrowing source also affected the likelihood of seropositivity (*p* = 0.015) (Table 2.3 & Appendix V).

2.4.2.2 IAV

Pigs were more likely to be seropositive for IAV at weaning (*p* < 0.001), the end of grower (*p* = 0.003), and the end of finisher (*p* < 0.001) compared to the end of nursery. Cohort two pigs were more likely to be seropositive than cohort one (*p* < 0.001) (Table 2.4). Pigs fed a low complexity nursery diet were more likely to be seropositive than conventionally fed pigs (*p* = 0.04) (Appendix VI). The variation in seropositivity due to the farrowing source and pig was 32 and 68 %, respectively.
2.4.2.3 M. hyopneumoniae

Pigs were more likely to be seropositive for *M. hyopneumoniae* at weaning compared to end of nursery (*p* < 0.001). Pigs that were seropositive for PRRSV were more likely to be seropositive for *M. hyopneumoniae* (*p* < 0.001), while pigs in cohort two were less likely to be seropositive (*p* < 0.001) (Table 2.5 & Appendix VII). The variation in seropositivity due to farrowing source and pig was 33 and 67 %, respectively.

2.5 Discussion

This study aimed to investigate antibody responses to porcine reproductive and respiratory syndrome virus, influenza A virus, and *Mycoplasma hyopneumoniae* in pigs at different stages of production, to determine the interaction in antibody response between those pathogens, and to examine the effects of nursery diet complexity on antibody responses to those pathogens.

In general, seropositivity indicates that an animal has either absorbed maternally derived antibodies or been exposed to infectious agents through natural infection or vaccination. In this study, seropositivity proportions were high at weaning for all three pathogens, likely due to the absorption of antibodies through the sow’s colostrum and milk (Klobasa et al., 1985; Werhahn et al., 1981), low at weaning, and high again for PRRSV and IAV at the end of the grower and finisher stages. The decline in seropositivity observed from weaning to nursery in this study indicates the loss of maternal antibodies (Chase & Lunney, 2012), which also suggests that pigs may be particularly susceptible to pathogens post-weaning. Antibodies to both PRRSV and IAV appeared to be fairly prevalent in the high seropositivity groups, and thus the risk for infection from these agents is high throughout production. Because none of the groups included in the multivariable analyses were vaccinated for any of the pathogens of interest, it is largely assumed
that increases in antibody responses post-weaning were the result of natural infection.

Implementation of vaccination paradigms may be beneficial to enhance the development of the immune response. Additionally, monitoring for the presence of disease using techniques such as ELISA will help to identify specific pathogens present on a farm and reduce doubt of falsely diagnosing one disease for another.

Unlike PRRS and IAV, pigs were more likely to be seropositive for *M. hyopneumoniae* only at weaning compared to end of nursery in the high seropositivity groups. These results either indicate the vulnerability of weanlings to infection, as antibody responses did not seem to increase significantly in later stages of production, or the lack of *M. hyopneumoniae* infections in these stages. Due to the nature of *M. hyopneumoniae* and its tendency to produce chronic infections in the host, the assumption is that after the decline of maternal antibodies, the young pigs mount a slower immune response (Erlandson et al., 2005). This seems to suggest that the pathogen is not being cleared from production but rather the immune response is slower to respond.

The second objective of this study was to determine if infection with one infectious agent influences the activity of another. It was found that pigs seropositive for PRRSV were more likely to be seropositive for *M. hyopneumoniae*. The present study did not determine if co-infection with PRRSV and *M. hyopneumoniae* produced more severe disease, but these results have been reported in the past (Thacker et al., 1999; Thacker et al., 2001). Additionally, while managerial factors, such as pig density and pig flow, would affect the spread of disease on farm, it is possible that infection with one agent would increase susceptibility to the other agent(s). This suggests that while controlling for the presence of one infectious agent is important, in order to prevent more severe disease, care should be taken to prevent co-infection as much as possible.
Understanding which pathogens are a threat on a farm-specific basis using techniques such as ELISA may help in reducing the detrimental effects of co-infection.

The final objective of this study was to investigate whether nursery diet complexity had an impact on antibody responses to PRRSV, IAV, and *M. hyopneumoniae*. Pigs fed a low complexity nursery diet were more likely to be seropositive for PRRSV and may have been likelier to be seropositive for IAV. However, there was no significant association between nursery diet complexity and *M. hyopneumoniae* seropositivity. The diet complexity has also been previously found to have no effect on antibody responses to *Salmonella* (Schut et al., 2019). These results may suggest that the low complexity diet increased the susceptibility of pigs to PRRS and influenza viruses but had no effect on susceptibility to *M. hyopneumoniae* and *Salmonella*. Alternatively, these results may indicate that the LC diet enhanced antibody responses to PRRSV and IAV, resulting in higher antibody titres, while having no effect on the antibody responses to *M. hyopneumoniae* and *Salmonella*. Interestingly, nursery diet complexity only affected seropositivity to the viral pathogens but not the bacterial pathogens. The associations found between nursery diet complexity and antibody response to the pathogens tested in this study should be interpreted with caution and need to be investigated more thoroughly, for example, in a challenge setting, while evaluating innate and cell mediated immune responses in additional pigs. While the overall number of pigs used was reasonable, the number of infected pigs, especially PRRS-infected pigs, was less than ideal, and a challenge study may help to increase the power of the study. The potential for cost-saving benefits to producers may encourage further study into the effects of nursery diet complexity on immune responses. Further investigation into the effects of nursery diet complexity on antibody responses to other notable porcine pathogens may help shed more light on the effects of diet complexity on
immune development. Additionally, other branches of the immune system, such as innate and cell-mediated immune responses, could also be examined to determine if additional facets of the immune system are affected.

While seropositivity at the pig level was relatively high, a proportion of pigs remained seronegative throughout all stages of production. This indicates either that these pigs were never exposed to the infectious agents; that animals were exposed but the pathogens were unable to bypass the innate immune system in order to establish infection and activate the adaptive immune system; that an immune response was generated but was not robust enough to be read as seropositive by the ELISA kits; or that pigs had not yet seroconverted at the time of sample collection. However, there may have also been some variation in results based on the ELISA kits used for antibody detection. The IDEXX ELISA kits have been found to have 100 % sensitivity and 99.9 % specificity for PRRSV (Seo et al., 2016); 86 and 89 % for IAV (Tse et al., 2012); and relatively low sensitivity (63 %) but high specificity (100 %) for M. hyopneumoniae (Erlandson et al., 2005). However, as noted by Erlandson and colleagues (2005), the low sensitivity of the M. hyopneumoniae test is likely due to the nature of the infectious agent and the slow immune response produced by M. hyopneumoniae rather than the efficacy of the ELISA kits themselves. As such, there may have been false negatives generated in this study, but the classification of groups as high and low seropositivity and considering a pig “seropositive” if it tested seropositive at least once over the course of production likely worked to counteract this issue.

The ELISA kits used in this study were unable to differentiate between antibody responses to natural infections and vaccination. However, because only one farrowing source was vaccinated for M. hyopneumoniae and the corresponding groups were not included in the analyses, the seropositivity observed in the high seropositivity groups can be largely assumed to
be from maternal antibodies in the early stages of production and natural infection later in life. These results may help to encourage vaccination in post-weaning pigs, when the interaction between maternal antibodies and vaccine antigens is minimized (Chase & Lunney, 2012).

2.6 Conclusion

Understanding periods of vulnerability on farm is important for producers to be able to develop site-specific methods of disease prevention and control. Monitoring frequently for changes in the current pathogenic threats to a farm may help to confer broader protection, improve animal health and welfare, and increase producer profits, as well as ensure animals are not incorrectly treated for a different but clinically similar disease. Finally, while further research is needed to investigate the association between other components of immune system, such as innate and cell mediated immune responses, and low complexity nursery diets and the effect on disease susceptibility, this study suggests low complexity nursery diets, which offer cost-saving incentives, may be beneficial on farms with low disease pressures.

