Genetic and biological factors influencing host response to porcine reproductive and respiratory syndrome virus in growing pigs

by

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Porcine Reproductive and Respiratory Syndrome (PRRS) is a disease of major importance in the swine industry due to its economic impact and its negative effects on animal welfare. PRRS has been estimated to cost the US swine industry alone $664 million per year and impacts all stages of swine production. Current efforts to contain PRRS include biosecurity and vaccination strategies but these have not been widely successful, partly due to large genetic and antigenic variation observed between different PRRS virus (PRRSV) strains. The PRRS Host Genetics Consortium was created with the goal of increasing understanding of the host genetic response to PRRSV infection and evaluating the potential of genetic selection of pigs that are more resistant to PRRS. Approximately 200 commercial crossbred pigs in each of 15 trials were experimentally infected with either the NVSL-97-7895 (NVSL) or KS-2006-78109 (KS06) isolate of PRRSV at approximately 28 days of age. Serum was collected at 0, 4, 7, 11, 14, 21, 28, 35 and 42 days post infection (dpi). Weights were collected at 0 dpi and weekly thereafter. Tonsils and ear samples for genotyping were collected at 42 dpi. Serum viremia and tonsil virus levels were measured using a semi-quantitative PCR assay. The levels of serum PRRSV N protein specific immunoglobulin G was quantified at 42 dpi using fluorescent microsphere immunoassay, and measurements were reported as a sample to positive ratio (S:P). Monophasic or biphasic Wood’s curves were fitted to serum viremia records, and the parameters for these curves were used to derive serum curve characteristics for each animal. Additionally, 2nd order Legendre polynomials were fitted to weight measurements. Serum viremia and weight curve fittings allowed daily serum viremia and daily weights to be interpolated to
further explore the dynamics of host response to PRRSV infection. The objectives of this dissertation were to: 1) investigate the potential to select for pigs with increased resistance to PRRS that is independent of virus isolate; 2) assess the usefulness of antibody response as an indicator trait of increased PRRS resistance and characterize the relationship of host antibody response with viremia and weight gain; and 3) investigate whether there is a heritable genetic component to tonsil virus levels and identify factors associated with lower tonsil virus levels in order to reduce PRRSV persistence.

NVSL was found to be a more virulent isolate than KS06, but host genetic response to PRRSV influencing peak serum viremia, weight gain, and S:P is expected to be similar during infection with either of these isolates, as evidenced by the estimated strong positive genetic correlations of response to NVSL infection with KS06 infection. A SNP (WUR10000125) that was previously found to be associated with viral load and weight gain under PRRSV infection with the NVSL isolate was found to be associated with viral load and peak viremia in pigs infected with NVSL or KS06; however, there was no observed effect on weight gain in KS06 infected pigs. This suggests the effect of the quantitative trait locus (QTL) marked by WUR may be dependent on the virulence of the PRRSV variant. The relationships between serum viremia and three-day intervals of weight gain changed throughout the course of infection, and were particularly different for pre- and post-peak serum viremia levels. A dynamic relationship was also observed between S:P and serum viremia or three-day intervals weight gain, suggesting that in order to fully explore the relationship between different PRRS response traits, they need to be considered at multiple time points throughout infection. Three QTL, located in the major histocompatibility complex (MHC), were found to be associated with S:P. These QTL
were not associated with serum viremia levels or weight gain, suggesting that the genetic correlations of S:P with these traits are due to regions outside of the MHC. Peak viremia, S:P and weight gain during PRRSV infection were identified as traits which have the most potential for successful selection for improved response to PRRSV infection due to high heritability estimates, high genetic correlations with PRRS serum viral load and the ability to measure these traits relatively easily in an industry setting.
CHAPTER I

INTRODUCTION

Motivation

Porcine Reproductive and Respiratory Syndrome (PRRS) is one of the foremost concerns in the swine industry due to its economic impact and its negative effects on animal welfare. PRRS has been prevalent in swine herds across the world for over 25 years and has been estimated to cost the US swine industry alone $664 million per year (Holtkamp et al., 2013). PRRS affects all stages of swine production because, as its name suggests, it is both a reproductive and a respiratory disease. In breeding age pigs, PRRS causes reduced fertility and increased piglet mortality, while in growing pigs the respiratory aspect is of major concern, with decreased performance and increased mortality rates (Christianson and Joo, 1994). Current efforts to contain PRRS include biosecurity and vaccination strategies but these have not been widely successful (Darwich et al., 2010). Genetic selection of pigs that are more resistant to PRRS may help mitigate the costs and animal welfare concerns associated with the disease (Lewis et al., 2007).

PRRS is caused by a positive, single strand RNA virus called PRRS virus (PRRSV) that belongs the Arteriviridae family of viruses. Other viruses in this family include equine arteritis virus, lactate dehydrogenase elevating virus, and simian hemorrhagic fever virus, which infect horses, mice and humans, respectively (Wensvoort et al., 1992). A defining characteristic of the Arteriviridae family is the large genetic and antigenic variability within
each species of virus (Han et al., 2006; Van Doorsselaere et al., 2012). The source of this genetic variation is the virus’ ability to rapidly mutate and is further diversified by the ability of different variants to recombine, creating new variants (Kappes and Faaberg, 2015). As a result, the clinical pathology can vary substantially between PRRSV isolates (Halbur et al., 1995; Halbur et al., 1996).

If a pig recovers from infection from one PRRSV isolate it may not be fully protected upon re-exposure to a heterologous PRRSV isolate due to the large genetic and antigenic variation across PRRSV variants. The most frequently used PRRS vaccine is an attenuated live virus, modified from the parent PRRSV isolate VR-2332 (Storgaard et al., 1999). While most pigs successfully produce antibodies in response to this vaccine, antibodies against the vaccine do not guarantee cross-protection to genetically distinct PRRSV variants (Roca et al., 2012; Trus et al., 2014).

PRRSV is able to remain present in a herd for an extended period of time due to its ability to withstand varying environmental conditions and its persistence within tissues. PRRSV has been shown to survive outside its host for years under the right conditions (e.g. low temperatures with low relative humidity) (Bloemraad et al., 1994; Zimmerman et al., 2010) and within tissues such as the tonsils and lymph nodes for several months after infection, potentially resulting in pigs that are shedding virus despite the lack of clinical signs of PRRS (Wills et al., 2003). These factors particularly impact naïve pigs that have been introduced to the herd following an outbreak because these pigs will not have the protective immunity achieved from exposure to the isolate of PRRSV that is circulating in the herd (Rowland and Morrison, 2012; Wills et al., 1997).
The PRRS Host Genetics Consortium, a multi-institutional effort involving several North American breeding companies and universities, was established with the goal of increasing understanding of the host genetic response to PRRSV infection (Lunney et al., 2011). Genetic selection for pigs with increased resistance to PRRS is desirable due to its economic and animal welfare impacts on the swine industry, the genetic variability of the virus, the lack of a cross-protective vaccine, and the ability of the virus to persist in herds for an extended period of time (Wiese, 2005).

The initial studies from the PHGC have shown much promise in the potential of genetic selection for pigs with increased resistance to PRRS. Both viral load, defined as area under the curve of log10 serum viremia from 0-21 days post infection (VL), and weight gain during a 42 day trial period (WG) following experimental infection of nursery piglets with PRRSV isolate NVSL-97-7895 were shown to be moderately heritable and negatively correlated, both genetically and phenotypically (Boddicker et al., 2012). Furthermore, a large effect quantitative trait locus (QTL) for both these traits was identified on chromosome 4. All of the genetic variation in this region associated with VL and WG was captured by one single nucleotide polymorphism (SNP), WUR10000125, for which AB animals had lower viral load and higher weight gain, compared to AA animals (Boddicker et al., 2012). The observed effect of this QTL was shown to be conserved across breeds, and the causal mutation was shown to be in linkage disequilibrium (LD) with the tag SNP WUR10000125 (Boddicker et al., 2014a; Boddicker et al., 2014b). A putative causal mutation has since been identified in the gene GBP5, which plays a role in pro-inflammatory response (Koltes et al., 2015).
Research Objectives

While genetic selection for increased resistance to PRRS appears promising based on these initial studies, further characterization is necessary before implementation in the swine industry. One area of major concern is whether the genetics associated with host response to infection is consistent between genetically distinct PRRSV isolates. To this end, additional PHGC trials were conducted in which animals were infected with a different isolate of PRRSV: KS06-72109 (Ladinig et al., 2015). Also, while serum viremia and weight gain have been explored, the genetic correlation of antibody response with these traits during PRRSV infection has not been assessed. Despite the importance of measuring serum viremia, a major problem surrounding PRRSV is its ability to remain in swine herds due to its ability to persist in tissues, such as in the tonsils. Animals with a persistent infection have the potential to shed PRRSV despite the lack of clinic signs of PRRS. Therefore, identifying a heritable genetic component to this trait and/or correlates that are more easily measured, would be useful for identifying animals with persistent viremia.

The objectives of this thesis were to: 1) investigate the potential to select for pigs with increased resistance to PRRS that is independent of virus isolate; 2) assess the usefulness of antibody response as an indicator trait of increased PRRS resistance and characterize the relationship of host antibody response with viremia and weight gain; and 3) investigate whether there is a heritable genetic component to tonsil virus levels and identify factors associated with lower tonsil virus levels in order to reduce PRRSV persistence.
Organization

The organization of this thesis is as follows: Chapter 2 is a review of the current literature on PRRS, which gives an overview of the contributing factors to PRRSV genetic variation, the challenges associated with containment of the disease, a description of the humoral immune response to PRRSV infection, an explanation of why vaccination has not been successful, and the current body of knowledge surrounding the potential of genetic selection for increased resistance to PRRS. Chapter 3 compares host response to experimental infection with the NVSL and KS06 PRRSV isolates, both phenotypically and genetically, and evaluates the WUR SNP as a genetic marker for increased resistance to PRRS. Chapter 4 explores the potential of antibody response to be used as a biomarker for increase disease resistance, through its relationship with viremia and weight gain, as well as the identification of QTL associated with antibody response. Chapter 5 estimates the heritability of tonsil virus levels and identifies possible predictors of tonsil virus levels. Chapter 6 discusses the current studies from the PHGC trials and how the results can be utilized to establish an effective selection program for increased resistance to PRRSV infection. Chapter 7 is the final discussion of the research in this dissertation and identifies further areas of research to increase our understanding of the biological mechanisms of PRRSV infection.

References


Porcine Reproductive and Respiratory Syndrome

Impact of porcine reproductive and respiratory syndrome

Porcine Reproductive and Respiratory Syndrome (PRRS) is a disease of major concern from both an economic and animal welfare standpoint. Since it was first identified, PRRS has become a global swine disease, with only Australia, New Zealand, and Switzerland still PRRS free (fao.org, 2015). Although a global cost assessment of the impact of PRRS is not available, the estimated annual cost of the disease to the United States swine industry is $664 million (Holtkamp et al., 2013). PRRS affects all stages of production. In reproductive age pigs, sows infected with PRRSV have an increased incidence in premature farrowing and a higher proportion of piglets are mummified, born dead, or weaker than normal (Mengeling et al., 2000). In boars, a reduction in libido may also be observed, as well as a reduction in sperm quality (Christianson and Joo, 1994). In general, infection is associated with inappetance, depression, and lethargy, as well as pyrexia. Interstitial pneumonia, dyspnea, stunted growth, and increased incidence of mortality are also common in growing pigs (Christianson and Joo, 1994).

Cause of porcine reproductive and respiratory syndrome

The causal agent of PRRS, a virus, was isolated in separate efforts in the Netherlands and the United States (Collins et al., 1992; Wensvoort et al., 1992b). The virus
was identified in alveolar macrophages as being a positive-sense single-stranded RNA virus with similar characteristics as the Lactate Dehydrogenase Virus and the Equine Arteritis Virus (Wensvoort et al., 1992b). These three viruses are in the viral family Arteriviridae and order nidovirales (Meulenberg et al., 1993), which also includes the families coronaviridae and roniviridae. Two major subtypes of PRRSV have been identified, represented by prototype Lelystad (Type 1 or European strains) and prototype VR-2332 (Type 2 or North American strains) (Wensvoort et al., 1992a). PRRSV has been isolated from lung, tonsil, lymph node, thymus, spleen, heart, brain, and kidney tissues of pigs, and primarily infects monocytes, macrophages, and dendritic cells (Halbur et al., 1996a; Halbur et al., 1995; Magar et al., 1993). The tropism of PRRSV for macrophages was confirmed soon after identification of the virus (Duan et al., 1997b), particularly for alveolar macrophages (Duan et al., 1997a).

**Viral entry into the host cell**

Entry of PRRSV into macrophages is mediated by a number of protein interactions between the host cell and the virus. Heparan sulphate was the first identified mediator of infection (Delputte et al., 2002). Heparan sulphate is a proteoglycan, with covalently attached glycosaminoglycans (GAGs). Virions are thought to concentrate on the cell surface by attaching to GAGs, which stabilizes them and permits a more efficient infection of that cell (Delputte et al., 2005). The viral M/GP5 complex is the likely PRRSV ligand for heparin sulphate (Delputte et al., 2002; Vanderheijden et al., 2001). This ligand is not restricted to macrophages, thus is not the sole mediator of PRRSV infection (Bernfield et al., 1999). Sialoadhesin, another a mediator of PRRSV infection (Vanderheijden et al., 2003), is a macrophage-restricted type 1 transmembrane glycoprotein and is a member of
a family of sialic acid-binding lectins called siglecs (Munday et al., 1999). This protein is sufficient for PRRSV entry into cells via clathrin-mediated endocytosis but is not a sufficient for the disassembly of the nucleocapsid (Vanderheijden et al., 2003). Entry of PRRSV via this receptor is mediated through α2-3-linked and α2-6-linked sialic acids on the surface of the virion. The virus travels to early endosomes (pH 6.0-6.5) and is released (Van Gorp et al., 2009). Scavenger receptor CD163 is a type 1 transmembrane glycoprotein mainly expressed on cells of a monocyte/macrophage lineage (Van Gorp et al., 2008, 2009). CD163 is responsible for viral uncoating and genome release once PRRSV has been internalized, through interactions with GP2 and GP4 (Das et al., 2010). Aspartic protease cathepsin E and serine proteases have also been implicated as being involved in PRRSV infection, although their exact role is unclear (Lee and Yoo, 2006). Van Breedam et al. (2010) developed a model based on the aforementioned information to describe viral entry into macrophages.

**PRRSV replication and genome structure**

Upon entry into the cell, the PRRSV particles migrate to the vesicles of the endoplasmic reticulum and the golgi apparatus (Thanawongnuwech et al., 1997), where they then rearrange host cell membranes to establish a viral replication complex (RC). The RC plays a structural and/or functional role by offering a suitable microenvironment for viral RNA synthesis, or may facilitate the recruitment of membrane associated host cell proteins for the purpose of viral replication and transcription (Pedersen et al., 1999).

Viruses have evolved to have small genomes in order to replicate as quickly as possible. As such, any host cell machinery that can be utilized results in fewer required viral genes and a smaller genome that can be replicated faster. Positive-sense single-
stranded RNA viruses are able to use the host cell machinery to translate the viral genome into the required proteins for virus replication. The PRRSV genome ranges from 14.9 to 15.5 kb (Conzelmann et al., 1993) and is organized into overlapping open reading frames (ORF) 1 to 7. The first PRRSV genes that are translated are ORF1a and ORF1b, which encode at least 16 replicase non-structural proteins (nsps) (Kappes and Faaberg, 2015). These proteins are required because the host cell does not have the ability to replicate RNA from an RNA template, which is achieved by the virus through a replication complex, which includes an RNA-dependent RNA polymerase (nsp9). The nsps provide the necessary replication machinery and are responsible for replication of the viral genome (gRNA) for new virion particles and also replicates ORFs 2-7 in the form of subgenomic RNA (sgRNA) via a negative-strand intermediate (Snijder and Meulenberg, 1998). This is done through an abortive disjoining/rejoining discontinuous transcription strategy (Meng et al., 1996).

The sgRNAs encode eight structural proteins that are involved in formation on the new virion particles (Kappes and Faaberg, 2015). ORF2 encodes 2 proteins, with ORF2a encoding a surface protein glycoprotein 2 (GP2), and ORF2b encoding the envelope protein (E). ORFs 3-5 produce the surface proteins GP 3-5; with GP2, GP3 and GP4 form a trimeric complex resulting in the minor glycoprotein complex which functions in viral entry into the cell. ORF5a encodes the ORF5a protein, which is required for virion viability. ORF6 encodes the membrane protein (M), which forms a heterodimer with GP5. The nucleocapsid is encoded by ORF7, which is the major structural element with the PRRSV virion which functions to package the viral genomic RNA (gRNA). GP5, M, and N make up the majority of the protein content of PRRSV (Kappes and Faaberg, 2015).
**Variation in PRRSV**

PRRSV, similar to other arteriviruses, is characterized by a large amount of genetic and antigenic variability. Molecular evolution studies have reported the level of genetic diversity between variants within each subtype at greater than 15% when comparing whole genomes (Han et al., 2006; Van Doorselaere et al., 2012), compared to a ~40% difference between subtypes (Forsberg, 2005). Genetic differences between PRRSV variants result in varying degrees of pathogenicity among PRRSV variants (Halbur et al., 1995; Halbur et al., 1996b). The primary source of this diversity is the virus' ability to mutate, presumably due to the lack of proofreading ability of the PRRSV RNA-dependent RNA polymerase, resulting in a high error rate during replication of the gRNA (Kappes and Faaberg, 2015). Another source of the genetic variation observed in PRRSV is the ability of one isolate to recombine with other PRRSV isolates, creating mosaic isolates (Martin-Valls et al., 2014). The large polycistronic RNA genome of PRRSV and the transcription of a nested set of 5’, 3’ co-terminal sgRNAs through a discontinuous transcription strategy, contributes to the complexity of PRRSV, along with other arteriviruses, compared to other positive strand RNA viruses (Gorbalenya et al., 2006; van Hemert et al., 2008). Emerging isolates rapidly spread in swine herds and are characterized by insertions or deletions (e.g. in nsp2) (Brockmeier et al., 2012) and may contain detectable recombination breakpoints (Shi et al., 2013). The flexibility of the PRRSV genome drives many of the difficulties associated with eradication of this virus from the pig industry, particularly through vaccination efforts.

Clinical manifestations of PRRS disease differ between PRRSV isolates in quality and quantity. Several other factors such as age of the host and viral or bacterial co-infections can influence PRRSV replication and clinical outcome (Van der Linden et al.
2003). The infectious dose also differs by isolate, with ranges from $1 \times 10^{0.26}$ 50% tissue culture infectious dose (TCID50) for MN-184 to $1 \times 10^{3.1}$ TCID50 for VR-2332.11 (Cutler et al., 2011). Additionally, route of exposure appears to play a role, and higher doses may be required for oral versus intranasal exposure ($1 \times 10^{5.3}$ and $1 \times 10^{4.0}$, respectively) and lower doses for intramuscular exposure (as few as 20 viruses) (Hermann et al., 2005; Yoon et al., 1999). PRRSV is highly susceptible to inactivation by heat and drying and infective virus was not recovered from a variety of common surfaces and materials at temperatures from 25º to 27ºC. However, PRRSV is more viable at lower temperatures and in less humid conditions (Hermann et al., 2007). At temperatures between –20ºC and –80ºC, PRRSV can be stable for months to years, but infectivity was quickly lost when the pH of the cell culture medium was < 6.0 or > 7.5 (Bloemraad et al., 1994). PRRSV can survive for several days in cell culture if kept at 4ºC, a temperature often observed during the fall and winter months in North America, which coincides with peak observed clinical signs of PRRS (Bloemraad et al., 1994; Zimmerman et al., 2010).

All of the factors referred to above influence the basic reproduction ratio (R0) of the PRRSV, which is the number of secondary infections in a fully susceptible population given contact with 1 infectious individual for the duration of the infective period (Dietz, 1993). An R0 value of 3.0 (95% CI, 1.5 to 6.0) has been estimated for PRRSV transmission (Nodelijk et al., 2000). Modelling the spread of PRRSV within a farm has demonstrated that herd size is negatively associated with the probability of achieving stabilized status (defined as having PRRSV negative 3-week-old piglets, evaluated by PCR) and that repeated mass vaccination for the purpose of gilt acclimation is more effective for disease control than a single exposure (Jeong et al., 2014).
**PRRS Containment Efforts**

Current PRRS control strategies such as vaccination and biosecurity practices are not consistently successful and, thus, new methods for control must be examined. Primary factors that contribute to transmission of PRRSV between herds include herd ownership, gilt source, and market trucks (Kwong et al., 2013). However, studies have reported evidence of airborne dispersion of PRRSV over long distances (Otake et al., 2010) and air filtration has been found to significantly reduce the incidence of new-strain PRRSV incursions (Alonso et al., 2013).

**Challenges to containment**

Prolonged persistence of PRRSV in individual pigs has been reported exceeding 150 days (Allende et al., 2000). Viremia and persistence are among the most critical factors for the spread of the virus in and between herds and for the endemic character of the disease. Pigs with higher levels of circulating virus have a higher propensity to shed PRRSV and infect neighboring pigs (Yoon et al., 1993). Routes of shedding for PRRSV include saliva, nasal secretions, semen, urine, feces, and mammary gland secretions (Rossow et al., 1994; Swenson et al., 1994; Wagstrom et al., 2001; Wills et al., 1997b). Thus, PRRSV transmission occurs through multiple potential routes. The amount of viral shedding depends on strain type, with less shedding occurring with less virulent strains (Cho et al., 2007; Cutler et al., 2011). This is also likely affected by such factors as age of the host, the presence of other pathogens, and route of infection (Cho et al., 2006; van der Linden et al., 2003). Persistently infected pigs are often asymptomatic, making them hard to identify, despite high levels of PRRSV in tissues such as the lymph nodes and tonsils (Wills et al., 2003). Due to stress, persistent pigs can experience a resurgence of circulating
virus, which can trigger a secondary outbreak if the virus has mutated, such that the pigs lack protective immunity from the previous outbreak. Persistently infected pigs are also of concern for naïve pigs that were introduced into the herd after the PRRSV outbreak (Rowland and Morrison, 2012; Wills et al., 1997a). PRRSV may persist on a farm for up to 3.5 years, with 2% variation in ORF5 (Larochelle et al., 2003). The presence of genetic variability among pigs within farms and within individual pigs (Goldberg et al., 2003) can result in cyclical recurrence of disease. Herd closure, however, which typically exceeds 200 days, is usually successful in eliminating PRRSV (Schaefer and Morrison, 2007).

**Herd PRRS status**

Holtkamp et al. (2011) developed a 5-category classification system to define a herd’s PRRS status, ranging from positive unstable (1) through positive stable (2a or 2b), provisionally negative (3), and negative (4). Category 1 herds are defined as any virus on the farm and clinical signs associated with PRRS. Category 2a and 2b herds must have had a 90 day period of sustained lack of viremia in weaning-age pigs and no clinical signs of PRRS in breeding age pigs. Category 2a herds have no intention to eliminate the virus. This may be favorable in high-density regions where infection pressure is high, in which case it may be preferable to maintain a uniform level of immunity through vaccination, strategic exposure to live virus from the herd, or both (Perez et al., 2015). Vaccination strategies include vaccinating all sows with a modified-live-virus (MLV) PRRS vaccine, or challenge exposure of sows with serum containing live virulent PRRSV recovered from the herd (Fano et al., 2005; Lambert et al., 2012a). Acclimatization of gilts to provide immunity to PRRSV by intentional exposure to wild-type PRRSV, or vaccination followed by a period sufficient to ensure they are no longer shedding virus have also been reported
to reduce spread of the virus (Dee, 1997). In studies that compared animals that received a modified-live virus vaccine to those that received serum, exposure to field virus through serum promoted virus elimination from the sow herd, whereas exposure via the vaccine promoted a quicker return to baseline production levels (Linhares et al., 2012). However, exposure to the modified live vaccine reduced viral shedding compared to unvaccinated animals (Linhares et al., 2012).

Category 2b, 3, and 4 herds have the goal of eliminating PRRSV from the sow herd. There are several methods to eliminate PRRSV from a sow herd. Category 3 herds are provisionally negative, meaning 60 days have passed since the introduction of negative breeding replacements during a herd rollover, with evidence that these animals remained negative. Furthermore, all growing pigs that are present must be PRRSV negative. Category 4 starts when all infected animals have been removed from the herd, or 1 year after the herd was classified as category 3 if all animals are seronegative for PRRS (Holtkamp et al., 2011). Whole-herd depopulation and repopulation is the most effective means of eliminating a virus from a herd but this disruption in production comes at a financial cost (Schaefer and Morrison, 2007). Long-term financial gains from this method are only realized if PRRSV-free replacement pigs are available and the farm remains uninfected. Partial depopulation has been offered as an alternative to complete depopulation-repopulation and has had success in eradicating disease while mitigating the effects on production, pig flow, and profit (Bruna et al., 1997). Test and removal methods, which aim to identify infected animals and remove them from the herd, are also effective, although this requires extensive testing of individual animals (Dee et al., 2001).
Regional eradication programs

Producers and veterinarians in some regions of North America have recognized the importance of a united approach to combating PRRSV infection (Corzo et al., 2010; Lambert et al., 2012b). An important aspect of this has been the communication and mapping of the PRRS status of farms in a region and the associated PRRSV genotypes. Maps of this information, including dynamic maps that visualize changes over time, are a valuable resource to study how PRRSV is spreading and may allow the detection of biosecurity gaps that may be specific to a region. A collaborative effort between farmers and veterinarians within a region allows for accurate data collection through questionnaires that are given to the producer by veterinarians. In return, the producer receives reports on disease status and risks in the region. An example of such a program is the Production Animal Disease Risk Assessment Program (www.padrap.org).

Cost is one of the major factors in the establishment and continuation of regional eradication programs. Surveillance of PRRS status in a region is not cheap and it requires the participation of a large number of producers, for whom it must be beneficial. It is important to clearly define the population of interest, the objective for testing and the approach that can achieve the objective in the population of interest for the lowest cost. It is also important to identify achievable milestones to quantify the success of the program in order to efficiently prove that a state, region or country is free of PRRSV, which is the ultimate goal (Arruda et al., 2015; Perez et al., 2015; Tousignant et al., 2015).

Sample collection for PRRS detection

Sample collection and measurement of PRRS status needs to be fast, accurate and cost effective in order to be widely used. Herd PRRS status is frequently assessed using
serum collected on individual pigs then quantified using antibody- or PCR-based assays, which measure antibody or virus levels, respectively (Kittawornrat et al., 2012). Oral fluids provide a higher probability of detection and require fewer samples than serum. In addition, collection of oral fluids places minimal stress on pigs or people but are usually only taken on a pen (rather than individual) basis by hanging a rope in the pen and allowing the pigs to chew on it (Olsen et al., 2013). This method cannot be used to collect samples from individual animals unless they are individually housed (Fetzer et al., 2006; Kittawornrat et al., 2012). Antibodies can be easily detected in both serum and oral fluid (Prickett and Zimmerman, 2010). Analysis of oral fluid is a good way for producers to determine whether PRRSV is present on their farm. If identification of the particular PRRSV variant is important, a PCR-based assay is needed because antibody assays do not provide this information. However, if a producer is interested in monitoring the immune status of the herd, an antibody assay would be more useful than a PCR-based assay because it provides information on both recent and past exposure history. The ideal sample collection and detection method for PRRS status depends on the goal of the test.

**Humoral Immune Response to PRRSV Infection**

Early after exposure to PRRSV, a vigorous anti-PRRSV antibody response can be measured in the host, with initial detection at 7–9 days post-infection, which typically coincides with peak viremia (Loemba et al., 1996). The first antibodies produced are of the IgM isotype, which is characterized by a rapid response within a week post infection and a rapid decay to undetectable levels 3-4 weeks post infection (Labarque et al., 2000). IgG levels are high and maintained for an extended period of time after 1 week post infection.
(Labarque et al., 2000; Loemba et al., 1996) and can be detected in excess of 36 weeks post infection (Meier et al., 2003). The earliest antibodies detected are of the IgM isotype and are directed against the 15 kDa N protein, followed by the 19 kDa M protein, then the 26 kDa GP5 envelope glycoprotein (Loemba et al., 1996). Despite early production of antibodies that recognize PRRSV, there is no evidence that early antibody response plays a role in the protection of the host against PRRSV infection, as neutralizing antibodies do not appear until at least 3-4 weeks post infection and may not be detected until 8 weeks post infection (Labarque et al., 2000; Loemba et al., 1996; Meier et al., 2003). Neutralizing antibody titers greater than 1:8 (i.e. an eight-fold dilution of serum is sufficient to prevent cytopathic effects) were able to protect against serum viremia but did not prevent viral replication in tissues and, as a result, viral shedding. Passive transfer may be effective if the neutralizing antibody titer is greater than 1:32, which represents a greater number of neutralizing antibodies than a 1:8 titer (Lopez et al., 2007).

Neutralizing Antibody Response

An effective vaccine causes the pig to produce antibodies that neutralize both homologous and heterologous variants of the virus. GP5 was the first PRRSV structural protein that was reported to have a major linear neutralizing epitope (Pirzadeh and Dea, 1997, 1998), and variants that escape immune response have been found to differ in GP5 sequence (Weiland et al., 1999). This was postulated to be a conformational epitope because immunizing against PRRSV with a DNA vaccine encoding GP5 elicited the production of serum neutralizing antibodies, while immunization with recombinant GP5 protein produced in bacteria from the same ORF5 DNA construct failed to induce the production of neutralizing antibodies (Pirzadeh and Dea, 1998), which was confirmed in
later studies (Vanhee et al., 2011). There is no consistent evidence for linear epitopes (i.e. epitopes based on amino acid sequence) in the ectodomain of GP5. However, it is likely that the neutralizing epitope(s) that exist on the PRRSV GP5 is (are) conformational (Fan et al., 2015) and that the M protein, when forming the dimer with GP5, contributes to the conformational nature of the neutralization epitope on GP5 (Trible et al., 2015).

While most initial studies focused on GP5, more contemporary studies have turned to assessing whether or not neutralizing epitopes are present on other PRRSV proteins. Neutralizing epitopes have been identified by a monoclonal antibody on the envelope’s GP4 structural protein and another one on the membrane protein (Yang et al., 2000). Recently, a single amino acid in the M protein was shown to be necessary for a polyclonal antibody to mediate broadly neutralizing activity (Trible et al., 2015). Peptide scanning technology has led to the identification of peptides in GP2, GP3 and GP4 that define linear neutralizing epitopes in these three minor glycoproteins but, interestingly, none were identified in GP5 (Vanhee et al., 2011). Non-structural proteins have also been investigated. Interestingly, nsp2 contains a cluster of non-neutralizing epitopes, suggesting an immunodominant role for this major nonstructural protein in clearance of viremia (Mulupuri et al., 2008).

The most robust antibody responses are against viral proteins without neutralizing epitopes, as the nucleocapsid also does not contain a neutralizing epitope (Chand et al., 2012). Furthermore, antibody response to proteins with neutralizing epitopes, such as GP5, tend to be delayed and weak (Chand et al., 2012; Mulupuri et al., 2008). Since the initial reports that the N-glycan moieties in GP5 of type-II PRRSV are important for the virus to escape the effect of NAbs (Ansari et al., 2006), glycan shielding has been implicated as an
important mechanism employed by the virus to evade neutralizing antibody response, ensuring in vivo persistence of virus. A recent study on neutralizing response to PRRS vaccination indicated that neutralizing antibody response levels are not always indicative of vaccine-induced protection for PRRSV (Li and Murtaugh, 2012).

**Vaccine cross-protective action**

While neutralizing antibodies are not required for immunological protection against PRRSV infection, their presence can prevent viremia (Li et al., 2014), perhaps primarily by mitigating the impact of re-exposure to the virus (Lopez and Osorio, 2004). However, usually only partial protection to heterologous strains of PRRSV is observed (Roca et al., 2012; Trus et al., 2014) because most neutralizing antibodies are specific to the homologous vaccine strain (Han et al., 2014). However, vaccination does reduce clinical pathology of PRRS and viral shedding (Roca et al., 2012; Scortti et al., 2006; Trus et al., 2014). Significant titers of cross-neutralizing antibodies are rare (Zhou et al., 2012), although some animals have shown the ability to neutralize against heterologous isolates (Trible et al., 2015). Pigs vaccinated with MLV vaccine may be partially protected against secondary heterologous challenge. Thus, certain PRRSV epitopes are capable of inducing the production of cross-neutralizing antibodies (Osorio et al., 1998). Among pigs that produce neutralizing antibodies in response to the MLV vaccine, there is a robust but strain-specific increase in serum NAb titers following heterologous challenge, which does provide some protection against the secondary challenge (Osorio et al., 1998; Scortti et al., 2006). This phenomenon can be interpreted as evidence that protective cross-neutralization can occur with PRRSV, not that it always occurs, as isolates differ in their ability to be neutralized (Javier Martinez-Lobo et al., 2011). This suggests that the MLV primes the
immune system but that secondary challenge is required for NAb to be detected systemically (Osorio et al., 1998).

**Genomics of Host Response to PRRSV Infection**

Disease resistance may be defined as the ability of the individual host to resist infection or to control the pathogen life cycle, e.g., to limit proliferation or transmission of infection to other hosts. Resistance is best understood from an ecological consideration of the interaction between the host and the pathogen species (Bishop, 2012; Bishop and Stear, 2003; Grenfell and Dobson, 2008). There are several mechanisms that can cause an animal to be more resistant: less likely to become infected, reduced pathogen proliferation once infected, or reduced shedding or transmission of infection. Tolerance can be defined as the ability of a host to tolerate infection and show little or no measurable detriment, that is, minimal effects of disease (Bishop, 2012). If the underlying the genetic mechanisms of resistance and tolerance can be teased apart, then genetic improvement of these two traits could have markedly different impacts. For example, improving resistance should also reduce transmission of infection between animals, whereas improving tolerance will reduce clinical signs of disease but may not reduce transmission of infection. With selection for resistance, unselected animals introduced into the population would benefit from the improved herd-level resistance, whereas with selection for tolerance, unselected animals in the same environment would be at risk from disease (Lewis et al., 2007).

**GBLUP and GWAS**

In assessing the potential of genetic selection for increased resistance, genetic parameter estimation and genome-wide association studies are often the first steps taken.
Estimation of genetic parameters of traits can be performed using pedigree or genotype information (such as single nucleotide polymorphism (SNP) genotypes) if available. Using marker genotypes, a genomic relationship matrix can be constructed (VanRaden, 2008) as follows:

\[
G = \frac{Z Z'}{\sum_{i=1}^{k} 2p_i(1 - p_i)}
\]

where \( G \) is the \( n \times n \) genomic relationship between all \( n \) animals; \( Z \) is the centered \( n \times k \) array of \( n \) animals and their genotypes (coded -1/0/1) at \( k \) SNPs; \( p_i \) is the frequency of the major allele at SNP \( i \). Using the \( G \)-matrix is advantageous over the pedigree-based relationship matrix, particularly when pedigree relationships are sparse. The \( G \)-matrix allows relationships between animals that are not linked by pedigree to be estimated. Furthermore, for animals that are linked by pedigree, the relationships between animals can be better estimated using genotypes by capturing the Mendelian sampling term (VanRaden, 2008).

Genome Wide Association Studies (GWAS) involve the use of genetic markers, such as SNPs, to test for statistical associations between genomic regions and a phenotype, which may serve to better understand the biological mechanisms influencing a genetically inherited trait (Visscher et al., 2012). GWAS can be used to assess whether the trait of interest is primarily driven by a few loci with large effects, or by many loci with smaller effects. Bayesian methods have been developed for GWAS, including BayesB and BayesC (Kizilkaya et al., 2010; Meuwissen et al., 2001). BayesB and BayesC assume a mixture distribution of SNP effects, whereby a proportion, \( \pi \), of the SNPs are assumed to have an effect of zero, and the effects of the remaining \( 1-\pi \) SNPs come from either a t-distribution (BayesB) or a Normal distribution (BayesC) (Kizilkaya et al., 2010; Meuwissen et al., 2001).
2001). In BayesB, each SNP has its own variance, while BayesC fits a single effect variance across all SNPs (Kizilkaya et al., 2010; Meuwissen et al., 2001). These differences result in BayesB to be more appropriate for traits that have large QTL underlying them, while BayesC is more appropriate for traits that are extremely polygenic with no large-effect QTL.

**PRRS Host Genetics Consortium studies**

Profound differences in innate immune response (Ait-Ali et al., 2008), histopathology (Halbur et al., 1998; Petry et al., 2005), antibody production, weight gain, viral burden, etc. (Petry et al., 2005; Reiner et al., 2010) have been observed between breeds of pigs during infection with the same PRRSV isolate, providing strong evidence of a heritable genetic component to resistance to PRRS. This was further supported by the increase in heritability estimates of sow reproduction traits during a PRRS outbreak compared to a healthy environment (Lewis et al., 2009). These observations prompted the recognition that host genetic studies could provide insight into novel methods for containing PRRS (Lunney and Chen, 2010) and the formation of the PRRS Host Genetics Consortium (PHGC), a collaborative North American research project with the goal of understanding the role of host genetics in resistance to PRRSV infection, and the impacts of PRRS on pig health and growth (Lunney et al., 2011). In these studies, rebound, defined as an at least 2 \( \log_{10} \) increase in viremia after the initial decline, was identified in ~30% of the pigs. The PHGC reported a substantial heritable genetic component to viral load (defined as area under the curve of \( \log_{10} \) serum viremia from 0 to 21 days post infection (dpi)) and weight gain (total weight gain during the infection trial period of 35 or 42 days) in nursery pigs during experimental infection with PRRSV isolate NVSL-97-7985.
These traits were also found to be antagonistically related to each other, both phenotypically and genetically.

A genome-wide association study identified a genomic region on chromosome 4 that was associated with both VL and WG, and explained 12% (VL) and 9% (WG) of the total genetic variation for these respective traits. A marker in this region, SNP WUR10000125, was further characterized, and it was found that animals carrying the B allele had lower viral load and higher weight gain during PRRSV infection with NVSL-97-7895 (Boddicker et al., 2012). Further characterization of the association of this SNP with VL and WG provided no evidence of breed of origin or parent of origin effects (Boddicker et al., 2014b). The marker was expected to be in high linkage disequilibrium with the causal mutation and provide adequate prediction of disease response to PRRS (Boddicker et al., 2014a). Subsequently, a putative quantitative trait nucleotide was identified in GBP5 (Koltes et al., 2015), a gene involved in the formation of the inflammasome via NLRP3 (Shenoy et al., 2012). Expression network analysis of animals with the AA and AB genotype for WUR revealed that AB animals recover from the impact of PRRSV infection sooner and place more emphasis on the innate immune response, which is consistent with the role of GBP5 (Schroyen et al., 2015). Conversely, AA animals place more energy into fighting the infection and place more emphasis on the adaptive immune system (Schroyen et al., 2015). While WUR explains a substantial amount of genetic variation for VL and WG, it is also worth noting that there is a substantial polygenic component to these traits (Boddicker et al., 2014a), which is consistent with what is expected for disease resistance traits (Wilkie and Mallard, 1999).
Other genomic studies on PRRS

Other studies have also demonstrated a heritable genetic component to host response to PRRSV infection. QTL were identified that were associated with classical and alternative complement activity, Haptaglobin concentration and C3c serum concentration at 0 and 10 dpi in a Duroc/Berlin Miniature pig F2 population of pigs, following vaccination with a modified live PRRS vaccine (Wimmers et al., 2009). Several serum cytokines levels in addition to pattern recognition receptors (i.e. Toll-like receptors (TLRs)) were measured in Duroc/Pietrain F2 animals following vaccination with the same vaccine, and QTL for IL-10, IFNG, and TLR9 were identified but no QTL were identified for IL-2 or TLR2 (Uddin et al., 2011).

Multiple lines of evidence support the presence of a heritable genetic component to PRRSV-specific total antibody response to PRRSV infection, for which the MHC, known as the Swine Leukocyte Antigen Complex in pigs, plays a crucial role. Between-breed differences in antibody response to PRRSV, measured by serum neutralization tests and the commercial ELISA (measures all antibody directed towards PRRSV N protein), infection have been identified (Halbur et al., 1996a; Petry et al., 2007). In the study discussed above, Wimmers et al. (2009) found an association between DQB MHC class II gene variants and antibody response at 10 days after vaccination with a modified live PRRS vaccine in an F2-population of reciprocal Berlin Miniature and Duroc crossbred piglets. Uddin et al. (2011), identified a region at 14 cM on SSC3 to be associated with antibody response using the commercial ELISA kit at 10 days post vaccination in 15 week old Duroc/Pietrain F2 crossbred pigs. Serao et al. (2014) identified two large QTL on SSC7 in
a PRRSV outbreak sow herd using the commercial ELISA kit. One of these QTL was in the MHC class II region and explained 25% of the genetic variation in antibody response.

Modeling disease phenotypes

Using statistical models to estimate parameters that describe the shape of repeated measures over time is a powerful method to extract information about how a process (i.e. response to infection) changes over time. Such models can be used to better understand the dynamics of a biological process, while reducing the noise that accompanies a single measurement. Akin to this, parameters from model fitting can be used to interpolate fitted values to the data that otherwise could not be collected, due to such reasons as cost, time, and stress to the animal. Such models have been used to fit lactation and growth curves (Grossman and Koops, 2003; Huisman et al., 2002). Recently, the Wood’s curve (Wood, 1967) was shown to be an appropriate model for serum viremia levels during experimental PRRSV infection in the PHGC trials (Islam et al., 2013). In this study, either a Wood’s curve, or an Extended Wood’s curve was fitted to repeated serum viremia measurements collected at various time points during the 42 day PHGC trials to objectively distinguish between animals that experienced a rebound in viremia and those that did not, and classified 17% of animals as having experienced a rebound in serum viremia. Estimates of parameters obtained from fitting the Wood’s curve to each individual’s serum viremia data can be used to better understand the dynamics of PRRSV infection and what is happening at specific time points. However, a limitation of fitting a model is that the number of parameters estimated is confined by the number time points that data are collected on each animal, as over parameterization could result in incorrect interpretation of the data.
Selection for Resistance to PRRSV Infection

The ultimate goal of the PRRS Host Genetics Consortium is to develop methods to select pigs with improved response to PRRS. Previous studies, mentioned above, suggest that selection for resistance to PRRS is possible, due to moderate heritabilities for resistance phenotypes and the identification of the WUR10000125 SNP (Boddicker et al., 2014a). However, several steps must be taken before selection of pigs with increased PRRS resistance can be implemented. In order for selection for resistance to PRRS to be both cost effective and successful, decisions such as which phenotypes to collect and on which animals are crucial.

Direct vs. indirect selection for disease resistance

Rothschild (1985) reviewed approaches to selection for disease resistance in the pig and distinguished direct and indirect methods. Possible direct approaches he outlined were to i) observe the breeding stock under dirty conditions ii) challenge the breeding stock, iii) challenge the sibs or progeny of breeding stock, or iv) challenge clones. Given the lack of public support for cloning, the latter option is not feasible at this time. The first two options are also not likely to be favored by the swine industry because a crucial aspect of the swine industry is to keep breeding stock pathogen free. Thus, challenging breeding stock for the purpose of recording disease performance, especially to PRRS infection, is unlikely. Observing the breeding stock is faced with the same problem. Measurement of disease resistance phenotypes in breeding stock is usually not an option because of efforts to keep these herds disease free. Therefore, the most desirable option is to make decisions based on the performance of challenged sibs or progeny of breeding stock at the commercial level, where response to PRRSV infection can be monitored and recorded. Even in the
context of a multiplier herd, numerous problems involved with disease challenge or selection under challenge exist. Exposure to PRRSV is not equal among herds, with the incidence of PRRS being much more frequent in some herds than others. Furthermore, during a PRRSV outbreak, the timing and level of exposure to PRRSV, as well as the control of the PRRSV variant the pigs are exposed to, cannot be controlled. These are all factors that would impact the ability to detect animals that are better responders to PRRSV infection. Therefore, while disease performance recording during an outbreak is useful, there are serious limitations to this approach.

Assessment of the potential for selection typically starts with estimation of the heritability of the trait and/or of the effects of genetic markers associated with a trait, which may be lower in an outbreak herd due to the limited ability to control the environmental variation (Bishop and Woolliams, 2010). Factors that can contribute to environmental variation in an outbreak herd include incomplete recording, incomplete exposure, and imperfect sensitivity and specificity of diagnosis. Thus, the true heritability for disease resistance, were it to be measured under ideal circumstances, is likely to be much higher than is typically estimated in field settings (Bishop and Woolliams, 2010). The ability to estimate host genetic variation in disease resistance may be further complicated by the relationship between the pathogen’s infectivity and the host’s susceptibility to the disease, which can only be assessed by measuring response given a known level of infection (Pooley et al., 2014). Therefore, experimental infections provide a more powerful means for estimating the true genetic variation underlying host resistance to disease.

Alternatively, indirect methods can be applied for selection of animals that respond better to disease, such as vaccination, in vitro testing, use of genetic markers, or
construction of resistant genotypes (Rothschild, 1985). Genetic markers, such as WUR10000125, may be useful to select for increased resistance to PRRS, either from the perspective of genomic selection or marker assisted selection. Construction of resistant genotypes, using technologies such as gene editing, may be a feasible approach in the future. It would be possible to gene edit the causal mutation in GBP5 marked by WUR, for example, to increase the frequency of the favorable causal mutation, which may reduce the average PRRS viral load in the event of an outbreak; however the majority of the host genetics influencing response to infection is polygenic so gene editing a mutation such as the causal WUR variant will only provide some benefit. Recently, pigs with CD163 edited were created which conferred complete resistance to PRRS by preventing entry of PRRSV into macrophages and, thus, preventing infection. However, the edited pigs will need substantial testing to ensure there are no other undesirable effects in pigs harboring the edited gene (Whitworth et al., 2016). Furthermore, if this is achieved, this method will still take time to be utilized at the commercial level, due to foreseeable legal and cost barriers, as well as a possible lack of consumer acceptance. Vaccination at the commercial level is, however, akin to experimental infection, as this practice gives breeders control of variables such as genetic background of the virus, dosage, and time of infection. Response to vaccination of animals at the commercial level can then be used to make selection decisions at the nucleus or multiplier level through connections via the pedigree or by genotyping. The success of the use of vaccination for genetic selection purposes will, however, depend on the level with which host genetic resistance is sustained between different variants of the pathogen, and the vaccine strain used. In vitro test, or tests that can be used as indicators (e.g. certain blood parameters) of performance during infection are also a desirable method
Complicating factors associated with such an approach include the decision of which immune response parameters to choose, and, furthermore, when during the animal’s life to collect phenotypes, as age may play a factor in these parameters (Rothschild, 1985).

**Conclusions**

According to Wiese (2005) breeding for improved disease resistance is justified if 1) the disease is of major importance; 2) current control strategies are not adequate, sustainable, or cost effective; and 3) available animals do not cope with these disease challenges (i.e. the disease has an obvious impact on the animal’s wellbeing). This review has shown that selection for improved resistance to PRRSV infection is justified based on these criteria; PRRS is of major importance to the global swine industry due to the large, ongoing costs associated with it; current vaccination and biosecurity practices have not been successful at eradicating PRRS from the North American swine herd, which has a major impact of the welfare and production of these animals when faced with PRRSV challenges. In addition to the points made by Wiese (2005), sizeable genetic variation in the pigs’ response to PRRSV infection must exist, which has been shown in multiple genomic and quantitative genetic studies. And finally, selection for improved disease resistance must be effective across different variants of PRRSV.

While current genomic and quantitative genetic studies have shown promising results in terms of the likely success of selection of pigs that have increased resistance to PRRSV infection, there are still gaps in our knowledge. Some of these gaps that will be addressed in this dissertation, including whether response to PRRSV infection is consistent
between isolates; whether the effect of the WUR marker is similar under infection with distinct PRRSV isolates and whether the WUR region has an effect on other PRRS resistance phenotypes; whether serum antibody levels is a useful phenotype to measure resistance to PRRS in the growing pig; and to what extent persistence, as measured by tonsil virus level, is influenced by serum viremia and host genetics.

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CHAPTER III

COMPARISON OF HOST GENETIC FACTORS INFLUENCING PIG RESPONSE TO INFECTION WITH TWO NORTH AMERICAN ISOLATES OF PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS

Submitted to Genetics Selection Evolution


Contribution from Authors

Andrew Hess: Fitted weight curves, conducted the statistical analyses, interpretation of results, and wrote the manuscript.

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Melanie Hess: Aided in the interpretation of results and draft of manuscript.

Bob Rowland: Conceived the study and led the animal infection trials and sample collection.

Joan Lunney: Conceived the study and coordinated the handling, storage, and sample preparation for DNA.

Andrea Doeschl-Wilson: Oversaw the fitting the Wood’s curves to the viremia data and estimation of the Wood’s curve parameters. Provided insight into statistical analysis of the data and contributed to interpretation of results and writing the manuscript.

Steven Bishop: Oversaw the fitting the Wood’s curves to the viremia data and estimation of the Wood’s curve parameters.

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All co-authors reviewed and contributed to development of the manuscript.
Abstract

Background

Porcine Reproductive and Respiratory Syndrome (PRRS) is one of the most important swine diseases in the world and genetic selection of pigs for increased resistance to PRRS is an attractive method to improve health status of the swine herd. Pigs were experimentally infected with one of two genetically distinct type 2 PRRS virus (PRRSV) isolates: NVSL-97-7895 (NVSL; ~1500 pigs) and KS-2006-72109 (KS06; ~700 pigs). This study compared phenotypic and genetic responses to infection with these isolates and evaluated whether a genetic marker WUR10000125 (WUR) on chromosome 4 that was associated with viral load and weight gain under infection with NVSL also has an effect on response to infection across North American PRRSV isolates. The Wood’s lactation curve was fitted to repeated viremia measurements to derive five curve characteristics that were evaluated.

Results

Infection with NVSL was characterized by reaching a 14±2% higher peak viremia (PV) 2.5±0.6 days earlier (time to peak; TP) than KS06, followed by 36±14% faster virus clearance (Vmax), which occurred 3.9±0.7 days sooner (Tmax). Weight gain from 0-42 days post infection (WG) tended to be higher under infection with KS06 than NVSL (3.7±1.5 kg). Estimates of heritability were moderate for both PRRSV isolates for viral load from 0-21 days post infection (VL) (NVSL: 0.31±0.06; KS06: 0.51±0.09) and WG (NVSL: 0.33±0.06; KS06: 0.31±0.09). Strong negative genetic correlations were observed between VL and WG for both NVSL (-0.74±0.10) and KS06 (-0.52±0.17) infected pigs. Pigs with the AB genotype at the WUR SNP had a more desirable phenotype than AA pigs.
for all traits under NVSL infection (VL\textsubscript{AA}-VL\textsubscript{AB}=4.5±0.4%; WG\textsubscript{AA}-WG\textsubscript{AB}=-2.0±0.2 kg; PV\textsubscript{AA}-PV\textsubscript{AB}=2.8±0.4%; TP\textsubscript{AA}-TP\textsubscript{AB}=0.20±0.09 days; Tmax\textsubscript{AA}-Tmax\textsubscript{AB}=0.68±0.16; Vmax\textsubscript{AA}-Vmax\textsubscript{AB}=-3.8±1.5%), but only for VL and PV for KS06 (VL\textsubscript{AA}-VL\textsubscript{AB}=4.2±0.9%; PV\textsubscript{AA}-PV\textsubscript{AB}=3.4±0.7%); effects on other traits were smaller and not significantly different from zero (p>0.05). Genetic correlations of host response between isolates were strong for VL (0.86±0.19), WG (0.86±0.27), and PV (0.94±0.28). Accounting for WUR genotype had little impact on these correlations, suggesting a substantial polygenic component to response to PRRSV infection that is common between these two PRRSV isolates.

Conclusions

These results suggest that KS06 is a less virulent PRRSV isolate than NVSL but that genetic selection for increased resistance to either of these genetically distinct isolates is expected to increase resistance to the other isolate.

Background

Porcine Reproductive and Respiratory Syndrome (PRRS) costs the U.S. swine industry $664 million per year (Holtkamp et al., 2013). Past efforts to contain PRRS have had limited success, in large part due to the high mutation rate and antigenic variability of PRRS virus (PRRSV), which have encumbered efforts to produce vaccines that are cross-protective to different PRRSV isolates (Darwich et al., 2010). PRRSV isolates are classified into two types: Type 1 or European isolates and Type 2 or North American isolates (Wensvoort et al., 1992). These two types are distinct both genetically (Dea et al., 2000) and pathogenically (Han et al., 2013; Choi et al., 2015).
Genetic selection of pigs that are more resistant to PRRS can be an attractive method to improve the health status of the swine herd (Lewis et al., 2007). The goal of the PRRS Host Genetics Consortium (PHGC) is to identify host genes or genomic regions associated with increased resistance of pigs to PRRSV infection (Lunney et al., 2011). Previous studies using multiple contemporary North American crossbred weaner pigs that were experimentally infected with a North American isolate of PRRSV, NVSL-97-7895 (NVSL), identified heritable genetic components to viral load and weight gain following infection, and found a strong association of the single nucleotide polymorphism (SNP) WUR10000125 (WUR) on chromosome 4 with these two host response traits (Boddicker et al., 2012; 2014a; 2014b). A putative causative mutation that is in high linkage disequilibrium with WUR, in the guanylate binding protein 5 (GBP5) gene, was identified by Koltes et al. (2015). The protein produced by the GBP5 gene plays a crucial role in NLRP3-mediated formation of the inflammasome, which is involved with inflammatory response (Shenoy et al., 2012). It is currently not known whether selection for improved host resistance to a single PRRSV isolate will result in pigs that are resistant to other PRRSV isolates.

The objectives of this study were to 1) compare host responses to infection with NVSL and the genetically distinct North American PRRSV isolate KS-2006-72109 (KS06); 2) estimate the genetic parameters of response to infection when pigs are infected with either NVSL or KS06; and 3) estimate the associations of the WUR SNP with response following infection with NVSL or KS06. It was hypothesized that host genetics influencing response to infection are highly correlated between the two virus isolates and that associations of the WUR SNP with host response to infection are similar for these two
isolates. The Wood’s lactation function has previously been shown to appropriately model PRRS serum viremia following experimental infection (Islam et al., 2013). Thus, curve characteristics of the fitted viremia profiles derived from the Wood’s curve parameters were used to quantify different aspects of the dynamics of host response to PRRSV infection with these two isolates.

**Methods**

**Study design**

A detailed description of the design, data collection and molecular techniques used in the PHGC trials is in Lunney et al. (2011). The Kansas State University Institutional Animal Care and Use Committee approved all experimental protocols for these trials. Pigs used for this study were from 14 PHGC trials of ~200 weaner pigs (Table 3.1). Pigs were provided from commercial breeding programs in the United States and Canada. Within each challenge trial, pigs were from a single high health farm and genetic background, except for trials 5, 8 and 12, which each included pigs from one genetic background but two farms. All source farms were free of PRRS, *Mycoplasma hyopneumoniae*, and swine influenza. Four breeding companies supplied pigs of the same breed cross for more than one trial, with pigs in one trial infected with KS06 and in one or more trials with NVSL (Table 3.1). Pigs from the same breeding company and the same breed cross were from the same genetic background.

For each trial, animals were transported to Kansas State University at weaning (average age of 21 days) and randomly placed into pens of 10 to 15 pigs. After a 7-day acclimation period, pigs were experimentally infected, both intramuscularly and
intranasally, with $10^5$ (TCID50) of NVSL-97-7985, a highly virulent type 2 PRRSV isolate (Truong et al., 2004), for trials 1 to 8 and 15 and of KS-2006-72109, a more contemporary type 2 PRRSV isolate, for trials 10 to 14. NVSL and KS06 are 89% similar at the glycoprotein (GP)5 nucleotide sequence level (Ladinig et al., 2015). Blood samples were collected at −6, 0, 4, 7, 11, 14, 21, 28, 35, and 42 days post-infection (dpi). Body weight was measured at 0, 7, 14, 21, 28, 35, and 42 dpi. Pigs were euthanized at 42 dpi. Trials 7 and 8 were stopped at 35 dpi due to unavailability of the facility.

Serum viremia was measured using a semi-quantitative TaqMan polymerase chain reaction assay for PRRSV ribonucleic acid (RNA), as described in Boddicker et al. (2012; 2014a; 2014b) and Ladinig et al. (2015). Assay results were reported as the log_{10} of PRRSV RNA copies per mL of serum. A time course of viremia levels for each animal within a trial was plotted in order to provide an initial assessment of response to infection and to confirm all animals were initially infected (Figure S3.1). Trial 13 was excluded from further analyses due to unusual viremia profiles that were not observed in any other PHGC trial; some trial 13 animals showed delayed presence of serum viremia and all pigs had low and highly variable viremia levels over time compared to the other trials, suggesting the virus was attenuated or the pigs were not naïve. The latter could be due to the presence of maternal antibodies resulting from previous infection or vaccination in the source herd (Geldhof et al., 2013).

Across all nine trials infected with NVSL, 12% of pigs died or were euthanized for humane reasons before 42 dpi. Mortality rate was similar in the five KS06 trials, with 9% pigs dying or euthanized before 42 dpi. Dead pigs were necropsied and subsequent gross and microscopic pathology by a board-certified pathologist identified PRRS associated
disease as the major source of mortality, except for trial 6. Death loss was high in trial 6 (46% by day 42), due to secondary bacterial infections, as identified by pathology, including *Escherichia coli*, *Streptococcus suis*, *Staphylococcus aureus*, and *Mycoplasma hyopneumoniae*[7], and these have subsequently been traced to the pig supplier. The impact of this coinfection on pig performance was further investigated by Boddicker et al. (Boddicker, 2013). The animals from all other trials remained negative of secondary infections.

**Genotyping and pedigree**

Ear tissue was collected from all pigs for DNA isolation. DNA samples from trials 1 to 10 were genotyped with Illumina’s Porcine SNP60 Beadchip (Ramos et al., 2009) v1 (San Diego, California) at GeneSeek Inc. (Lincoln, Nebraska) and samples from trials 11 to 15 were genotyped with Illumina’s Porcine SNP60 Beadchip v2 (San Diego, California) at Delta Genomics (Edmonton, Alberta). Only SNPs that were included on both versions of the Illumina’s Porcine SNP60 Beadchip were used in this study. SNPs were removed if they were fixed within a trial, or if they were unmapped or mapped to a sex chromosome in build 10.2 of the swine genome (GenBank Accession: GCA_000003025.4); this left 48,164 SNPs. No additional filters were applied to the animals or genotypes. The animal with the least number of called SNPs had a call rate of 0.82 (99th percentile: 0.98), while the SNP with the lowest call rate among animals was 0.62 (99th percentile: 0.97). Missing genotypes were assigned the average genotype (on 0, 1, 2 scale) for animals in that trial for that SNP. This set of SNPs will be referred to as 60k SNPs.

Pedigree information was available for all pigs in all trials. Trials 1-3, which consisted of animals from the same breeding company in consecutive parities, had the most
extensive pedigree information, with records up to two generations back, while only sire and dam were available for the other trials. As such, there were no relationships between animals in different trials, except for trials 1-3. Pedigree was corrected using parental genotypes for trials 1 through 8, as described by Boddicker et al. (2014b). The 1250 highest quality 60k SNPs, based on GC score and call rate, were used in Cervus 3.0 [15] to verify pedigree information for trials 11 and 15, and assign sire for trials 12 and 13, which used pooled semen (Marshall et al., 1998). Parental genotypes were not available for trials 10 and 14, therefore, the pedigree provided by the breeding company was assumed to be correct.

Viremia curve characteristics

In previous studies, Boddicker et al. (2012; 2014a; 2014b) used viral load, defined as area under the curve of log$_{10}$ viral copies/ml of serum from 0-21 dpi, as a measurement of response to PRRSV infection. Area under the curve is a summary phenotype of the viral burden but it does not explicitly capture the dynamics of an individual animal’s curve that can influence this viral burden; two animals that have different viremia curves can have the same viral load. Analysis of different aspects of the viremia curve may aid in the understanding of differences in virulence in the two virus isolates, as well as provide insight into the role host genetics plays in response to infection (Islam et al., 2013). The genetic mechanisms for one curve characteristic may be conserved across isolates, while another curve characteristic may be variable across isolates.

The Wood’s curve, an incomplete gamma function often used to model lactation yield in dairy cattle (Wood, 1967; Boujenane and Hilal, 2012; Maiwashe et al., 2013), was shown to appropriately model viremia profiles in PHGC trials 1 to 8 (Islam et al., 2013):
\[ V(t) = a_1 t^{b_1} e^{-c_1 t} \]

where \( V(t) \) is serum viremia on the log_{10} scale at time \( t \) dpi, \( a_1 \) is a parameter that impacts the magnitude of all points on the curve, \( b_1 \) is an indicator of the initial rate of increase to peak viremia, and \( c_1 \) is an indicator of the rate of decline after the peak and dominates the viremia profile at later stages of infection. These three function parameters were estimated for each individual that had at least five time points measured using Bayesian inference with a likelihood framework, implemented using a Markov chain Monte Carlo method, as described in Islam et al. (2013).

The raw viremia profiles of some pigs appeared bi-modal, so an extended Wood’s curve was also fitted for each piglet using the same methodology (Islam et al., 2013):

\[ V(t) = a_1 t^{b_1} e^{-c_1 t} + \max\left(0, a_2 (t - t_0)^{b_2} e^{-c_2 (t-t_0)}\right) \]

where \( t_0 \) denotes the time of onset of the second phase of the profile, which is assumed to follow the same Wood’s shape as the primary phase and is thus defined by a second set of Wood’s model parameters. A piglet was classified as experiencing viremia rebound based on the Akaike’s Information Criterion (AIC) if \( AIC_{\text{WOOD'S}} - AIC_{\text{EXTENDEDWOOD'S}} \) was greater than 1.488, which corresponds to the 95% significance level of the likelihood ratio test between these models (Islam et al., 2013). A summary of the raw estimates of the curve parameters \((\hat{a}_1, \hat{b}_1, \hat{c}_1, \hat{a}_2, \hat{b}_2, \hat{c}_2)\), as well as proportion of the animals classified as having cleared, rebound, or persistent serum viremia profile, is given in Table S3.1. A more detailed description of the fitting of the Wood’s and Extended Wood’s curve, including a visualization of a Wood’s and extended Wood’s curve, is provided in Islam et al. (2013).

Using the estimates of the curve parameters \((\hat{a}_1, \hat{b}_1, \hat{c}_1)\) of the single or the extended Wood’s curve for each pig, five curve characteristics were derived to describe the viremia...
profile of each pig. For pigs with extended Wood’s curves, only estimates of parameters of the primary phase were used in this study because this phase was previously shown to have a heritable genetic component, while heritability for rebound was previously estimated to be 0.03, suggesting that rebound is largely governed either by viral escape or other environmental factors (Islam et al., 2013).

The first characteristic evaluated, area under the Wood’s curve, hereafter referred to as viral load (VL), was given by the definite integral:

\[
VL_t = \int_0^{21} \hat{a}_{1i} t^{\hat{b}_{1i}} e^{-\hat{c}_{1i} t} \, dt
\]

VL is a measure of both the level of viremia and the extent to which viremia is maintained. The range 0-21 dpi was chosen to capture the uni-modal phase of infection common to all pigs. Previous analyses of viremia from trials 1 to 8 fitted a LOESS curve through viremia and integrated to obtain area under the curve from 0-21 dpi (Boddicker et al., 2012; Boddicker et al., 2014a; Boddicker et al., 2014b). This measure will be denoted by VL_B. Since it was not known how similar VL_B and VL were to each other, which may impact interpretation and comparisons with previous studies, a bivariate model using pedigree information was fitted to the VL and VL_B data, separately for the KS06 and NVSL trials, using ASReml 3.0 (Gilmour et al., 2009). Based on similar heritabilities and high genetic and phenotypic correlations between VL and VL_B for both isolates, it was concluded that VL based on the Wood’s curve describes the same biological trait as VL_B (Table 3.2). Therefore, VL derived from the Wood’s curve was used for all remaining analyses.

The second curve characteristic evaluated was time in dpi to peak viremia (TP), derived by setting the first derivative of the Wood’s equation to zero and solving for t, resulting in:
The third curve characteristic was peak viremia (PV), which was calculated by setting $t = TP$ in the expression for the Wood’s curve:

$$PV_i = \hat{a}_{1i} \left( \frac{\hat{b}_{1i}}{\hat{c}_{1i}} \right)^{\hat{b}_{1i}} e^{-\hat{b}_{1i}}$$

TP and PV are related to the host’s ability to respond during the replication-dominant phase of early PRRSV infection (Beyer et al., 2000). PV is reached when the rate of virus clearance from serum is equal to the number of virus particles released into the bloodstream. TP is the time it takes to reach PV, with animals that can mount a response early in infection expected to have a shorter TP.

Curve characteristics that relate to the host’s response during the post-peak, clearance-dominant phase of PRRSV infection were also evaluated. The maximal decay rate ($V_{\text{max}}$) is reached when the rate of viral clearance from serum is highest compared to the rate of viral replication. Time to maximal decay ($T_{\text{max}}$) was derived by setting the second derivative of the Wood’s equation to zero and solving for $t$:

$$T_{\text{max}} = \frac{\hat{b}_{1i} + \sqrt{\hat{b}_{1i}}}{\hat{c}_{1i}}$$

Substituting this value for $t$ in the first derivative and taking the absolute value gives $V_{\text{max}}$:

$$V_{\text{max}} = \left| -\hat{a}_{1i} \sqrt{\hat{b}_{1i}} \left( \frac{\hat{b}_{1i} + \sqrt{\hat{b}_{1i}}}{\hat{c}_{1i}} \right)^{\hat{b}_{1i} - 1} e^{-(\hat{b}_{1i} + \sqrt{\hat{b}_{1i}})} \right|$$

$V_{\text{max}}$ was defined as the absolute value, such that an animal with a larger $V_{\text{max}}$ cleared viremia more quickly from the serum.
**Body weights**

Body weights were collected weekly and used to interpolate daily weights. To obtain separate weight gain curves for each pig, a random regression model was fitted to the weight data of all animals, separately for the NVSL and KS06 trials, using second order Legendre polynomials in the following model that was implemented in ASReml 3.0 (Gilmour et al., 2009):

$$W_{ijklnmp}(t) = \sum_{n=0}^{2} L_n(t) + P_j + A_k + S_l + \sum_{n=0}^{2} L_n(t) \ast R_m + \sum_{n=0}^{2} L_n(t) \ast A_n + Tr_o$$

$$+ \text{Pen(Tr)}_{po} + \epsilon_{ijklnmp}$$

where $L_n(t)$ denotes the $n^{th}$ order Legendre polynomial at $t$ dpi for individual $i$. $L_n(t)$, $P$, $A$, $S$ and $L_n(t) \ast R$ were fitted as fixed effects. $L_n(t)$ was fitted as a covariate, with $t$ ranging from 0 to 42 dpi. $P$ is the parity of dam, classified as first, second, or later parities, $A$ is the age of the individual at inoculation, $S$ is the sex of the individual, and $L_n(t) \ast R$ is the interaction between the $n^{th}$ order Legendre polynomial at $t$ dpi ($L_n(t)$) and rebound status ($R$). $L_n(t) \ast A$, $Tr$, and $Pen(Tr)$ were included as random effects and denote the interaction between the $n^{th}$ order Legendre polynomial at $t$ dpi and animal, trial, and the nested effect of pen within trial, respectively. $L_n(t) \ast A$ models an individual’s weight at each time point and captures both genetic and permanent environmental effects, and used an unstructured variance-covariance structure for polynomial parameters of a given animal and independence of parameters between animals. Residual variances were modeled separately for each dpi, in order to allow for an increase in variance over time. Trial and Trial*Pen were included to capture systematic environmental effects. This model was then used to
obtain fitted values of each pig’s weight for each dpi (0-42) ($\hat{W}(t)$), using all coefficients estimated from the above model.

**Genomic relationship matrices**

Due to the limited pedigree information and availability of genotypes on all animals with phenotypes, a genomic relationship matrix ($G$) was constructed from the 60k SNP genotype data, using the VanRaden method (VanRaden, 2008). The $G$-matrix included relationships among all animals across trials. In some cases, fitting relationships between breeds can absorb between-breed differences that could be due to selection, which can overestimate the genetic variance because the “base population” is the population from which the breeds subsequently diverged (Hayes et al., 2009). Thus, a block diagonal $G$-matrix was also constructed ($G_B$) that only considered relationships between animals from the same genetic background, with zero relationships between animals from different companies. Results from analyses with $G_B$ are expected to be similar to what would be found with a pedigree-based analysis of these data if the pedigree was more extensive. A third $G$-matrix was constructed that was the same as $G_B$ but only included animals from trials that were paired across isolates ($G_P$), to assess whether the estimates of correlations of traits between NVSL and KS06 infected pigs could be biased due to different breed crosses being evaluated for each isolate. In order to assess the impact of the WUR region on these genetic correlations, matrices $G$, $G_B$ and $G_P$ were also constructed after excluding the 118 SNPs in the 5Mb region surrounding the WUR SNP. These new matrices are designated $G_W$, $G_B-W$ and $G_P-W$, respectively.
Statistical Models for Phenotypic and Genetic Comparisons of NVSL and KS06

All analyses used to evaluate responses to NVSL and KS06 infections were conducted using an animal model in ASReml 3.0 (Gilmour et al., 2009). The univariate model was:

$$Y_{ijkmnopq} = \mu + P_i + A_j + W_k + S_l + R_m + An_n + Li_o + Tr_p + Pen(Tr)_{qp} + \varepsilon_{ijkmnopq}$$

where $Y$ is the dependent variable of daily fitted viremia values, fitted weights, VL, TP, PV, Tmax, Vmax, or weight gain from 0-42 dpi (WG). Parity of the dam ($P$), classified as first, second, or later parity, and sex of the piglet ($S$) were fitted as a fixed class effects. To account for potential model differences in curve fittings between rebound and non-rebound pigs, the fixed class effect of rebound ($R$) was included in the model. Age ($A$) and weight ($W$) of the piglet at infection (0 dpi) were included as linear covariates. Random effects included animal genetic effects ($An$; using the full G-matrix), litter ($Li$), trial ($Tr$), Pen nested within trial ($Pen(Tr)$), and $\varepsilon$ as the residual. The random effect of animal, $An$, was assumed to have a variance-covariance proportional to the genomic relationship matrix generated from SNP genotypes. The distribution of the error was assumed $\sim N(0, \sigma_\varepsilon)$ and animal $\sim N(0, \sigma_\text{g})$. The phenotypic variance was obtained by summing the animal, litter, and pen within trial variance components. Heritability was obtained by dividing the animal variance component by the phenotypic variance.