2.7 Acknowledgements

This project was supported by NSERC, OMAFRA, Swine Innovation Porc, the Canadian Centre for Swine Improvement, Alliance Genetics Canada, and Ontario Pork. Thank you to participating pork producers, Heather Reinhardt and Jane Newman for sample collection, Margaret Ainslie-Garcia and Corinne Schut for sample analysis, and Dr. Jutta Hammermüller for laboratory assistance.
2.8 Tables and figures

Figure 2.1. Percentage of pigs testing seropositive for PRRSV at least once from weaning to end of finisher

This figure depicts the percentage of pigs in 14 groups that tested seropositive for porcine reproductive and respiratory syndrome virus (PRRSV) at least once from weaning to the end of the finisher stage. Sera collected from 618 pigs were analyzed for PRRSV antibodies by commercial ELISA. Note: farrowing sources 2 and 7 included only one cohort.
Figure 2.2. Percentage of pigs testing seropositive for IAV at least once from weaning to end of finisher

This figure depicts the percentage of pigs in 14 groups that tested seropositive for influenza A virus (IAV) at least once from weaning to the end of the finisher stage. Sera collected from 618 pigs were analyzed for IAV antibodies by commercial ELISA. *Note:* farrowing sources 2 and 7 included only one cohort.
Figure 2.3. Percentage of pigs testing seropositive for *M. hyopneumoniae* at least once from weaning to end of finisher

This figure depicts the percentage of pigs in 14 groups that tested seropositive for *Mycoplasma hyopneumoniae* at least once from weaning to the end of the finisher stage. Sera collected from 590 pigs were analyzed for *M. hyopneumoniae* antibodies by commercial ELISA. *Note:* farrowing sources 2 and 7 included only one cohort.
Figure 2.4. Percentage of pigs testing seropositive for PRRSV, IAV, and *M. hyopneumoniae* at each production stage from weaning to the end of finisher

This figure depicts the percentage of pigs that were seropositive for porcine reproductive and respiratory syndrome virus (PRRSV), influenza A virus (IAV), and *Mycoplasma hyopneumoniae* in high seropositivity groups at each stage of production from weaning to the end of the finisher stage. *Note:* Three, 14, and 5 groups were classified as high seropositivity for PRRSV, IAV, and *M. hyopneumoniae*, respectively. Seropositivity was determined by commercial ELISA.

*Significantly different from end of nursery (p < 0.05).
**Figure 2.5.** Number of times pigs tested seropositive for PRRSV, IAV, and *M. hyopneumoniae*

This figure depicts the number of times individual pigs tested seropositive for porcine reproductive and respiratory syndrome virus (PRRSV), influenza A virus (IAV), and *M. hyopneumoniae* in high seropositivity groups of pigs from weaning to the end of the finisher stage. *Note:* Three, 14, and 5 groups were classified as high seropositivity. Seropositivity was determined by commercial ELISA.
Figure 2.6. PRRSV, IAV, and *M. hyopneumoniae* seropositivity in pigs fed conventional or low complexity nursery diets

This figure depicts the percentage of pigs seropositive for porcine reproductive and respiratory syndrome virus (PRRSV), influenza A virus (IAV), and *Mycoplasma hyopneumoniae* in high seropositivity groups of pigs fed high complexity (*i.e.*, high animal protein content) or low complexity (*i.e.*, mainly plant-based) nursery diets. *Note*: Three, 14, and 5 groups were classified as high seropositivity. Seropositivity was determined by commercial ELISA.

* *p < 0.05*
Table 2.1. Seropositivity to PRRSV, IAV, and *M. hyopneumoniae* in 14 groups of pigs

<table>
<thead>
<tr>
<th>Seropositivity (%)</th>
<th>Cohort</th>
<th>Farrowing source</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>PRRSV</td>
<td></td>
<td></td>
</tr>
<tr>
<td>One</td>
<td>3.33</td>
<td>0.00</td>
</tr>
<tr>
<td>Two</td>
<td>65.22</td>
<td>65.38</td>
</tr>
<tr>
<td>IAV</td>
<td></td>
<td></td>
</tr>
<tr>
<td>One</td>
<td>36.67</td>
<td>100.00</td>
</tr>
<tr>
<td>Two</td>
<td>86.96*</td>
<td>11.54</td>
</tr>
<tr>
<td><em>M. hyopneumoniae</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>One</td>
<td>78.95*</td>
<td>2.33</td>
</tr>
<tr>
<td>Two</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

This table describes the percentage of individual pigs that were seropositive for porcine reproductive and respiratory syndrome virus (PRRSV), influenza A virus (IAV), or *Mycoplasma hyopneumoniae* in 14 groups of pigs from 8 farrowing sources. Highlighted (grey) cells indicate high seropositivity groups, defined as groups with 20% or greater pig-level seropositivity from weaning to the end of finisher. Seropositivity was determined by commercial ELISA.

*These groups were vaccinated for *M. hyopneumoniae*. 
### Table 2.2. Seropositivity profiles in pigs tested for PRRSV, IAV, and *M. hyopneumoniae*

<table>
<thead>
<tr>
<th>Number (%) of pigs</th>
<th>Production stage (age in days)</th>
<th>At weaning (19-33)</th>
<th>End of nursery (52-70)</th>
<th>End of grower (96-115)</th>
<th>End of finisher(^\wedge) (130-159)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PRRSV 1 (0.3)</td>
<td>IAV 48 (13.2)</td>
<td>10 (2.8)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>0 (0.0)</td>
<td>5 (1.4)</td>
<td>2 (0.6)</td>
<td></td>
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</tr>
<tr>
<td>0 (0.0)</td>
<td>24 (6.6)</td>
<td>22 (6.1)</td>
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</tr>
<tr>
<td>21 (5.8)</td>
<td>54 (14.9)</td>
<td>34 (9.4)</td>
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</tr>
<tr>
<td>0 (0.0)</td>
<td>14 (3.9)</td>
<td>1 (0.3)</td>
<td></td>
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</tr>
<tr>
<td>1 (0.3)</td>
<td>19 (5.2)</td>
<td>12 (3.3)</td>
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</tr>
<tr>
<td>10 (2.8)</td>
<td>42 (11.6)</td>
<td>12 (3.3)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 (0.0)</td>
<td>14 (3.9)</td>
<td>4 (1.1)</td>
<td></td>
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</tr>
<tr>
<td>1 (0.3)</td>
<td>8 (2.2)</td>
<td>16 (4.4)</td>
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<td>13 (3.6)</td>
<td>4 (1.1)</td>
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<td>3 (0.8)</td>
<td>2 (0.6)</td>
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<tr>
<td>1 (0.3)</td>
<td>11 (3.0)</td>
<td>2 (0.6)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11 (3.0)</td>
<td>31 (8.5)</td>
<td>5 (1.4)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 (0.6)</td>
<td>4 (1.1)</td>
<td>2 (0.6)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15 (4.1)</td>
<td>11 (3.0)</td>
<td>27 (7.4)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>299 (82.6)</td>
<td>62 (17.1)</td>
<td>188 (51.8)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>362 (100)</td>
<td>363 (100)</td>
<td>343 (100)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Seropositivity profiles were generated for pigs that were tested at 4 visits. This table depicts the number of pigs that were seropositive for porcine reproductive and respiratory syndrome virus (PRRSV), influenza A virus (IAV), and *Mycoplasma hyopneumoniae* at each or multiple stage(s) of production (weaning, end of nursery, end of grower, and/or end of finisher), e.g. row 1, with black squares at all 4 timepoints, shows the number and percentage of pigs seropositive at all 4 times for PRRSV (column 1), IAV (column 2) and *M. hyopneumoniae* (column 3), while row 2 shows the number and percentage of pigs seropositive for the first 2 time points and negative for the other 2 time points.

\(^\wedge\) animals at the end of finisher time-point were sampled one-week prior to slaughter.
**Table 2.3.** Mixed-effects multi-level logistic regression analysis for porcine reproductive and respiratory syndrome virus (PRRSV) seropositivity

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Odds ratio</th>
<th>Standard error</th>
<th>95% confidence interval</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nursery diet</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HC&lt;sup&gt;A&lt;/sup&gt;</td>
<td>Referent</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>LC&lt;sup&gt;A&lt;/sup&gt;</td>
<td>18.33</td>
<td>8.90</td>
<td>7.08 - 47.45</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Farrowing source</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Referent</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>15.12</td>
<td>14.33</td>
<td>2.36 - 96.91</td>
<td>0.004</td>
</tr>
</tbody>
</table>

This table displays the mixed-effects multi-level logistic regression analysis for porcine reproductive and respiratory syndrome virus (PRRSV) seropositivity with sow and pig as random effects in 3 high seropositivity groups. Seropositivity was determined by commercial ELISA.

<sup>A</sup>HC = high complexity diet, LC = low complexity diet.
Table 2.4. Mixed-effects multi-level logistic regression analysis for influenza A virus (IAV) seropositivity

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Odds ratio</th>
<th>Standard error</th>
<th>95% confidence interval</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nursery diet</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HC&lt;sup&gt;A&lt;/sup&gt;</td>
<td>Referent</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>LC&lt;sup&gt;A&lt;/sup&gt;</td>
<td>1.34</td>
<td>0.19</td>
<td>1.01 - 1.77</td>
<td>0.04</td>
</tr>
<tr>
<td>Cohort</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>One</td>
<td>Referent</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Two</td>
<td>12.28</td>
<td>4.25</td>
<td>6.23 - 24.18</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

This table displays the mixed-effects multi-level logistic regression analysis for influenza A virus (IAV) seropositivity with farm, sow, and pig as random effects in 14 high seropositivity groups. Seropositivity was determined by commercial ELISA.