Comparison of host response to NVSL and KS06 infection

Data from paired trials (Table 3.1) and the full G-matrix were used to estimate the effect of isolate on daily fitted viremia values, weekly weights, VL, TP, PV, Tmax, Vmax or WG by including isolate as a fixed class effect into the above model. Phenotypic
differences between virus isolates were assessed using the t-statistic reported by ASReml 3.0 (Gilmour et al., 2009) with a significance cutoff of $\alpha=0.05$.

**Genetic parameters by isolate**

Heritabilities, and phenotypic and genetic correlations were estimated separately for each isolate for VL, WG, TP, PV, Tmax or Vmax using the full G-matrix in order to quantify relationships between the response traits. Heritabilities and litter effects were estimated using a univariate model. A multivariate model using all traits was attempted for genetic and phenotypic correlations between traits but this model did not achieve convergence, so bivariate models were used instead. Estimates of correlations were considered statistically significant at $\alpha=0.05$ based on a t-test with 1496 degrees of freedom for NVSL and 670 degrees of freedom for KS06.

The genetic correlation between viremia and weight gain was expected to change during the course of infection. Thus, the genetic correlation between $V(t)$ and three day weight gain at day $t$ ($WG(t)$) were estimated for every other dpi (i.e. 1, 3, 5, ..., 41), separately for each isolate, using a bivariate model. Three-day weight gain was derived from the fitted daily weights and included weight three days prior was fitted as a covariate instead of weight at day zero. Weight gain at 1 dpi was adjusted for weight at infection. These bivariate analyses resulted in two $21 \times 21$ matrices of genetic correlations between viremia and three-day weight gain, which were visualized in heat maps.

**Association of WUR genotype with response to NVSL and KS06 infection**

The association of genotype at the WUR SNP with VL, WG, TP, PV, Tmax and Vmax were estimated separately for infection with NVSL and KS06 by including the interaction of isolate with WUR genotype into the above model, with the full G-matrix
representing the relationships between animals. This model was also fitted to daily fitted viremia values and fitted weights to generate viremia and weight curves for each isolate and WUR genotype. For these analyses, the alleles at the WUR locus were reported using the Illumina A/B genotype reference system, as was used in the original studies reporting on the association of this SNP with weight gain and viral load during PRRSV infection (Boddicker et al., 2012; Boddicker et al., 2014a; Boddicker et al., 2014b). Statistical differences between each isolate by WUR combination were assessed using the t-statistic reported in ASReml (Gilmour et al., 2009) and the residual degrees of freedom from the model, with a significance cutoff of α=0.05.

Genetic correlations of response between isolates

The different G-matrices described above were used to estimate genetic correlations of VL, WG, TP, PV, Tmax and Vmax between the two virus isolates using a bivariate model. Genetic correlations were evaluated for statistical significance based on a t-test with 2168 degrees of freedom when using $G$, $G_w$, $G_b$ and $G_{b-w}$, and with 1378 degrees of freedom when using only paired trials ($G_p$ and $G_{p-w}$).

Results

Comparison of host response to infection with NVSL and KS06

Raw viremia profiles suggested differences in pig response to infection with the NVSL versus the KS06 PRRSV isolate (Figure S3.1). To statistically quantify these differences, a selection of curve characteristics were derived from the Wood’s function parameters and compared between isolates using data from trials that were paired by genetic background to remove confounding between isolate and genetic background (Table
Pigs infected with NVSL had 16±2% higher VL than pigs infected with KS06 (Table 3.3, Figure 3.1). Pigs infected with NVSL had 14±2% higher PV and reached PV 2.5±0.6 days earlier (TP) than pigs infected with KS06 (Table 3.3, Figure 3.1). Both TP and PV are related to the host’s ability to respond during the replication-dominant phase of PRRSV infection. Compared to pigs infected with KS06, NVSL infected animals reached maximal PRRSV clearance 3.9±0.7 days earlier (Tmax) and cleared at a 36±14% faster maximal rate (Vmax) than their KS06 infected counterparts (Table 3.3, Figure 3.1). Tmax and Vmax are both related to the host’s ability to clear PRRSV. When comparing the impact that infection had on weight gain, pigs infected with the NVSL isolate had a tendency to grow slower than their KS06 infected counterparts (Table 3.3, Figure 3.1). This comparison of viremia characteristics and WG between isolates (Table 3.3, Figure 3.1) indicated that NVSL is more virulent than KS06 because it reached a higher PV more rapidly and resulted in higher VL and slower growth of the pigs. KS06 appears to be more persistent than NVSL, as shown by a longer time to maximal decay rate, lower maximal decay, and a larger percentage of pigs classified as persistently infected, defined as a non-rebound pig with a fitted log10 serum viremia value greater than 1 at 42 dpi (56% for KS06 vs 40% for NVSL; Table S3.1).

**Heritability estimates for viremia curve characteristics and weight gain**

All evaluated traits were estimated to be moderately to highly heritable, except for Vmax under infection with NVSL (Table 3.3). The traits with the highest estimated heritability under infection with NVSL were VL and WG and these traits also had high heritability estimates under infection with KS06. Vmax had the lowest estimated heritability under infection with NVSL compared to the other PRRSV curve
characteristics, but a moderate heritability under infection with KS06 (Table 3.3). The estimated genetic variance for Vmax was similar under infection with NVSL and KS06 (0.00053 vs. 0.00072), so the difference in heritability was primarily driven by a larger estimated environmental variance under infection with NVSL compared to KS06 (0.0057 vs. 0.0027).

Heritability estimates were similar between NVSL and KS06 for WG, TP, and Tmax (Table 3.3). These traits also had similar estimates of the litter component for NVSL and KS06 infected pigs. Traits VL, PV, and Vmax had lower heritabilities and larger litter components for NVSL compared to KS06. Summing the heritability and litter components gave similar results for the two isolate for VL and PV, although the heritabilities were quite different between isolates (Table 3.3). The larger number of animals infected with the NVSL isolate compared to KS06 may result in more accurate separation of the heritability and litter components for NVSL.

**Genetic parameter estimates using different G matrices**

Estimates of the genetic correlation between isolates for PV were very similar when using the full G matrix (G) or the G matrix containing information about genetic relationships within genetic background (G_B; Table 3.4), indicating that host genetic factors influencing PV were conserved across genetic backgrounds. The genetic correlation between isolates for WG was slightly higher when using G_B compared to G (Table 3.4), suggesting that WG of pigs within the same genetic background are more similar than between pigs of different genetic backgrounds. The estimate of the genetic correlation of VL between pigs infected with NVSL and KS06 dropped substantially when G_B was used, compared to using G (Table 3.4). The estimate of genetic correlation between isolates for
TP increased when using $\mathbf{G}_b$ rather than $\mathbf{G}$ (Table 3.4), such that the estimate based on $\mathbf{G}_b$ was no longer significantly different from one but also not significantly different from zero. Estimates of the genetic correlation between isolate for Tmax and Vmax were not significantly different from zero or one when using either $\mathbf{G}_b$ or $\mathbf{G}$ (Table 3.4). In general, estimates from matrices that only included relationships between animals from the same genetic background were similar whether all animals were used ($\mathbf{G}_b$) or only those from trials that were paired across isolate ($\mathbf{G}_p$).

**Genetic correlations among viral load and viremia curve characteristics**

VL, defined as area under the Wood’s curve from 0-21 dpi, was largely driven by PV, as shown by the high genetic and phenotypic correlations between these two traits for both isolates (Tables 3.5 and 3.6). PV had the highest genetic correlation between PRRSV isolates and was not significantly different from one (Table 3.4). No other curve characteristic had a between virus isolate genetic correlation estimate that was significantly different from zero. This suggests that the observed genetic correlation between isolates for VL is primarily due to the high genetic correlation observed between isolates for PV.

Tmax and Vmax had strong negative genetic correlations with each other for both isolates but they were only highly correlated with VL for NVSL (Tables 3.5 and 3.6). Time to maximal decay rate (Tmax) was 19.3 days for KS06 but 15.4 days for NVSL (Table 3.3). Thus, Vmax was expected to play a larger role in VL for NVSL than for KS06, because VL was calculated from 0-21 dpi. No conclusions can be drawn about the genetic correlations between isolate for Tmax or Vmax because the estimates were not significantly different from zero or one due to large standard errors (Table 3.4).
The two time-related traits TP and Tmax had strong positive genetic and phenotypic correlations with each other for both isolates (Tables 3.5 and 3.6) because TP \( \frac{b}{c} \) is a component of Tmax \( \left( \text{Tmax} = \text{TP} + \frac{\sqrt{TP}}{\sqrt{c}} \right) \). The genetic correlation between isolates was significantly different from one for TP (0.25±0.33, Table 3.4), indicating that host genetic control of the time until maximal virus decay rate may differ between virus isolates.

**Genetic correlations of weight gain with viremia curve characteristics**

PV had a moderate negative genetic correlation with WG for NVSL (Table 3.5) infected pigs but this genetic correlation was not significantly different from zero for KS06 infected pigs (Table 3.6), due to a larger standard error and a less negative estimate. Vmax also had a significant genetic correlation with WG. These results suggest that the reduction in growth is caused by an overall high viremia level over a prolonged period of time, which is further supported by the finding that WG had the highest estimated genetic and phenotypic correlations with VL for both isolates (Tables 3.5 and 3.6).

**Genetic correlations of viremia with weight gain**

The genetic and phenotypic correlations between VL and WG were negative and of similar magnitude for the two isolates (Tables 3.5 and 3.6). Between isolate genetic correlation estimates for VL and WG were high and not significantly different from one (Table 3.4), indicating that host genetic control of VL and WG was very similar under infection with either the NVSL or the KS06 isolate.

A more thorough exploration of the relationship between PRRS viremia and weight gain was accomplished by estimating genetic correlations between fitted viremia and three-day weight gain across the infection period (Figure 3.2). Genetic correlations generally showed a similar pattern between NVSL and KS06, however correlations for NVSL were
more extreme (range = -1 to 0.43) than KS06 (range = -0.75 to 0). A more detailed explanation of these results can be found in the Discussion: Genetic correlation of viremia with weight gain across time section.

**Associations of WUR genotype with response to infection with NVSL and KS06**

Least square means of daily viremia and weight were estimated by fitting the interaction between isolate and WUR genotype for all trials simultaneously and are shown in Figure 3.3. Very few pigs had the BB genotype at the WUR locus, so estimates of least square means for the BB genotype had high standard errors. For VL, both AB and BB animals were significantly different from AA, while BB animals were not significantly different from AB animals, suggesting complete dominance, as previously reported by Boddicker et al. (Boddicker et al., 2012; Boddicker et al., 2014a; Boddicker et al., 2014b). Results for BB animals will not be discussed further in the following section.

Pigs with the AA WUR genotype had 4.5±0.4% higher VL (P<0.001; Figure 3.4A) and grew 2.0±0.2 kg less than pigs with the AB genotype after infection with NVSL (P<0.001; Figure 3.4B). These estimates are consistent with previous estimates of the association of WUR under infection with NVSL (Boddicker et al., 2014b). Genotype at the WUR SNP was also found to be associated with VL under infection with KS06, for which VL was 4.2±0.9% higher in AA animals than in AB animals (P<0.001) (Figure 3.4A). In contrast to infection with NVSL; however, WUR genotype did not have a significant association with WG (P=0.32), although the direction of the effect was consistent, with AA pigs 0.4±0.4 kg less than pigs with the AB genotype (Figure 3.4B).

Genotype at the WUR locus was associated with all viremia curve characteristics in pigs infected with NVSL (Figures 3.3 and 3.4A). Compared to AA animals, AB animals
had 2.8±0.4% lower PV (P<0.001; Figure 3.4D), which was reached 0.20±0.09 days earlier (P<0.02; Figure 3.4C). AB animals also had a 3.8±1.5% faster maximal decay rate (P<0.02; Figure 3.4F), which was reached 0.68±0.16 days sooner (P<0.001; Figure 3.4E). In KS06 trials, WUR was associated with 3.4±0.7% higher PV in AA animals compared to AB animals (P<0.001; Figure 3.4D) but no association was found with Vmax (P=0.36; Figure 3.4F) and the direction of the effect for Vmax was opposite to that of NVSL infected animals, with AB having a 3.1±3.4% slower maximal decay rate than AA animals. Compared to AA animals, AB animals tended to reach peak viremia 0.30±0.16 (P=0.052) days sooner (Figure 3.4C) and the maximal decay rate 0.47±0.29 (P=0.078) days later (Figure 3.4E). The effect of WUR genotype was significantly different between the NVSL and KS06 isolates for both WG (P=0.001) and Vmax (P=0.041; Figure 3.4).

Plotting average weight curves and viremia using the Wood’s curve parameters from the primary phase of infection (i.e. not including rebound) for pigs with the AA and AB genotypes at WUR by isolate (Figure 3.3), provided a visualization of the overall differences in the shape of the viremia and weight curves. For KS06, the effect of WUR on VL was mainly driven by differences in PV but the difference in viremia level between AA and AB was not maintained due to a slightly lower rate of clearance in AB compared to AA animals, resulting in similar viremia levels at 42 dpi. Conversely, for NVSL, the difference in viremia levels between AA and AB animals first appeared around peak viremia and became larger during the primary stages of infection due to a faster clearance rate for AB animals. In general, the WUR genotype had a lower association with response to infection in pigs infected with KS06 than pigs infected with NVSL, suggesting that the magnitude of the effect of SSC4 QTL depends on virulence of the PRRSV isolate.
Impact of the WUR region on heritabilities and genetic correlations

Heritabilities of response traits were estimated by including all SNPs in the full G-matrix (Table 3.4) and also by excluding SNPs in the 5 Mb region surrounding the WUR SNP (G.w; Table 3.7) in order to assess how much of estimates of heritability and genetic correlations between isolates were attributed to the WUR region. In the NVSL trials, estimated heritabilities were lower for all traits when the G.w was used, except for Vmax, which remained the same. Heritability estimates for WG and Tmax were not affected by using G.w, while the estimates dropped for all other traits when G.w was used, with the largest drops for PV and VL.

Genetic correlations for the response traits between isolates were also estimated using full G (Table 3.4) and G.w (Table 3.7). Estimates of genetic correlations between isolates for WG and Vmax were slightly larger when using G.w compared to G, while the estimates for all other response traits decreased, with the largest decreases for PV and VL. Genetic correlation estimates between isolates were significantly different from zero and not significantly different from one for VL, WG and PV when using either G or G.w (Tables 3.4 and 3.7). This indicates that host genetic control of response to infection is highly conserved across isolates for these three traits. The high genetic correlation estimates for these traits when using G.w indicates that the conserved host genetic response between isolate for VL and PV did not solely depend on the SSC4 QTL but has a large polygenic component. The increase in the genetic correlation between isolates for WG when using G.w compared to G is consistent with the observed effect of WUR in pigs infected with NVSL and the much smaller effect in pigs infected with KS06. Estimates of genetic correlations between isolates for Tmax and Vmax had large standard errors when
using either G or G-W, so no conclusions can be drawn because these estimates were not significantly different from zero or one. Estimates of the genetic correlations between isolates for TP were significantly different from one but not significantly different from zero for either G or G-W, indicating that host genetic control of TP is not highly conserved across isolate.

GB and GP were also constructed without the 5 Mb WUR region (GB-W and GP-W, respectively). The results obtained using these matrices were as expected based on the differences between G and G-W and were also consistent with the differences in the estimates when comparing the use of GB and GP to the use of G (Tables 3.4 and 3.7).

Discussion

Consistent with the previously conducted studies of the PHGC, the animals used in this study were inoculated with PRRSV both intramuscularly and intranasally. The protocol used in the study is a standard challenge protocol designed to give every pig a consistent amount of virus. It also simulates the most likely routes for infection through needle sticks and intranasal exposure. Previous studies comparing route of exposure have shown that the dosage used to inoculate the pigs impacted the level of viremia in the pigs independent of route of exposure, resulting in similar levels of antibody production between the two routes of exposure (Yoon et al., 1999). Similarly, growth and antibody response was similar between intranasally and simultaneously intranasally/intramuscularly vaccinated pigs (Sornsen et al., 1998). Given that no differences in host response are expected to be observed between these methods, this approach was the most appropriate to ensure consistent levels of infection between pigs and allowed more power to distinguish
between animals that differ in their genetic merit regarding response to PRRSV infection. Relatley, this method would closely mimic practices employed when vaccinating the animals, potentially providing insight into response to vaccination, although further studies are needed to confirm.

Our results suggest that KS06 is a less virulent PRRSV isolate than NVSL but, importantly, that genetic selection for pigs with improved weight gain and reduced viral load under either PRRSV infection is expected to be effective across these PRRSV isolates. This study also affirmed the important influence of the WUR10000125 genomic region on SSC4 on host response to PRRSV. The effect of genotype at this locus was consistent between isolates for traits related to viremia. While AB animals gained slightly more weight than AA animals during infection with KS06, a significant difference between WUR genotypes was only observed in NVSL for weight gain, suggesting that the influence of this QTL on weight gain may depend on virulence of the PRRSV isolate.

Modelling viremia using the Wood’s curve

This study has demonstrated the utility of mathematical functions to assess the impact of host genetics and virus isolate on PRRS viremia kinetics. The Wood’s curve uses three parameters, a, b, and c, which are related to the overall level of viremia (a) and describe the shape of the curve (b, which is dominant pre-peak, and c, which is dominant post-peak) (Wood, 1967). While other mathematical functions may more adequately model PRRS viremia during infection, the number of data points collected during these trials limited the fitting of more complex models. Fitting a Wood’s curve is a more powerful method for comparing viremia kinetics than the LOESS smoothed fit used in previous analyses of these data (Boddicker et al., 2012; 2014a; 2014b). The LOESS smoothed fit
uses a parameter indicating the degree of polynomial to fit to the data and a smoothing parameter for curve fitting (Jacoby, 2000), with the primary intent of filtering out noise from the data. The limitation of the LOESS fit, however, is that it does not lend itself to extracting fitted parameters that specify particular physical properties of a system that have important implications in understanding the dynamics of PRRSV infection. Although both methods adequately fitted the data, Wood’s curve parameters describe both the magnitude and shape of the curve, which can be used to explore different characteristics of the viremia curves. Exploring Wood’s curve characteristics can provide insight into important biological questions, such as which aspects of host response are under strongest genetic control and how selection for one curve characteristic may affect others, and thus the entire profile. The Wood’s function can also be used to explore the relationship between curve characteristics and other phenotypes, such as growth under infection. Furthermore, comparison of the extended Wood’s and Wood’s curve functions allowed for an objective method for separating primary infection from rebound infection viremia curves (Islam et al., 2013).

While the advantages of fitting a Wood’s curve to model the dynamics of PRRS viremia are clear, care needs to be taken in the interpretation of the correlations between curve characteristics because strong correlations between these curve characteristics are likely to arise, partly as an artifact of the Wood’s function and partly as they reflect true correlations between curve characteristics that are independent of the Wood’s function. One example for which the genetic correlation between traits is partially driven by the Wood’s function rather than by the same genetic mechanisms is the high genetic correlation
observed between Tmax and Vmax, because both rely heavily on the b parameter of the Wood’s function.

**Genetic parameter estimates using different G matrices**

Three different G matrices were constructed for both the full G matrix and the G-W matrix. GB only contained relationships between animals from the same genetic background, with zeros for relationships between animals from different companies. Thus, while G contains information about genetic variance that exists within genetic background as well between genetic backgrounds, GB only contains information about genetic variance within genetic background and is, therefore, more similar to the pedigree-based relationship matrix because there was no pedigree information between animals from different genetic backgrounds. GP was a block diagonal matrix that used only pigs from the same genetic background that were paired across isolates and was used to avoid biases in estimates that could result from including different breeds in the analyses for each isolate. In general, estimates using GB considered only animals from trials that were paired across isolate (GP) were consistent with estimates using GB. This suggests that, while the genetic correlation between isolates for VL may be moderate within genetic backgrounds, some genetic backgrounds have high VL under infection with both NVSL and KS06, while some genetic backgrounds have low VL under infection with both NVSL and KS06, such that when the relationships between genetic backgrounds are considered (using G), the genetic correlation between isolates for VL increases. Selecting for improved VL during infection with one PRRSV isolate is likely to improve VL during infection with another PRRSV isolate, but the extent to which such selection is successful across-isolate will likely differ between genetic backgrounds.
Comparison of genetic parameter estimates for viral load and weight gain to previous estimates

Estimates of heritability of VL and WG during NVSL infection were slightly different from previously reported estimates using these data (Boddicker et al., 2014b) (VL: $0.31 \pm 0.06$ vs $0.44 \pm 0.13$; WG: $0.33 \pm 0.06$ vs $0.29 \pm 0.11$) (Table 3.3). Differences between estimates can be attributed to the use of genomic rather than pedigree-based relationships, the inclusion of trial 15, and the addition of age and weight at infection as covariates in the model used in this study. Age and weight at infection are important to include in the analysis because pigs that are older or heavier at infection tend to be able to mount a stronger immune response, independent of the host’s genetic ability to combat the virus (Thanawongnuwech et al., 1998; Klinge et al., 2009). The use of genomic instead of pedigree-based relationships halved the standard errors of estimates because the G matrix more accurately captures relationships between animals, especially with limited pedigree information available.

The genetic correlation estimate was more negative for the NVSL infected pigs than previously reported ($r_g$: $-0.74 \pm 0.10$ vs $-0.46 \pm 0.20$; $r_p$: $-0.33 \pm 0.03$ vs $-0.29 \pm 0.03$) (Boddicker et al., 2014b), which can be attributed to the combination of using genomic versus pedigree relationships, the addition of trial 15, and the inclusion of age and weight at infection as covariates. Strong genetic correlations between VL and WG suggest that there are common genes or pathways that affect both of these traits, likely through more resistant pigs having less viremia and therefore less infection-induced reduction in WG.
Impact of PRRSV genetic diversity on host response to PRRSV infection

Differences in viremia and weight gain during infection with NVSL versus KS06

PRRSV glycoprotein 5 (GP5) is a major envelope protein that plays a vital role in the virion’s formation and infectivity and harbors a major neutralizing epitope (Ostrowski et al., 2002). This gene is often used to assess genetic differences between PRRSV isolates and is suggestive of differences in virulence between isolates (Ansari et al., 2006; Chand et al., 2012). Variation in GP5 impacts the pig’s ability to produce neutralizing antibodies, which may not be protective against different isolates or different quasispecies that may be existing within a pig (Pirzadeh et al., 1998; Rowland et al., 1999; Ansari et al., 2006). NVSL and KS06 were isolated from different geographic regions nearly ten years apart and are 89% similar at the GP5 nucleotide and amino acid sequence level (Ladinig et al., 2015; Trible et al., 2015) and they are clustered into two distinct branches based on molecular phylogeny (Ladinig et al., 2015). Forsberg et al. (2002) found that, on average, PRRSV isolates have a substitution rate of 0.073 per nucleotide across isolates at open reading frame 5, while the maximum substitution rate between two isolates was 0.153 substitutions per nucleotide; NVSL and KS06 have a substitution rate of 0.11 per nucleotide at the GP5 level (Trible et al., 2015).

This study has demonstrated that these virus isolates differ in both their virulence and in resulting viremia profile characteristics. Infection with NVSL was characterized by reaching high peak viremia early, followed by a quick clearance of the virus, whereas the KS06 virus accumulated more slowly towards a lower peak viremia and took longer to clear from serum. Pig growth tended to be less stunted when pigs were infected with the KS06 isolate compared to the NVSL isolate (Table 3.3). This may be because piglets
infected with the KS06 isolate do not need to put as much energy into eliminating the virus, thus allowing them to place more emphasis on growth. These results are consistent with resource allocation theory, which hypothesizes that trade-offs between competing traits (e.g. health and growth) are a consequence of limited resources (i.e. energy availability) (Rauw, 2008). Genetic correlations between viremia and weight gain changed during the course of infection and tended to be more extreme in NVSL infected pigs than in KS06 infected pigs, suggesting that more energy is required to fight infection with NVSL (Figure 3.2), which is supported by the lower weight gain observed in NVSL infected pigs (Figure 3.1). These findings are consistent with those of Doeschl-Wilson et al (2009), who showed that a temporary reduction in growth, due to greater investment in immune response during early infection, will benefit growth in the long-term due to a shorter or less severe infection, or both. The investment that the animal diverts to immune response and its impact on weight gain depends on the pathogen load that the animal experiences and thus on virulence of the isolate (Doeschl-Wilson et al., 2009).

**Genetic Correlations of viremia with weight gain across time**

In animals infected with NVSL, animals that had high viremia from 0-7 dpi tended to have high WG later on in the trial but low WG early on. During the early stages of infection, pigs with high viremia may need to allocate more energy to fighting the infection and away from growth, resulting in a negative genetic correlation between early viremia and early weight gain. It appears that under infection with NVSL, pigs that have higher viremia and lower weight gain early in infection may reap the benefits in terms of improved weight gain later in infection, as a result of reducing PRRS viral burden. Thus, animals with higher early viremia divert more resources to fighting infection early on, which pays
off in the long run with higher weight gain at the end of the trial. This notion is supported by the weaker genetic correlations between early viremia and weight gain under infection with the less virulent isolate, KS06 (Figure 3.2B), which resulted in lower pre-peak viremia (Figure 3.1). It is also likely that pigs with high early viremia suffer a loss in appetite (Xiao et al., 2010), which may further reduce early weight gain in these animals. The positive genetic correlation between early viremia and late weight gain could reflect a return to homeostasis after infection in these pigs (Exton, 1997). These observations are consistent with findings from a modeling approach that systematically investigated the short- versus long-term effects of infection and genetic resistance on growth, and the role of nutrient allocation on the relationship between growth and pathogen load (Doeschl-Wilson et al., 2009).

While the relationship between weight gain and early viremia may differ between isolates, the ability of the animal to effectively clear the virus from serum is crucial for maintaining growth. This is evident from a block of highly negative correlations of viremia at 15 to 28 dpi with weight gain from 22 dpi onward in pigs infected with NVSL (Figure 3.2A). The time period from 15 to 28 dpi corresponds to the time when pigs are clearing PRRSV from serum the most rapidly. Strong genetic correlations were identified of viremia after 28 dpi with later weight gain, in particular for viremia at 33 dpi. This negative genetic correlation is likely the result of rebound pigs, as this time point corresponds to the average time that rebound pigs reach secondary peak viremia.

Similar to NVSL, KS06 infected pigs showed strong negative genetic correlations of viremia with weight gain at approximately the time of maximal viral clearance (Figure 3.2). In KS06 infected pigs, this critical period seemed to be viremia after 17-28 dpi and
weight gain after 17 dpi. This time period corresponds to the time in which antibodies, specifically IgG, are produced at the highest rates, suggesting that the ability to clear the virus effectively may depend on the pig’s ability to mount a successful adaptive immune response (Lopez and Osorio, 2004).

**Viral rebound**

Rebound (i.e. a bi-modal viremia profile) was observed more frequently when pigs were infected with the NVSL isolate than with the KS06 isolate. One possible explanation for the observed rebound in viremia is the presence of quasispecies within a host. PRRSV has a very high mutation rate, estimated to be between 4.7 and $9.8 \times 10^{-2}$ nucleotides/year, which is the highest reported for an RNA virus (Murtaugh et al., 2010). This high mutation rate causes within-animal variation in the PRRSV genome (Goldberg et al., 2003), with each population of common variants termed a quasispecies. Pigs infected with NVSL had higher viremia than pigs infected with KS06 throughout most of the pre-rebound phase (Figure 3.1). This greater level of viremia means that more replications and, thus, more mutations have occurred for NVSL, so there is likely a greater number of quasispecies present in animals infected with NVSL than KS06. The greater the number of quasispecies, the greater the chance that a variant is able to escape host immune response and cause viral rebound (Cостерс et al., 2010). NVSL could also have a greater number of quasispecies, if the mutation rate is higher for NVSL than for KS06. Animals in the same pen can also transfer quasispecies between each other, whereby a quasispecies from one pig could be transferred to another pig and cause reinfection and viral rebound (Goldberg et al., 2003).

Alternately, NVSL may avoid host immune response more effectively than KS06, possibly escaping humoral immune response by localizing to certain tissues. Previous
research has identified that the tonsils are a primary source of PRRSV persistence (Wills et al., 1997; Wills et al., 2003). This may be due to an abundance of memory B-cells in the tonsil but absence of effector, plasma-producing, B-cells (Mulupuri et al., 2008). An abundance of PRRSV in tonsils may result in cyclical reappearance of circulating virus. If the ability of the virus to localize to tissue to escape immune response differs between isolates, this will be reflected in the tonsil viremia levels. Studies are underway to address this possibility.

Impact of the SSC4 QTL on PRRS disease resistance

Consistent with previous reports, the WUR SNP was significantly associated with VL during PRRSV infection, in that animals with the AB genotype had lower VL than animals with the AA genotype (Boddicker et al., 2012; 2014a; 2014b). The effect of WUR on VL appeared to be primarily driven by the pig’s ability to control the rate of virus replication, as made evident by the large effect the marker had on PV. This was the only curve characteristic for which WUR had significant effect for both NVSL and KS06 infected pigs (Figure 3.4), which is likely due to the role the putative causative gene, GBP5, plays in the host’s immune response. The GBP5 gene showed allele specific expression based on WUR genotype, and animals that have the AA genotype appear to produce no functional GBP5 (Koltes et al., 2015). GBP5 plays a role in the innate immune response during infection. Specifically, NLRP3 interacts with tetrameric GBP5 to promote inflammasome assembly (Shenoy et al., 2012).

Although the SSC4 QTL appears to play a significant role in host response to PRRSV infection, there is a substantial polygenic component beyond the SSC4 region for VL and PV. WUR genotype explained 13% of the genetic variance for VL (Boddicker et
al., 2012; 2014a; 2014b). Consistent with this finding, accounting for WUR genotype did not remove all of heritability of VL and, although the genetic correlation between isolates dropped when accounting for WUR genotype, it remained high and not significantly lower than one (Table 3.7).

Interestingly, WUR genotype was not found to be significantly associated with WG in pigs infected with KS06, although the effect was in the same direction as for NVSL, only smaller. The difference in VL between isolates was greater than the difference in VL between AA and AB genotypes, thus AA individuals infected with KS06 had lower VL than AB individuals infected with NVSL. This may mean that less energy is needed to fight the virus during infection with the KS06 isolate and, as a result, weight gain was less affected, as evidenced by the higher weight gain during infection observed when pigs were infected with KS06 compared to NVSL. Thus, the effect of WUR on weight gain may only be substantial during infection with more virulent isolates of PRRSV due to the increased severity of infection. Isolate-specific QTL for resistance have been identified in a number of infection (fungal, bacterial, and viral) studies in plants (Leonardsschippers et al., 1994; Caranta et al., 1997; Liu et al., 2011) and in a study on Dengue Virus in mosquitoes (Fansiri et al., 2013).

It is likely that the SSC4 QTL affects the severity of infection and its effect on WG is through the increased resources that have to be allocated to fighting the infection when viremia is higher. The relationship between VL and reduction in WG may be non-linear, which may explain why the direction of the WUR effect on WG was the same for both PRRSV isolates but the magnitude of the effect differed. A high genetic correlation between isolates was observed for WG, despite the lack of a significant WUR effect for
KS06 (Table 3.7). Given the number of genetic factors that can influence WG, it is likely that what is in common for WG between these two isolates are the polygenic effects, which would explain the high genetic correlation between isolates despite the lack of a QTL in the WUR region in the KS06 trails. The WUR region explained 9% of the genetic variance for weight gain for the NVSL trials, while few other genomic regions explained more than 1% of the genetic variance (Boddicker et al., 2014a).

**Potential avenues of selection for increased resistance to PRRS**

**Selection on WUR genotype**

This study has shown that genotype at the WUR SNP is associated with VL and PV (Figure 3.4A and D) across two distinct PRRSV isolates, indicating that selection to increase the frequency of the B allele, corresponding to increased PRRS resistance, may help with reducing PRRS VL across isolates. Reducing viral burden has the potential to decrease the costs associated with PRRS by reducing PRRS incidence because a lower viral burden may also reduce virus shedding, which reduces the chance that other pigs will be infected. Although WUR did not have a significant effect on WG under infection with KS06, the direction of the effect was the same as for the NVSL isolate, thus selection to increase the frequency of the B allele at WUR is expected to improve WG under infection with more virulent isolates of the virus, with no negative effect on WG with less virulent isolates (Figure 3.4B). Given the suspected dominant nature of this SNP (Boddicker et al., 2012; Boddicker et al., 2014a; Boddicker et al., 2014b) and the putative QTN that it marks (Koltes et al., 2015), increasing the frequency of the B allele is expected to help improve PRRSV resistance by increasing the number of AB and BB animals, which are expected to perform similarly for VL and WG.
Selecting for the AB genotype at WUR has the potential to reduce VL and PV across breeds and isolates, however the amount of response is limited because the SSC4 region explained only a portion of the genetic variance in host response. The response traits VL and PV, however, also have large polygenic effects that appear to be conserved across isolates and breeds and independent of WUR (Table 3.4 and 3.7). Thus, genomic selection for VL or PV in combination with marker assisted selection on WUR may hold the greatest potential for improved resistance to PRRS.

Potential for genomic selection

Genomic selection uses markers spaced throughout the genome to predict the genetic merit of an individual. All host response traits investigated here had a moderate-to-high heritable genetic component (Table 3.3), suggesting that genomic selection for different aspects of host response to PRRSV infection is feasible. There were high genetic correlations between VL, WG and PV for both isolates, except for PV and WG for KS06 (Tables 3.5 and 3.6), suggesting that genomic selection for one trait is likely to improve response in the other two. Genetic correlations of host response between isolates were also high for VL, WG and PV (Table 3.4), suggesting that genomic selection for response to one isolate will result in improvement across isolates.

A limitation of genomic selection is the size of the data that will need to be generated on a continual basis in order to ensure accurate prediction of breeding values. Genomic predictions tend to be more accurate the more animals are in the training set. It has also been shown, however, that prediction accuracy decreases as the number of generations between the training and prediction sets increases (Wolc et al., 2011), so periodic re-training on new phenotypes and genotypes will be necessary.
Potential for selection on response to vaccination

While genomic selection for host response to PRRSV sounds appealing, quality infection data on many animals are needed to get accurate predictions. In principle, information on naturally infected commercial pigs can be fed back into the nucleus in order to make selection decisions based on host response in commercial pigs. However, several factors cannot be controlled in a natural infection setting, such as virus dosage, time since infection, and the age and weight of the pig at infection, which all have an effect on how the pig responds to infection.