<sup>A</sup>HC = high complexity diet, LC = low complexity diet.
Table 2.5. Mixed-effects multi-level logistic regression analysis for *Mycoplasma hyopneumoniae* seropositivity

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Odds ratio</th>
<th>Standard error</th>
<th>95% confidence interval</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>PRRSV(^\text{A}) seropositivity</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>Referent</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Yes</td>
<td>11.43</td>
<td>11.24</td>
<td>1.66 - 78.59</td>
<td>0.013</td>
</tr>
<tr>
<td>Cohort</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>One</td>
<td>Referent</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Two</td>
<td>0.12</td>
<td>0.08</td>
<td>0.03 - 0.45</td>
<td>0.002</td>
</tr>
</tbody>
</table>

This table displays the mixed-effects multi-level logistic regression analysis for *Mycoplasma hyopneumoniae* seropositivity with farm, sow, and pig as random effects in 5 high seropositivity groups. Seropositivity was determined by commercial ELISA.

\(^\text{A}\)PRRSV = Porcine reproductive and respiratory syndrome virus
Chapter 3: Genetic variants associated with antibody responses to porcine reproductive and respiratory syndrome virus, influenza A virus, and *Mycoplasma hyopneumoniae* in swine

3.1 Abstract

Respiratory diseases on swine farms can have detrimental effects not only on animal health, drug use, and welfare, but also on production costs and profits. The growing concern of antimicrobial resistance is adding pressure to reduce the use of antimicrobials on farm, and to move to alternative disease control measures. Using a genome wide association study approach can help to identify single-nucleotide variants (SNVs) associated with disease resistance or susceptibility, allowing for animals with more favourable immune responses to be selected to produce immunologically robust offspring. The goal of this study was to determine whether there are SNVs associated with antibody responses to porcine reproductive and respiratory syndrome virus, influenza A virus, or *Mycoplasma hyopneumoniae* in swine. Fourteen groups of pigs (n = 618) from 8 farrowing sources in Ontario were tested for antibody responses to those three pathogens multiple times. The pigs were also genotyped in order to conduct a genome wide association study using a custom 54K Affymetrix chip. Two SNVs in *RAB38* (p = 2.85 x 10^{-5} and 5.20 x 10^{-5}) and one SNV near *BRN53* (p = 5.78 x 10^{-5}) were approaching a suggestive association (p < 1 x 10^{-5}) for PRRSV seropositivity. A SNV near *PARP14* (p = 2.79 x 10^{-5}), an upstream variant of *ADCY6* (p = 1.08 x 10^{-4}), and a SNV in the *TG* gene (p = 1.33 x 10^{-4}) were approaching a suggestive association for IAV seropositivity. A SNV near a currently unidentified gene, *ENSSSCG00000051043*, (p = 1.90 x 10^{-4}), a SNV in *DNASE1L3* (p = 2.11 x 10^{-4}), and an upstream variant of *TEKT2* (p = 2.20 x 10^{-4}) were nearing a suggestive association for *M. hyopneumoniae* seropositivity. These results may identify some candidate genes for
disease resistance studies and may help to elucidate potential targets for breeding for disease resistance in the future.

### 3.2 Introduction

Porcine reproductive and respiratory syndrome (PRRS), swine influenza, and mycoplasmal pneumonia are three of the most significant diseases affecting swine farm productivity. While there are methods of controlling for the incidence and severity of these diseases, their effectiveness is varied, and these infectious diseases continues to cause economic losses for the industry. In addition, immune responsiveness is a complex, multifactorial trait, and certain animals are more likely to develop more severe infection than others (Petry et al., 2007). As such, using a genome-wide association study (GWAS) approach, which widely screens the entire genome for single-nucleotide variants (SNVs) that affect certain phenotypic traits, is a technique that is becoming more widely accessible to identify potential selection markers for disease resistance. In pork production, the traits that are usually selected for are performance traits such as meat quality and average daily gain, as they are directly correlated with increased profits (Fontanesi et al., 2014; Reyer et al., 2017). However, the finding that selecting for disease resistance can be done without resulting in a negative impact on other physical production traits is promising (Geraci et al., 2019). This makes breeding for disease resistance a relatively risk-free method for enhancing herd health and reducing the burden of infectious disease on farm, provided selecting for one trait does not negatively influence another.

Selectively breeding animals with desirable phenotypes for immune responses results in herds that are more immunologically robust and at a lower risk for developing disease when compared to low immune responders (Crawley et al., 2005; Mallard et al., 2015; Wagter-Lesperance et al., 2011). While these effects have largely been investigated in cattle, they can
also be seen in swine. These results are promising, as increasing immune robustness would allow for the reduction of antimicrobial use on farm, decreasing costs and potential harm caused by antimicrobial resistant pathogens. Importantly, traits such as milk quality and reproductive fitness in cattle also improve (Mallard et al., 2015; Wilkie & Mallard, 1999). In a similar vein, investigating immune response at the genotypic level would allow for precise selection and breeding of animals with favourable immune responses to pathogen exposure. Additionally, increasing the overall immunity may also result in improving other desirable features associated with raising livestock.

Selecting genes that enhance the adaptive immune response would enhance immunity to a limited number of pathogens, and selecting for resistance to particular pathogens may also increase susceptibility to others, making selection for the resistance of one pathogen potentially harmful (Kaiser, 2010). In contrast, selecting for genes that broadly enhance the innate immune response should in turn increase responses to a wider range of pathogens, and because the adaptive immune response is driven by the innate response, this may also increase the activity of the humoral and cell-mediated branches of the immune response, in-kind (Kaiser, 2010).

While infectious agents are detrimental on their own, the presence of multiple pathogens on a farm is likely to greatly reduce the health of the animals and profitability of the farm. For instance, the PRRS virus can reduce the immune response through the destruction of porcine alveolar macrophages (PAMs), which disrupts their ability to kill other infectious agents (Solano et al., 1998), and it also interferes with type I interferon (IFN) responses which increases the length of time available for transmission to other naïve individuals (Dotti et al., 2013; Huang et al., 2015). Influenza infections can also be intensified by co-infections with other bacteria or viruses (Kothalawala et al., 2006), and the colonization of M. hyopneumoniae reduces the
activity of respiratory cilia, which inhibits the clearance of cellular debris from the respiratory tract, also increasing the potential for co-infections (Bin et al., 2014; Livingston et al., 1972). Co-infection is dangerous as the results are often more severe than infection by either pathogen alone. Infection with *M. hyopneumoniae* prolongs and worsens PRRS-associated pneumonia (Thacker et al., 1999) and co-infection with *M. hyopneumoniae* and IAV also worsens clinical signs compared to single infection with either agent (Thacker et al., 2001). As such, increasing resistance to a wide array of pathogens is desirable, but by increasing resistance to one or more immunosuppressive pathogens, this will also decrease the likelihood of infection with other infectious agents.

The consequences of SNVs on disease resistance have been studied in regard to a highly virulent strain of PRRSV using viral load (VL) and weight gain (WG) as phenotypes for disease (Boddicker et al., 2012). A SNV on *Sus scrofa* chromosome 4 (WUR10000125) was found to influence both factors, and pigs with the favourable SNV had reduced VL and increased WG compared to pigs with the unfavourable genotypes (Boddicker et al., 2014). This suggests that SNVs in this region could have a major impact on the physiological response to PRRSV infection (Boddicker et al., 2012). Another study investigating SNVs in the natural resistance-associated macrophage protein 1 (*NRAMP1*) gene, whose protein product plays a crucial role in immune responses against intracellular pathogens, found that SNVs in this region were associated with increased susceptibility to infectious diseases (Ding et al., 2014; Wu et al., 2008). These findings suggest that *Nramp1* may also be a desirable candidate gene for further study.

Researchers investigating SNVs associated with antibody responses to a number of infectious diseases, including IAV and *M. hyopneumoniae*, found no genes associated with
antibody responses to IAV and 1 gene on Sus scrofa chromosome (SSC) 7 associated with antibody responses to M. hyopneumoniae (Sanglard et al., 2019). To our knowledge, no other GWAS have been carried out that examined antibody responses to IAV and M. hyopneumoniae. However, resistance to M. hyopneumoniae infection, as measured by phenotypic traits of immune response, has been shown to be a heritable trait (Kadowaki et al., 2012; Okamura et al., 2016), and these results support investigating genes for the improvement of disease resistance.

This study was designed to broadly investigate SNVs spread across the genome, as well as some specific innate immune gene variants previously identified, through the implementation of a genome-wide association study. Identifying certain SNVs that broadly increase disease resistance may help to selectively breed pigs with more robust immune responses. However, as noted by Davies and colleagues (2009), breeding for improved immune responses should be viewed as part of a larger disease management strategy. As such, this study aimed to identify SNVs associated with PRRSV, IAV, and M. hyopneumoniae antibody responses in pigs from weaning to the end of the finisher stage.

3.3 Materials and methods

3.3.1 Study design

Farm and pig selection have been described previously (Ainslie et al., 2018; Schut et al., 2019). To summarize, fourteen groups of 50 – 60 pigs from eight farrowing sources were selected for use in this study. A total of 832 pigs were used, and a subset (n = 618) were selected for the present study on the basis that samples were collected from these pigs from at least weaning and end of nursery. From six of the eight farrowing sources, two cohorts (Cohort 1 and 2) were included in the study, while the remaining farrowing sources included only one cohort. Pigs in Cohort 1 were born between May and August, while pigs in Cohort 2 were born between
October and January. Off-site nursery and finishing were used on all but one source. Pigs were divided and tagged as part of a larger study to receive either a standard diet (high complexity; HC) or an experimental lower cost, low complexity (LC) nursery diet in which the majority of the animal protein was replaced with corn and soybean. The diets for all pigs were the same at all other production stages. A survey was distributed to producers to collect data regarding farm management practices.

### 3.3.2 Sample collection

Blood samples were collected from either the jugular vein or suborbital sinus at weaning and at the end of the nursery, grower, and finisher stages in all 14 groups except for one, where samples were not collected at the end of the finisher stage. Blood samples were centrifuged at 1500 x g for 20 minutes and sera was stored at -20 °C. Ear and tail tissue samples were collected from each pig for genotyping purposes and stored at -20 °C.

### 3.3.3 Antibody detection

Sera were analyzed for the presence of anti-PRRSV antibodies, anti-IAV antibodies, and anti-\(M.\) \textit{hyopneumoniae} antibodies using three commercially-available ELISA antibody test kits (IDEXX Laboratories, Inc., Westbrook, Maine, USA): porcine reproductive and respiratory syndrome virus antibody test kit, swine influenza virus antibody test kit, and \textit{Mycoplasma hyopneumoniae} antibody test kit, respectively, as per the manufacturer’s instructions (Appendix I).

A sample-to-positive (S/P) ratio for PRRSV antibodies was calculated as follows:

\[
S/P = \frac{\text{Sample absorbance (650)} - \text{Mean}_{\text{negative control}}}{\text{Mean}_{\text{positive control}} - \text{Mean}_{\text{negative control}}}
\]

A pig was considered seropositive for PRRSV if the \(S/P\) ratio was \(\geq 0.4\).
A sample-to-negative (S/N) ratio for IAV was calculated as follows:

\[ S/N = \frac{\text{Sample absorbance (650)}}{\text{Mean}_{\text{negative control}}} \]

A pig was considered seropositive for IAV if the S/N ratio was < 0.6.

A S/P ratio for \textit{M. hyopneumoniae} was calculated as follows:

\[ S/P = \frac{\text{Sample absorbance (650)} - \text{Mean}_{\text{negative control}}}{\text{Mean}_{\text{positive control}} - \text{Mean}_{\text{negative control}}} \]

A pig was considered seropositive for \textit{M. hyopneumoniae} if the S/P ratio was > 0.4.

### 3.3.4 Phenotype designation

Binary case/control traits were defined for each pig and pathogen in which the cases were classified as pigs that were seropositive for said pathogen at least once from end of nursery to end of finisher. The controls were pigs that remained seronegative over the course of production for the same pathogen. Seropositivity at weaning was excluded due to the potential for maternal antibodies to confound the results.

### 3.3.5 DNA extraction and genotyping

DNA was extracted from tissue samples using the Qiagen DNEasy Blood and Tissue kit following the manufacturer’s recommendations. DNA was then sent to Eurofins Genomics for genotyping using a custom 54K Affymetrix Axiom \textsuperscript{®} myDesign\textsuperscript{™} microarray chip designed in consultation with the Canadian Centre for Swine Improvement (Ottawa, Ontario, Canada). The chip contained SNVs of interest from several unrelated research groups, some of which had proprietary labels that precluded their identification in the genome and thus they were excluded from the analysis.
3.3.6 Quality control (QC) and genome-wide association study

Any SNVs that could not be mapped onto Sus scrofa chromosomes and those that had no corresponding reference SNV (rs)IDs in Ensembl Sscrofa 11.1 were removed. Quality control was performed in PLINK v1.9. Pigs with SNV call rates of less than 90% were excluded and SNVs with a minor allele frequency lower than 5% or a call rate less than 95% were excluded from further analysis. The SNV call rates were compared between cases and controls, and Fisher’s exact test was used to exclude SNVs in which the missingness between a case and control was significantly different (p < 1.0 x 10^{-5}).

Covariates were selected for inclusion if significant (p < 0.05) in univariable analysis using a mixed-effects logistic regression model in Stata (Stata/MP 13.0 for Mac, StataCorp LP, Texas, USA). For the PRRSV seropositivity model, PRRSV seropositivity at weaning, farrowing source, and nursery diet complexity were included as covariates. For the IAV seropositivity model, IAV seropositivity at weaning, farrowing source, and cohort were included as covariates. For the M. hyopneumoniae seropositivity model, farrowing source and PRRSV seropositivity from end of nursery to end of finisher were included as covariates.

A genomic relatedness matrix (GRM) was calculated in GEMMA v0.96 to account for population structure and the relatedness of pigs as there were many siblings in the study population and no sire information. All association analyses were performed using a generalized logistic mixed model association test (GMMAT) for genome-wide analysis of case-control data using the GMMAT v0.9.3 package for R. The covariates analyzed in Stata and the GRM created in GEMMA were included in the GMMAT analysis. The p-value threshold for suggestive and significant associations was 1.0 x 10^{-5} and 5.0 x 10^{-7}, respectively (Burton et al., 2007). The Wald test was used for significance, and p-values were corrected for multiple testing using the
Benjamini-Hochberg procedure (Benjamini & Hochberg, 1995). Allele frequencies in case-control populations were calculated and significance was assessed using an allelic chi-squared ($\chi^2$) test in PLINK v1.9. The average genomic inflation factors ($\lambda$) were calculated for each model in order to determine if they were able to account for relatedness and population structure without test statistic inflation (Pearson & Manolio, 2008).

3.4 Results

Following QC filtering of the data, a total of 46,409 SNVs were retained for GWAS analysis from 598, 598, and 572 pigs for the PRRSV, IAV, and M. hyopneumoniae seropositivity models, respectively. Of the pigs retained for analysis, 10.9% (65/598) were seropositive for PRRSV at least once from the end of nursery to the end of the finisher stage (i.e., were classified as “cases”), 55.4% (331/598) were seropositive for IAV, and 35.5% (203/572) were seropositive for M. hyopneumoniae.

3.4.1 Genome-wide association study

Manhattan plots and quantile-quantile (qq) plots for each pathogen are presented in Figure 3.1 and Figure 3.2, respectively. The genomic inflation factors ($\lambda$) for the PRRSV, IAV, and M. hyopneumoniae GWAS analyses were 0.97, 0.99, and 1.01, respectively.

The SNVs were sorted based on significance and the 15 most significant SNVs for each pathogen are presented in Table 3.1, Table 3.2, and Table 3.3. While no SNVs in the present study are below the recommended threshold for either a significant ($p < 5.0 \times 10^{-7}$) or suggestive ($p < 1.0 \times 10^{-5}$) association, the top three candidates nearing this threshold for each model are described.

Two intron variants on Sus scrofa chromosome (SSC) 9 in the RAB38 gene were approaching a significant association in the PRRSV model ($p = 2.85 \times 10^{-5}$ and 5.20 $\times 10^{-5}$). An
intergenic variant on SSC 10 near *BRINP3* was also approaching significance in the PRRSV seropositivity model (p = 5.78 x 10^{-5}).

In the IAV seropositivity model, an intergenic variant on SSC 13 near *PARP14*, an upstream gene variant on SSC 5 near *ADCY6*, and an intron variant on SSC 4 near the *TG* gene were approaching significance (p = 2.79 x 10^{-5}; 1.08 x 10^{-4}; 1.33 x 10^{-4}).

An intergenic variant on SSC 2 near *ENSSSCG00000051043*, an intron variant on SSC 13 near *DNASE1L3* (p = 2.11 x 10^{-4}), and an upstream gene variant of *TEKT2* on SSC 6 were nearing significance in the *M. hyopneumoniae* seropositivity model (p = 1.90 x 10^{-4}; 2.11 x 10^{-4}; 2.20 x 10^{-4}).

### 3.5 Discussion

This study was designed to investigate SNVs associated with antibody responses to porcine reproductive and respiratory syndrome virus, influenza A virus, and *Mycoplasma hyopneumoniae* using a GWAS approach. This technique allows for the exploration of candidate genes that may reveal genetic pathways to producing more robust hosts, which, when selectively bred, would decrease disease susceptibility in these animals and reduce the costs associated with infectious disease on farm.