Response to vaccination may be an attractive alternative method for providing phenotypes on host response to PRRS because all of the above factors can be controlled with vaccination. A major current PRRS vaccine is a modified live virus, which has reduced virulence compared to commonly occurring wild type isolates. Thus, vaccinated pigs have circulating viremia that can be measured, similar to infection with any natural PRRSV isolate (Cano et al., 2007). Measuring response to vaccination overcomes the limitations of natural infections because it is a controlled infection. Before this can be implemented in industry, however, it is necessary to evaluate the genetic correlation between response to vaccination and response to natural infection with a variety of isolates.

Response to vaccination could be evaluated by using viremia measured on a single serum sample at the average time for pigs to reach peak viremia after vaccination, which may serve as an approximation for the peak of the individual. This has the advantage of needing only a single serum sample, rather than multiple samples throughout infection, as is needed for VL. Our results show that PV is highly correlated, genetically, with VL under infection with both NVSL and KS06 (Tables 3.5 and 3.6) and that genetic control of PV is
expected to be highly conserved between isolates, both within breeds and across the North American pig population (Table 3.4). To implement this, the expected time to peak after vaccination will need to be assessed, along with the genetic correlation of viremia at this time point with VL after vaccination.

**Further considerations**

When considering selection for increased disease resistance, particularly one specific disease, the consequences of selection needs to be assessed. A common question addressed in geneticists aiming to breed disease-resistant animals is whether or not the parasite will evolve so as to overcome the genetic changes in the host (i.e. parasite evolution). This is especially of concern with a rapidly mutating virus such as PRRSV. Simulation studies have shown that selection for a quantitative resistance (i.e. a continuous scale of levels of resistance) will result in selection of more virulent forms of the parasite (Gandon and Michalakis, 2000). Likewise, vaccines that have high efficiency, but less than 100%, will also select for more virulent forms of the parasite, as has been observed with Mareck’s disease (Read et al., 2015). Selection on a single disease resistance locus can be thought of in this same context if the resistance allele does not sufficiently reduce the spread of the parasite. This consequence can be alleviated by genomic selection, as the combination of genetic factors that confer a higher genetic resistance in one animal is less likely to be identical to that in another animal. This can further be alleviated by using multiple approaches to combat the disease (such as vaccination, described above). On another, although not unrelated, note, selection on general health, or a generalized immune response could be utilized (Wilkie and Mallard, 1999; Stear et al., 2001), which may be beneficial for infections with different pathogens, while presumably preventing the
skewing of the immune response in the animals toward to be strong towards specific types of pathogens, which could increase the vulnerability of the animals to others.

Another consideration is the impact that selection for increased resistance will have on production. Recently, studies have been published that discussed the impacts of WUR on production traits. Abella et al. (2016) reported that AA animals show more growth compared to AB animals in an uninfected setting, while another study (Niu et al., 2015) found that AB animals had equivalent or superior growth performance and meat quality compared to AA animals. However, further investigation on the broader impact of selection for improved response to PRRSV infection is needed.

There are two major types of PRRSV: Type 1 (European) and Type 2 PRRSV (North American), defined by the major genomic differences between them (Dea et al., 2000). These two types PRRSV of differ both in pathogenicity (Choi et al., 2015) and virulence (Han et al., 2013). Therefore, the influence that pig genetics has on response to type 1 PRRSV infection still remains to be elucidated; however, a recent study conducted by Abella et al. (2016) showed a favorable response of pigs with the AB genotype that were infected with a European PRRSV isolate. While these results are promising, the similarity in host genetics influencing response to type 1 and type 2 PRRSV isolates as a whole is something that merits further investigation.

Conclusions

Despite pronounced differences in viremia profiles between NVSL and KS06, the underlying genetic factors influencing host response to infection were found to be largely the same between these two PRRSV isolates for VL, PV, and WG. NVSL and KS06 are
diverse isolates, therefore these results suggest that genomic selection for VL, PV, or WG during infection with one isolate will improve these traits when infected with another virus isolate. The WUR SNP, previously identified to be associated with VL and WG under infection with NVSL, was also found to be associated with all curve characteristics in the NVSL trials; but only with VL and PV in KS06 trials, suggesting the effect of WUR may depend on virulence of the PRRSV isolate. Infection trials that involve more isolates of PRRSV are needed to confirm that genetic factors that influence host response to PRRSV infection are consistent across a range of PRRSV isolates. Genetic correlations of viremia and weight at multiple time points provided insight into how host genetic control of viremia and growth changes throughout the trial. Analysis of the relationship between viremia and weight gain via genome-wide association studies over the course of infection has the potential to identify additional genomic region that could improve host response to PRRSV infection across isolates if selected upon and will allow for a more comprehensive understanding of the host genes and genomic regions associated with response to PRRSV infection. Studies that are currently underway as part of the PHGC include field trials, and response to vaccination and coinfection with PRRSV and PCV2b.

Acknowledgements

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Newsham Choice Genetics, Fast Genetics, Genetiporc, Inc., Genesus, Inc., PigGen Canada, Inc., IDEXX Laboratories, and Tetracore, Inc. Contributions of the late Dr. Steve Bishop of the Roslin Institute are also acknowledged. Steve was involved in the design of the experiments and oversaw the fitting the Wood’s curves to the viremia data and estimation of the Wood’s curve parameters.

References


Boddicker, N. J. 2013. The genetic basis of host response to experimental infection with the porcine reproductive and respiratory syndrome virus in pigs, Iowa State University Ames, IA.


Choi, K., J. Lee, C. Park, J. Jeong, and C. Chae. 2015. Comparison of the Pathogenesis of Single or Dual Infections with Type 1 and Type 2 Porcine Reproductive and Respiratory Syndrome Virus. Journal of Comparative Pathology 152: 317-324.


Forsberg, R. et al. 2002. The genetic diversity of European type PRRSV is similar to that of the North American type but is geographically skewed within Europe. Virology 299: 38-47.


### Tables and Figures

Table 3.1 Animal Composition of the PHGC Trials

<table>
<thead>
<tr>
<th>PRRS Virus Isolate</th>
<th>Trial Number</th>
<th>Number of Animals</th>
<th>Breed Cross</th>
<th>Genetic Background</th>
</tr>
</thead>
<tbody>
<tr>
<td>NVSL-97-7895</td>
<td>1-3</td>
<td>507</td>
<td>LW x LR</td>
<td>A</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>191</td>
<td>Duroc x LW/LR</td>
<td>B</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>182</td>
<td>Duroc x LR/LW</td>
<td>C</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>109</td>
<td>LR x LR</td>
<td>D</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>186</td>
<td>Pietrain x LW/LR</td>
<td>E</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>158</td>
<td>Duroc x LW/LR</td>
<td>F</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>166</td>
<td>Pietrain x LW</td>
<td>G</td>
</tr>
<tr>
<td>KS-2006-72109</td>
<td>10</td>
<td>184</td>
<td>Pietrain x LW</td>
<td>G</td>
</tr>
<tr>
<td></td>
<td>11</td>
<td>177</td>
<td>LW x LR</td>
<td>A</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>146</td>
<td>LR x LW</td>
<td>H</td>
</tr>
<tr>
<td></td>
<td>13&lt;sup&gt;3&lt;/sup&gt;</td>
<td>173</td>
<td>Duroc x LW/LR</td>
<td>F</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>165</td>
<td>Duroc x LR/LW</td>
<td>C</td>
</tr>
</tbody>
</table>

1. LW = Large White; LR = Landrace
2. Genetic background is defined as pigs from the same breeding company and the same breed cross.
3. Trial 13 was excluded from analyses due to unusual viremia profiles as seen in Figure S3.1.
Table 3.2. Comparison of Viral Load of Boddicker (VL<sub>B</sub>) and Viral Load based on the Wood’s Curve (VL) for the two virus isolates (NVSL and KS06)

<table>
<thead>
<tr>
<th>VL&lt;sub&gt;B&lt;/sub&gt; vs VL&lt;sup&gt;1&lt;/sup&gt;</th>
<th>Heritability</th>
<th>Genetic correlation</th>
<th>Phenotypic correlation</th>
</tr>
</thead>
<tbody>
<tr>
<td>NVSL VL&lt;sub&gt;B&lt;/sub&gt;</td>
<td>0.23 (0.10)</td>
<td>0.98 (0.03)</td>
<td>0.90 (0.01)</td>
</tr>
<tr>
<td>NVSL VL</td>
<td>0.22 (0.10)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>KS06 VL&lt;sub&gt;B&lt;/sub&gt;</td>
<td>0.35 (0.09)</td>
<td>0.98 (0.02)</td>
<td>0.90 (0.01)</td>
</tr>
<tr>
<td>KS06 VL</td>
<td>0.35 (0.09)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1. All trials except trial 13 were used in the analysis. Data from NVSL and KS06 infection trials were analyzed separately.
Table 3.3. Least Square Means, Heritabilities, Litter Effects, and Phenotypic Standard Deviations of Responses to Infection with the NVSL and KS06 isolates.

<table>
<thead>
<tr>
<th>Trait</th>
<th>LSMeans (^2,^3)</th>
<th>P-value (^5)</th>
<th>Heritability (^3,^4)</th>
<th>Litter (^3,^4)</th>
<th>Phen. s.d. (^3,^4)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NVSL (s.e.)</td>
<td>KS06 (s.e.)</td>
<td>NVSL (s.e.)</td>
<td>KS06 (s.e.)</td>
<td>NVSL (s.e.)</td>
</tr>
<tr>
<td>WG</td>
<td>15.8 (1.1)</td>
<td>19.5 (1.4)</td>
<td>0.076</td>
<td>0.33 (0.06)</td>
<td>0.31 (0.09)</td>
</tr>
<tr>
<td>VL</td>
<td>110.5 (1.4)</td>
<td>95.0 (1.6)</td>
<td>&lt;0.001</td>
<td>0.31 (0.06)</td>
<td>0.51 (0.09)</td>
</tr>
<tr>
<td>TP</td>
<td>7.0 (0.4)</td>
<td>9.5 (0.4)</td>
<td>0.004</td>
<td>0.22 (0.05)</td>
<td>0.20 (0.09)</td>
</tr>
<tr>
<td>PV</td>
<td>6.6 (0.1)</td>
<td>5.8 (0.1)</td>
<td>&lt;0.001</td>
<td>0.17 (0.05)</td>
<td>0.45 (0.08)</td>
</tr>
<tr>
<td>Tmax</td>
<td>15.4 (0.5)</td>
<td>19.3 (0.6)</td>
<td>0.002</td>
<td>0.21 (0.05)</td>
<td>0.16 (0.09)</td>
</tr>
<tr>
<td>Vmax</td>
<td>0.30 (0.02)</td>
<td>0.22 (0.02)</td>
<td>0.033</td>
<td>0.09 (0.05)</td>
<td>0.26 (0.09)</td>
</tr>
</tbody>
</table>

1. WG: Weight Gain (kg); VL: Viral Load (Area Under the Wood’s Curve of log10 serum viremia from 0 to 21 days post infection; Viremia*Days); TP: Time to Peak Viremia (Days); PV: Peak Viremia (log10 serum viremia); Tmax: Time to maximal rate of viremia decay (Days); Vmax: Maximal rate of viremia decay (log10 serum viremia/day)
2. Full G-matrix used
3. Estimates were obtained by fitting isolate in the model and only included trials whereby pigs from the same genetic background were infected with both NVSL and KS06
4. Estimates were obtained by using the full G-matrix. NVSL and KS06 estimates were estimated separately, and included all animals infected with that isolate, except trial 13.
5. P-value for the difference in the estimated LSMeans between NVSL and KS06
Table 3.4. Estimates of Genetic Correlations (s.e.) between Response to infection with the NVSL versus KS06 Isolates and using different relationship matrices

<table>
<thead>
<tr>
<th>Trait</th>
<th>Full (G)</th>
<th>Block Diagonal (G_B)</th>
<th>Paired Block Diagonal (G_P)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Heritability</td>
<td>Genetic Correlation</td>
<td>Heritability</td>
</tr>
<tr>
<td></td>
<td>NVSL</td>
<td>KS06</td>
<td>NVSL</td>
</tr>
<tr>
<td>VL</td>
<td>0.32 (0.06)</td>
<td>0.53 (0.07)</td>
<td>0.86 (0.19)</td>
</tr>
<tr>
<td>WG</td>
<td>0.33 (0.05)</td>
<td>0.30 (0.09)</td>
<td>0.86 (0.27)</td>
</tr>
<tr>
<td>TP</td>
<td>0.22 (0.05)</td>
<td>0.21 (0.09)</td>
<td>0.25 (0.33)</td>
</tr>
<tr>
<td>PV</td>
<td>0.17 (0.05)</td>
<td>0.46 (0.07)</td>
<td>0.94 (0.28)</td>
</tr>
<tr>
<td>Tmax</td>
<td>0.21 (0.05)</td>
<td>0.14 (0.09)</td>
<td>0.82 (0.53)</td>
</tr>
<tr>
<td>Vmax</td>
<td>0.10 (0.05)</td>
<td>0.25 (0.09)</td>
<td>0.63 (0.51)</td>
</tr>
</tbody>
</table>

1. G: Full G-matrix with all relationships included; G_B: Block Diagonal G-matrix, with the relationships between animals from different genetic backgrounds set to zero; G_P: Paired Block Diagonal G-matrix, with the relationships between animals from different genetic backgrounds set to zero and only included trials whereby pigs from the same genetic background were infected with both NVSL and KS06
2. WG: Weight Gain (kg); VL: Viral Load (Area Under the Wood’s Curve of log10 serum viremia from 0 to 21 days post infection; Viremia*Days); TP: Time to Peak Viremia (Days); PV: Peak Viremia (log10 serum viremia); Tmax: Time to maximal rate of viremia decay (Days); Vmax: Maximal rate of viremia decay (log10 serum viremia/day)
3. NE: Was not able to be estimated because the model did not achieve convergence in ASReml
Table 3.5. Estimates of correlations\(^1\) (s.e.) of Response to Infection with PRRSV Isolate NVSL

<table>
<thead>
<tr>
<th>Trait(^2)</th>
<th>VL</th>
<th>WG</th>
<th>TP</th>
<th>PV</th>
<th>Tmax</th>
<th>Vmax</th>
</tr>
</thead>
<tbody>
<tr>
<td>VL</td>
<td></td>
<td>-0.33 (0.03)</td>
<td>0.10 (0.03)</td>
<td>0.66 (0.02)</td>
<td>0.36 (0.03)</td>
<td>-0.27 (0.03)</td>
</tr>
<tr>
<td>WG</td>
<td>-0.74 (0.10)</td>
<td></td>
<td>-0.02 (0.03)</td>
<td>-0.22 (0.03)</td>
<td>-0.16 (0.03)</td>
<td>0.12 (0.03)</td>
</tr>
<tr>
<td>TP</td>
<td>0.31 (0.15)</td>
<td>0.27 (0.16)</td>
<td></td>
<td>-0.09 (0.03)</td>
<td>0.72 (0.01)</td>
<td>0.12 (0.03)</td>
</tr>
<tr>
<td>PV</td>
<td>0.85 (0.07)</td>
<td>-0.73 (0.13)</td>
<td>0.05 (0.19)</td>
<td></td>
<td>-0.23 (0.03)</td>
<td>0.40 (0.03)</td>
</tr>
<tr>
<td>Tmax</td>
<td>0.81 (0.10)</td>
<td>-0.11 (0.16)</td>
<td>0.83 (0.07)</td>
<td>0.50 (0.21)</td>
<td></td>
<td>-0.51 (0.02)</td>
</tr>
<tr>
<td>Vmax</td>
<td>-0.72 (0.21)</td>
<td>0.45 (0.22)</td>
<td>-0.11 (0.26)</td>
<td>-0.27 (0.33)</td>
<td>-0.57 (0.19)</td>
<td></td>
</tr>
</tbody>
</table>

1. Phenotypic correlations (above diagonal), Genetic correlations (below diagonal) were estimated using an Animal model in ASReml and the full G-matrix.
2. WG: Weight Gain (kg); VL: Viral Load (Area Under the Wood’s Curve of log10 serum viremia from 0 to 21 days post infection; Viremia*Days); TP: Time to Peak Viremia (Days); PV: Peak Viremia (log10 serum viremia); Tmax: Time to maximal rate of viremia decay (Days); Vmax: Maximal rate of viremia decay (log10 serum viremia/day)
Table 3.6. Estimates of correlations\(^1\) (s.e.) of Response to Infection with PRRSV Isolate KS06

<table>
<thead>
<tr>
<th>Trait(^1)</th>
<th>VL</th>
<th>WG</th>
<th>TP</th>
<th>PV</th>
<th>Tmax</th>
<th>Vmax</th>
</tr>
</thead>
<tbody>
<tr>
<td>VL</td>
<td></td>
<td>-0.23 (0.05)</td>
<td>-0.06 (0.05)</td>
<td>0.76 (0.02)</td>
<td>0.13 (0.05)</td>
<td>-0.16 (0.05)</td>
</tr>
<tr>
<td>WG</td>
<td>-0.52 (0.17)</td>
<td>-0.05 (0.05)</td>
<td>-0.13 (0.05)</td>
<td>-0.06 (0.06)</td>
<td>0.13 (0.05)</td>
<td></td>
</tr>
<tr>
<td>TP</td>
<td>-0.08 (0.22)</td>
<td>-0.10 (0.24)</td>
<td>0.02 (0.05)</td>
<td>0.80 (0.02)</td>
<td>0.02 (0.05)</td>
<td></td>
</tr>
<tr>
<td>PV</td>
<td>0.91 (0.05)</td>
<td>-0.30 (0.18)</td>
<td>-0.08 (0.25)</td>
<td>-0.19 (0.05)</td>
<td>0.52 (0.04)</td>
<td></td>
</tr>
<tr>
<td>Tmax</td>
<td>0.19 (0.23)</td>
<td>-0.42 (0.23)</td>
<td>0.69 (0.19)</td>
<td>-0.24 (0.28)</td>
<td>-0.52 (0.04)</td>
<td></td>
</tr>
<tr>
<td>Vmax</td>
<td>-0.01 (0.20)</td>
<td>0.42 (0.21)</td>
<td>-0.12 (0.28)</td>
<td>0.51 (0.13)</td>
<td>-0.75 (0.18)</td>
<td></td>
</tr>
</tbody>
</table>

1. Phenotypic correlations (above diagonal), Genetic correlations (below diagonal) were estimated using an Animal model in ASReml and the full G-matrix.
2. WG: Weight Gain (kg); VL: Viral Load (Area Under the Wood’s Curve of log10 serum viremia from 0 to 21 days post infection; Viremia*Days); TP: Time to Peak Viremia (Days); PV: Peak Viremia (log10 serum viremia); Tmax: Time to maximal rate of viremia decay (Days); Vmax: Maximal rate of viremia decay (log10 serum viremia/day)
### Table 3.7. Estimates of Genetic Correlations of Response to infection between PRRSV Isolates when Excluding the 5 Mb WUR Region from the G Matrix

<table>
<thead>
<tr>
<th>Trait</th>
<th>Full (G-W)</th>
<th>Block Diagonal (G_B-W)</th>
<th>Paired Block Diagonal (G_P-W)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NVSL</td>
<td>KS06</td>
<td>NVSL</td>
</tr>
<tr>
<td>VL</td>
<td>0.25 (0.06)</td>
<td>0.49 (0.09)</td>
<td>0.76 (0.22)</td>
</tr>
<tr>
<td>WG</td>
<td>0.28 (0.06)</td>
<td>0.30 (0.09)</td>
<td>0.89 (0.29)</td>
</tr>
<tr>
<td>TP</td>
<td>0.21 (0.05)</td>
<td>0.20 (0.09)</td>
<td>0.18 (0.34)</td>
</tr>
<tr>
<td>PV</td>
<td>0.13 (0.05)</td>
<td>0.40 (0.08)</td>
<td>0.79 (0.34)</td>
</tr>
<tr>
<td>Tmax</td>
<td>0.19 (0.05)</td>
<td>0.14 (0.09)</td>
<td>0.80 (0.54)</td>
</tr>
<tr>
<td>Vmax</td>
<td>0.10 (0.05)</td>
<td>0.23 (0.09)</td>
<td>0.70 (0.53)</td>
</tr>
</tbody>
</table>

1. G: Full G-matrix with all relationships included; G_B: Block Diagonal G-matrix, with the relationships between animals from different genetic backgrounds set to zero; G_P: Paired Block Diagonal G-matrix, with the relationships between animals from different genetic backgrounds set to zero and only included trials whereby pigs from the same genetic background were infected with both NVSL and KS06.
2. Subscript _W denotes that the 5 Mb WUR Region was excludes in construction of the G-matrix.
3. WG: Weight Gain (kg); VL: Viral Load (Area Under the Wood`s Curve of log10 serum viremia from 0 to 21 days post infection; Viremia*Days); TP: Time to Peak Viremia (Days); PV: Peak Viremia (log10 serum viremia); Tmax: Time to maximal rate of viremia decay (Days); Vmax: Maximal rate of viremia decay (log10 serum viremia/day).
4. NE: Was not able to be estimated because the model did not achieve convergence in ASReml.
Figure 3.1. Comparison of Response to Infection when Pigs are infected with NVSL (red) or KS06 (blue) PRRSV isolates. For comparison between viruses, trials were matched based on genetic background. Least Square Means of daily viremia, predicted using the monophasic Wood’s curve parameters, and weights were estimated using ASReml with the full G-matrix. Viremia, Weight, Time to Peak, Peak Viremia, Time to Maximal Decay, and Maximal Decay were compared when pigs were infected with either NVSL or KS06 PRRSV isolate.
Figure 3.2. Heat Map of time course genetic correlations between viremia and weight gain in pigs infected with the (A) NVSL or (B) KS06 PRRSV isolate. Genetic correlations from fitting a bivariate animal model in ASReml using the full G-matrix. NVSL and KS06 were analyzed separately. All trials, except trial 13, were used in the analysis. Each square in the heat map represents the genetic correlation between viremia at a given time point t (X-axis) and the 3-day weight gain at time point t* (Y-axis).
Figure 3.3 Least Square Means of WUR genotype effects on predicted viremia and weight in pigs infected with either the NVSL or KS06 PRRSV isolate. Least Square Means of the WUR genotype for predicted viremia and weight for NVSL (A) and KS06 (B) when fitting the Isolate*WUR interaction into the Animal model in ASReml using the full G-matrix. All trials, except trial 13, were used for the analysis.
Figure 3.4. Least Square Means of WUR genotype effects on weight gain and viremia curve parameters in pigs infected with either the NVSL or KS06 PRRSV isolate. Least Square Means of the WUR genotype for VL (A), WG42 (B), TP (C), PV (D), Tmax (E), and Vmax (F) when fitting the Isolate*WUR interaction into the Animal model in ASReml using the full G-matrix. All trials, except trial 13, were used for the analysis. Estimates with different letter assignments are significantly different (P≤0.05).
Figure S3.1. Raw Viremia Curves for Each Trial
Table S3.1. Raw means (s.d.) of Estimated Wood’s Curve Parameters and Proportion of Animals that were Classified as having Cleared, Rebound, or Persistent Serum Viremia Profile

<table>
<thead>
<tr>
<th>PRRSV Isolate</th>
<th>$\hat{a}_1$</th>
<th>$\hat{b}_1$</th>
<th>$\hat{c}_1$</th>
<th>$\hat{a}_2$</th>
<th>$\hat{b}_2$</th>
<th>$\hat{c}_2$</th>
<th>Cleared</th>
<th>Rebound</th>
<th>Persistent</th>
</tr>
</thead>
<tbody>
<tr>
<td>NVSL</td>
<td>3.52 (1.21)</td>
<td>0.74 (0.33)</td>
<td>0.10 (0.04)</td>
<td>5.34 (2.64)</td>
<td>5.28 (2.86)</td>
<td>1.99 (1.04)</td>
<td>0.44</td>
<td>0.17</td>
<td>0.40</td>
</tr>
<tr>
<td>KS06</td>
<td>2.27 (1.02)</td>
<td>0.86 (0.33)</td>
<td>0.09 (0.03)</td>
<td>4.45 (2.87)</td>
<td>4.48 (2.78)</td>
<td>1.64 (0.94)</td>
<td>0.38</td>
<td>0.06</td>
<td>0.56</td>
</tr>
</tbody>
</table>
CHAPTER IV

GENETIC RELATIONSHIPS OF ANTIBODY RESPONSE, VIREMIA LEVEL AND WEIGHT GAIN IN PIGS EXPERIMENTALLY INFECTED WITH PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS

_A paper to be submitted to Journal of Animal Science_


**Contribution from Authors**

**Andrew Hess:** Fitted weight curves, conducted the statistical analyses, interpretation of results, and wrote the manuscript.

**Ben Trible:** Performed the antibody assays and collected antibody results

**Yu Wang:** Developed and help perform the antibody assays

**Melanie Hess:** Aided in the interpretation of results and draft of manuscript

**Bob Rowland:** Conceived the study and led the animal infection trials and sample collection.

**Joan Lunney:** Conceived the study and coordinated the handling, storage, and sample preparation for DNA.

**Graham Plastow:** Conceived the study and coordinated the sample preparation for DNA.

**Jack Dekkers:** coordinated and oversaw statistical analysis of the data and contributed to interpretation of results and writing the manuscript.

**Abstract**

Porcine Reproductive and Respiratory Syndrome (PRRS) is one of the costliest global diseases in pigs. The potential of genetic selection on PRRSV antibody response to improve resistance to PRRSV infection was assessed by experimentally infecting
commercial crossbred weaner pigs across eight trials with one of two genetically distinct
PRRSV isolates, NVSL-97-7895 (~750 pigs) and KS-2006-72109 (~450 pigs). This study
1) estimated the genetic parameters of antibody response, measured using Fluorescent
Microsphere Immunoassay as sample to positive ratio (S:P) of PRRSV N-protein specific
IgG in serum at 42 days post infection (dpi); 2) assessed the relationship of antibody
response with serum viremia and growth under infection; and 3) identified genomic regions
associated with antibody response. The estimated heritability of antibody response under
PRRSV infection was 0.31±0.09 (NVSL) and 0.40±0.10 (KS06). Host control of S:P under
infection with distinct PRRSV isolates appeared to be under similar genetic control, with
an estimated genetic correlation of 0.73±0.39. Genetic correlations of S:P with either viral
load (NVSL: -0.20±0.18; KS06: -0.69±0.20), measured as area under the Wood’s Curve
of log10 serum viremia 0-21dpi, or weight gain from 0-42 dpi (NVSL: -0.38±0.19; KS06:
-0.08±0.25), were generally weak. However, genetic correlations of S:P at 42 dpi with daily
serum viremia across the experimental period (NVSL range: 0.56 to -0.45; KS06 range: -
0.22 to -0.80) and 3-day weight gain (NVSL range: -0.48 to 0.21; KS06 range: -0.20 to
0.78) revealed dynamic relationships. The WUR10000125 SNP on SSC4 (WUR),
previously identified to be associated with viremia and weight gain during PRRSV
infection, did not have a significant effect on antibody levels (P>0.05). Genotype-specific
genetic correlations of S:P with daily viremia and 3-day weight gain suggest that the
differences in weight gain observed in pigs with the unfavorable WUR genotype may be
due to the utilization of an alternative, and likely more energetically costly response, in
pigs with the AA genotype compared to pigs with the favorable allele. Genome-wide
association studies identified 3 SNPs in the Major Histocompatibility Complex associated
with antibody response that explained ~10 and 45% of genetic variance for S:P for NVSL and KS06, respectively, but were not associated with viremia or weight gain. These findings suggest antibody response to PRRSV infection may be a feasible biomarker for selection of pigs with increased resistance to PRRSV infection.

**Introduction**

Porcine reproductive and respiratory syndrome (PRRS) is a disease of major global concern from both an economic and animal welfare standpoint (Holtkamp et al., 2013). Development of an effective PRRS vaccine has been largely unsuccessful because they are not cross-protective to heterologous strains of PRRSV (Darwich et al., 2010). Pigs genetically selected for improved resistance to multiple isolates of PRRSV may aid in containment of PRRS (Lewis et al., 2007; Hess et al., 2016).

A robust PRRSV-specific non-neutralizing antibody response, such as nucleocapsid (N)-specific IgG, is observed early in infection and is sustained long after serum clearance of PRRSV (Chand et al., 2012). The role that this response plays in providing protection of the pig to PRRSV infection is unclear (Lopez and Osorio, 2004); however, PRRSV N-protein specific antibody response was favorably genetically correlated with sow reproductive performance during a PRRSV outbreak (Serao et al., 2014). N-specific antibody response may also be genetically correlated with performance in weaner pigs infected with PRRSV, although this may change over time as different host genes are expressed at different levels throughout the course of infection (Schroyen et al., 2015).
PRRSV N-protein specific IgG response at 42 days post infection (dpi), as well as periodic measurements of weights and serum viremia were available for weaner age pigs experimentally infected with either NVSL-97-7895 (NVSL) or KS-2006-72109 (KS06) PRRSV isolates. The objectives of this study were to: 1) estimate the genetic parameters of antibody response; 2) assess the relationship of antibody response with the dynamics of viremia and growth following PRRSV infection; and 3) identify regions associated with antibody response to PRRSV infection. Pigs infected with either NVSL or KS06 were analyzed separately and compared to assess whether the genetic basis of PRRSV N-protein specific IgG response was consistent between PRRSV isolates.

Materials and Methods

Study design

A detailed description of the design, data collection and molecular techniques used in the PRRS Host Genetics Consortium (PHGC) trials can be found in (Lunney et al., 2011). The Kansas State University Institutional Animal Care and Use Committee approved all experimental protocols for these trials.

Each of the 8 PHGC challenge trials used in this study (PHGC 1-5 and 10-12) consisted of ~200 commercial crossbred pigs used in North America that were transported at weaning to Kansas State University (average age of 28 days). Pigs within a trial were from the same high health farm, except for two trials (5 and 12), which each included pigs from two farms. All farms were free of PRRSV, Mycoplasma hyopneumoniae, and swine influenza virus. Upon arrival, pigs were randomly placed into pens of 10 to 15 pigs. Following a 7-day acclimation period, the pigs were experimentally infected, both
intramuscularly and intranasally, with 105 (TCID50) of either PRRSV isolate NVSL-97-7985 (PHGC 1-5) or KS-2006-72109 (PHGC 10-12). NVSL and KS06 are 89% similar at the GP5 nucleotide sequence level (Ladinig et al., 2015). One breeding company supplied pigs of the same breed cross for four trials; PHGC 1-3 were infected with NVSL and PHGC 11 with KS06. Each of the other trials used in this study consisted of pigs from a different breed cross. Hess et al. (2016) provide a further description of these PHGC trials.

Body weights were measured weekly throughout each trial, from 0 to 42 days post infection (dpi). Serum viremia was measured from blood samples collected at 0, 4, 7, 11, 14, 21, 28, 35, and 42 dpi using a semi-quantitative TaqMan PCR assay for PRRSV RNA, as described in Boddicker et al. (2012). PRRSV N-protein-specific serum Immunoglobulin G (IgG) levels were measured at 42 dpi using a fluorescent microsphere immunoassay (FMIA) (Christopher-Hennings et al., 2013; Stephenson et al., 2015). Pigs were euthanized at 42 dpi and ear notches were collected for genotyping on Illumina’s Porcine SNP60 Beadchip (Ramos et al., 2009) v1 (San Diego, California) at GeneSeek Inc. (Lincoln, Nebraska) for all trials except 11 and 12, which were genotyped on Porcine SNP60 Beadchip v2 (San Diego, California) at Delta Genomics (Edmonton, Alberta).

Across all five trials, 6% of pigs died before 42 dpi when infected with NVSL. Mortality rate was similar in the KS06 trials, with 7% of pigs dying before 42 dpi across the three trials. Dead pigs were necropsied and subsequent gross and microscopic pathology performed by a board-certified pathologist who identified PRRS-associated disease as the major cause of mortality.
PRRSV N-protein specific antibody response

FMIA uses multiple fluorescent microspheres and each bead set is conjugated to different antigens or antibodies as the solid phase for the detection of antibodies or antigens in biological samples (Christopher-Hennings et al., 2013). Results were reported as mean fluorescence intensity (MFI). Assays were conducted on a total of 21 96-well plates. MFI were standardized using a sample to positive ratio (S:P), calculated for each animal as:

$$S:P = \frac{\text{Sample} - \text{Negative}}{\text{Positive Control} - \text{Negative}}$$ \[1\]

where sample is the MFI of an individual. An animal that fell within the linear phase of the standard curve (i.e. had a MFI value between 15,000 and 30,000) and that had ample serum was included as a positive control for every plate. A blank well (negative) was used as a negative control on each plate to correct for background noise. Due to the high level of repeatability between technical replicates (Wang, 2013; Stephenson et al., 2015), each animal was only measured once.

Models for viremia and weight over time

In order to capture the dynamics of changes in viremia over time, a single Wood’s curve (Equation 2) or an Extended Wood’s Curve (Equation 3) was fitted to the viremia data of each animal, as described by Islam et al. (2013) and Hess et al. (2016):

$$V(t) = a_1 t^{b_1} e^{-c_1 t}$$ \[2\]

$$V(t) = a_1 t^{b_1} e^{-c_1 t} + \max(0, a_2(t-t_0)^{b_2} e^{-c_2(t-t_0)})$$ \[3\]

where $V(t)$ is the viremia on the log10 scale (reported as log10 of PRRSV RNA copies per ml of serum) at t dpi, $a_1$ impacts the general level of viremia across time, $b_1$ is an indicator of the initial rate of increase to peak viremia, and $c_1$ is an indicator of the rate of decline after the peak and dominates the function as $t \to \infty$. In the extended Wood’s curve of
equation [3], \( t_0 \) denotes the onset of the second phase of the profile, which was assumed to follow the same Wood’s shape as the primary phase and was thus defined by a second set of Wood’s curve parameters (Islam et al., 2013).

Wood curve parameter estimates \((\widehat{a}_1, \widehat{b}_1, \widehat{c}_1)\) for each pig were then used to obtain fitted viremia values for each pig for each day during infection. If the Extended Wood’s Curve fitted a pig’s viremia profile better than the single Wood’s Curve, as determined by AIC (Islam et al., 2013), that pig was classified as a rebound pig and estimates from the Extended model \((\widehat{a}_1, \widehat{b}_1, \widehat{c}_1, \widehat{a}_2, \widehat{b}_2, \widehat{c}_2)\) were used to obtain fitted values. Non-rebound pigs were further classified as cleared or persistent if their fitted viremia at 42 dpi was less than or greater than 1 log\(_{10}\), respectively. Of the pigs used in this study, 22% of pigs from the NVSL trials and 6% of pigs from the KS06 trials were classified as rebound and 33 and 53% of pigs infected with NVSL and KS06, respectively were classified as persistent, with the remaining 45 and 41% classified as cleared.