For the model of PRRSV antibody responses, the ratio of cases to controls was low and as such, the results found should be regarded with caution. Two SNVs in the *RAB38* gene were nearing a suggestive association with PRRSV seropositivity. *RAB38* is a member of the RAS oncogene family and is expressed in alveolar type II cells, melanocytes, and platelets. In rats, *RAB38* is expressed most in the lung (Osanai, 2018), particularly in cells that produce lung surfactant, which lowers surface tension and ensures alveolar lumens are maintained (Osanai et al., 2008). The protein product, Ras-related protein Rab-38, is involved in melanin production
and melanosome biogenesis and is also associated with Hermansky-Pudlak syndrome (HPS) in humans, which usually culminates in fatal interstitial pneumonia by middle age (Osanai, 2018). While there are no studies in pigs showing a relationship between *RAB38* mutations and PRRSV susceptibility, it is important to note the expression of *RAB38* in alveolar type II cells and the preferred tropism of PRRSV for alveolar macrophages, as this may suggest that there is in fact an association between SNVs in *RAB38* and susceptibility to PRRSV. Additionally, it is possible that SNVs that alter the normal activity of *RAB38* may also disrupt the secretion of lung surfactant and change the environment of the lung, increasing the potential for amplified infectivity with PRRSV (Osanai et al., 2008).

An intergenic variant near the *BRINP3* gene, which produces BMP/retinoic acid-inducible neural-specific protein 3, was also approaching a suggestive association with PRRSV seropositivity, and variants in or near *BRINP3* were a frequent recurrence in the output from this model. In humans, this gene is mostly expressed in the brain and small intestine but is also expressed to a lesser degree in the lung (NCBI, 2020a). Under-expression of *BRINP3* is associated with ulcerative colitis and is thought to predispose or propagate the chronic inflammation seen in this condition (Smith et al., 2014). Because *BRINP3* is expressed in the intestine as well as the lung, it is possible that under-expression of the gene would produce the same result in both areas, and therefore SNVs that reduce the expression of *BRINP3* may also encourage inflammation in the lung. *BRINP3* was also implicated in a study investigating SNVs associated with meat quality in beef cattle (Xia et al., 2016). Animals that are classified as high immune responders may also display phenotypically favourable characteristics in other traits, such as milk quality and reproductive health (Crawley et al., 2005). It is postulated that if phenotypic physical traits can be markers of immune responsiveness, then genes associated with
production traits may indicate an animal will produce a robust immune response. As this gene is associated with meat quality in beef cattle, it may also have a role in meat quality in pigs, and as such, there may be a need to explore the link between these traits and immune response. However, these results are mostly speculative and would need to be explored further to elucidate the meaning behind this result.

An intergenic variant near PARP14 was nearing a suggestive association with IAV seropositivity. The PARP14 gene encodes PARP14, a member of the poly(ADP-ribose) polymerase (PARP) protein family, which promotes the expression of cellular antiviral genes while inhibiting the transcription and translation of viral RNA in humans, along with a number of other genes (Shim et al., 2017). As this gene is involved with encoding antiviral proteins, it would make an enticing target for future studies to determine if this SNV enhances immune responses to a variety of viral pathogens in addition to IAV.

An upstream variant of ADCY6, which encodes a member of the adenylyl cyclase family of proteins, was approaching a suggestive association with IAV seropositivity. The adenylyl cyclase proteins are involved in transmembrane signaling pathways. When SNVs were analyzed for a number of erythrocyte-related traits, ADCY6 was discovered to be a gene that was highly expressed in the bone marrow and could therefore have a role in red blood cell related parameters (Bovo et al., 2019). While this gene is highly expressed in the heart and kidney, it is postulated that haematological parameters may act as indicators of the health status of animals (Bovo et al., 2019). As such, SNVs that alter the activity of ADCY6 may be possible candidates for general health status and therefore disease resistance.

The TG gene is protein coding and produces thyroglobulin, and an intron variant of this gene was nearing a suggestive association with IAV seropositivity. Diseases associated with
variants in TG in humans include thyroid dyshormonogenesis 3 and autoimmune thyroid disease 3. The concept of the thyroid hormones 3,3',5,5'-tetraiodo-L-thyroxine (T4) and 3,3',5-triiodo-L-thyronine (T3) being modulators of immune responses is likely an important relationship in this case. Thyroid hormones are not suspected to be involved in the development of the immune response but they are believed to be involved in maintaining immune function (Davis, 1998; Dorshkind & Horseman, 2000). Specifically, cell-mediated immunity, natural killer cell activity, and the production of antiviral entities by interferon (IFN) are thought to be affected by thyroid hormones (De Vito et al., 2011). These findings suggest that in addition to cell-mediated immunity, humoral immunity may also be modulated either directly or indirectly by the thyroid hormone, and thus genetic variants in the gene would alter immune responses to infection.

An intergenic variant between a protein-coding gene (ENSSSCG000000051043) and a long non-coding RNA (lncRNA) gene (ENSSSCG00000049953), was found to be nearing a suggestive association with M. hyopneumoniae seropositivity. ENSSSCG00000051043 is a member of the gene tree ENSGTT0100000218776 along with ENSSSCG00000042744. Unfortunately, not much is known about any of the genes near this SNV, but further investigation into these genes may indicate their involvement in immune responses.

An intron variant in the DNASE1L3 gene, which is involved with several biochemical functions such as DNA binding, calcium ion binding, and deoxyribonuclease activity, was nearing a suggestive association with M. hyopneumoniae seropositivity. The protein product is an endonuclease which, when secreted into body fluids, mediates the breakdown of DNA during cellular apoptosis, and mutations in DNASE1L3 cause systemic lupus erythematosus-16 in humans (Onuora, 2016). Previous investigations into this gene have also found it to be upregulated in patients with the inflammatory asthma phenotype (Baines et al., 2014; Sánchez-
Ovando et al., 2020). Also, analyzing gene expression in children with severe Mycoplasma pneumoniae pneumonia (MPP) found DNASE1L3 to be among the top 10 most down-regulated genes in children with severe MPP (Wang et al., 2017). This indicates that either up or downregulation of this gene has the potential to alter the environment of the lung. Further investigation into this variant and its effects on endonuclease activity in regard to M. hyopneumoniae infection may help explain the results found in the present study.

An upstream variant of COL8A2 gene, which encodes the alpha 2 chain of type VIII collagen, was nearing a suggestive association with M. hyopneumoniae seropositivity. While typically associated with corneal abnormalities, the protein product is also required for the migration and proliferation of vascular smooth muscle cells and may therefore have a role in the maintenance of vessel wall integrity and structure. However, in examining the variant in its genomic context, this SNV is also near the TEKT2 gene, whose protein product, Tektin 2, is a structural component of ciliary and flagellar microtubes and plays a role in the attachment of the inner dynein arm to microtubules in tracheal cilia (NCBI, 2020b). While primarily expressed in the testis, TEKT2 is also expressed in the lung, and defects in TEKT2 include primary ciliary dyskinesia which is characterized by chronic respiratory tract infections (NCBI, 2020b). Animals with variants near this gene may therefore be particularly vulnerable to prolonged infection with M. hyopneumoniae, as ciliary dyskinesia due to abnormal gene functioning combined with ciliostasis due to M. hyopneumoniae infection would increase the immunosuppressive effects of infection (DeBey & Ross, 1994).

The total number of pigs in the present study, and therefore the number of cases and controls for each seropositivity model, likely resulted in a reduced ability to detect significant associations, as the number of recommended cases and controls is in the thousands (Anderson et
al., 2010). The lower number of overall subjects would reduce the likelihood that significant SNVs will be found, which may explain the lack of statistically significant SNVs found in the present study. This study also used a fairly stringent significance threshold which would reduce the likelihood of uncovering significant SNVs. However, controlling for covariates in the present study likely reduced the amount of variation seen and therefore strengthened the analysis. Additionally, the repeated testing of pigs throughout the course of production would also increase the strength of the analysis as pigs were more likely to be identified as seropositive at different points depending of production rather than if single-point testing were used. As such, we believe that these results offer a promising starting point for the investigation of candidate genes related to immune responses to PRRSV, IAV, and *M. hyopneumoniae*.

### 3.6 Conclusion

While no significant SNVs were found during the course of this study, several promising candidates were nearing a suggestive association with immune responses to PRRSV, IAV, and *M. hyopneumoniae*. Because disease resistance involves so many different factors, these SNVs may benefit health and immune responses to a wide array of pathogens if careful selection is carried out. As such, further investigation into these candidate genes, and additional genome-wide association studies that widely screen for desirable health characteristics, may help to uncover a piece of the multifaceted pathway to disease resistance.

### 3.7 Acknowledgments

Thank you to NSERC, OMAFRA, Swine Innovation Porc, the Canadian Centre for Swine Improvement, Alliance Genetics Canada, and Ontario Pork for financial support. Thank you also to Margaret Ainslie-Garcia and Corinne Schut for sample analysis, and participating pork producers.
3.8 Tables and figures

Figure 3.1. Manhattan plots of the GWAS analyses for seropositivity from end of nursery to end of finisher for PRRSV (A), IAV (B), and *M. hyopneumoniae* (C)

The horizontal solid and dashed red lines indicate the threshold for significant (*p = 5.0 x 10^{-7}*) and suggestive (*p = 1.0 x 10^{-5}*) associations, respectively.
Figure 3.2. Quantile-quantile plots for PRRSV (A), IAV (B), and *M. hyopneumoniae* (C) seropositivity from end of nursery to end of finisher

λ = the average genomic inflation factor
Table 3.1. Top 15 SNVs ranked by significance for the GWAS analysis of PRRSV seropositivity from end of nursery to end of finisher

<table>
<thead>
<tr>
<th>SNV ID</th>
<th>SSC</th>
<th>Location (bp)</th>
<th>Variant type</th>
<th>Gene</th>
<th>Gene location</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs81303379</td>
<td>9</td>
<td>21603270</td>
<td>Intron</td>
<td>RAB38</td>
<td>9: 21577172-21640115</td>
<td>2.85 x 10^-5</td>
</tr>
<tr>
<td>rs81314727</td>
<td>9</td>
<td>21586430</td>
<td>Intron</td>
<td>RAB38</td>
<td>9: 21577172-21640115</td>
<td>5.20 x 10^-5</td>
</tr>
<tr>
<td>rs339953027</td>
<td>10</td>
<td>3398590</td>
<td>Intergenic</td>
<td>BRINP3</td>
<td>10: 2963527-3367623</td>
<td>5.78 x 10^-5</td>
</tr>
<tr>
<td>rs81402382</td>
<td>8</td>
<td>88296664</td>
<td>Intergenic</td>
<td>ENSSSCG00000048383</td>
<td>8: 88433002-88437262</td>
<td>6.62 x 10^-5</td>
</tr>
<tr>
<td>rs322812546</td>
<td>10</td>
<td>3387068</td>
<td>Intergenic</td>
<td>BRINP3</td>
<td>10: 2963527-3367623</td>
<td>1.06 x 10^-4</td>
</tr>
<tr>
<td>rs343644537</td>
<td>10</td>
<td>3279972</td>
<td>Intron</td>
<td>BRINP3</td>
<td>10: 2963527-3367623</td>
<td>1.34 x 10^-4</td>
</tr>
<tr>
<td>rs331759559</td>
<td>8</td>
<td>74031613</td>
<td>Non-coding transcript exon</td>
<td>ENSSSCG00000047296</td>
<td>8: 74030037-74033237</td>
<td>1.61 x 10^-4</td>
</tr>
<tr>
<td>rs81476748</td>
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<td>88161441</td>
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<td>1.38 x 10^-4</td>
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<tr>
<td>rs81466361</td>
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<td>35410133</td>
<td>Intron</td>
<td>BCL2L1</td>
<td>17: 35366141-35415359</td>
<td>2.01 x 10^-4</td>
</tr>
<tr>
<td>rs81451792</td>
<td>15</td>
<td>4960376</td>
<td>Intergenic</td>
<td>No nearby genes</td>
<td>No nearby genes</td>
<td>2.08 x 10^-4</td>
</tr>
<tr>
<td>rs81302342</td>
<td>10</td>
<td>21726062</td>
<td>Intron</td>
<td>PTPRC</td>
<td>10: 21466373-21764560</td>
<td>2.30 x 10^-4</td>
</tr>
<tr>
<td>rs81345850</td>
<td>17</td>
<td>45916454</td>
<td>Intergenic</td>
<td>ENSSSCG00000047462</td>
<td>17: 44714620-45880822</td>
<td>3.13 x 10^-4</td>
</tr>
<tr>
<td>rs81426148</td>
<td>10</td>
<td>56950786</td>
<td>Intergenic</td>
<td>PARD3</td>
<td>10: 56967424-57600466</td>
<td>3.25 x 10^-4</td>
</tr>
<tr>
<td>rs343122135</td>
<td>17</td>
<td>37780566</td>
<td>Intergenic</td>
<td>AHCY</td>
<td>17: 37726077-37744461</td>
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</tr>
<tr>
<td>rs80808333</td>
<td>1</td>
<td>41471270</td>
<td>Intergenic</td>
<td>TBC1D32</td>
<td>1: 41057838-41265375</td>
<td>3.88 x 10^-4</td>
</tr>
</tbody>
</table>

aSSC = Sus scrofa chromosome  
bLocation in Ensembl Sscrofa 11.1  
cIf the variant was intergenic, the closest gene within a 1 Mbp window was identified
Table 3.2. Top 15 SNVs ranked by significance for the GWAS analysis of IAV seropositivity from end of nursery to end of finisher

<table>
<thead>
<tr>
<th>SNV ID</th>
<th>SSC</th>
<th>Location (bp)</th>
<th>Variant type</th>
<th>Gene</th>
<th>Gene location</th>
<th>p-value</th>
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<tbody>
<tr>
<td>rs80887016</td>
<td>13</td>
<td>137871580</td>
<td>Intergenic</td>
<td>PARP14</td>
<td>13: 137809503-137865383</td>
<td>2.79 x 10^{-5}</td>
</tr>
<tr>
<td>rs326099980</td>
<td>5</td>
<td>14854205</td>
<td>Upstream</td>
<td>ADCY6</td>
<td>5: 14834426-14850728</td>
<td>1.08 x 10^{-4}</td>
</tr>
<tr>
<td>rs80889028</td>
<td>4</td>
<td>8168520</td>
<td>Intron</td>
<td>TG</td>
<td>4: 8158396-8382435</td>
<td>1.33 x 10^{-4}</td>
</tr>
<tr>
<td>rs335864253</td>
<td>6</td>
<td>155682261</td>
<td>Intron</td>
<td>PRKAA2</td>
<td>6: 155681781-155754272</td>
<td>1.40 x 10^{-4}</td>
</tr>
<tr>
<td>rs341436291</td>
<td>11</td>
<td>30800313</td>
<td>Intergenic</td>
<td>PCDH17</td>
<td>11: 30511261-30611727</td>
<td>1.77 x 10^{-4}</td>
</tr>
<tr>
<td>rs341382366</td>
<td>11</td>
<td>30778570</td>
<td>Intergenic</td>
<td>PCDH17</td>
<td>11: 30511261-30611727</td>
<td>2.27 x 10^{-4}</td>
</tr>
<tr>
<td>rs81347177</td>
<td>9</td>
<td>133644258</td>
<td>Upstream</td>
<td>BRINP1</td>
<td>1: 259350367-259544576</td>
<td>2.34 x 10^{-4}</td>
</tr>
<tr>
<td>rs81295200</td>
<td>11</td>
<td>14357170</td>
<td>Intron</td>
<td>NHLRC3</td>
<td>11: 14184090-14381617</td>
<td>2.39 x 10^{-4}</td>
</tr>
<tr>
<td>rs341834386</td>
<td>11</td>
<td>30832645</td>
<td>Intergenic</td>
<td>PCDH17</td>
<td>11: 30511261-30611727</td>
<td>2.39 x 10^{-4}</td>
</tr>
<tr>
<td>rs81373910</td>
<td>3</td>
<td>95160980</td>
<td>Intergenic</td>
<td>SRRBD1</td>
<td>3: 94909127-95138960</td>
<td>2.40 x 10^{-4}</td>
</tr>
<tr>
<td>rs80866638</td>
<td>11</td>
<td>30730002</td>
<td>Intergenic</td>
<td>PCDH17</td>
<td>11: 30511261-30611727</td>
<td>2.79 x 10^{-4}</td>
</tr>
<tr>
<td>rs342563198</td>
<td>15</td>
<td>5514782</td>
<td>Intergenic</td>
<td>No nearby genes</td>
<td>No nearby genes</td>
<td>3.05 x 10^{-4}</td>
</tr>
<tr>
<td>rs81326533</td>
<td>2</td>
<td>144946273</td>
<td>Intron</td>
<td>NRC31</td>
<td>2: 144822937-144956095</td>
<td>3.19 x 10^{-4}</td>
</tr>
<tr>
<td>rs328668992</td>
<td>11</td>
<td>30805782</td>
<td>Intergenic</td>
<td>PCDH17</td>
<td>11: 30511261-30611727</td>
<td>3.72 x 10^{-4}</td>
</tr>
</tbody>
</table>

\( ^a \text{SSC} = \text{Sus scrofa chromosome} \)

\( ^b \text{Location in Ensembl Suscrofa 11.1} \)