Previous analyses also evaluated viral load (VL), defined as the area under the Wood’s curve of log viremia from 0-21 dpi (Hess et al., 2016), which is a measure of both the level of viremia and the rate and extent of clearance of the virus in the blood following infection:

\[
VL_i = \int_0^{21} \widehat{a}_{1i} t \widehat{b}_{1i} e^{-\widehat{c}_{1i} t} \, dt
\]  

[4]

Body weights were collected weekly and used to interpolate daily weights. A random regression model was fitted to the weight data of all pigs simultaneously, but separately for animals infected with NVSL and KS06, using second order Legendre polynomials in the following model that was implemented in ASReml (Gilmour et al., 2009):
\[ W_{ijklmop}(t) = \sum_{n=0}^{2} L_{ni}(t) + P_j + A_k + S_l + \sum_{n=0}^{2} L_{ni}(t) \ast R_m + \sum_{n=0}^{2} L_{ni}(t) \ast A_n \]

\[ + Tr_o + Pen(Tr)_{po} + \varepsilon_{ijklmop} \]  

where \( L_{ni}(t) \) denotes the nth order Legendre polynomial at t dpi for individual i. \( L_{n}(t) \), P, A, S and \( L_{ni}(t) \ast R \) were fitted as fixed effects. \( L_{ni}(t) \) was fitted as a covariate, with t ranging from 0 to 42 dpi, P is the parity of dam, classified as first, second, or later parities, A is the age of the individual at inoculation, S is the sex of the individual, and \( L_{ni}(t) \ast R \) is the interaction between the nth order Legendre polynomial at t dpi and rebound status. \( L_{ni}(t) \ast A_n \), Tr, and Pen(Tr) were included as random effects and denote the interaction between the nth order Legendre polynomial at t dpi and animal, trial, and the interaction between trial and pen, respectively. \( L_{n}(t) \ast A_n \) models an individual’s weight at each time point and captures both genetic and permanent environmental effects, and used an unstructured variance-covariance structure for polynomial parameters of a given animal and independence of parameters between animals. Residual variances were modeled separately for each measured dpi, in order to allow for an increase in variance over time. Trial and Trial*Pen were included to capture systematic environmental effects. This model was then used to obtain fitted values of each pig’s weight for each dpi (0-42) \(((W(t))^\hat{)})\), using all coefficients estimated from the above model. Fitted weights were used to model three-day weight gain based on equation [6], with W representing the fitted weight of an individual three days prior to the one being fitted as a dependent variable, except for weight gain from 0-1 dpi, for which weight at infection was fitted. This method was also used to get a 42 day weight gain (WG), by analyzing the fitted weight at 42 dpi with weight at infection included as a covariate.
Parameter estimation for antibody response

The model used for analysis of S:P was:

\[ Y_{ijklmnopqr} = \mu + P_i + A_j + W_k + S_l + R_m + An_n + Li_o + Tr_p + Pen(Tr)_{qp} + \\
\] 

\[ Pl_r + \varepsilon_{ijklmnopqr} \]  \[ 6 \]

where \( Y \) is the dependent variable of S:P. Parity of the dam (P), classified as first, second, or later parity, and sex of the piglet (S) were fitted as a fixed class effects. To account for potential model differences in curve fittings between rebound and non-rebound pigs, the fixed class effect of rebound (R) was included in the model. Age (A) and weight (W) of the piglet at infection (0 dpi) were included as linear covariates. Random effects included animal genetic effects (An), litter (Li), trial (Tr), Pen nested within trial (Pen(Tr)), the plate the assay was run on (Pl), and \( \varepsilon \) as the residual. Animal genetic effects were estimated using the genomic relationship matrix (G-matrix), including relationships between all animals, which was constructed using the VanRaden method (VanRaden, 2008), based on 48,164 autosomal SNPs (Build 10.2 http://www.ncbi.nlm.nih.gov/genome/guide/pig/, accessed August 13, 2015) that were segregating on both versions 1 and 2 of the Illumina Porcine SNP60 Beadchip. The heritability of S:P was estimated using model [6], separately for NVSL and KS06, using ASReml v3.0 (Gilmour et al., 2009). The genetic correlation between S:P for the two isolates was estimated using a bivariate model using equation [6], with S:P as the dependent variable separated by isolate. The phenotypic variance was obtained by summing the animal, litter, and pen within trial variance components. Heritability was obtained by dividing the animal variance component by the phenotypic variance.
A region on chromosome 4 of the swine genome (SSC4) has previously been identified to be associated with VL and WG (Boddicker et al., 2012). A tag SNP, WUR10000125 (WUR), was identified that explained all of the genetic variance in this region for both traits (Boddicker et al., 2012; Boddicker et al., 2014a; Boddicker et al., 2014b). The numbers of animals with each genotype for WUR in the current study are given in Table 4.1. Using model [6], LS Means for S:P at 42 dpi were estimated for the two isolates, WUR genotype (AA or AB; BB animals were not considered for this analysis due to low frequency), and viremia status at 42 dpi. To estimate LS Means for the two isolates, isolate was added to model [6] using a univariate model and joint analysis of all data. To estimate the association of WUR genotype with S:P, the interaction of isolate*WUR was also added to model [6]. To estimate the association between viremia status and S:P, the interaction of isolate*viremia status was added to model [6] and rebound (R) was removed.

**Genetic correlations of antibody response with weight gain and viremia**

Genetic correlations of S:P at 42 dpi with three-day weight gain (WG0-1, WG0-3, WG2-5…WG38-41) and with fitted viremia at every other dpi (i.e. 1, 3, 5, …, 41) were estimated in order to assess how the relationships of antibody response with weight gain and viremia changed over the course of infection. Genetic correlations were estimated using a bivariate model in ASReml that was the same as Equation [6] but without fitting plate for weight gain and viremia. The dependent variables were S:P and either a daily viremia or 3-day weight gain measurement. For S:P and viremia, W in model [6] denotes weight at infection.
In NVSL infected pigs, genetic correlations of S:P with viremia and weight gain were also estimated separately for animals with the AA versus the AB genotype for the WUR SNP to assess the impact genotype at this SNP has on the relationships between these traits. The KS06 trials were not used for this analysis due to the limited number of animals. BB animals were grouped with AB animals, because of low numbers and because they were expected to perform similarly to AB animals, due to the dominant nature of this quantitative trait locus (Boddicker et al., 2014b). However, estimates for the AB/BB animals did not converge due to low numbers of animals. Thus, instead, estimates of the genetic covariance between traits for this group were obtained by assuming that the covariance between traits for the full data set (including AA, AB, and BB animals) was equal to a weighted sum of the covariances for the two genotype groups:

$$\text{Cov}(S:P,T)_{\text{Full}} = p_{\text{AA}} \text{Cov}(S:P,T)_{\text{AA}} + p_{\text{AB/BB}} \text{Cov}(S:P,T)_{\text{AB/BB}}$$

where T denotes either viremia or 3-day weight gain. The genetic correlation for AB/BB animals was then estimated by assuming equal genetic variances of S:P, viremia, and weight gain for AA and AB/BB animals.

**Genome wide association study**

Method Bayes B, as implemented in GenSel 4.73 (Fernando and Garrick, 2012), was used to test associations of the 60k SNPs with S:P based on the following model:

$$Y_{ijklmnopqr} = \mu + P_i + A_j + W_k + S_l + R_m + A_n + L_o + Tr_p + \text{Pen}(Tr)_{qp} + \sum_{s=1}^t z_{ns} \alpha_s \delta_s + \varepsilon_{ijklmnopqr}$$

where Y is the dependent variable of S:P and P, S, A, W, R, Tr, Pen(Tr) and Pl were as described in Equation [6] but all fitted a fixed effects because GenSel does not accommodate random effects other than SNP effects; t is the number of SNPs fitted; z is
the genotype of SNP s for animal n, α is the allele substitution effect for SNP s, and δ indicates whether SNP s was included (δ = 1) or excluded (δ = 0) in the model for a given iteration of the Monte Carlo Markov Chain. The prior probability of δ = 0 was set equal to π = 0.99. A total of 50,000 iterations were run, including a burn-in of 5000. A total of 0.19% of genotypes were missing and these were replaced with the mean genotype for that SNP within a trial. For each iteration, breeding values were calculated for each non-overlapping 1 Mb region, or window, to obtain samples of the breeding values for each animal for each window. These values were summed across the genome to get the breeding value of an individual. The additive genetic variance in each iteration was calculated as the variance of the breeding values of all animals for that iteration. The percent genetic variance explained by a window was calculated by the variance of the breeding values of all animals for that window divided by the total genetic variance multiplied by 100. The estimate of the percent genetic variance explained by a window was the posterior mean percent genetic variance explained by that window across all iterations (excluding 5000 burn-in) (Garrick and Fernando, 2013).

Windows that explained more than 5% of the total genetic variance for S:P were deemed to have a strong association with antibody response. LS Means were estimated for the SNP or SNPs within each of these windows with the highest Posterior Probability of Inclusion (PPI), which was derived as the proportion of iterations of the MCMC chain that the given SNP was included in the model (Wolc et al., 2012). The LS Means for S:P were estimated for the genotypes of these SNPs by fitting SNP genotype in model [6].
Results

Effects of isolate, WUR genotype and viremia status

The LS Mean estimates for S:P for pigs infected with NVSL and KS06 were not significantly different from each other (P=0.55; Table 4.1), nor was S:P significantly different between animals that had genotypes AA or AB for WUR, when infected with either NVSL (P=0.28) or KS06 (P=0.78). The effect of viremia status was significant, with rebound animals on average having a higher S:P ratio than persistently infected or cleared animals when infected with either NVSL or KS06 (P<0.05), which may be indicative of a host memory response or viral escape from host immune response.

Estimates of genetic parameters for S:P

Moderate to high heritabilities were estimated for S:P ratio in both NVSL and KS06 infected animals (NVSL: 0.31±0.09; KS06: 0.40±0.10). In an attempt to gain a better understanding as to whether these are genetically similar traits following infection with different isolates, the genetic correlation between isolates was estimated to be 0.73±0.39. Although this is a moderately high correlation, the large standard error prevented conclusions as to whether S:P can be considered the same trait during infection with these two PRRSV isolates due to the large standard error. An attempt to improve the estimate by only analyzing trials with the same genetic background (i.e. same breeding company and same breed cross) was unsuccessful due to model convergence issues.

Correlations of antibody response with viremia and growth

Genetic and phenotypic correlations between S:P, VL, and WG were estimated separately for NVSL and KS06 (Table 4.2). All phenotypic correlations were negative and significantly different from zero (P<0.05), with the exception of the correlation between
S:P and WG in KS06, which was estimated to be 0.00. Phenotypic correlations of VL and WG with S:P tended to be weak, while the phenotypic correlation between VL and WG was moderately negative for both NVSL and KS06. For NVSL, the genetic correlation between VL and S:P was not significantly different from zero (P=0.27); however, a strong negative genetic correlation between these traits was observed when pigs were infected with KS06 (rg=-0.69±0.20, P<0.001). The genetic correlation between WG and S:P was moderately negative for pigs infected with NVSL (rg=-0.38±0.19, P<0.05) but was not significantly different from zero for pigs infected with KS06 (P=0.75). Genetic correlations between VL and WG were strongly negative for both virus isolates (P<0.01). It was suspected that the relationship of S:P with viremia or weight gain was more dynamic than was captured using VL and WG, therefore these traits were broken down into viremia at different time points during infection and 3-day weight gain.

**Relationship of antibody response with viremia and weight gain over the course of infection**

Phenotypic standard deviations were similar between pigs infected with NVSL and KS06 for both daily viremia and 3-day weight gain (Figures 4.1A-D). Heritability estimates for viremia ranged from 0.12 (at 7 dpi) to 0.54 (at 13 dpi) for pigs infected with NVSL and had a similar range for KS06: 0.19 (at 27 dpi) to 0.47 (at 9 dpi). Heritability estimates for three-day weight gain ranged from 0.11 (at 33 dpi) to 0.42 (at 1 dpi) for pigs infected with NVSL and from 0.17 (at 41 dpi) to 0.40 (at 19 dpi) for KS06.

Genetic correlations of S:P at 42 dpi with daily viremia based on the Wood’s curve were estimated for all odd-numbered dpi. For NVSL, pre-peak viremia (before 7 dpi) was strongly positively correlated with S:P, was significantly different from zero on days 1, 3
and 5 (P<0.05), and peaked at 5 dpi (rg=0.58±0.26) (Figure 4.1A). After this point, the genetic correlation became negative, with estimates significantly less than zero for 13 to 21 dpi (P<0.05) and the lowest genetic correlation occurring at 17 dpi (rg= -0.46±0.19). However, for pigs infected with KS06, the genetic correlation between viremia and S:P was negative at all time points and significantly less than zero from 5 to 33 dpi, with the lowest genetic correlation at 21 dpi (rg=-0.78±0.22) (Figure 4.1B).

For pigs infected with either NVSL or KS06, the genetic correlation between 3-day weight gain and S:P at 42 dpi changed from negative early in infection to positive late in infection (Figure 4.1C/D). In general, genetic correlations were higher for KS06 than for NVSL (Range: rg(dpi1)=-0.20±0.27 to rg(dpi41)= 0.78±0.29). The only genetic correlations that were significantly different from zero for KS06 were positive and occurred at 21 to 41 dpi (Figure 4.1D). For NVSL (range: rg(dpi1)=-0.48±0.18 to rg(dpi41)= 0.39±0.29), however, correlations significantly lower than zero were observed at 1 to 13 dpi, while none of positive correlations were significantly different from zero (Figure 4.1C). For both daily viremia and 3-day weight gains, phenotypic correlations with S:P at 42 dpi tended to be weak, but showed a similar trend as the genetic correlations (Figures 4.1A-D).

Genetic correlations of S:P at 42 dpi with viremia and weight gain across the experimental period were also estimated by WUR genotype in NVSL infected pigs. When considering genetic correlations for animals with the AA genotype at the WUR SNP, the relationship of S:P with viremia and weight gain was more extreme than for the whole dataset (Figures 4.1A & 4.2A). For AA animals, stronger negative correlations were observed between S:P and viremia than for all animals (Figures 4.1A & 4.2A), with genetic
correlations significantly different from zero from 11 to 27 dpi. Similar to using all animals, the genetic correlation between S:P and viremia was lowest at 17 dpi but the genetic correlation at this dpi was much more negative for AA animals (rg=-0.86±0.24; Figure 4.2A) than for all animals (rg=-0.46±0.19; Figure 4.1A). Estimates of genetic correlations for AB and BB animals showed a consistent but generally low positive genetic correlation of S:P with viremia (Figure 4.2A).

Stronger negative genetic correlations were also observed between S:P at 42 dpi with weight gain for AA animals than for all animals, with a persistent negative correlation between these traits of ~-0.6 from 1 to 17 dpi (P<0.05), which then went positive at 29 dpi. Conversely, AB and BB animals started with a negative correlation between S:P and weight gain, which switched to positive at 11 dpi, and remained positive thereafter, with a maximum at 23 dpi of 0.57 (Figure 4.2B).

**Genome wide association study**

GWAS for S:P were conducted separately for the NVSL and KS06 data, as well as combined. All analyses showed peaks on chromosome 7 (SSC7), in the Major Histocompatibility Complex (MHC) region (Figure 4.3). The 29 Mb window was identified in all three GWAS and explained 10.3% of the genetic variance for NVSL, 43.1% for KS06 and 29.9% for the combined analyses. The SNP ALGA0039771 (ALGA) consistently had a high PPI compared to other SNPs in the window (NVSL: 0.30; KS06: 1.00; Joint: 1.00). Another SNP in this window, MARC0058875 (MARC), had a high PPI for NVSL but a low PPI in the other GWAS (NVSL: 0.40; KS06: 0.01; Joint: 0.06). The SNP DIAS0000349 (DIAS) in the 26 Mb window was identified in the joint analysis
(explaining <1% of genetic variance for NVSL and KS06 but 13.8% for the joint analysis) but had a low PPI in the NVSL and KS06 analyses (NVSL: 0.05; KS06: 0.01; Joint: 1.00).

The LS Means for S:P at 42 dpi were estimated for each SNP identified by the GWAS (Table 4.3). DIAS had a significant association with S:P at 42 dpi for both NVSL and KS06 infected animals (NVSL: P<0.0001; KS06: P=0.0002), whereby animals with the AB DIAS genotype had significantly lower S:P than AA animals. MARC, which was identified only in the GWAS of animals infected with NVSL, had a significant association with S:P at 42 dpi in NVSL infected animals (P<0.0001) but not in KS06 infected animals (P=0.16). Animals with the AA genotype for this SNP had significantly higher S:P than AB or BB animals. ALGA, which was identified in all GWAS, was associated with S:P for both NVSL and KS06 infected animals (P<0.0001). In NVSL infected pigs, animals with the ALGA AA genotype had the highest S:P, followed by AB animals. In KS06 infected pigs, however, a significant difference in S:P was not observed between animals with the ALGA AA and AB genotypes.

Daily viremia and 3-day weight gains were also evaluated for an association with the three SNPs identified in the GWAS in order to assess whether the observed genetic correlations between S:P and viremia or weight gain were due to the differences in their SSC7 MHC genotypes. However, estimates of SNP effects were always small and only significantly different from zero at a couple of time points (Tables S4.1 and S4.2).

**Discussion**

The results of our study suggest that antibody response to PRRSV infection may be a useful biomarker for selection for more virus resistant pigs. Antibody response to PRRSV
infection, measured as S:P of PRRSV N-protein specific IgG at 42 dpi, had a moderate-high estimated heritability; the estimate of the genetic correlation of S:P between isolates was also moderate to high, although it had a large standard error. The generally moderate to high negative estimates of genetic correlations of antibody response at 42 dpi with viremia, as well as positive genetic correlations of antibody response with 3-day weight gain at later stages of infection, suggest that selection for improved antibody response at the late stage of infection will improve the host’s genetic ability to fight PRRSV infection. The MHC is expected to play a major role in antibody response to infection, which was confirmed by this region explaining a large proportion of the genetic variance; however, the MHC did not appear to play a role in the genetic correlations of antibody response with weight gain or viremia. The estimated correlations of S:P with both viremia and weight gain using animals with the AA WUR genotype showed a stronger negative genetic correlation of S:P with both of these traits compared to AB/BB animals, suggesting that a poor antibody response to infection is energetically more costly in AA animals.

A major challenge with containing PRRS is the level of genetic and antigenic variability observed between different PRRSV isolates (Darwich et al., 2010). Antibody response can be categorized into a neutralizing antibody response, where the antibody directly inhibits the function of the virus in some way (Lopez and Osorio, 2004) and non-neutralizing antibody response, where the antibody binds to the virus in order to tag it for virolysis or viral clearance via phagocytosis (Burton, 2002). Following PRRSV infection, the earliest and strongest antibody response is a non-neutralizing IgG response against the virus nucleocapsid (N) protein (Chand et al., 2012), which is the antibody response measured in this study. Virus neutralizing antibodies are typically weak and delayed during
PRRSV infection but once an antibody is produced that successfully neutralizes the virus, this response is very effective (Lopez and Osorio, 2004). This effective neutralizing antibody response is generally specific to homologous, but not heterologous, isolates of PRRSV due to the high genetic and antigenic variability observed in PRRSV. Thus, vaccination with modified live virus (MLV) or previous natural infection generally results in only homologous protection against closely related PRRSV isolates (Huang and Meng, 2010). Understanding the host’s ability to mount a non-neutralizing PRRSV-specific antibody response, such as PRRSV N-protein specific IgG, may afford a better understanding of host response to PRRSV infection that is not specific to the PRRSV isolate.

**Fluorescent microsphere immunoassay**

In this study, antibody response was measured using FMIA, a protein detection assay that utilizes fluorescent microspheres as the binding surface for antigen-antibody complexes, rather than the standard commercialized technique for detection of antibodies against PRRSV, enzyme-linked immunosorbent assay (ELISA). The commercial ELISA was designed around a 0.4 S:P as a positive-negative cutoff and, therefore, not designed to be quantitative measure of antibody activity. FMIA has been shown to have higher specificity and sensitivity than ELISA (Langenhorst et al., 2012), with the threshold for identifying PRRSV infected animals set by Wang (2013) for FMIA at 0.2, which balanced sensitivity with specificity. The FMIA assay was also able to detect antibodies sooner after infection than ELISA, with 4% of animals tested showing antibodies at 4 dpi when assayed with FMIA, compared to 0% when using the ELISA kit (Wang, 2013). Another advantage
of FMIA over ELISA is that it allows uniform detection of multiple antigens or antibodies simultaneously within a small volume of a single sample.

**Antibody response at 42 dpi**

Serum antibody levels were only assayed at 42 dpi. It has been reported that antibodies against PRRSV N protein peak after 28 dpi and remain high for several months after infection, with low levels of antibody still detected in some animals for up to a year after infection (Yoon et al., 1995; Loemba et al., 1996; Johnson et al., 2011). The end of the experimental trial, at 42 dpi, was chosen here in order to evaluate the final antibody level achieved. However, due to resources available, we did not determine how quickly IgG accumulated in individual pigs or whether it accumulated linearly. Further research that includes assessing data from more time points would be useful to gain a better understanding of the genetic and biological processes that are involved with the kinetics of antibody response, such as the time after infection when antibodies start to be produced, the rate at which antibodies are produced, and when the antibody level plateaus (e.g. area under the curve). At a minimum, the recommended time points for further analysis include 14 and 28 dpi. 14 dpi is an attractive time point because most animals have seroconverted at this point (Cano et al., 2007) but it is an early time point for antibody response. An intermediate time point (i.e. 28 dpi) between seroconversion and the predicted 42 dpi plateau would allow assessment of the rate of antibody accumulation.

The FMIA assay used in this study could also measure IgM (Wang, 2013). However, IgM levels were either undetectable or low because the animals had already undergone class switching at 42 dpi (Di Noia, 2015). Thus, the IgM data were not analyzed in this study. This is consistent with previous reports on the kinetics of IgM antibody
response to PRRSV infection (Loemia et al., 1996; Venteo et al., 2012). Measurement of antibody levels at 14 dpi would also allow IgM antibody levels to be evaluated; IgM levels are expected to be low for most pigs at 28 dpi (Venteo et al., 2012).

**Comparison of NVSL and KS06 isolates**

NVSL and KS06 are two genetically distinct PRRSV isolates (Ladinig et al., 2015; Trible et al., 2015). KS06 was isolated from a farm that had recently experienced an abortion storm (Ladinig et al., 2015). Among PRRSV isolates, NVSL is considered a highly virulent isolate. (Truong et al., 2004). Comparison of reproductive performance of pregnant gilts following infection with these isolates showed that they are similar in virulence (Ladinig et al., 2015). However, for the data used in this study, KS06 infected growing pigs had lower viral burden and had less stunted growth than NSVL infected pigs (Hess et al., 2016). Johnson et al. (2004) showed that there is a strong positive correlation between the virulence of the PRRSV used and antibody response. However, S:P levels at 42 dpi were not significantly different between NVSL and KS06 PRRSV infected pigs in our study, perhaps due to the dpi selected or because these two isolates were not different enough in virulence to observe a difference in immunogenicity.

Estimates of heritability of S:P at 42 dpi were 0.31±0.09 for NVSL and 0.40±0.10 for KS06. These are similar to the estimated heritability from a commercial multiplier sow herd that experienced a PRRS outbreak (Serao et al., 2014), which used the commercial ELISA kit. Both our study and the study by Serao et al. (2014) targeted virus N-protein. While the commercial kit measures all antibody isotypes, Serao et al. (2014) measured antibody response at ~46 days after the start of the outbreak, when IgG is the predominant antibody isotype (Loemia et al., 1996).
To assess whether the genetics influencing S:P is similar when infected with different isolates of PRRSV, the genetic correlation between NVSL S:P and KS06 S:P was estimated. Unfortunately, due to large standard errors, this analysis was inconclusive. Supporting the finding of a high genetic correlation of S:P between NVSL and KS06, a region was identified that had an effect on S:P in the GWAS of each of the two isolates in our study. This region explained 10% of the genetic variance for pigs infected with NVSL and 43% for KS06. It is conceivable that there is a substantial portion of the polygenic variance is also in common between isolates, as was identified for viral load and weight gain during PRRSV infection (Hess et al., 2016; Waide, 2015).

**Antibody response in cleared, rebound and persistently infected pigs**

Within PRRSV populations there are subpopulations termed quasispecies, which are characterized by subtle variations from the consensus sequence, and these may be responsible for PRRSV escape from immune response (Rowland et al., 1999; Costers et al., 2010). Rebound in viremia is likely due to immune escape by the virus, which the pig recognizes as re-exposure to the virus. Rebound pigs had the highest S:P at 42 dpi. Therefore, pigs that have a rebound in viremia are expected to have greater stimulation of memory B cells, resulting in a higher level of antibody in these animals than those that didn’t have a rebound (Kurosaki et al., 2015). It is also possible that pigs with higher antibody levels put more selective pressure on the virus and are, therefore, more likely to show rebound (Costers et al., 2010; Delisle et al., 2012; Trible et al., 2015). Whether rebound versus antibody response is the cause versus effect is unclear because antibody response was only measured at the end of the experimental period.
Animals that had persistent viremia had similar S:P as pigs that had cleared the virus by 42 dpi, although they were expected to have lower S:P values due to a failure to mount a successful immune response. It may be that these animals had a delayed antibody response but a similar antibody plateau level at 42 dpi (Yoon et al., 1995; Loemba et al., 1996; Johnson et al., 2011). Measuring antibody response at multiple time points would help to elucidate these relationships between viremia status and antibody response.

**Genetic correlations of antibody response with weight gain and viremia**

Resource allocation theory relates traits competing for the limited energy resources that an animal has to the trade-offs that are often observed between traits (Van Noordwijk and Dejong, 1986; Roff, 2007) and can explain negative relationships between traits. Genetic correlations between traits are commonly used to assess trade-offs between traits at the genetic level (Roff, 2007). Studies investigating growth and immune function have observed trade-offs between these traits through negative genetic relationships (van der Most et al., 2011). Selection for production traits has resulted in reduced immunological fitness in multiple species of livestock (Rauw et al., 1998; van der Most et al., 2011). However, selection for improved immune response has been shown to not have as large of a negative impact on growth, perhaps because immune response is less costly than growth (Coop and Kyriazakis, 1999; Wilkie and Mallard, 1999; van der Most et al., 2011) or because healthy animals do not divert as many resources to immune response (Rauw, 2012).

In this study, weak genetic correlations of S:P at 42 dpi with VL and WG42 were observed (Table 4.2). VL and WG42 are both summary measures of the cumulative infection severity and impact on growth over time. The relationship between immune
response and growth is, however, expected to change over time, as the animal places
different levels of emphasis on these traits throughout the course of infection (Coop and
Kyriazakis, 1999; Hess et al., 2016). This has also been demonstrated using simulation
studies (Doeschl-Wilson et al., 2009). Therefore, the low genetic correlations of S:P with
either VL or WG42 could be a consequence of genetic correlations between traits at
different time points being averaged out. Fitting curves to both viremia, using a Wood’s
Curve, and weight, using Legendre polynomials, allowed the assessment changes in the
relationships S:P at 42 dpi with viremia and weight gain changed over time.

Results showed that the genetic correlations of S:P at 42 dpi with weight gain and
viremia indeed changed substantially over time. The genetic correlation estimates of S:P
with weight gain were negative early after infection but positive later on (Figure 4.1 C/D).
The strongest negative genetic correlation estimates between antibody response and daily
viremia were observed during the time that IgG antibody production is expected to start
(10-20 dpi; Figure 4.1A/B) (Chand et al., 2012; Venteo et al., 2012). Combined with
correlations with weight gain, these results suggest that animals that placed more emphasis
on immune response early in infection and therefore suffered with lower early weight gain,
reaped the benefits of this later in infection by more effectively clearing the virus (Figure
4.1), which is supported by findings from Doeschl-Wilson et al. (2009).

In NVSL infected animals, the genetic correlation of S:P at 42 dpi with weight gain
shifted from negative to positive shortly after 25 dpi, which corresponded to the time when
pigs started to clear viremia. Thus, the transition from negative to positive genetic
correlations of antibody response with weight gain in NVSL infected pigs may be due to
the pig recovering from infection. During infection, animals experience anorexia,
presumably in order to promote an effective immune response (Exton, 1997; Kyriazakis et al., 1998; Langhans, 2000). Once the animal has produced an effective immune response, the animal will return to a normal feed intake level, resulting in a return to homeostasis (Kyriazakis et al., 1998), or will increase feed intake, resulting in compensatory growth (Diaz et al., 2005).

In KS06 infected pigs, the genetic correlation of S:P at 42 dpi with weight gain was weak and negative early in infection but rapidly changed to positive at 7 dpi (Figure 4.1D). The genetic correlation of S:P at 42 dpi with daily viremia was negative at all time points for animals infected with KS06 (Figure 4.1B), whereas NVSL infected pigs showed a strong positive correlation between S:P and viremia early in infection, which switched to a negative correlation at ~7 dpi, and was most negative between 14 and 21 dpi, when production of IgG is expected to start (Figure 4.1A). Previous analyses of these viremia and weight data showed that weight gain was greater for animals infected with KS06 than NVSL (Hess et al., 2016). Thus, differences in genetic correlations of S:P with either weight gain or viremia when comparing NVSL and KS06 infections may be due to KS06 being less virulent and, thus, requiring less energy to be diverted to combat the infection, which could be reflected in the weaker genetic correlations of S:P with early weight gain traits for KS06.

**Impact of the SSC4 QTL on the relationship of S:P with viremia and weight gain**

The WUR10000125 SNP did not have a direct effect on the level of antibody produced in NVSL or KS06 infected animals (Table 4.1). The genotype at this SNP did appear to have an impact on the genetic correlation of S:P with both viremia and weight gain under infection with NVSL (Figure 4.2), but this was not tested for KS06 due to the
already limited number of animals for breaking the set into AA and AB/BB animals. Compared to all NVSL infected animals, for pigs with the unfavorable AA genotype S:P at 42 dpi had greater negative genetic correlations with viremia and more persistent negative relationships with weight gain over time (Figures 4.1 and 4.2). While direct values of the genetic correlations of S:P with viremia and weight gain could not be estimated for AB/BB animals, estimates of the genetic correlations for these animals derived by comparing all animals to AA animals, revealed that S:P of AB/BB animals generally had high positive genetic correlations with weight gain (Figure 4.1B) and lower and more sporadic genetic correlations with viremia, compared to their AA counterparts (Figure 4.1A).

The GBP5 gene, for which WUR is a tag SNP, is involved in the formation of the inflammasome and animals with the AA WUR genotype do not produce functional GBP5 protein (Koltes et al., 2015). The GBP5 protein binds to NLRP3 to promote ASC protein oligomerization and inflammasome assembly to activate caspase-1 (Shenoy et al., 2012). This pathway has been shown to induce an increase in IgG response (Kumar et al., 2009; Ellebedy et al., 2013). Gene expression profiling studies have shown that AA animals maintain higher expression of immune-related genes longer after infection than their AB counterparts (Schroyen et al., 2015). Furthermore, genes that were differentially expressed between 4 and 0 dpi showed greater enrichment of genes associated with humoral immune response for AA versus AB pigs (Schroyen et al., 2015). This suggests that animals with the AA genotype require more energy to produce an effective antibody response than AB animals (either earlier, greater quantity, or both). Effective antibody response reduces viremia over time but the production of these antibodies may also come at a cost of weight
gain. Lee (2006) discussed that patterns in constitutive immunity among individuals will depend on the cost of the defense, and individuals at the nonspecific inflammatory end of innate immune response spectrum should maintain higher levels of constitutive protection in order to minimize the use of costly inflammatory responses. Therefore, AA animals may also rely more heavily on alternate, likely non constitutive, inflammatory responses than AB or BB animals, resulting in a greater energetic cost to fighting PRRSV infection. The higher VL and lower WG observed in AA animals compared to AB animals (Boddicker et al., 2014b; Hess et al., 2016) may be due to the time or energetic resources it takes to mount an effective alternative inflammatory response.

Further analyses by WUR genotype would be useful for understanding the differences in the response between the animals with different genotypes at this QTL. In addition, body temperature and feed intake could be included as additional traits to explore response to PRRSV infection, which may provide insight into the early innate immune response to PRRSV infection of AA versus AB animals. Also, cytokines could be investigated as other blood parameters involved in response to infection, while cytokine levels at day zero might serve as useful predictors of response to infection.

**Genes affecting antibody response**

The MHC, known as the Swine Leukocyte Antigen Complex in pigs, plays a crucial role in the host’s ability to mount an antibody response to infection (Lunney et al., 2009). The MHC is a gene-rich region containing a cluster of genes associated with the immune system. One of the better characterized functions of the gene products of the MHC is the processing and presentation of antigens to T cells; however, many other immune-related genes and genes with unknown functions also map within this region (Janeway et al.,
2001). Between-breed differences in antibody response to PRRSV infection have been identified (Halbur et al., 1996; Petry et al., 2007). Wimmers et al. (2009) found an association of DQB MHC class II gene variants with antibody response at 10 days after vaccination with a modified live PRRS vaccine in an F2-population of reciprocal Berlin Miniature and Duroc crossbred piglets. Uddin et al. (2011) identified a region at 14 cM on SSC3 at 10 days post vaccination to be associated with antibody response in 15 week old Duroc/Pietrain F2 crossbred pigs. Serao et al. (2014) identified two large QTL on SSC7 for S:P measured by ELISA in a PRRSV outbreak sow herd. One of these QTL was in the MHC class II region and explained 25% of the genetic variation in antibody response.

As expected, the GWAS in our study also identified the MHC as being associated with antibody response at 42 dpi, using the NVSL, KS06, or combined datasets. The 1 Mb window starting at 29 Mb on SSC7 was identified in all three GWAS and explained 10.3% of the genetic variance for NVLS, 43.1% for KS06, and 29.9% for the combined analyses. The SNP DIAS0000349 (DIAS) in the 26 Mb window was identified as being associated with S:P in the joint analysis (explaining <1% of genetic variance for NVSL and KS06 and 13.8% for the joint analysis). DIAS may not have been identified in the NVSL GWAS due to potential non-additive effects (Table 4.3); while it may not have been identified in the KS06 GWAS due to a low minor allele frequency (Table 4.3) and corresponding low number of BB animals, resulting in a greater level of shrinkage in the Bayes-B analysis that was employed in the GWAS. Two of these SNPs, ALGA and MARC, were located in the MHC class II region of SSC7, while DIAS was located at the distal end of the MHC class I region of SSC7. The function of MHC I and II molecules is to bind peptide fragments from pathogens and present these on the surface of the cell for recognition by
the appropriate T cells (Janeway et al., 2001). Once the antigen is presented on the surface with an MHC class II molecule, it is recognized by CD4+ T-cells, which release cytokines that amplify the immune response, for example, by stimulating B cell differentiation. B cell differentiation into plasma B cells is responsible for the production of antibodies (Kurosaki et al., 2015). Therefore, it is not surprising that the MHC class II region shows a strong association with S:P. One SNP in this region, MARC, was previously identified as being associated with S:P ratio in sows that experienced a PRRS outbreak (Serao et al., 2014). Interestingly, in this study, this SNP was only associated with S:P for pigs infected with NVSL. The location of DIAS at the MHC I/III juncture makes it difficult to provide a good candidate gene for this SNP. Over all, the linkage disequilibrium in this region was low, and the LD between ALGA, MARC, and DIAS was also low (Supplemental Figure 4.1). This suggests that the QTL is in close proximity to the SNPs identified, and that the SNPs likely mark independent QTL.

Despite the strong genetic correlations of S:P with both viremia and weight gain, the SNPs identified for S:P by the GWAS either did not have a significant association with viremia and weight gain or only a small effect, indicating that these QTL play a role in antibody response but not in viremia or weight gain. GWAS of viral load and weight gain following infection with NVSL (Boddicker et al., 2012; Waide, 2015) and KS06 (Waide, 2015) have concluded that the majority of the genetic variance of these traits is explained by polygenic effects, as was also found with antibody response in this study. Serao et al. (in preparation) have also found that the majority of the genetic correlation between antibody response and reproductive traits following a PRRS outbreak is due to the non-MHC portion of the genome. Taken together, these results indicate that the majority of the
genetic correlation of antibody response with viral load and weight gain may be explained by many small QTL spread across the genome, rather than by the QTL located within the MHC. If this is the case, genomic selection for improved resistance to PRRSV may be more effective at improving multiple aspects of host response to PRRSV infection, than selection on a limited number of SNPs in the MHC region.

Conclusions

Antibody response, measured by PRRSV N-protein specific serum IgG levels at 42 dpi, was estimated to have moderate-high heritability for both the NVSL and KS06 PRRSV isolates. The genetic correlation of antibody response between isolates was also moderate to high, suggesting similar host genetic control, although a large standard error was observed in this study. The results of this study are consistent with resource allocation theory, in that pigs that put more energy into fighting infection early on have higher antibody levels and lower viremia during later stages of infection. This was at a cost of early growth but showed a benefit later in infection, presumably as animals recovered from infection. Animals with the unfavorable AA genotype at the WUR locus on SSC4 showed a stronger negative correlation of antibody response with viremia and a more persistent negative genetic correlation with growth. This suggests that more energy is required for AA animals to fight infection, resulting in lower growth. Genetic correlations of antibody response with either viremia or weight gain likely also depend on the virulence of the PRRSV isolate.

Antibody response has the potential to be used as a biomarker for selection for improved resistance to PRRS, however the genetics influencing host antibody response to
infection will need to be assessed at more time points in order to explore how antibody response changes over time and how this correlates with changes in other traits over time. GWAS identified three SNPs associated with antibody response located on SSC7 in the MHC but these SNPs were not significantly associated with viremia or weight gain.

Response to vaccination may provide an attractive alternative to natural infection, because all animals can be vaccinated at the same dose/age/time and phenotypes (e.g. viremia, weight gain, S:P, etc.) can be collected with higher consistency. Measuring PRRS resistance based on response to vaccination may have the added benefit of reducing the circulating level of PRRSV during natural infection, as the vaccine provides partial protection to subsequent PRRSV exposure (Osorio et al., 1998). Further studies are, however, needed to estimate the genetic correlation between response following vaccination and response following natural PRRSV infection. It is also important to assess the desirable, or optimal, level of antibody response that would increase resistance or decrease susceptibility to PRRSV, while not negatively impacting the ability of the pig to combat other infections.

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Table 4.1: LS Means (s.e.) of S:P ratio by WUR genotype and Viremia Status for Pigs Infected with NVSL or KS06

<table>
<thead>
<tr>
<th>Factor</th>
<th>Level</th>
<th>NVSL (n = 746)</th>
<th>KS06 (n = 443)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall</td>
<td></td>
<td>0.87 (0.02)</td>
<td>0.90 (0.04)</td>
</tr>
<tr>
<td>WUR Genotype²</td>
<td>AA</td>
<td>0.88 (0.02)</td>
<td>0.90 (0.03)</td>
</tr>
<tr>
<td></td>
<td>AB</td>
<td>0.86 (0.03)</td>
<td>0.90 (0.05)</td>
</tr>
<tr>
<td>Viremia Status</td>
<td>Cleared</td>
<td>0.84 (0.03)</td>
<td>0.85 (0.04)</td>
</tr>
<tr>
<td></td>
<td>Rebound</td>
<td>0.90 (0.03)</td>
<td>0.97 (0.05)</td>
</tr>
<tr>
<td></td>
<td>Persistent</td>
<td>0.85 (0.03)</td>
<td>0.88 (0.04)</td>
</tr>
</tbody>
</table>

1. Estimates that share a letter within the same factor and isolate are not significantly different from each other at p<0.05
2. NVSL: AA (n=552), AB (n=177), and BB (n=17); KS06: AA (n=382), AB (n=60), and BB (n=1)
Table 4.2: Genetic and Phenotypic Correlations (s.e.) among Viral Load (VL), Weight Gain (WG) and S:P ratio in Pigs Infected with NVSL or KS06

<table>
<thead>
<tr>
<th>Traits</th>
<th>Genetic Correlation</th>
<th>Phenotypic Correlation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NVSL</td>
<td>KS06</td>
</tr>
<tr>
<td>VL, S:P</td>
<td>-0.20 (0.18)</td>
<td>-0.69 (0.20)</td>
</tr>
<tr>
<td>WG, S:P</td>
<td>-0.38 (0.19)</td>
<td>-0.08 (0.25)</td>
</tr>
<tr>
<td>VL, WG</td>
<td>-0.74 (0.10)</td>
<td>-0.52 (0.17)</td>
</tr>
</tbody>
</table>
Table 4.3: Least Square Means of S:P ratio for Three SNPs on chromosome 7 for infection with one of two isolates of the PRRS virus

<table>
<thead>
<tr>
<th>Marker (Chr; Pos)</th>
<th>Isolate</th>
<th>Genotype</th>
<th>Minor allele frequency (allele)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>AA</td>
<td>AB</td>
</tr>
<tr>
<td>DIAS (SSC7; 26Mb)</td>
<td>NVSL</td>
<td>0.88 (0.04)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.77 (0.04)&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>KS06</td>
<td>0.89 (0.04)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.77 (0.05)&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>MARC (SSC7; 29Mb)</td>
<td>NVSL</td>
<td>0.93 (0.04)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.82 (0.04)&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>KS06</td>
<td>0.86 (0.06)&lt;sup&gt;a&lt;/sup&gt;</td>
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Estimates with different letters within a row are significantly different from each other at P<0.05
Figure 4.1. Estimates of Heritabilities, Phenotypic Standard Deviations and Genetic and Phenotypic Correlations with S:P at 42 dpi for viremia (A,B) or 3-Day Weight Gain (C,D) at different dpi following Infection with NVSL (A,C) or KS06 (B,D)
Figure 4.2. Estimates of Genetic Correlations of Antibody Response at 42 dpi with Viremia over time (A) and Weight Gain over time (B) for Animals with the AA (Red) or AB and BB (Yellow) WUR Genotype

A NVSL Viremia WUR

B NVSL Weight Gain WUR
Figure 4.3: Manhattan Plots of Percent of Total of Genetic Variance explained by each 1 Mb window of the Swine Genome for S:P Ratio in Pigs Infected with NVSL (A), KS06 (B), and the combined Analysis (C)
Figure S4.1 Linkage Disequilibrium Plot of for SNPs in the 26-30 Mb region of chromosome 7
| DPI | Viremia | | | | Weight Gain | | | |
|-----|---------|-----|-----|-----|---------|-----|-----|
|     | NVSL    | KS06 | NVSL | KS06 | NVSL    | KS06 |
|     | AA      | AB   | BB   | AA   | AB   | BB   | AA   | AB   | BB   |
| 1   | 2.97(0.24) | 2.81(0.23) | 2.75(0.24) | 2.09(0.21) | 2.20(0.20) | 7.34(0.02) | 7.33(0.02) | 7.32(0.02) | 7.07(0.02) | 7.08(0.02) |
| 3   | 5.44a(0.04) | 5.29ab(0.04) | 5.31b(0.04) | 4.23a(0.02) | 4.29a(0.03) | 4.34a(0.03) | 7.73(0.07) | 7.76(0.07) | 7.78(0.07) | 7.51(0.07) | 7.55(0.07) |
| 5   | 6.55a(0.06) | 6.47ab(0.06) | 6.51b(0.06) | 5.39a(0.04) | 5.41a(0.05) | 5.47a(0.05) | 8.17(0.07) | 8.20(0.07) | 8.22(0.07) | 8.03(0.06) | 8.07(0.06) |
| 7   | 6.87(0.05) | 6.84(0.05) | 6.91(0.05) | 5.91(0.04) | 5.91(0.05) | 5.96(0.05) | 8.64(0.06) | 8.67(0.06) | 8.68(0.06) | 8.60(0.06) | 8.64(0.06) |
| 9   | 6.72(0.05) | 6.74(0.06) | 6.80(0.06) | 5.99(0.05) | 5.98(0.06) | 6.04(0.05) | 9.14(0.07) | 9.17(0.07) | 9.18(0.07) | 9.21(0.07) | 9.24(0.07) |
| 11  | 6.32(0.05) | 6.35(0.05) | 6.40(0.05) | 5.81(0.05) | 5.77(0.04) | 5.85(0.04) | 9.68(0.06) | 9.70(0.06) | 9.71(0.06) | 9.85(0.06) | 9.88(0.06) |
| 13  | 5.78(0.05) | 5.81(0.05) | 5.85(0.05) | 5.46(0.04) | 5.41(0.05) | 5.49(0.05) | 10.28(0.06) | 10.27(0.06) | 10.28(0.06) | 10.54(0.04) | 10.56(0.04) |
| 17  | 4.60(0.06) | 4.62(0.06) | 4.64(0.06) | 4.57(0.05) | 4.50(0.04) | 4.60(0.04) | 11.48(0.05) | 11.50(0.05) | 11.51(0.05) | 12.04(0.03) | 12.05(0.03) |
| 19  | 4.04(0.06) | 4.05(0.06) | 4.05(0.06) | 4.10(0.06) | 4.03(0.05) | 4.14(0.05) | 12.14(0.04) | 12.16(0.04) | 12.17(0.04) | 12.85(0.03) | 12.86(0.03) |
| 21  | 3.52(0.07) | 3.53(0.07) | 3.52(0.07) | 3.64(0.05) | 3.58(0.06) | 3.69(0.06) | 12.85(0.04) | 12.86(0.04) | 12.87(0.04) | 13.69(0.03) | 13.70(0.03) |
| 23  | 3.06(0.08) | 3.06(0.08) | 3.03(0.08) | 3.21(0.06) | 3.15(0.07) | 3.26(0.07) | 13.58(0.04) | 13.59(0.04) | 13.60(0.04) | 14.58(0.03) | 14.59(0.03) |
| 25  | 2.65(0.08) | 2.64(0.08) | 2.60(0.08) | 2.82(0.06) | 2.77(0.07) | 2.88(0.07) | 14.34(0.04) | 14.36(0.04) | 14.36(0.04) | 15.51(0.03) | 15.52(0.03) |
| 27  | 2.28(0.08) | 2.27(0.08) | 2.22(0.08) | 2.51(0.06) | 2.46(0.07) | 2.59(0.07) | 15.14(0.03) | 15.15(0.03) | 15.16(0.03) | 16.48(0.03) | 16.48(0.03) |
| 29  | 1.97(0.09) | 1.95(0.09) | 1.89(0.09) | 2.17(0.07) | 2.13(0.08) | 2.23(0.08) | 16.84(0.03) | 16.85(0.03) | 16.85(0.03) | 18.54(0.03) | 18.54(0.03) |
| 31  | 1.60(0.10) | 1.67(0.10) | 1.61(0.10) | 1.87(0.08) | 1.84(0.09) | 1.92(0.09) | 17.73(0.03) | 17.74(0.03) | 17.74(0.03) | 19.63(0.03) | 19.63(0.03) |
| 33  | 1.46(0.10) | 1.43(0.10) | 1.36(0.10) | 1.62(0.08) | 1.59(0.09) | 1.67(0.09) | 18.66(0.03) | 18.67(0.03) | 18.67(0.03) | 20.76(0.03) | 20.76(0.03) |
| 35  | 1.26(0.11) | 1.24(0.11) | 1.15(0.11) | 1.40(0.09) | 1.38(0.10) | 1.45(0.10) | 19.63(0.03) | 19.63(0.03) | 19.63(0.03) | 21.93(0.05) | 21.93(0.05) |
| 37  | 1.08(0.12) | 1.05(0.12) | 0.98(0.12) | 1.21(0.10) | 1.20(0.11) | 1.26(0.11) | 20.62(0.03) | 20.62(0.03) | 20.62(0.03) | 23.15(0.05) | 23.14(0.05) |
| 39  | 0.93(0.12) | 0.90(0.12) | 0.83(0.12) | 1.05(0.11) | 1.04(0.12) | 1.09(0.12) | 21.65(0.03) | 21.65(0.03) | 21.65(0.03) | 24.40(0.05) | 24.39(0.05) |
| 41  | 0.80(0.12) | 0.77(0.12) | 0.70(0.12) | 0.91(0.12) | 0.90(0.13) | 0.94(0.13) | 24.40(0.05) | 24.39(0.05) | 24.38(0.05) | 24.40(0.05) | 24.39(0.05) |
Table S4.2: SNP Effects of MARC0058875 on Viremia and Weight Gain in NVSL and KS06 Infected Pigs

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Table S4.3: SNP Effects of DIAS0000349 on Viremia and Weight Gain in NVSL and KS06 Infected Pigs

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CHAPTER V

IDENTIFICATION OF FACTORS ASSOCIATED WITH TONSIL VIRUS LEVELS IN PIGS EXPERIMENTALLY INFECTED WITH PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS

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Abstract

Porcine Reproductive and Respiratory Syndrome (PRRS) is one of the most important global swine diseases from both an economic and animal welfare standpoint. PRRS has plagued the US swine industry for over 25 years and containment of PRRS virus (PRRSV) has been unsuccessful to date. The primary phase of PRRS serum viremia typically clears between 21 and 42 days post infection (dpi); however, tonsils are a main site of PRRSV persistence and PRRSV can be detected in tonsils in excess of 150 dpi. Measuring tonsil virus levels at 42 dpi can be used to assess tonsil persistence, as levels of virus in tonsil virus at this time are likely to influence how long the virus will remain in the tissue. Tonsil virus levels (TV) were measured on pigs experimentally infected with either the NVSL-97-7895 (NVSL; n=524) or KS-2006-72109 (KS06; n=328) PRRSV isolate across five trials. The objectives of this study were to 1) estimate the heritability of tonsil virus levels at 42 dpi; 2) assess whether there is a relationship between serum viremia and tonsil virus levels; 3) conduct a genome-wide association study to identify genomic regions associated with tonsil virus levels; and 4) compare the results for NVSL and KS06. TV was lowly heritable for both isolates (NVSL: 0.05±0.06; KS06: 0.11±0.10). Low TV was phenotypically associated with traits related to viral clearance from serum: earlier and faster rate of maximal viral clearance, lower total serum viral load and lower viremia level at 35 or 42 dpi. Although no QTL with major effects were identified, several genomic
regions showed some association (>0.10% of the total genetic variance in three GWAS: the NVSL-infected dataset, the KS06-infected dataset and the complete dataset). These regions contained the genes CCL1, CCL2, CCL8, HS3ST3B1, GALNT10, TCF7, C1QA/B/C, HPSE, G0S2 and CD34, which are involved in viral infiltration/replication and viral clearance from tissue. Results were similar between PRRSV isolates NVSL and KS06. Selection for viral clearance traits may reduce PRRSV persistence in the tonsil across PRRSV isolates, however genetic correlations need to be calculated to determine whether this will be successful.

**Introduction**

Porcine reproductive and respiratory syndrome (PRRS) is a viral disease of major concern to the global swine industry from both an animal welfare and an economic standpoint. Adult pigs infected with PRRSV are severely compromised in their reproductive abilities and growing pigs weakened by PRRSV infection are at higher risk of preweaning mortality. Pigs and piglets infected with PRRSV also suffer from anorexia, depression, and respiratory distress, resulting in slowed growth and reduced well-being (Pejsak et al., 1997). In the United States, PRRS costs the swine industry $664 million per year, of which over half can be attributed to the growing pig (Holtkamp et al., 2013).

PRRSV is characterized by rapid mutation rates and high antigenic variability, which has encumbered efforts to contain PRRSV through vaccination and biosecurity measures (Darwich et al., 2010). Once infected with PRRSV, painstaking efforts are necessary to return a herd to a PRRSV negative status. These efforts are often impeded by pigs that are persistently infected with PRRSV. Pigs are expected to clear PRRSV from
serum between 3 and 8 weeks post infection (Hess et al., in preparation-a; Wills et al., 2003); however some pigs may still be PRRSV positive in tissue long after this acute phase of infection – even 150 to 250 days post infection (Allende et al., 2000; Wills et al., 2003). Pigs with PRRSV present in tonsils or lymph nodes after the acute phase of PRRSV infection are classified as persistently infected pigs (Benfield et al., 1998). Persistent pigs can experience a resurgence of circulating virus, which can trigger a secondary outbreak if the virus has mutated, such that the pigs lack the protective immunity from the previous outbreak. Persistently infected pigs are also of concern to naïve pigs that were introduced into the herd after the initial PRRSV outbreak (Rowland and Morrison, 2012). Successful identification and removal of persistently infected pigs could substantially help with containment of PRRS and improve herd health. However, persistently infected pigs are often asymptomatic, which makes it hard to identify them, despite high levels of PRRSV in tissues such as lymph nodes and tonsils (Benfield et al., 1998; Wills et al., 2003).

This study measured the amount of virus present in tonsils 42 days following experimental infection, rather than measuring persistence as the presence or absence of PRRSV in tonsil because most animals are expected to have PRRSV present in their tonsils at 42 dpi (Wills et al., 2003). Level of viremia in tonsils at 42 dpi may be an indicator of persistence, as higher levels of PRRSV in the tonsils at 42 dpi are likely to result in prolonged presence of PRRSV in tonsils up to 250 dpi. Tonsil levels were analyzed at 42 dpi because this time point covers both the later stages of acute infection as well as the early stages of persistent infection (Rowland et al., 2012). The tonsil was chosen because it is an active site of PRRSV replication (Rowland et al., 2003) and PRRSV is known to persist in this tissue (Allende et al., 2000; Wills et al., 2003).
Genetic selection of pigs that are more resistant to PRRS is an attractive method to improve the health status of the swine herd (Lewis et al., 2007). The goal of the PRRS Host Genetics Consortium (PHGC) is to identify host genes or genomic regions associated with increased resistance of pigs to PRRSV infection (Lunney et al., 2011). Previous analyses identified a heritable genetic component to PRRS serum viremia kinetics and weight gain during infection with one of two genetically distinct PRRSV isolates: NVSL-97-7895 (NVSL) and KS-2006-72109 (KS06) (Boddicker et al., 2012; Hess et al., in preparation-a). In these studies, a major quantitative trait locus (QTL) was identified by genome-wide association studies (GWAS), WUR10000125 (WUR), whereby animals with the B allele had reduced serum viremia and increased weight gain during PRRSV infection (Boddicker et al., 2012; Hess et al., in preparation-a). A putative causal mutation was identified for this QTL in GBP5 by Koltes et al. (2015), which is a gene that is involved with the formation of the inflammasome mediated by NLRP3 (Shenoy et al., 2012). It is likely that the ability of an animal to clear serum viremia has an impact on tonsil virus levels (TV). Therefore, we hypothesized that animals that clear serum viremia levels faster, and thus have lower serum viremia at 42 dpi, will also have lower levels of TV at that time, and that tonsil virus levels will have a heritable genetic component. In addition, a GWAS can provide insight into the genetic mechanisms involved with response to infection influencing tonsil virus levels. Thus, the objectives of this study were to 1) estimate the heritability of tonsil virus levels at 42 dpi; 2) assess whether there is a relationship between serum viremia and tonsil virus levels; 3) conduct a genome-wide association study to identify genomic regions associated with tonsil virus levels; and 4) compare the results for NVSL and KS06.
Materials and Methods

Study design

A detailed description of the design, data collection and molecular techniques used in the PHGC infection trials is in Lunney et al. (2011). The Kansas State University Institutional Animal Care and Use Committee approved all experimental protocols for these trials. Pigs used for this study were from 5 PHGC trials of ~200 weaner pigs: trials 3, 5, 7, 11 and 14. A more detailed description of these trials is in Hess et al. (in preparation-a). Pigs were provided from commercial breeding programs in the United States and Canada. Trials 3 and 11, as well as trials 5 and 14 were from the same genetic background. Within each challenge trial, pigs were from a single high health farm and genetic background, except for trial 5, which included pigs from one genetic background but two farms. All source farms were free of PRRS, *Mycoplasma hyopneumoniae*, and swine influenza. Animals were transported at weaning (average age of 21 days) to Kansas State University and randomly placed into pens of 10 to 15 pigs. After a 7-day acclimation period, pigs were experimentally infected, both intramuscularly and intranasally, with $10^5$ (TCID50) of either NVSL-97-7985 (trials 3, 5, and 7), a highly virulent PRRSV isolate (Truong et al., 2004); or with KS-2006-72109 (trials 11 and 14), a more contemporary PRRSV isolate. NVSL and KS06 are 89% similar at the GP5 nucleotide sequence level (Ladinig et al., 2015). Blood samples were collected at −6, 0, 4, 7, 11, 14, 21, 28, 35, and 42 days post-infection (dpi). Body weight was measured at 0, 7, 14, 21, 28, 35, and 42 dpi. Pigs were euthanized at 42 dpi. Trial 7 was stopped at 35 dpi due to facility unavailability. Tonsil samples were collected at euthanization. Across the NVSL trials, 5% of pigs died or were euthanized for humane reasons before 35/42 dpi. Mortality rate was similar in the
KS06 trials, with 7% pigs dying or euthanized before 42 dpi across the two trials. Dead pigs were necropsied and subsequent gross and microscopic pathology by a board-certified pathologist identified PRRS associated disease as the major source of mortality.

**Serum viremia and tonsil virus levels**

Serum viremia and tonsil virus levels were measured using a semi-quantitative TaqMan PCR assay for PRRSV RNA, as described in Boddicker et al. (2012; 2014a; 2014b), whereby samples were run in duplicate using 96-well plates. Serum assay results were reported as the log_{10} of PRRSV RNA copies per ml of serum, while tonsil virus levels (TV) were reported as the log_{10} of PRRSV RNA copies per mg of tissue. NVSL trials (3, 5 and 7) used the AgPath ID NA & EU PRRSV reagents (Applied Biosystems), while KS06 trials (11 and 14) used the Tetracore U.S. and EURO PRRSV Master Mix reagents (Tetracore), herein referred to as “AB” and “Tetracore” assays, respectively. The Tetracore reagents were used due to the failure of the AB primers to amplify cDNA of the KS06 isolate. The two probe sets gave consistent serum viremia measurements (Rowland, personal comment); however it is possible that they do not give consistent tonsil virus measurements. Thus, tonsil samples from a subset of 35 NVSL-infected animals were also evaluated using the Tetracore kit to evaluate the consistency between assays. Results showed that measurements using the Tetracore assay were, on average, 1.34 log_{10} lower than those using AB reagents (4.79 vs. 3.45), with measurements 1 log_{10} apart using the AB reagents, on average only being 0.68 log_{10} apart using the Tetracore reagents based on linear regression (Figure 5.1) ($R^2=0.76$). The resulting linear regression equation can be used to adjust the NVSL tonsil virus levels for differences between the kits, in order to obtain the expected TV of an animal if it were assayed using the Tetracore kit.
In this study, tonsil viremia data were available on 524 NVSL infected animals and on 328 KS06 infected animals. RNA integrity number (RIN) scores of the RNA extracted from the tonsil samples were determined by an Agilent bioanalyzer, which serves as a measurement of the quality of the RNA in a sample. 14 animals were removed due to an unreadable RIN, which is interpreted as a low quality RNA sample (Schroeder et al., 2006). Virus levels were assigned to a sample using a standard curve when performing the TaqMan qPCR assay. This standard curve has a linear portion and samples within this portion can be accurately measured, while samples falling in the non-linear range are not accurately measured at the dilution used. In addition to the animals that did not have RIN, 30 NVSL infected animals and 26 KS06 infected animals were identified as having low-confidence measurements, including samples whose virus values fell outside the standard curve (too high (28) or too low (19)) or for which no virus was detected (9) due to either assay failure or lack of virus in the sample. Two approaches were taken to edit the dataset for analysis: one that included animals with low-confidence tonsil virus values (Liberal) and one that excluded these animals (Conservative). For the liberal approach, animals whose measured virus levels fell above the standard curve were given tonsil virus values that were double the maximum of the linear portion of the standard curve for the plate the sample was run on. Animals whose measured virus levels fell below the standard curve or were undetermined, were given tonsil virus values equal to half the minimum of the linear portion of the standard curve.

**Genotypes**

Ear tissue was collected from all pigs for DNA isolation. Tissue or DNA samples for trials 3, 5 and 7 were genotyped with Illumina’s Porcine SNP60 Beadchip (Ramos et
al., 2009) v1 (San Diego, California) at GeneSeek Inc. (Lincoln, Nebraska) and samples from trials 11 and 14 were genotyped with Illumina’s Porcine SNP60 Beadchip v2 (San Diego, California) at Delta Genomics (Edmonton, Alberta). Only SNPs that were included on both versions of the Illumina’s Porcine SNP60 Beadchip were used for analysis. SNPs were also removed if they were fixed within trial, if they were unmapped or if they mapped to a sex chromosome in build 10.2 of the swine genome (http://www.ncbi.nlm.nih.gov/genome/guide/pig/, accessed August 13, 2015); this left 48,164 SNPs. For these SNPs, missing genotypes (0.19%) were assigned the average genotype (on a 0/1/2 scale) for animals in that trial for that SNP. This set of SNPs will be referred to as 60k SNPs.

Due to the limited pedigree information and availability of genotypes on almost all animals, the 60k SNP genotype data was used to construct a genomic relationship matrix (G-matrix) among all animals, using the method of Van Raden (2008). The G-matrix also included relationships between animals from different trials and genetic backgrounds.

**Modelling viremia profiles with the Wood’s function**

Previous studies have utilized the Wood’s curve to model serum viremia profiles in the PHGC trials (Hess et al., in preparation-a; Hess et al., in preparation-b; Islam et al., 2013):

\[ V(t) = a_1 t^{b_1} e^{-c_1 t} \]

where \( V(t) \) is serum viremia on the \( \log_{10} \) scale at time \( t \) dpi, \( a_1 \) impacts the overall viremia level across time, \( b_1 \) is an indicator of the initial rate of increase to peak viremia, \( c_1 \) is an indicator of the rate of decline after the peak and dominates the viremia profile at the later stages of infection. These three function parameters were estimated for each individual that
had at least five time points using Bayesian inference with a likelihood framework, implemented using a Markov chain Monte Carlo method (Islam et al., 2013).

The raw viremia profiles of some pigs appeared bi-modal, so an extended Wood’s curve was also fitted for each pig using the same methodology:

$$V(t) = a_1 t^{b_1} e^{-c_1 t} + \max(0, a_2 (t - t_0)^{b_2} e^{-c_2 (t-t_0)})$$

where $t_0$ denotes the onset of the second phase of the profile, which is assumed to follow the same Wood’s curve shape as the primary phase and is thus defined by a second set of Wood’s model parameters. A pig was classified as experiencing viremia rebound based on the Akaike’s Information Criterion (AIC) if $AIC_{WOOD’S} - AIC_{EXTENDEDWOOD’S}$ was greater than 1.488, corresponding to the 95% significance level of the likelihood ratio test between these models (Islam et al., 2013). In this study, 15.5% of NVSL infected pigs and 4.6% of KS06 infected pigs were classified as rebound pigs. Previously, NVSL was determined to be more virulent, while KS06 was characterized as being more persistent in serum (Hess et al., in preparation-a). A total of 45.2% of NVSL infected pigs and 65.2% of KS06 infected pigs were classified as having persistent PRRSV levels in serum, defined as having an estimated log$_{10}$ serum value that exceeded 1 at the end of the trial (Islam et al., 2013), as determined by fitted Wood’s curve. The remaining animals were assumed to have cleared viremia from serum by the end of the trial.

Five curve characteristics were derived to describe individual viremia profiles using the estimates of the curve parameters $(\hat{a}_1, \hat{b}_1, \hat{c}_1)$ of the single or the extended Wood’s curve in order to elucidate the relationship between serum and tonsil virus levels. The first
characteristic evaluated, area under the Wood’s curve from infection until euthanasia, hereafter referred to as viral load (VLTotal), was given by the definite integral:

\[
VL_{\text{Total}} = \int_{0}^{T} \left[ \bar{a}_1 t^\bar{b}_1 e^{-\bar{c}_1 t} + \max(0, \bar{a}_2 (t - t_0)^\bar{b}_2 e^{-\bar{c}_2(t-t_0)}) \right],
\]

where \( T \) is the time of euthanasia, either 35 dpi (trial 7) or 42 dpi (trials 3, 5, 11 and 14). VLTotal is a measure that includes both the level of viremia and the extent to which viremia is maintained.

The remaining four curve characteristics have been described in Chapter 3 of this thesis. The second curve characteristic evaluated was time to (first – in the case of rebound) peak viremia (TP), derived by setting the first derivative of the Wood’s equation to zero and solving for \( t \), resulting in:

\[
TP = \frac{\bar{b}_1}{\bar{c}_1}
\]

The third curve characteristic was (first) peak viremia (PV), which was calculated by setting \( t = TP \) in the Wood’s equation:

\[
PV = \bar{a}_1 \left( \frac{\bar{b}_1}{\bar{c}_1} \right)^{\bar{b}_1} e^{-\bar{b}_1}
\]

Time to maximal decay (Tmax) was derived by setting the second derivative of the Wood’s equation to zero and solving for \( t \):

\[
T_{\text{max}} = \frac{\bar{b} + \sqrt{\bar{b}}}{\bar{c}}
\]

Plugging this value in for \( t \) in the first derivative and taking the absolute value gives Vmax:
\[ V_{\text{max}} = | - \hat{a}\sqrt{b} \left( \frac{\hat{b} + \sqrt{b}}{\hat{c}} \right)^{\hat{b}-1} e^{-\left(\hat{b}+\sqrt{b}\right)} | \]

**Other response phenotypes**

Body weights were collected weekly and used to interpolate daily weights as described in Chapters 3 and 4 of this thesis. Fitted weights from 0-7, 7-14, 14-21, 21-28, 28-35, and 35-42 were used for analysis in this study. Measured serum viremia at euthanasia (vEND) was also analyzed to evaluate the relationship between serum viremia and tonsil virus levels collected on the same day. Total PRRSV N-protein IgG (measured as an S:P ratio) was available on 207 NVSL (trials 3 and 5) and 156 KS06 (trial 11) infected pigs that had tonsil viremia data (Chapter 4).

Neutralizing antibody (NAb) titer data was available on 360 NVSL (trials 3, 5 and 7) and 157 KS06 (trial 11) infected animals that had sufficient serum available at 42 dpi. Serum samples collected at 42 dpi were assayed for NAb response by incubating 200 50% tissue culture infectious dose (TCID\textsubscript{50}) of the virus with 1:2 dilutions of serum and then transferring to tissue culture plates with confluent MARC-145 cells; PRRSV cytopathic effects were assayed 4 days later (Trible et al., 2015). The inverse of the highest serum dilution without cytopathic effects was recorded as the NAb titer and this ranged from \(<8\) to \(>1024\). For statistical analysis, NAb response was converted to an adjusted \(\log_2\) scale (0-8).

**Statistical analyses**

**Plate effects**

Tonsil viremia assays were run on 96-well plates and plate was partially confounded with both pen and family – with individuals from the same pen tending to be
run on the same plate and half- or full-sibs also often run on the same plate. The following
two models were fitted to tonsil virus levels, separately for each PRRSV isolate, using
ASReml 3.0 (Gilmour et al., 2009), to evaluate the effect of including plate in the model:
one that included plate, and the other that excluded plate. The full model was:

\[
Y_{ijklmnopqrs} = \mu + Tr_i + P_j + S_k + A_l + W_m + R_n + RIN_o + An_p + Li_q + Pen(Tr)_{ir} + Pl_s + \epsilon_{ijklmnopqrs}
\]

where Y is the dependent variable of tonsil virus level. Parity of the dam (P), classified as
first, second, or later parity, sex of the pig (S), and trial (Tr) were fitted as a fixed class
effects. To account for potential differences between rebound and non-rebound pigs based
on serum viremia, the fixed class effect of rebound (R) was also included in the model.
Age (A) and weight (W) of the piglet at infection, and the RIN of the tonsil sample were
fitted as linear covariates. Random effects included animal genetic effects (An; using the
full G-matrix), litter (Li), Pen nested within trial (Pen(Tr)), Plate (Pl), and ε as the residual.
Forty-three animals that did not have plate information were not used in this part of the
study.

Results showed that the variance attributed to plate was partitioned between the
animal, pen and residual components when plate was not included in the model (Table
S5.1). It is expected that the component of plate that is partitioned into the animal
component when plate is not included in the model is a combination of true genetic
variance and true plate variance due to confounding between plate and family. It is not
known what proportion of this is true genetic variance compared to true plate variance so
the resulting heritability estimates are expected to be either be an over-estimate (excluding
plate) or under-estimate (including plate) of the true heritability. The decision was made to
exclude plate from the analyses because including plate information has the potential to account for some of the genetic variation in TV and influence genetic correlations between TV and viremia, weight and antibody traits.

**Heritability and association analyses**

Estimation of the heritability of TV and of phenotypic associations of TV with other PRRS response traits were conducted using the model described above without plate effects. The relationships of TV with serum viremia characteristics, weekly weight gains, and antibody response were estimated by separately including each of these traits as a fixed covariate in the model. The coefficient of determination of the independent variable was used to assess the proportion of TV explained by the covariate. When estimating the association between TV and WUR genotype, WUR genotype was added as a fixed effect.

**Genome Wide Association Study**

The Bayes C method implemented in GenSel 4.73 (Fernando and Garrick, 2012) was used to test associations of 60k SNPs with TV based on the following model:

\[
Y_{ijklmnopqt} = \mu + Tr_i + P_j + S_k + A_l + W_m + R_n + RIN_o + Li_p + Pen(Tr)_{1q} + \sum_{r=1}^{s} z_r \alpha_r \delta_r + \epsilon_{ijklmnopqt}
\]

where \(Y\) is the dependent variable of TV; \(P, S, W, R, RIN, Tr, \) and Pen(Tr) were as described above but all fitted as fixed effects because GenSel does not accommodate random effects other than SNP effects; \(s\) is the number of SNPs fitted; \(z_r\) is the genotype of SNP \(r\) for each animal \((t)\), \(\alpha_r\) is the allele substitution effect for SNP \(r\), and \(\delta_r\) indicates whether SNP \(r\) was included \((\delta_r = 1)\) or excluded \((\delta_r = 0)\) in the model for a given iteration of the Monte Carlo Markov Chain. The prior probability of \(\delta_r = 0\) was set equal to \(\pi = \)
0.995. A total of 500,000 iterations were run, including a burn-in of 50,000. Breeding values were calculated for each non-overlapping 1 Mb region (window) for each iteration to obtain a sample of the breeding value for each animal for each window. The total breeding value of an animal was computed as the sum of the animal’s window breeding values across the genome. The percent genetic variance explained by a window in a given iteration was calculated by the variance of the breeding values of all animals for that window divided by the variance of total breeding values, multiplied by 100. The estimate of the percent genetic variance explained by a window was the posterior mean percent genetic variance explained by that window across all iterations (excluding 50,000 burn-in) (Garrick and Fernando, 2013).