\( ^c \text{If the variant was intergenic, the closest gene within a 1 Mbp window was identified} \)
### Table 3.3. Top 15 SNVs ranked by significance for the GWAS analysis of *M. hyopneumoniae* seropositivity from end of nursery to end of finisher

<table>
<thead>
<tr>
<th>SNV ID</th>
<th>SSC</th>
<th>Location (bp)</th>
<th>Variant type</th>
<th>Gene</th>
<th>Gene location</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs346069120</td>
<td>2</td>
<td>110919886</td>
<td>Intergenic</td>
<td>ENSSSCG00000051043</td>
<td>2: 111154780-1111611113</td>
<td>1.90 x 10^{-4}</td>
</tr>
<tr>
<td>rs81445130</td>
<td>13</td>
<td>39968087</td>
<td>Intron</td>
<td>DNASE1L3</td>
<td>13: 39954804-39975946</td>
<td>2.11 x 10^{-4}</td>
</tr>
<tr>
<td>rs319409139</td>
<td>6</td>
<td>92275595</td>
<td>Upstream</td>
<td>TEKT2*</td>
<td>6: 92233586-92237900</td>
<td>2.20 x 10^{-4}</td>
</tr>
<tr>
<td>rs80821715</td>
<td>14</td>
<td>70874378</td>
<td>Intron</td>
<td>CTNNA3</td>
<td>14: 69200215-70938204</td>
<td>2.39 x 10^{-4}</td>
</tr>
<tr>
<td>rs80891253</td>
<td>4</td>
<td>274403</td>
<td>Upstream</td>
<td>C8orf82</td>
<td>4: 275917-278547</td>
<td>2.68 x 10^{-4}</td>
</tr>
<tr>
<td>rs81304138</td>
<td>2</td>
<td>141877566</td>
<td>Upstream</td>
<td>ENSSSCG00000014361</td>
<td>2: 141879128-142010238</td>
<td>2.70 x 10^{-4}</td>
</tr>
<tr>
<td>rs343926712</td>
<td>12</td>
<td>42585364</td>
<td>Intron</td>
<td>ZNF207</td>
<td>12: 42571406-42612396</td>
<td>2.78 x 10^{-4}</td>
</tr>
<tr>
<td>rs80798251</td>
<td>4</td>
<td>116861512</td>
<td>Intron</td>
<td>ENSSSCG00000048322</td>
<td>4: 116800929-116917677</td>
<td>5.37 x 10^{-4}</td>
</tr>
<tr>
<td>rs330773844</td>
<td>17</td>
<td>54153176</td>
<td>Intergenic</td>
<td>ENSSSCG00000046693</td>
<td>17: 53824361-53829207</td>
<td>6.03 x 10^{-4}</td>
</tr>
<tr>
<td>rs80969821</td>
<td>1</td>
<td>221303393</td>
<td>Intron</td>
<td>DOCK8</td>
<td>1: 221256009-221491953</td>
<td>6.08 x 10^{-4}</td>
</tr>
<tr>
<td>rs81274107</td>
<td>2</td>
<td>112115880</td>
<td>Intergenic</td>
<td>EFNA5</td>
<td>2: 112534917-112815628</td>
<td>6.42 x 10^{-4}</td>
</tr>
<tr>
<td>rs80897004</td>
<td>4</td>
<td>125150795</td>
<td>Intron</td>
<td>TGFBR3</td>
<td>4: 125101190-125316796</td>
<td>6.54 x 10^{-4}</td>
</tr>
<tr>
<td>rs81325155</td>
<td>4</td>
<td>1232672</td>
<td>Splice region</td>
<td>ENSSSCG00000043108</td>
<td>4: 1232480-1233063</td>
<td>7.62 x 10^{-4}</td>
</tr>
<tr>
<td>rs80896266</td>
<td>5</td>
<td>84801267</td>
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<td>17</td>
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<td>Intergenic</td>
<td>ZFP64</td>
<td>17: 53369672-5345999</td>
<td>7.91 x 10^{-4}</td>
</tr>
</tbody>
</table>

*a*SCC = *Sus scrofa* chromosome  
*b*Location in Ensembl Sscrofa 11.1  
*c*If the variant was intergenic, the closest gene within a 1 Mbp window was identified  
*d*This gene was not associated with the variant in Ensembl 11.1 but was determined to be potentially affected by the SNV
GENERAL DISCUSSION AND CONCLUSIONS

Porcine reproductive and respiratory syndrome virus, influenza A virus, and *Mycoplasma hyopneumoniae* are three respiratory pathogens with the potential to have devastating impacts on swine health, production costs and profits, and, in the case of IAV, a potential for viral reassortment in the pig and the introduction of novel influenza viruses. In addition, the desire to reduce the use of antimicrobials in order to minimize the potential for antimicrobial resistance increases the risk of transmitting and contracting infectious diseases, making the need for alternate methods of disease control of paramount importance (Davies et al., 2009; Kaiser, 2010). Understanding which diseases are prevalent on-farm and at which stages of production will help to identify specific periods of vulnerability on a farm-to-farm basis. In addition, the concept of breeding for disease resistance is a growing field of interest and may help to broadly enhance immunity and reduce the need for antimicrobial use and the costs associated with infectious disease on-farm (Davies et al., 2009).

The first objective of this thesis was to characterize patterns of antibody responses to PRRSV, IAV, and *M. hyopneumoniae* in 14 groups of pigs in Ontario from weaning to the end of the finisher stage. Antibodies were detected at high levels in 3, 14, and 5 out of 14 groups for PRRSV, IAV, and *M. hyopneumoniae*, respectively. For all three pathogens, antibody levels were high at weaning due to high levels of maternal antibodies. After pigs were weaned, the antibody levels dropped by the end of the nursery stage as maternal antibodies declined (Butler et al., 2006; Chase & Lunney, 2012), indicating a period of particular disease susceptibility for weanlings. Antibody levels for PRRSV and IAV then began to rise again at the end of grower stage, and continued until the end of finisher stage, indicating exposure to the viruses and the development of active immune responses. However, as there was no significant change in
antibody levels for *M. hyopneumoniae* after the end of nursery, these results seem to suggest that pigs were not being exposed or that their active adaptive immune responses were low, slow, or absent (Erlandson et al., 2005). Due to the immunosuppressive effects of *M. hyopneumoniae*, the probability of the immune response being delayed after pigs were exposed is high (Caruso & Ross, 1990). Understanding the pathogenic threats to a herd, as well as the immune responses to specific pathogens, on a farm-by-farm basis is important in reducing the burden of infectious disease, as exposure can change depending on geographical region, time of year (Chiari et al., 2014; Rosendal et al., 2014), and biosecurity practices (Desrosiers, 2011; Levis et al., 2015), and immune responses can change in response to infection based on age (Klinge et al., 2009; Markowska-Daniel et al., 2011), breed (Reiner et al., 2010), and genetics (Dunkelberger, 2017), among many other factors. Frequent monitoring of the pathogens afflicting individual farms would help ensure control measures can be carefully tailored while helping to reduce the site-specific disease pressures.

Determining the effects of feeding a low complexity nursery diet on antibody responses to PRRSV, IAV, and *M. hyopneumoniae* was the second objective of this thesis. Previously, nursery diet complexity has been shown to have no detrimental effects on growth rate, carcass quality (Skinner et al., 2014), or antibody responses to Salmonella (Schut et al., 2019). Nursery diet complexity had no effect on antibody responses to *M. hyopneumoniae* in the present study, but pigs that were fed a low complexity nursery diet were more likely to be seropositive for both PRRSV and IAV. This may indicate that these pigs had weakly effective innate immune responses and therefore more pigs were seropositive, or that they were more capable of mounting a robust adaptive immune response. As such, further investigation into the effects of feeding a
low complexity diet are needed to determine if the immune response is affected for other pathogens, and if the diet is safe to feed on farms with moderate disease pressures.

The final objective of this thesis was to identify single-nucleotide variants associated with antibody responses to PRRSV, IAV, and *M. hyopneumoniae* using a genome-wide association study approach. Two SNVs in *RAB38*, whose protein product is found in high concentrations in the lungs, were found to be nearing a suggestive association with PRRSV antibody responses. Mutations in this gene are thought to cause unusual alveolar structure and abnormal lung surfactant homeostasis in mice (Osanai et al., 2008). The tropism of the PRRS virus to alveolar macrophages may indicate that variants in or near this gene may increase susceptibility to infection. A third SNV that was nearing a suggestive association with PRRSV seropositivity was an intergenic variant near *BRIP3*. This gene has been found to be associated with meat quality markers such as colour and muscle area in beef cattle (Xia et al., 2016). Animals that have high antibody and cell-mediated immune responses also exhibit other favourable production traits, such as improved weight gain in infected animals, and as such, there may be a link between immune response and productivity traits (Mallard et al., 2015; Wilkie & Mallard, 1999). This indicates that genes associated with general health traits, such as reproductive health and meat and milk quality, may also be associated with more favourable responses to infectious disease.