**Results**

**Heritability Analyses**

TV was estimated to be lowly heritable for either isolate and both heritabilities were not significantly different from zero (Table 5.1). Heritability estimates using the conservative dataset were higher than estimates using the liberal dataset, although differences were not significantly different from zero.

**Associations of TV with Host Response Traits**

**Isolate and serum viremia status**

Using the conservative dataset, NVSL infected pigs had 43% higher TV than KS06 infected pigs (P<0.001); however, after adjusting NVSL levels to their Tetracore equivalents, NVSL infected pigs had 2% higher TV than KS06 infected pigs and this difference was not significant (P<0.1; Table 5.3). For NVSL, pigs that were classified as
having persistent serum viremia levels had significantly higher tonsil virus levels than pigs that had cleared serum viremia (P<0.001). Rebound animals had an intermediate value of tonsil virus levels that were not significantly different from cleared or persistent animals (P>0.1). For KS06, the direction of the effect between TV and serum viremia status was the same as NVSL, although differences were not as significant (Table 5.3). Adjusting NVSL to their Tetracore equivalent would result in differences between cleared and persistent animals that are similar to those in KS06 infected animals.

**Serum viremia**

Associations of different serum viremia curve characteristics with TV were assessed based on regression coefficients and coefficients of determination (R²; Table 5.4). Neither TP nor PV were significantly associated with TV for either the NVSL or KS06 data. An earlier and faster virus clearance (Tmax and Vmax) was, however, associated with lower TV for both NVSL and KS06 infected pigs for both the conservative and liberal datasets, except for Vmax in pigs infected with KS06 in the liberal dataset. After adjustment of NVSL to their Tetracore equivalents, the regression coefficients for Tmax and Vmax would be more similar for NVSL and KS06 infected pigs. Animals that had lower levels of serum viremia throughout the trial (VLTotal) or on the day that the tonsils were collected (vEND) had lower tonsil virus level at 42 dpi for all datasets, except for VLTotal in pigs infected with KS06 in the liberal dataset (Table 5.4A/B).

**Weight gain**

Analysis of weekly weight gain did not reveal any associations with TV (Table 5.4A). However, there was a marginally significant (P=0.04) positive association of weight gain during the last week of the trial with TV, suggesting that pigs that gained more weight
at the end of the trial had higher TV. However, this was only seen in NVSL infected pigs in the conservative dataset.

**Antibody response**

A significant association was identified between TV and both S:P and NAb response in KS06 infected pigs in the conservative dataset, but no association was identified for NVSL infected pigs in either the liberal or conservative dataset (Table 5.4). The regression coefficient suggests that animals that had a higher antibody levels at the end of the trial had lower TV, while animals with higher neutralizing antibody titers had higher TV.

**Genome wide association studies**

No significant association between TV and WUR genotype was identified for either isolate and in either the conservative or the liberal dataset (P>0.6; Table 5.3). The GWAS identified no major QTL for either isolate separately or jointly (Figures 5.1 and 5.2). The 1 Mb window with the greatest association explained only 0.14% of the genetic variance. One Mb regions that explained more than 0.1% of genetic variance when analyzing each isolate separately and together were further investigated for their biological relevance to PRRSV infection because they were identified in independent analyses (NVSL and KS06). In the conservative dataset, 21, 24, and 62 regions were identified that explained at least 0.1% of genetic variance in the NVSL, KS06, and combined data sets, respectively. Of these, 8 regions were identified in all three analyses, with 7 of these having functionally relevant genes within 1 Mb in either direction of the identified 1 Mb window (Table 5.2A).

In the liberal dataset, 17, 23, and 61 regions were identified that explained at least 0.1% of genetic variance in the NVSL, KS06, and combined data sets, respectively. The
liberal dataset identified only five regions that explained at least 0.1% of genetic variance in all three GWAS: NVSL, KS06 and the combined analysis. One region did not contain any obvious functionally relevant genes and three of the four remaining regions were also identified using the conservative dataset (Table 5.2B).

**Discussion**

**Differences between the Applied Biosystem and Tetracore Assays**

Ideally, the reported level of tonsil virus in a sample will be invariant of the probe set that is used for measurement, thus, a regression coefficient and an $R^2$ near 1 when comparing the same animals using two different probe sets. However, in this study the level of viremia reported on a set of 35 animals by the AB and Tetracore assays were different. A regression coefficient was calculated from these 35 NVSL-infected pigs to adjust the AB results to their Tetracore equivalent. This regression coefficient ($b=0.63$) can then be used to make comparisons between NVSL and KS06.

**Conservative vs. liberal dataset**

Results from the analyses that used the liberal dataset were generally consistent with results from the conservative dataset, however estimates tended to be less significant using the liberal dataset due to larger standard errors. The lower heritability estimate and the identification of fewer regions in the GWAS using the liberal dataset compared to the conservative dataset indicate that the conservative dataset is more appropriate for use in our analyses; therefore, this discussion will primarily focus on results from the conservative dataset.
Heritability estimates

Heritability estimates for TV were low and not significantly different from zero (Table 5.1). Bishop and Woolliams (2010) reviewed potential sources of variation in the macro environment that contribute to phenotypic variation that, if not controlled, have the potential to underestimate heritability. This may also be occurring in the microenvironment when measuring viremia in the tonsils. Tonsils are a site of PRRSV replication and PRRSV preferentially replicates in macrophages. Therefore, potential sources of variation include the size of tonsils and macrophage abundance. If available, accounting for these effects as covariates in the model may lead to a higher heritability estimate because this variation is likely to be part of the residual variance.

The quality of the assay that was used to measure the number of RNA copies is another potential source of variation. For example, plate-to-plate variation could contribute to the ability to accurately measure TV. In the trials evaluated in this study, plate accounted from 2 (Trial 14) to 73% (Trial 5) of the phenotypic variance, and explained on average 39% of the phenotypic variance across all trials. Ideally, plate would be fitted as a random effect into the model but it was not able to be included in these analyses due to the confounding of plate with family.

Our measurement of tonsil virus level contains both genomic and subgenomic RNA because the probes used in the TaqMan assays measure the amount of particular segments of RNA, rather than the whole strand of genomic RNA. This is not an issue when measuring serum viremia levels because PRRSV does not replicate in serum (Lawson et al., 1997). The tonsils, however, are an active site of PRRSV replication. Thus, only part of the RNA measured will be genomic RNA from fully formed PRRS virions, while the other part will
be from subgenomic RNA that is created during replication (Kappes and Faaberg, 2015). This is of importance because we are interested in the number of fully formed PRRS virions present in the tonsil and may have an impact on the conclusions of the analysis.

**Associations of tonsil virus levels with host response traits**

The estimated regression coefficients for TV on serum viremia traits provided evidence that there is a relationship between serum viremia and tonsil virus levels, although these relationships may be weak given the low $R^2$ values (Table 5.4A/B). The results suggest that a faster clearance of virus from serum results in a lower level of virus that localizes to the tonsil, most likely because a lower level of circulating virus in serum affords the virus less of an opportunity to localize to tissues in which it persists for extended periods of time. To our knowledge, this is the first time that such a relationship has been assessed during PRRSV infection. The relationship of TV with clearance of PRRSV from serum is of particular interest because these serum viremia characteristics explained the most variation in tonsil virus levels of all PRRSV response traits investigated when considering both isolates (Table 5.4A/B).

The identification of a relationship of high TV with low weight gain in the later weeks of the trial in pigs infected with NVSL suggests that, although persistently infected pigs are often asymptomatic (Benfield et al., 1998; Wills et al., 2003), low weight gain in the later stages of acute infection may be indicative of the likelihood of a pig becoming persistently infected with PRRSV. Unfortunately, weight gain late in infection did not explain a large proportion of the variance of TV (Table 5.4A).

A dynamic relationship has been observed between antibody response at 42 dpi and serum viremia levels throughout the course of infection (Hess et al., in preparation-b). The
relationship between S:P and TV was negative during infection with either NVSL or KS06; however, a significant association was only identified in KS06 infected animals. The stage of infection at 42 dpi may be different between NVSL infected pigs and KS06 infected pigs, because a greater proportion of NVSL infected pigs have already cleared serum viremia before 42 dpi, compared to KS06 infected pigs. Therefore, it would be beneficial to evaluate the relationship between antibody level and tonsil virus level at multiple time points during infection to gain a deeper understanding of the relationship between these two traits. In the conservative dataset of KS06, a positive association was identified between NAb and TV, but no association was identified in NVSL infected pigs. The positive association between neutralizing antibody and tonsil virus levels may be due to antibody dependent enhancement (ADE), a phenomenon in which virus-specific antibodies enhance the entry of virus, and in some cases the replication of virus, into monocytes/macrophages and granulocytic cells through interaction with Fc and/or complement receptors (Yang et al., 2000). ADE has been demonstrated in PRRSV, and has been shown to vary between virus isolates (Yoon et al., 1997). Neutralizing antibody titer was assessed using the inoculum. It is possible that the PRRS virions that are represented in the tonsil are immune escape variants, and the once neutralizing antibodies are aiding the current population of PRRSV in the tonsil. Therefore, in addition to differences due to the time that TV was collected in relation to its serum viremia curve of the measurement between, the viruses may differ in their ability to enhance viral replication in the tonsils via ADE. The negative (favorable) relationship observed between S:P and positive (unfavorable) relationship for NAb could represent different things. Non-neutralizing antibodies may be reducing viremia through the C1q complement mediated response
(Taylor et al., 2000), as discussed in further detail below, whereas NAb may be exacerbating virus levels due to ADE (Tirado and Yoon, 2003).

**Genome wide association studies**

The GWAS did not identify QTL with large effects and the WUR SNP that was found to be associated with serum viremia levels under infection with NVSL and KS06 isolates (Boddicker et al., 2012; Hess et al., in preparation-a) was not found to be associated with tonsil virus levels. Nevertheless, eight genomic regions explained at least 0.1% of the genetic variance in the GWAS for each isolate and combined in the conservative dataset. Three regions were identified in both the conservative and liberal datasets and these contained the genes HS3ST3B1, TCF7 and C1QA/B/C (Table 5.2A/B).

Genes that encode proteins that are responsible for modification of heparan sulfate, HPSE and HS3ST3B1, were located in regions identified in the GWAS (Table 5.2A). Heparan sulfate was the first identified mediator of infection (Delputte et al., 2002). Heparan sulfate is a proteoglycan, with covalently attached glycosaminoglycans (GAGs). Virions are thought to concentrate on the cell surface by attaching to GAGs, which stabilizes them and permits a more efficient infection of that cell (Delputte et al., 2005). Heparanase cleaves heparan sulfate proteoglycans, is expressed in immune-related tissues, and is upregulated in pigs infected with PRRSV (Tao et al., 2013). In vitro treatment of cells with HPSE has been shown to reduce PRRSV levels in infected porcine alveolar macrophages (Delputte et al., 2002). Heparanase is a hydrolase and cleaves between the saccharide subunits of the glycosaminoglycan chain. Heparanase activity is present in both endosomal and lysosomal compartments, but it can also occur at the cell surface, where it might digest adjacent HSPGs (Iozzo, 2001). HS3ST3B1 is involved in GAG sulfation of
the 3-OH position of glucosamine heparan sulfate, one of the final steps in biosynthesis of HS (Liu et al., 1999). Therefore, the ability of the animal to modify heparan sulfate may be an area of interest for future studies.

GALNT10 is another gene that is located in a region that was identified in the GWAS (Table 5.2A). The short cytoplasmic tails of all GalNAc-Ts contain basic residues that may interact with peripheral Golgi membrane protein tethering complexes (Smith and Lupashin, 2008). Upon entry into the cell, the PRRSV particles migrate to the vesicles of the endoplasmic reticulum and the Golgi apparatus (Thanawongnuwech et al., 1997), where they then rearrange host cell membranes to establish a viral replication complex. The latter plays a structural and/or functional role by offering a suitable microenvironment for viral RNA synthesis, or may facilitate the recruitment of membrane associated host cell proteins for the purpose of viral replication and transcription (Pedersen et al., 1999). Thus, GALNT10 may be involved in the establishment of the replication complex.

Other genes identified in the GWAS play a role in recruitment and activation of cells involved in the immune response related to T-cell responses. Such roles include transcriptional activation of T-cell differentiation (TCF7), recruitment of monocytes (which differentiate into macrophages) by chemotactic cytokines such as CCL1 (secreted by activated T-cells), CCL2, and CCL8, and T-cell migration into lymph nodes (CD34). The genes C1QA/B/C are also located in one of the eight regions identified in the GWAS. C1q is the first subcomponent of the classical pathway of activation of complement. Both immunoglobulin (IgG and IgM) and viruses are known to interact with C1q (Kishore and Reid, 2000), which has been identified as a mediator of the clearance of apoptotic cells by macrophages (Taylor et al., 2000). Therefore, the ability of the animal to recruit T-cells to
the site of infection and signal uninfected macrophages to the site of infection may play a crucial role in containing PRRSV infection in the tonsils by targeting infected cells for clearance by macrophages via C1q.

**Conclusions**

This study evaluated factors that could potentially be used to reduce PRRSV persistence in infected swine herds. Tonsil virus levels were estimated to be lowly heritable under infection with both NVSL and KS06 and no major QTL were identified for either PRRSV isolate. However, several genomic regions that explained a proportion of genetic variance and that harbored strong candidate genes were identified. Identified genomic regions included genes involved with the host’s ability to control viral infiltration/replication, as well as the ability to clear infected cells from tissue. These genes may be useful targets for future gene expression analyses on tonsil tissue in order insight into the host genetic control of tonsil virus levels and viral persistence. Clearance of viremia from serum and serum viremia levels at the end of the trial were phenotypically associated with tonsil virus levels. The results from the GWAS analyses suggest that TV levels are likely to be regulated by both the host’s ability to control viral infiltration/replication, as well as its ability to clear infected cells from tissue, which may be associated with serum viremia levels. This suggests that there may be a genetic correlation of tonsil virus level with Tmax, Vmax or vEND, in which case selection that reduces serum viremia late in infection may also decrease tonsil virus levels and persistence. Unfortunately, genetic correlations were unable to be calculated for these data due to convergence issues, which may be resolved by accounting for tonsil size or number
of macrophages present in the tonsil. In studies involving the same animals used in this study, heritability estimates of serum curve characteristics were moderate-high (Hess et al., in preparation-a), so if serum viremia clearance characteristics are genetically correlated with tonsil virus levels, selection on those serum viremia characteristics may decrease viral persistence in tonsils.

Acknowledgements

This project was funded by Genome Canada, USDA-NIFA grant 2013-68004-20362 and National Pork Board grants #12-061 and #14-223. We would like to acknowledge contributions from members of the PRRS Host Genetics Consortium.

References


Table 5.1: Estimates of heritability for tonsil viremia using the conservative and liberal datasets for two PRRSV isolates.

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Dataset</th>
<th>Heritability (s.e.)</th>
<th>Phen. St. Dev.</th>
</tr>
</thead>
<tbody>
<tr>
<td>NVSL</td>
<td>Conservative</td>
<td>0.05 (0.06)</td>
<td>1.03</td>
</tr>
<tr>
<td></td>
<td>Liberal</td>
<td>0.04 (0.06)</td>
<td>1.13</td>
</tr>
<tr>
<td>KS06</td>
<td>Conservative</td>
<td>0.11 (0.10)</td>
<td>0.89</td>
</tr>
<tr>
<td></td>
<td>Liberal</td>
<td>0.06 (0.08)</td>
<td>1.08</td>
</tr>
<tr>
<td>BOTH</td>
<td>Conservative</td>
<td>0.09 (0.06)</td>
<td>0.98</td>
</tr>
<tr>
<td></td>
<td>Liberal</td>
<td>0.08 (0.05)</td>
<td>1.11</td>
</tr>
</tbody>
</table>

Litter variance was zero for all analyses
Phenotypic Standard Deviation includes trial, pen, animal and residual standard deviations
Table 5.2: LS Means (s.e.; number of animals) of Tonsil Virus Levels by Isolate, Serum Viremia Status and WUR Genotype in the liberal and conservative datasets

<table>
<thead>
<tr>
<th>Trait</th>
<th>Level</th>
<th>NVSL</th>
<th>KS06</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CON</td>
<td>LIB</td>
<td>CON</td>
</tr>
<tr>
<td>Isolate</td>
<td>Unadjusted*</td>
<td>4.69</td>
<td>4.65</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(0.09; 480)</td>
<td>(0.10; 510)</td>
</tr>
<tr>
<td></td>
<td>Adjusted</td>
<td>3.38</td>
<td>3.37</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(0.07; 480)</td>
<td>(0.08; 510)</td>
</tr>
<tr>
<td>Serum Viremia</td>
<td>Cleared</td>
<td>4.45(^a)</td>
<td>4.44(^a)</td>
</tr>
<tr>
<td>Status</td>
<td></td>
<td>(0.09; 203)</td>
<td>(0.09; 210)</td>
</tr>
<tr>
<td></td>
<td>Rebound</td>
<td>4.62(^a,b)</td>
<td>4.55(^a,b)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(0.13; 71)</td>
<td>(0.14; 77)</td>
</tr>
<tr>
<td></td>
<td>Persistent</td>
<td>4.88(^b)</td>
<td>4.82(^b)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(0.09; 206)</td>
<td>(0.09; 223)</td>
</tr>
<tr>
<td>WUR Genotype</td>
<td>AA</td>
<td>4.63</td>
<td>4.61</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(0.09; 270)</td>
<td>(0.09; 288)</td>
</tr>
<tr>
<td></td>
<td>AB</td>
<td>4.71</td>
<td>4.66</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(0.10; 181)</td>
<td>(0.10; 191)</td>
</tr>
<tr>
<td></td>
<td>BB</td>
<td>4.65</td>
<td>4.46</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(0.21; 29)</td>
<td>(0.22; 31)</td>
</tr>
</tbody>
</table>

CON = Conservative Dataset; LIB = Liberal Dataset
Unadjusted: NVSL and KS06 values were analyzed as measured
Adjusted: NVSL values were adjusted to their Tetracore equivalent
Estimates within the same isolate and dataset that have different letters are significantly different from each other
* Estimates for NVSL and KS06 were significantly different from each other in both the liberal and conservative datasets
Table 5.3B: Associations of Tonsil Virus Levels with Serum Viremia Characteristics, Weight Gain and Antibody Levels in the Liberal Dataset for the NVSL and KS06 isolate data

<table>
<thead>
<tr>
<th>Statistic</th>
<th>TP</th>
<th>PV</th>
<th>Tmax</th>
<th>Vmax</th>
<th>VLTotal</th>
<th>vEND</th>
<th>W7</th>
<th>W14</th>
<th>W21</th>
<th>W28</th>
<th>W35</th>
<th>W42</th>
<th>WGEND</th>
<th>S:P</th>
<th>NAb</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>NVSL</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>302</td>
<td>302</td>
<td>302</td>
<td>302</td>
<td>302</td>
<td>302</td>
<td>302</td>
<td>302</td>
<td>302</td>
<td>302</td>
<td>302</td>
<td>302</td>
<td>302</td>
<td>302</td>
<td>302</td>
</tr>
<tr>
<td>Reg.Coeff.</td>
<td>0.043</td>
<td>-0.183</td>
<td>0.094</td>
<td>-3.244</td>
<td>0.013</td>
<td>0.164</td>
<td>0.151</td>
<td>0.185</td>
<td>0.174</td>
<td>0.108</td>
<td>0.065</td>
<td>-0.022</td>
<td>0.117</td>
<td>-0.09</td>
<td>-0.006</td>
</tr>
<tr>
<td>s.e.</td>
<td>0.038</td>
<td>0.14</td>
<td>0.021</td>
<td>0.64</td>
<td>0.003</td>
<td>0.036</td>
<td>0.087</td>
<td>0.123</td>
<td>0.137</td>
<td>0.121</td>
<td>0.102</td>
<td>0.128</td>
<td>0.055</td>
<td>0.31</td>
<td>0.038</td>
</tr>
<tr>
<td>P-value</td>
<td>0.26</td>
<td>0.19</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
<td>0.09</td>
<td>0.14</td>
<td>0.21</td>
<td>0.38</td>
<td>0.62</td>
<td>0.86</td>
<td>0.04</td>
<td>0.78</td>
<td>0.86</td>
</tr>
<tr>
<td>R²</td>
<td>0</td>
<td>0</td>
<td>0.05</td>
<td>0.05</td>
<td>0.06</td>
<td>0.04</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.01</td>
<td>0.00</td>
</tr>
<tr>
<td><strong>KS06</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>328</td>
<td>328</td>
<td>328</td>
<td>328</td>
<td>328</td>
<td>328</td>
<td>328</td>
<td>328</td>
<td>328</td>
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<td>328</td>
<td>328</td>
<td>328</td>
<td>156</td>
<td>151</td>
</tr>
<tr>
<td>Reg.Coeff.</td>
<td>0.075</td>
<td>0.109</td>
<td>0.051</td>
<td>-1.16</td>
<td>0.008</td>
<td>0.25</td>
<td>0.087</td>
<td>0.112</td>
<td>0.054</td>
<td>0.009</td>
<td>-0.01</td>
<td>-0.012</td>
<td>-1.519</td>
<td>0.058</td>
<td></td>
</tr>
<tr>
<td>s.e.</td>
<td>0.04</td>
<td>0.143</td>
<td>0.023</td>
<td>1.182</td>
<td>0.004</td>
<td>0.061</td>
<td>0.139</td>
<td>0.177</td>
<td>0.175</td>
<td>0.152</td>
<td>0.132</td>
<td>0.119</td>
<td>0.119</td>
<td>0.45</td>
<td>0.057</td>
</tr>
<tr>
<td>P-value</td>
<td>0.06</td>
<td>0.44</td>
<td>0.03</td>
<td>0.33</td>
<td>0.03</td>
<td>&lt;.001</td>
<td>0.53</td>
<td>0.52</td>
<td>0.75</td>
<td>0.95</td>
<td>0.95</td>
<td>0.91</td>
<td>0.001</td>
<td>0.32</td>
<td></td>
</tr>
<tr>
<td>R²</td>
<td>0.01</td>
<td>0</td>
<td>0.02</td>
<td>0.02</td>
<td>0.05</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.07</td>
<td>0.01</td>
<td></td>
</tr>
</tbody>
</table>

**Notes:**
- TP: Time to peak serum viremia (Days);
- PV: Peak serum viremia (Log_{10} serum viremia);
- Tmax: Time to maximal decay rate of serum viremia (Days);
- Vmax: Rate of maximal decay rate of serum viremia (Log_{10} serum viremia/Day);
- VLTotal: Total viral load (Log_{10} serum viremia*Day);
- vEND: measured serum viremia at the end of the trial (Log_{10} serum viremia); W7: Weight gain from 0 to 7 dpi (kg); W14: Weight gain from 7 to 14 dpi (kg); W21: Weight gain from 14 to 21 dpi (kg); W28: Weight gain from 21 to 28 dpi (kg); W35: Weight gain from 28 to 35 dpi (kg); W42: Weight gain from 35 to 42 dpi (kg); WGEND: Weight gain in the last week of the trial (kg); S:P: Virus N-protein specific IgG response at 42 dpi (S:P); Nab: Neutralizing antibody response at 42 dpi (log2 endpoint titer)
<table>
<thead>
<tr>
<th>GWAS Region (Chr_Mb)</th>
<th>Candidate Genes</th>
<th>Relative Location (Chr_Mb)</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>12_42</td>
<td>CCL2</td>
<td></td>
<td>CCL2: displays chemotactic activity for <strong>monocytes</strong> and basophils.</td>
</tr>
<tr>
<td></td>
<td>CCL1</td>
<td>12_42.6</td>
<td>CCL1: <strong>secreted by activated T cells</strong> and displays chemotactic activity for <strong>monocytes</strong>.</td>
</tr>
<tr>
<td></td>
<td>CCL8</td>
<td></td>
<td>CCL8: Chemotactic factor that attracts <strong>monocytes</strong>, lymphocytes, basophils and eosinophils; This protein can bind heparin</td>
</tr>
<tr>
<td>12_60</td>
<td>HS3ST3B1</td>
<td>12_61.0</td>
<td>Catalyzes the addition of sulfate groups at the 3-OH position of glucosamine in <strong>heparan sulfate</strong></td>
</tr>
<tr>
<td>16_75</td>
<td>GALNT10</td>
<td>16_74.6</td>
<td>Membrane-bound polypeptide that catalyzes the first step in mucin-type O-glycosylation of peptides in the <strong>Golgi apparatus</strong>.</td>
</tr>
<tr>
<td>2_141</td>
<td>TCF7</td>
<td>2_142.0</td>
<td>Transcriptional activator - <strong>T-cell lymphocyte differentiation</strong>.</td>
</tr>
<tr>
<td>6_74</td>
<td>C1QA/B/C</td>
<td>6_74.6</td>
<td>C1q associates with C1r and C1s in order to yield the first component of the <strong>serum complement system</strong>.</td>
</tr>
<tr>
<td>7_39</td>
<td>?</td>
<td>7_39</td>
<td></td>
</tr>
<tr>
<td>8_145</td>
<td>HPSE</td>
<td>8_144.3</td>
<td>Cleaves <strong>heparan sulfate</strong> proteoglycans to permit cell movement through remodeling of the extracellular matrix</td>
</tr>
<tr>
<td>9_147</td>
<td>G0S2</td>
<td>9_146.5</td>
<td>G0S2: Promotes <strong>apoptosis</strong> by binding to BCL2</td>
</tr>
<tr>
<td></td>
<td>CD34</td>
<td>9_148.4</td>
<td>CD34: important adhesion molecule and is required for <strong>T cells</strong> to enter lymph nodes; It also interacts with L-selectin, important in <strong>inflammation</strong></td>
</tr>
</tbody>
</table>

All windows explained between 0.10% and 0.14% of genetic variance for GWAS including NVSL infected animals, KS06 infected animals, or both in the liberal dataset
<table>
<thead>
<tr>
<th>GWAS Region (Chr_Mb)</th>
<th>Candidate Genes</th>
<th>Relative Location (Chr_Mb)</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>12_34</td>
<td>TRIM25</td>
<td>12_34.1</td>
<td>Involved in innate immune defense against viruses by mediating ubiquitination of DDX58. Mediates Lys-63-linked polyubiquitination of the DDX58 N-terminal CARD-like region which is crucial for triggering the cytosolic signal transduction that leads to the production of interferons in response to viral infection.</td>
</tr>
<tr>
<td>12_60</td>
<td>HS3ST3B1</td>
<td>12_61.0</td>
<td>Catalyzes the addition of sulfate groups at the 3-OH position of glucosamine in heparan sulfate</td>
</tr>
<tr>
<td>2_141</td>
<td>TCF7</td>
<td>2_142.0</td>
<td>Transcriptional activator - T-cell lymphocyte differentiation.</td>
</tr>
<tr>
<td>6_7</td>
<td>?</td>
<td>6.7</td>
<td>C1q associates with C1r and C1s in order to yield the first component of the serum complement system.</td>
</tr>
<tr>
<td>6_74</td>
<td>C1QA/B/C</td>
<td>6_74.6</td>
<td>All windows explained between 0.10% and 0.13% of genetic variance for GWAS including NVSL infected animals, KS06 infected animals, or both in the liberal dataset</td>
</tr>
</tbody>
</table>
Figure 5.1A: Manhattan plots for tonsil virus levels in pigs infected with NVSL or KS06 PRRSV isolates using the conservative dataset.
Figure 5.1B: Manhattan plots for tonsil virus levels in pigs infected with NVSL or KS06 PRRSV isolates using the liberal dataset.
Table S5.1: Variance component estimates with and without plate in the model

<table>
<thead>
<tr>
<th>Variable</th>
<th>NVSL With Plate</th>
<th>NVSL Without Plate</th>
<th>KS06 With Plate</th>
<th>KS06 Without Plate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Residual</td>
<td>0.49</td>
<td>0.91</td>
<td>0.59</td>
<td>0.61</td>
</tr>
<tr>
<td>Animal</td>
<td>0.01</td>
<td>0.05</td>
<td>0.07</td>
<td>0.09</td>
</tr>
<tr>
<td>Litter</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Pen</td>
<td>0.00</td>
<td>0.06</td>
<td>0.00</td>
<td>0.09</td>
</tr>
<tr>
<td>Plate</td>
<td>0.70</td>
<td>–</td>
<td>0.12</td>
<td>–</td>
</tr>
<tr>
<td>Phenotypic</td>
<td>1.20</td>
<td>1.02</td>
<td>0.78</td>
<td>0.79</td>
</tr>
<tr>
<td>$h^2$</td>
<td>0.01 (0.08)</td>
<td>0.05 (0.07)</td>
<td>0.09 (0.09)</td>
<td>0.11 (0.10)</td>
</tr>
</tbody>
</table>

Phenotypic variance includes all variables.
Figure S5.1: Concordance between tonsil virus levels obtained using the Applied Biosystems and Tetracore assays.

\[ y = 0.6753x + 0.2157 \]

\[ R^2 = 0.7651 \]
CHAPTER VI

POSSIBLE AVENUES FOR SELECTION OF PIGS WITH IMPROVED RESPONSE TO PRRS: LESSONS LEARNED FROM THE PHGC

Introduction

Breeding for improved disease resistance is justified if 1) the disease is of major importance; 2) current control strategies are not adequate, sustainable, or cost effective; and 3) available animals do not cope with the disease challenge (i.e. reduction in animal welfare is apparent) (Wiese, 2005). Porcine reproductive and respiratory syndrome (PRRS) has been prevalent in the global swine population for over 25 years (fao.org, 2015). This disease has serious consequences from both an economic and an animal welfare standpoint (Holtkamp et al., 2013). Despite serious efforts to combat PRRS, through both vaccination and biosecurity approaches, containment efforts have not been widely successful to date (Darwich et al., 2010). This is in large part due to the genetic and antigenic variability and persistent nature of the causal agent: PRRS virus (PRRSV) (Wills et al., 1997). Therefore, an attractive method to aid in containment of the disease is selection of pigs with improved response to PRRSV infection. The goal of the PRRS Host Genetics Consortium (PHGC) is to identify host genes or genomic regions that are associated with increased resistance or reduced susceptibility of pigs to PRRSV infection (Lunney et al., 2011). This chapter will review the current knowledge gained from analyses using the PHGC trials that included pigs infected with either NVSL-97-7895 (NVSL) or KS-2006-72109 (KS06), two
genetically distinct PRRSV isolates. The primary focus will be on the role host genetics plays in response to PRRSV infection in growing pigs, and provide insight into potential ways to implement this information into a breeding program to select for pigs with improved response to PRRSV infection.

Traits Investigated as Part of the PHGC

Serum Viremia

The initial PHGC studies (Boddicker et al., 2012; Boddicker et al., 2014a; Boddicker et al., 2014b) analyzed data from infection with PRRSV isolate NVSL-97-7985 and characterized serum viremia by fitting a LOESS curve to the serum viremia data collected during infection. Viral load (VL) was defined as the area under the LOESS curve from 0-21 dpi. This was an effective phenotype to measure overall response to PRRSV infection because it reduced the noise of single measurements of viremia and provided a summary phenotype for response to PRRSV infection. The heritability of VL was moderate to high, estimated to be 0.44 ± 0.13 by Boddicker et al. (2014b).

The Wood’s curve, developed to model lactation curves in dairy cattle (Wood, 1967), was shown to appropriately model PRRS serum viremia data during the course of infection and objectively identify pigs that experienced a rebound in serum viremia (Islam et al., 2013). In Chapter 3, the Wood’s curve was used to estimate different aspects of the serum viremia curve of individual animals, such as peak viremia (PV), time to peak viremia (TP), maximal rate of decay (Vmax), time to maximal decay rate (Tmax), as well as viral load (VL), defined as the area under the Wood’s curve from 0-21 dpi. Parsing out the different aspects of serum viremia kinetics is crucial for and gaining a deeper understanding
of the biological mechanisms of host response to PRRSV infection and identifying aspects that may be most suitable for selection. The genetic and phenotypic correlations between VL derived from fitting a LOESS curve and a Wood’s curve were very high and not significantly different from one (Genetic: 0.98 ± 0.03; Phenotypic: 0.90 ± 0.01). The fitting of the Wood’s curve to serum viremia measurements also allowed the interpolation of daily viremia levels.

Heritability estimates for VL fitting the Wood’s curve were estimated to be 0.31 ± 0.06 in animals infected with NVSL and 0.51 ± 0.09 for animals infected with KS06 (Chapter 3). These estimates differed slightly from those using the LOESS curve due to additionally fitting age and weight at infection, using the G matrix instead of the A matrix to model covariances between animals, and the addition of Trial 15 (Chapter 3). Heritability estimates for the curve characteristics were all moderate, ranging from 0.09 to 0.22 for NVSL infected animals and 0.16 to 0.45 for KS06 infected animals (Chapter 3). The heritability of daily fitted viremia levels changed throughout the course of infection, ranging from 0.12 to 0.54 in NVSL infected pigs and 0.19 to 0.47 in KS06 infected pigs (Chapter 4).

**Weight Gain**

Weights were collected weekly throughout the infection trial, from the day of infection (0 dpi) through to 42 dpi. Boddicker et al. (2012, 2014a, 2014b) analyzed weight gain from 0-21 dpi and 0-42 dpi, and found that these traits were moderate to highly heritable (0.30±0.11 and 0.30±0.18, respectively) and were estimated to have a high genetic correlation (0.99±0.06). In chapters 3 to 5, Legendre polynomials were fitted to the collected weekly weights for each animal to reduce the amount of noise involved with a
single measurement and, more importantly, get a fitted weight value for each individual throughout the course of the trial. Heritability of weight gain 0-42 dpi from fitting the Legendre polynomials was estimated to be $0.33 \pm 0.06$ during infection with NVSL and $0.31 \pm 0.09$ during infection with KS06. Modeling the dynamics of both serum viremia and weight allowed for the interpolation of daily viremia and weight values, providing a more in-depth analysis of how these response traits change throughout infection (Chapter 3). These daily measurements would be difficult to collect because they are labor intensive and daily serum viremia measurements would be very stressful for the pigs. The heritability of 3-day weight gain from the daily fitted weights changed throughout the course of infection, ranging from 0.11 to 0.42 in NVSL infected pigs and 0.17 to 0.40 in KS06 infected pigs (Chapter 4).

**Antibody Response**

Total antibody response to PRRSV (S:P) (i.e. antibody levels that include both non-neutralizing and neutralizing antibodies) is a useful indicator of a pig’s ability to cope with disease because it is a measurement of the pig’s ability to mount a strong adaptive immune response. In PHGC Trials 1-5 and 10-12, S:P was measured at 42 dpi using Fluorescent Microsphere Immunoassay (FMIA) (Christopher-Hennings et al., 2013) This dpi was chosen because it is when antibody levels are expected to be maintained at high levels. S:P was moderately heritable in both NVSL infected ($0.31\pm0.09$) and KS06 infected ($0.40\pm0.10$) pigs. These were similar to heritability estimates obtained from a sow herd that experienced a PRRSV outbreak (Serao et al., 2014).