A SNV near *PARP14*, which promotes the expression of antiviral genes, was found to be associated with IAV seropositivity. Future research into the effects of this variant on other significant viral pathogens affecting swine farms may benefit from further research into this variant. Two other SNVs, an upstream variant of *ADCY6* and an intron variant in *TG*, were also found to be approaching significance to IAV seropositivity. *ADCY6* is highly expressed in the bone marrow and is thought to be a haematological marker of health status (Bovo et al., 2019),
and the TG gene, which produces thyroglobulin, may be involved in maintaining immune function (Davis, 1998). As such, these genes may be useful targets for further elucidation into their effects on immune response and their potential for disease resistance.

An intergenic variant near a currently unidentified gene, ENSSSCG00000051043, along with an intron variant in DNASE1L3 and a variant near TEKT2 were nearing a suggestive association with M. hyopneumoniae seropositivity. Because not much is known about ENSSSCG00000051043, further research is needed to determine its function and its potential involvement in disease resistance. DNASE1L3 is downregulated in children with severe Mycoplasma pneumoniae pneumonia (Wang et al., 2017) and may therefore be involved in susceptibility to M. hyopneumoniae infections in pigs. Variation in the expression of TEKT2, which causes ciliary dyskinesia, may exacerbate the ciliostasis that is associated with M. hyopneumoniae and could potentially produce more severe disease in animals with this variant.

The work outlined in this thesis may help pork producers gain a better understanding of the potential threats to the health of their herd and may encourage more frequent testing for and vaccination against the pathogenic risks on a farm-by-farm basis. The variants described herein appear to all play a role in health or immunity in pigs and may therefore be worthwhile to investigate further as candidate genes for disease susceptibility and resistance. Understanding which SNVs increase susceptibility to various pathogens may allow for these genes to be avoided, if possible, in breeding programs, while selecting for SNVs that broadly enhance immunity would allow for breeding more robust hosts, increasing general herd health and producer profits.
SUMMARY

1. Pigs in most groups were seropositive for IAV and *M. hyopneumoniae* infection, but PRRSV seropositivity was rare. However, if PRRSV was present in a group, most pigs in that group were PRRSV seropositive.

2. Seropositivity for PRRSV, IAV, and *M. hyopneumoniae* was high at weaning due to the presence of maternal antibodies, and the lowest at the end of nursery, indicating periods of increased disease susceptibility on farm. Seropositivity for PRRSV and IAV began to rise after the end of nursery but did not increase significantly for *M. hyopneumoniae* for the rest of production.

3. Nursery diet complexity may be associated with antibody responses to PRRSV and IAV.

4. Two intron variants in *RAB38* and an intergenic variant near *BRINP3* were nearing a suggestive association with PRRSV seropositivity.

5. An intergenic variant near *PARP14*, an upstream variant of *ADCY6*, and an intron variant in *TG* were nearing a suggestive association with IAV seropositivity.

6. An intergenic variant near *ENSSSCG00000051043*, an intron variant in *DNASE1L3*, and an upstream variant of *TEKT2* were nearing a suggestive association with *M. hyopneumoniae* seropositivity.
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APPENDICES

Appendix I: ELISA principles

The following describes the principles for the ELISA antibody test kits used in this study:

The IDEXX PRRS X3, Swine Influenza, and *M. hyo* antibody test kits are enzyme immunoassays designed to detect antibodies to PRRS virus, influenza A virus, or *M. hyopneumoniae*, respectively, in swine serum. The procedures are as follows: samples are added to 96-well microtitre plates coated with PRRS virus antigen, avian influenza virus nucleoprotein (NP) antigen, or *M. hyopneumoniae* antigen. Samples are incubated and unbound material is washed away. An anti-porcine: horseradish peroxidase conjugate is added to samples in the PRRS and *M. hyopneumoniae* kits, which binds to any attached porcine antibody in the wells, while an anti-NP monoclonal antibody enzyme conjugate is added to samples in the IAV kits, and is blocked from binding to the NP antigen if antibodies are present in the serum. Unbound conjugate is then washed away and 3,3`,5,5`-tetramethylbenzidine (TMB) substrate is added to the wells. Colour development is directly related to the amount of PRRSV and *M. hyopneumoniae* antibodies present in the test sample, and inversely proportional to the amount of IAV antibodies in the test sample.
**Appendix II:** Combination frequency of pigs that were seropositive for porcine reproductive and respiratory syndrome virus (PRRSV) at different stages of production

This image displays pigs that were tested at four visits for PRRSV and were seropositive at least once over the course of production. The number of pigs that were seropositive for each intersection of stages are shown above the corresponding bar.
Appendix III: Combination frequency of pigs that were seropositive for influenza A virus (IAV) at different stages of production

This image displays pigs that were tested at four visits for IAV and were seropositive at least once over the course of production. The number of pigs that were seropositive for each intersection of stages are shown above the corresponding bar.
Appendix IV: Combination frequency of pigs that were seropositive for *Mycoplasma hyopneumoniae* at different stages of production.

This image displays pigs that were tested at four visits for *M. hyopneumoniae* and were seropositive at least once over the course of production. The number of pigs that were seropositive for each intersection of stages are shown above the corresponding bar.
### Appendix V: Mixed-effects multi-level logistic regression analysis for porcine reproductive and respiratory syndrome virus (PRRSV) seropositivity

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Odds ratio</th>
<th>Standard error</th>
<th>95% confidence interval</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Production stage</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>At weaning</td>
<td>11.40</td>
<td>5.35</td>
<td>4.54 - 28.58</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>End of nursery</td>
<td>Referent</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>End of grower</td>
<td>15.70</td>
<td>7.49</td>
<td>6.16 - 40.00</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>End of finisher</td>
<td>30.08</td>
<td>15.09</td>
<td>11.25 - 80.41</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Nursery diet</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HC^A</td>
<td>Referent</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>LC^A</td>
<td>4.61</td>
<td>1.26</td>
<td>2.68 - 7.88</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Farrowing source</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Referent</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>8</td>
<td>3.71</td>
<td>2.00</td>
<td>1.28 - 10.66</td>
<td>0.015</td>
</tr>
</tbody>
</table>

This table displays the mixed-effects multi-level logistic regression analysis for porcine reproductive and respiratory syndrome virus (PRRSV) seropositivity with sow as random effect in 3 high seropositivity groups. Seropositivity was determined by commercial ELISA.

^HC = high complexity diet, LC = low complexity diet.
**Appendix VI**: Mixed-effects multi-level logistic regression analysis for influenza A virus (IAV) seropositivity

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Odds ratio</th>
<th>Standard error</th>
<th>95% confidence interval</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Production stage</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>At weaning</td>
<td>6.42</td>
<td>0.98</td>
<td>4.76 - 8.66</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>End of nursery</td>
<td>Referent</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>End of grower</td>
<td>1.56</td>
<td>0.24</td>
<td>1.16 - 2.11</td>
<td>0.003</td>
</tr>
<tr>
<td>End of finisher</td>
<td>2.45</td>
<td>0.40</td>
<td>1.78 - 3.37</td>
<td>&lt; 0.001</td>
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<tr>
<td>Cohort</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>One</td>
<td>Referent</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Two</td>
<td>4.09</td>
<td>0.91</td>
<td>2.65 - 6.31</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

This table displays the mixed-effects multi-level logistic regression analysis for influenza A virus (IAV) seropositivity with farrowing source and sow as random effects in 14 high seropositivity groups. Seropositivity was determined by commercial ELISA.
**Appendix VII: Mixed-effects multi-level logistic regression analysis for *Mycoplasma hyopneumoniae* seropositivity**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Odds ratio</th>
<th>Standard error</th>
<th>95% confidence interval</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Production stage</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>At weaning</td>
<td>7.85</td>
<td>2.11</td>
<td>4.63 - 13.31</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>End of nursery</td>
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<td>-</td>
<td>-</td>
</tr>
<tr>
<td>End of grower</td>
<td>0.72</td>
<td>0.18</td>
<td>0.44 - 1.17</td>
<td>0.185</td>
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<td>0.33</td>
<td>0.73 - 2.06</td>
<td>0.449</td>
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<tr>
<td><strong>PRRSV^ seropositivity</strong></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>Referent</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Yes</td>
<td>6.77</td>
<td>3.19</td>
<td>2.69 - 17.03</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td><strong>Cohort</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>One</td>
<td>Referent</td>
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<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Two</td>
<td>0.30</td>
<td>0.09</td>
<td>0.17 - 0.53</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

This table displays the mixed-effects multi-level logistic regression analysis for *Mycoplasma hyopneumoniae* seropositivity with farrowing source and sow as random effects in 5 high seropositivity groups. Seropositivity was determined by commercial ELISA.

^PRRSV = Porcine reproductive and respiratory syndrome virus