Despite moderate to high heritability estimates for PRRSV specific total antibody response, heritability estimates for PRRSV neutralizing antibody response (nAb) using
animals included in this dissertation (NVSL: trials 1-7 (n=949); KS06: trials 10-12 (n=349) were low (NVSL: 0.03±0.04; KS06: 0.14±0.10). This may have been a consequence of the assay used, which was a serum neutralization assay, with measurements reported as log2(endpoint titer of neutralizing antibody without cytopathic effects) (Trible et al., 2015). Resulting discrete values ranged from 0-8, and were approximately normally distributed. However, the errors associated with the assay are likely to be larger at higher titers than at lower titers because the dilutions used in the assay are multiplicative. Neutralizing antibodies are considered to be an important indicator of protective immunity against PRRSV (Lopez and Osorio, 2004) and neutralizing antibody levels have been found to be heritable for other swine diseases using a similar assay (Rothschild et al., 1984a; Rothschild et al., 1984b; Meeker et al., 1987). A more sensitive assay may increase heritability estimates.

**Tonsil Virus Level**

The persistent nature of PRRSV means that we are not only interested in reducing serum viremia but also the level of virus that resides in the tissue, as PRRSV can persist in tissues such as the tonsils and lymph nodes for several months (Allende et al., 2000). Ideally, host response to infection, measured by serum viremia, would be genetically correlated with the virus levels in tissue. Tonsil virus level was measured at 42 dpi for PHGC trials 3, 5, 7, 11 and 14 and was found to be lowly heritable in both NVSL infected (0.05±0.06) and KS06 infected (0.11±0.10) animals (Chapter 5).

According to Bishop and Woolliams (2010), the ability to parse the heritable genetic component from the environmental component depends on the level of noise in the system that the trait is being measured. While this noise may have been controlled in the
macro environment in these trials, it is possible that it is not controlled for in the internal environment of the animal (i.e. that of the tonsil). Replication occurs in macrophages (Duan et al., 1997) and the tonsil is an active site of PRRSV replication (Rowland et al., 2003). Thus, the measurement of PRRSV copies in the tonsil can be influenced by tonsil size and the number of macrophages in the tonsil. Accounting for these factors may increase heritability estimates.

**Cytokines**

Cytokines are small glycoproteins that regulate immunity, inflammation and hematopoiesis. They play a crucial role in innate and adaptive response to infectious disease (Lau, 1994; Khan, 2008). A particular type of cytokine, called chemokines, play a crucial role in cell migration, specifically recruitment of immune cells to a site of infection or injury (Luster, 1998). Cytokines provide a link between the innate and adaptive immune system and mounting a robust innate immune response can influence the success of the adaptive immune response (Luster, 2002). As such, cytokines have been considered as strong candidates for adjuvants in vaccine development (Lin et al., 1995). The use of chemokines IL-8 and CCL2 with vaccines have been shown to enhance adaptive immune response to HIV-1 (Kim et al., 1998). Type I IFN (IFNα/β) administered with an influenza virus vaccine enhanced antibody response (Bracci et al., 2005). Serum cytokine levels, either pre-infection or during infection, may serve as useful biomarkers for response to PRRSV infection.

In the PHGC trials evaluated in this thesis, 229 pigs infected with NVSL from trials 3 (n=35), 5 (n=77), and 7 (n=117) were chosen for cytokine analysis based on extreme (>1 standard deviation from population mean) levels of viral load and total weight gain and
based on WUR genotype. Serum cytokine levels (CCL2, IL-8, IFNα, IL-12, IL-10, IL-4, and IL-1β) were measured using FMIA at the time points for which serum was available, which were reported as Log10(pg/ml) (Choi et al., 2013). These cytokines were chosen to represent early innate immune response (IL-1β, IL-8, IFNα), T helper 1 immune response (IL-12), T helper 2 immune response (IL-4), a cell migration chemokine (CCL2) and regulatory immune response (IL-10).

Days 0 and 7 post infection were chosen to assess whether serum cytokine levels can be used to predict host response to PRRSV infection (Day 0), or be used as an additional measurement for improved response to PRRSV infection (Day 7). Cytokine levels at day zero are indicative of pre-infection cytokine levels and if cytokine levels at day 0 have a high genetic correlation with VL or WG, then selection can occur in the absence of infection, and thus phenotypes could be collected on the selection candidates themselves (i.e. in the nucleus). Day 7 was chosen because this is the average day that pigs reach peak serum viremia (Chapter 3) and if pigs are selected based on their serum viremia level at average peak, cytokine levels could also be measured based on the same serum sample.

Of these cytokines, CCL2 and IL-8 had normally distributed phenotypes at day zero, and showed a response that changed over the course of PRRSV infection (Figure 6.1). Additionally, IFNα changed in response to infection (Figure 6.1) but did not show substantial levels of variation at 0 dpi. Heritabilities were high for IL-8, CCL2 and IFNα levels (Table 6.1).
Figure 6.1: Raw means of CCL2 (Black), IFNα (Red) and IL-8 (Green) Serum Cytokine Levels 0-35 days post infection

Table 6.1: Parameter Estimates (se) for Cytokine Levels in 229 NVSL Infected Pigs

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Days Post Infection</th>
<th>$h^2$</th>
<th>Phenotypic St. Dev.</th>
<th>Genetic Correlation between Days</th>
<th>Correlation with VL</th>
<th>Correlation with WG</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-8 (pg/ml)</td>
<td>0</td>
<td>0.33 (0.17)</td>
<td>0.31</td>
<td>-0.13 (0.47)</td>
<td>0.05 (0.32)</td>
<td>0.61 (0.52)</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>0.38 (0.17)</td>
<td>0.37</td>
<td>0.73 (0.27)</td>
<td>0.56 (0.45)</td>
<td></td>
</tr>
<tr>
<td>CCL2 (pg/ml)</td>
<td>0</td>
<td>0.44 (0.16)</td>
<td>0.46</td>
<td>0.72 (0.17)</td>
<td>0.36 (0.23)</td>
<td>0.39 (0.39)</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>0.65 (0.15)</td>
<td>0.48</td>
<td>NA</td>
<td>0.42 (0.21)</td>
<td>0.60 (0.26)</td>
</tr>
<tr>
<td>IFNα (pg/ml)</td>
<td>7</td>
<td>0.41 (0.15)</td>
<td>0.47</td>
<td>NA</td>
<td>0.46 (0.24)</td>
<td>-0.44 (0.36)</td>
</tr>
</tbody>
</table>

Numbers in bold are significantly different from zero.

Comparisons between PRRSV Isolates

Whether selection for improved response to PRRSV infection will be effective across different isolates is an important question due to the high level of genetic variability observed between isolates (Pirzadeh et al., 1998). The studies from the PHGC have
currently focused on two genetically distinct isolates of PRRSV: NVSL and KS06 to
come back the different aspects of host response to PRRSV. These PRRSV isolates were
isolated ~10 years apart and had an 11% difference in GP5 nucleotide sequence (Ladinig
et al., 2015), which is similar to the average level of divergence in GP5 that is found in
North American PRRSV isolates (Shi et al., 2010). Therefore, similarities in host response
to these two virus isolates may be indicative of host response to North American PRRSV
variants.

When analyzing VL, measured as area under the Wood’s curve from 0-21 dpi, it
was found that pigs infected with NVSL had 16±2% higher VL than pigs infected with
KS06. The Wood’s curve characteristics revealed that these VL differences were driven by
differences in the shape of the viremia curve under infection with NVSL and KS06. Pigs
infected with NVSL had 14±2% higher PV and reached PV 2.5±0.6 days earlier than pigs
infected with KS06. Maximal decay rate was reached 3.9±0.7 days earlier and was 36±14%

t faster under infection with NVSL than KS06 (Chapter 3). The profile for KS06 was
consistent with what would be expected from a less virulent isolate of PRRSV (Beyer et
al., 2000), which was further supported by the 3.7±1.5 kg higher weight gain that was
observed for the KS06 infected pigs (Chapter 3).

The estimated genetic correlations of response traits between isolates were greater
than 0.6 for all traits except TP, for which had a genetic correlation estimate of 0.25
between isolates (Chapter 3). The genetic correlation could not be estimated for TV
(Chapter 5). Although they had large standard errors, these high genetic correlations
suggest that the genetic mechanism utilized by the host is similar between different isolates
of PRRSV. Specifically, VL, WG and PV can each be considered the same trait under
infection with either the NVSL or the KS06 PRRSV isolate (Chapter 3). Thus, selection on any of these traits under infection with one PRRSV variant is likely to improve response to infection with another PRRSV variant.

**Genetic Correlations between Traits**

In the studies by Boddicker et al. (2012; 2014a; 2014b) serum viral load and weight gain were found to be antagonistically related, both phenotypically and genetically in pigs infected with NVSL. A number of genetic correlations between response traits within isolate were consistent across isolates, and all of these genetic correlations were in the favorable direction (Chapter 3), e.g. a lower VL was genetically correlated with higher WG (NVSL: -0.74±0.10; KS06: 0.52±0.17), indicating that animals with lower overall serum viremia levels gain more weight during PRRSV infection. The strong positive genetic correlation between VL and PV that was observed for both isolates (NVSL: rg=0.85±0.07; KS06: rg=0.91±0.05) suggests that host genetic response to infection is largely driven by the ability of the animal to respond to the early stages of infection. As such, higher WG and lower VL were genetically correlated with lower PV. Viremia curve characteristics were found to have favorable genetic correlations with WG, whereby animals with higher WG have lower overall viral load, lower and sooner peak viremia as well as sooner and faster serum viremia clearance (Chapter 3). These favorable relationships are encouraging for the potential of genetic selection for improved response to PRRSV infection because they indicate that selection on one response trait is likely to improve other response traits i.e. selection for increased WG under PRRSV infection is likely to decrease serum viremia/serum viral load.
The genetic correlations between serum viremia and weight traits likely capture the aspects of host resistance to PRRSV because it represents the extent to which the genetic variation in WG that is in common with the host’s ability to control serum viremia levels (a resistance trait). Weight gain is typically considered a measurement of tolerance to infection and tolerance measurements have been found to be negatively correlated with resistance measurements (Raberg et al., 2007). As measured in the PHGC trials, WG is a measure of resilience and is comprised of both tolerance and resistance aspects, because it is based on the ability of the pig to clear the virus (resistance), as well as its ability to grow despite being infected (tolerance). Therefore, selecting for improved weight gain during infection would have a substantial impact on selecting pigs that are able to decrease viral burden (\(rg(VL,WG): NVSL = -0.74\pm0.10; KS06 = -0.52\pm0.17\)) and/or perform well under a given viral burden (not tested in this study).

Although tonsil virus was found to have a low heritability and genetic correlations were not able to be estimated due to convergence issues, lower total viral load and faster virus clearance was phenotypically associated with lower tonsil viremia (Chapter 5). Selecting for reduced serum viremia, particularly during the later stages of infection, may thus have a favorable impact on tonsil virus levels and PRRSV persistence because reducing the amount of circulating virus may reduce the amount of PRRSV that localizes to and infects the tissues where PRRSV persists. Selection for reduced serum viremia would be successful at reducing tonsil virus levels if these two traits were also found to be positively correlated at the genetic level.

Antibody response, viremia, and weight gain following infection are all complex processes. Complex relationships were observed when genetic correlations between
response traits were estimated across different time points, which revealed different relationships between S:P, viremia and weight gain pre-peak viremia versus post-peak viremia (Chapters 3 and 4). Thus, it’s important to consider these changing relationships when considering which traits to select upon to optimize response to selection for improved resistance to PRRS.

The genetic correlation of CCL2 levels at 0 dpi with CCL2 levels at 7 dpi were strongly positive, while the genetic correlation of IL-8 levels at 0 dpi with IL-8 levels at 7 dpi was slightly negative genetic correlation and was not significantly different from zero (Table 6.1). IL-8 levels at 7 dpi had a high genetic correlation with VL and a moderate genetic correlation with WG, although this was not significantly different from zero (Table 6.1). CCL2 levels at 7 dpi were moderately correlated with both VL and WG (Table 6.1). Interestingly, these genetic correlations of IL-8 and CCL2 with VL and WG were all positive, so any selection to decrease IL-8/CCL2 is likely to decrease VL but also decrease WG during infection. IFNα had moderate genetic correlations with both VL and WG, although not significantly different from zero (Table 6.1). However, selection to decrease IFNα is likely to decrease VL and increase WG during infection.

Genomic Regions Associated with Response to PRRSV Infection

Genome Wide Association Studies (GWAS) scan the genome to find regions that are associated with the trait of interest. These regions can then be explored to identify the causal mutation that affects these traits. Several GWAS have been conducted as part of the PHGC, identifying a number of genomic regions associated with response to PRRSV
infection that could potentially assist with successful selection for pigs that have improved response to PRRSV infection in the future.

**WUR10000125**

A genetic marker on chromosome 4, WUR10000125 (WUR), explained 12% of the genetic variation in VL and 9% for WG, with animals carrying the favorable B allele having lower viral load and higher weight gain under PRRSV infection with NVSL-97-7895 (Boddicker et al., 2012). This SNP was associated with VL and WG in all evaluated breeds, which comprise the majority of the breeding stock in North America, however the B allele was also at a low frequency in all breeds. A putative quantitative trait nucleotide was identified in GBP5 (Koltes et al., 2015), a gene involved in the formation of the inflammasome via NLRP3 (Shenoy et al., 2012). The marker WUR was found to be in complete LD with the causal mutation (Boddicker et al., 2014a; Koltes et al., 2015). Therefore, Marker Assisted Selection (MAS) instead of genomic or phenotypic selection has the advantage that genetic selection can occur without the collection of phenotypes, such as for the SSC4 QTL.

During infection with either NVSL or KS06, animals with the AB genotype at WUR had a lower peak viremia than their AA counterparts (Chapter 3). The association between PV and WUR genotype is consistent with the role of GBP5 in the innate immune response, which plays an important role early in infection. GBP5 is involved in the formation of the inflammasome via NLRP3 (Shenoy et al., 2012), and animals with the AA genotype do not produce functional GBP5 (Koltes et al., 2015). As a consequence, these animals have higher serum viremia levels, compared to animals with the AB or BB genotype (Boddicker et al., 2012). Of the curve characteristics, PV was the only trait with
which WUR genotype had a consistent and highly significant association across the two PRRSV isolates. This information may be of particular importance for implementation of selection for improved PRRSV resistance because understanding the mechanisms by which GBP5 acts and the time during it has the strongest effect gives us a better understanding of host response to infection and allows us to delve deeper into the inflammasome pathway that GBP5 is involved in and potentially identify other useful targets of selection of pigs for improved response to PRRSV infection.

While this SSC4 QTN is likely to be successful at improving host response to PRRSV infection, more research needs to be done on the other potential effects of this. For example, it is intriguing that the favorable B allele is in such a low frequency in the North American pig population, indicating that it may be negatively associated with traits in the current selection index. However, a recent study by Niu et al. (2015) suggested that animals with the AB genotype had better performance in terms of growth and meat quality than animals with the AA genotype.

In a recent GWAS, WUR explained 14.5% and 7.5% of the genetic variance for VL in NVSL and KS06 infected pigs, respectively. This marker also explained 10.5% of the genetic variance for WG in NVSL infected pigs, but was not identified as a large QTL in KS06 (0.5%) infected pigs (Waide, 2015). Consistent with these findings, in Chapter 3 pigs with the AA genotype had significantly lower WG for NVSL (-2.0±0.2), but not for KS06. The lack of an association between WUR and WG for KS06 may be related to the differences in virulence of the two isolates, whereby the impact of WUR on WG is only observed in more virulent isolates. In other organisms, primarily plants, isolate-specific QTL for disease resistance have also been identified (Leonardsschippers et al., 1994; Liu
et al., 2011). The genetic correlation between isolates for all traits were similar when accounting or not accounting for WUR (Chapter 3), suggesting that a large proportion of the host genetic response to PRRSV infection that is in common between isolates is polygenic. MAS will only improve host response to PRRSV infection while there is variation at this locus. Genomic selection may be a favorable alternative or concurrent method of selection for pigs with increased resistance to PRRS due to the large polygenic component that is common between isolates.

**Antibody Response**

GWAS identified strong associations of the MHC region with PRRSV N protein specific IgG response at 42 dpi (Chapter 4). The MHC contains a cluster of immune-related genes, mostly involved in the adaptive immune response (Flajnik and Kasahara, 2001). The strongest peak was located in the MHC class II region, a region that contains genes involved in the humoral immune response and, thus, antibody production. Interestingly, despite the strong genetic correlations of antibody response with both serum viremia and weight gain, the SNPs in the MHC that were identified to be associated with antibody response were not associated with serum viremia or weight gain. This suggests that the component that is in common between these traits is polygenic, and outside the MHC. This is consistent with what Serao (unpublished) has found for the genetic correlation between antibody response and reproductive performance in the sow during a PRRSV outbreak. When considering the use of antibody response, it is preferable to avoid specifically selecting on the MHC, as this may have undesirable consequences for immune response, such as increasing the susceptibility to another disease (Rauw, 2012). Selecting on the polygenic portion, which excludes the MHC, is expected to lower serum viremia and result
in higher weight gain after recovery from the initial exposure, while avoiding those potential undesired outcomes. Selective breeding for resistance to immunosuppressive diseases would reduce the prevalence of these diseases and enhance overall immune responsiveness (Stear et al., 2001). Given that PRRS is considered an immunosuppressive disease, it may be beneficial in the context of other diseases to select for increased antibody response to PRRS, although this remains to be explored.

**Tonsil Virus Level**

Genomic analysis of tonsil virus levels was able to identify genomic regions that, although explained only a small percentage of the genetic variance, may be biologically relevant (Chapter 5). Biological processes of genes that were in regions that were identified as being associated with tonsil virus levels included heparin sulfate processing, T-cell activation/signaling, the complement protein C1q, a cluster of CCL chemokines, and modification of the Golgi apparatus. Identification of processes such as these may help to identify causal mutations in the future, particularly if the tonsil virus level phenotype can be adjusted to account for the microenvironment.

**PRRS Response Phenotype Collection**

Recording phenotypes at the commercial production level is the most feasible approach for making selection decisions for improved disease resistance in pigs due to the requirement to keep the nucleus and multiplier stages of production disease free (Rajic et al., 2001). This method has the additional benefit of resulting in estimation of breeding values for disease response in nucleus animals that directly relate to the commercial level, where PRRS is most prevalent. An issue that then arises is linking the animals with
phenotypes to the selection candidates. Utilizing pedigree information alone may not be the most desirable approach, due to the large likelihood of missing information or mistakes in the pedigree. Using genotype information can alleviate these problems, as the information obtained by genotyping does not rely on accurate pedigree information (Meuwissen et al., 2001). Because it is not feasible to collect genotypes on all individuals, it may be more desirable to genotype a subset of animals and rely on pedigree information to link the other phenotyped individuals to the individuals with both phenotypes and genotypes. These can be combined with genotypes on the selection candidates to make selection decisions using single step genomic prediction methodologies (Chen et al., 2011).

Collecting phenotypes for selection using infection trials would be a powerful but very expensive way to obtain phenotypes to select for increased PRRS resistance (Rajic et al., 2001). Recording phenotypes during a PRRS outbreak in a commercial herd would overcome some of these costs, however the lack of control over infection timing, dosage and isolate will increase the noise of the phenotypes, and will make selection decisions difficult (Bishop and Woolliams, 2010). In addition, differences in management practices between herds would result in unequal representation of progeny of selection candidates in making selection decisions, as PRRS is more prevalent in some farms/herds than others (Kwong et al., 2013). Response to vaccination provides an attractive alternative to natural infection, because all animals can be vaccinated at the same dose/age/time and phenotypes can be collected with higher consistency. Measuring PRRS resistance based on response to vaccination may have the added benefit of reducing the circulating level of PRRSV during natural infection, as the vaccine provides partial protection to PRRSV exposure (Osorio et al., 1998).
Possible PRRS Response Traits for Selection

When deciding to select pigs that respond better to a disease, it is important to understand what mechanisms are involved in that trait response. Often used terms to describe an animal’s ability to respond to disease are resistance, tolerance, and resilience. Resistance is defined as the ability of the host to influence the life cycle of a pathogen. This can be achieved by inhibiting the pathogen’s ability to enter the host cell or to replicate once it has entered the host cell (Bishop, 2012). Therefore, there is likely an innate aspect to resistance as well as an adaptive component (Rauw, 2012). In the context of PRRS, peak viremia during an infection may capture innate immune aspects of resistance, whereas rate of clearance may capture the adaptive response. These responses can either come at a cost to animal productivity or be a benefit to animal productivity, depending upon which is greater: the cost of being resistant or the benefits of being resistant (Bishop, 2012; Rauw, 2012). In the context of PRRSV infection, it appears that the cost of being resistant is outweighed by its benefits, as evidenced by the negative genetic correlations observed between serum viremia traits and weight gain during infection. Resilience is defined as the level of productivity of an animal in the face of infection (Bishop, 2012). Weight gain during infection is an example of a resilience trait, however, weight gain during infection is likely a composite trait that includes aspects of the animal’s genetic ability to perform under normal conditions, as well as resistance and tolerance. As discussed by Bishop (2012), this is further complicated by the fact that covariances exist between these component traits of resilience, as evidenced by the relationship between viremia curve characteristics and weight gain. Comparing the performance of an animal in an uninfected setting to its performance at a given level of infection would provide insight into the host’s
ability to tolerate infection, whereby an animal that is less impacted by infection is more tolerant to the effects of infection. Thus, tolerance is defined as the ability to limit the amount of damage that is caused either by the pathogen or the host’s response to fight the pathogen (Bishop, 2012; Rauw, 2012). The most effective selection program for improved response to PRRSV infection will likely include traits that together cover resistance, tolerance and resilience.

**Serum Viremia Traits**

This dissertation has identified phenotypes that may be useful to select for increased PRRS resistance. Although viral load has a high heritability, indicating that selection for viral load is likely to be successful in selecting more resistant pigs, a major disadvantage of using VL as a phenotype for selection is that it is necessary to collect serum viremia at multiple time points in order to estimate VL. This is not a practical approach in the swine industry, and a single time point that captures the dynamics of infection would be most desirable, as this would be less costly to measure and less laborious to collect. PV is a trait that can be measured at a single time point and was highly correlated with VL for both NVSL and KS06 infected pigs. The limitation of this measurement is that PV is reached at a different time point in different animals, however there is generally a small window of time in which peak viremia occurs, so choosing a time point that would approximate peak viremia, such as average time to peak, may overcome this limitation.

In Chapter 3, we hypothesized that, since VL is largely driven by PV, the time point of raw viremia at the average peak for that isolate would have the highest genetic correlation with VL. In pigs infected with NVSL, peak viremia was estimated to be reached
at 7.0±0.4 dpi, whereas pigs infected with KS06 reached peak viremia at 9.5±0.4 dpi. During the first two

Table 6.2: Heritabilities ($h^2$), litter components ($c^2$) and genetic correlations ($r_g$) between raw viremia values and viral load

<table>
<thead>
<tr>
<th>Isolate</th>
<th>viremia4 (s.e.)</th>
<th>viremia7 (s.e.)</th>
<th>viremia11 (s.e.)</th>
<th>viremia14 (s.e.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NVSL</td>
<td>0.33 (0.06)</td>
<td>0.15 (0.05)</td>
<td>0.26 (0.05)</td>
<td><strong>0.36</strong> (0.05)</td>
</tr>
<tr>
<td>KS06</td>
<td>0.37 (0.09)</td>
<td><strong>0.53</strong> (0.08)</td>
<td>0.39 (0.09)</td>
<td>0.28 (0.09)</td>
</tr>
<tr>
<td>KS06</td>
<td>0.03 (0.04)</td>
<td>0.00 (0.02)</td>
<td>0.03 (0.04)</td>
<td><strong>0.10</strong> (0.04)</td>
</tr>
</tbody>
</table>

**Bold** indicates the time point that was most genetically correlated with VL.

weeks of infection, serum viremia was collected at 4, 7, 11 and 14 dpi, it was expected that the highest genetic correlation with VL would be observed at 7 dpi in NVSL infected animals and at 11 dpi in KS06 infected animals. The model used for bivariate analyses in Chapter 3 were used in this analysis. The time points that most closely approximated peak viremia had high genetic correlations with VL (Table 6.2), indicating that selection for decreased viremia at the average peak for an isolate is expected to reduce viral load. There was a high heritability and high genetic correlation between VL and viremia collected at 7 and 11 dpi in KS06, indicating viremia measured at average timing of peak viremia is likely to result in reduced VL under infection with KS06 (Table 6.2). This pattern was not observed with NVSL, where viremia at 14 dpi (closer to the timing of maximal rate of viremia decay) had a higher heritability and genetic correlation with viral load than viremia at 7 dpi (average time to peak viremia), although the litter component was much higher for
viremia at 7 dpi than 14 dpi (Table 6.2). Viremia at 14 dpi may represent a combination of both peak viremia and maximal decay rate.

**Antibody response**

Traits related to the adaptive immune response may also be useful for selection for improved disease resistance. Chapter 4 showed the strong negative correlation of antibody level at 42 dpi with serum viremia levels between 14 and 21 dpi, around the time of maximal viral clearance from serum. There was a dynamic relationship between antibody response and growth throughout infection. Genetically, individuals that had higher antibody levels at 42 dpi had lower growth early on but higher growth later in infection (Chapter 4). Considering the genetic correlations of S:P with weight gain and viremia together, animals that are genetically superior in their antibody response at 42 dpi (i.e. have higher S:P at 42 dpi) are more desirable, because they will also have higher weight gain in the later part of infection and have lower serum viremia. Serao et al. (2014) previously characterized antibody response in an outbreak herd and found that antibody response was moderately heritable and had high and favorable genetic correlations with sow reproductive performance during an outbreak. These findings suggest that measurement of PRRSV specific total antibody level is indicative of both increased resistance (viremia) and increased resilience (growth and reproductive success). Selection on antibody response to PRRSV is feasible is because the commercially available diagnostic test for PRRSV infection measures antibody response, which means the capability to measure antibody response during infection is already established at the commercial pig production level.

Therefore, further investigation into the heritability of PRRSV neutralizing antibody levels and genetic correlations of neutralizing antibody levels with other PRRS
response traits may reveal that selection for PRRSV neutralizing antibody levels will lead to pigs that respond better to PRRSV infection. Recently, an infectious clone of PRRSV that expresses green fluorescent protein was created (Wang et al., 2014). This may be used in future studies to obtain a more accurate measure of neutralizing antibody levels that is more continuous through measurement of the level of fluorescence. For example, the difference in or the proportion of fluorescence between a well containing just virus and containing virus and serum and virus after some incubation period could be used as a measurement of neutralizing antibody response.

**Weight gain**

Selection for increased weight gain during PRRSV infection is another important trait to consider. Recording weight gain after vaccination would be relatively easily implemented in the industry, as the equipment is already available to measure such a phenotype. Weight gain under PRRSV infection has been shown to be genetically correlated with serum viremia characteristics, such as VL, PV and Vmax (Chapter 3), suggesting that selection for improved weight gain under PRRSV infection will likely reduce serum viremia levels. The goal of using a vaccine is to provide protective immunity while minimizing the costs of establishing that protective immunity. Thus, PRRS vaccination may lead to minimal loss in weight gain. However, there is still expected to be genetic variation between individuals for weight gain after vaccination and further studies will be required to establish the potential of selecting for weight gain after vaccination, including the extent to which it genetically correlated with weight gain during infection with PRRSV and the optimal time frame over which weight gain should be collected.
Weight gain during PRRSV infection is likely to be of great concern to the producer because they can directly observe the economic benefits of improvement in this trait.

**Cytokines**

The results from the Cytokine analyses show the potential of cytokines as a genetic indicator of response to PRRSV infection. An analysis that evaluated a greater number of animals may provide more reliable estimates with lower standard errors. Selection on IL-8 or CCL2 levels prior to infection may result in lower viral load during PRRSV infection but are also unfavorably correlated with WG. The IFNα results suggest that, genetically, lower levels are indicative of a more desirable response to PRRSV infection (lower VL and higher WG). IFNα plays a vital role in regulating immune response to viral infection (Biron, 1998), thus, selection for reduced IFNα is not recommended as it could decrease the pig’s ability to respond to other diseases, although studying IFNα response may help to better understand the genetic mechanisms that are used to respond to PRRSV infection.

**Index Selection for PRRS Resistance**

Phenotypes on PV, WG, and S:P could be combined with WUR genotype to create a PRRSV health index. The concept of index selection is to select animals based on their overall genetic merit for a variety of traits simultaneously (Hazel, 1943). Selection for WUR genotype in an index rather than directly through MAS would lead to lower selection intensity on WUR, due to the performance of the individual for the other PRRS response traits being considered. This may be beneficial because it is currently unknown why the frequency of the favorable allele is so low in every breed cross that was evaluated in the
PHGC, which indicates there may be undesirable consequences from selecting directly on WUR.

Selection on an index, rather than for only one of the aforementioned traits, may improve our ability to manipulate the shape of multiple aspects of the serum viremia curve. Selection for lower peak viremia will reduce early viremia levels; while selection on the combination of S:P and WG may increase maximal decay rate and lower post-peak viremia. Decreasing serum viremia at all stages of infection through the index rather than direct selection on VL overcomes the issues associated with collecting numerous serum samples throughout infection in order to calculate VL.

**Future Directions**

**Antibody Response at Multiple Time Points**

The moderate genetic correlation estimate between antibody response to NVSL versus KS06 suggests that, although current vaccines are not cross-protective, selection on antibody response to the vaccine could lead to improved host response to infection with heterologous isolates of PRRSV. Further studies are, however, needed to estimate the genetic correlation between response following vaccination and response following natural PRRSV infection. Important phenotypes that may improve our understanding of response to PRRSV infection are the time it takes for PRRSV-specific antibodies (of different isotypes, including both neutralizing and non-neutralizing) to be detected in serum, rate of antibody production and the timing and level of the plateau of antibody level. In terms of phenotypes for selection, it would be ideal to identify a time point for serum collection that could be used for measurement of both serum viremia and antibody response to reduce the
number of times serum samples need to be collected. This could either be done with total antibody response or neutralizing antibody response, if neutralizing antibody response is found to be heritable and genetically correlated with serum viremia levels. If antibody response to the vaccine is under similar host genetic control to antibody response to distinct PRRSV isolates, it may be possible to select animals with increased resistance or reduced susceptibility to PRRS based on antibody response to the PRRS vaccine. Alternatively, it may be possible to select for resistance to several diseases by selecting for enhanced immune responsiveness (Wilkie and Mallard, 1999; Stear et al., 2001). This would reduce the need to collect phenotypes on infected or vaccinated animals and could occur in the nucleus herd. The efficacy of this approach in the context of PRRS and other diseases, however, would have to be assessed. Further analyses must, however, be conducted to confirm the desirability of S:P as an indicator trait for improved response to PRRS. It is important to assess the desirable, or optimal, level of antibody response that would increase resistance or decrease susceptibility to PRRSV, while not negatively impacting the ability of the pig to combat other infections.

**PRRSV Variants**

Identifying approaches of containment that are effective across different PRRSV variants is of major concern to the swine industry. The results reported in this dissertation provide promising results in regard to the potential of genetic selection that can be effective across different PRRSV variants. However, the effectiveness of genetic selection across additional PRRSV variants needs to be assessed to confirm these results. Keeping in mind that PRRS is a global swine disease, it would be useful to explore a suite of PRRSV isolates that are representative of different geographical regions. There are two major genotypes of
PRRSV with substantial differences in both their genome and pathology: North American and European (Wensvoort et al., 1992). Both of the isolates used in the studies of this dissertation were North American PRRSV isolates. Therefore, a study involving isolates of the European genotype would be useful for an assessment of the global impact of selection for increased PRRSV resistance.

**Response to Vaccination**

Vaccines are designed to confer resistance to natural infection with disease through the by priming the immune response. Currently, the most effective commercially available PRRS vaccine is a modified live vaccine (Huang and Meng, 2010). Response to PRRS vaccination is an attractive solution to obtaining phenotypes for selection, however the genetic correlations between response to vaccination and response to common field variants of PRRSV would need to be established. If there is a high genetic correlation between response after vaccination and response with field variants of PRRSV, the timing of average peak serum viremia after vaccination would need to be established, as well as the timing of antibody response after vaccination.

**Indicators of Inflammatory Response**

The level of viremia in blood is a combination of how well the virus replicates and how the host responds in an attempt to minimize viral replication and clear the virus from serum. The innate immune response to infection of the host, more specifically the inflammatory response, can be measured by the eye or rectal temperature of an individual during the early stages of infection (Galley and Webster, 1996). It is known that the NLRP3 mediated formation of the inflammasome is pyrogenic (Shenoy et al., 2012). Thus, animals that have higher body temperature during the early stages of infection may reflect the host’s
ability to fight PRRSV infection more effectively, for which a strong positive association with GBP5 would be expected. Inflammatory responses have also been shown to induce anorexia, so the return of an animal to normal temperature, or a change of eating habits back to normal, could also be good indicators of a pig that is more resistant to PRRSV infection (Colditz, 2002). These phenotypes may be more easily collected on a wider scale than viremia and, thus, genetic studies on these traits may help to understand the mechanisms that drive host response to PRRSV infection. Furthermore, these traits may have a benefit in production, as anorexia is a common clinical sign associated with PRRSV infection (Nodelijk, 2002).

**Potential Consequences of Selecting for Improved Response to PRRSV Infection**

A potential concern of selecting for improved response to one disease is that it may negatively impact response to other diseases. Therefore, the impact of genetic selection for improved response to PRRSV infection on response to other diseases also needs to be assessed. Further research is also necessary to examine the impact that selection for improved resistance to PRRS may have on production in a clean herd; if negative effects of selection for increased PRRS resistance are observed in a clean herd setting, appropriate weightings in a selection index may allow for increased resistance to PRRS with minimal costs to production performance.

**An Integrated Approach for Containment of PRRS**

Genetic selection for pigs with increased resistance to PRRS holds promise for reducing the economic impact of PRRS. While the studies reported in this dissertation, as well as previous studies on the potential of selection for increased PRRSV resistance, are
important for identifying tools to reduce the impact of PRRSV, it is imperative to keep in mind that genetic selection is only one tool in the toolkit for reducing disease in swine herds. It is important that we continue to collect data on herd management practices in order to take a collaborative approach to reduce the amount of PRRSV that is spread between herds. Also, continued efforts to develop more effective vaccines will likely play an important role in containment of disease, which will be bolstered by gaining a better understanding of host response to disease, for example through an integrative approach employing such methods as genomics and transcriptomics. Therefore, a multi-faceted, integrative approach to efforts to contain PRRS is necessary, as discussed by Lewis et al. (2007), which termed such an approach an Integrated Herd Health Management program, and recognized that while any single part of such a program may not be sufficient for disease control on its own, the complementary nature of multiple components should make an impact.

References


CHAPTER VI
GENERAL DISCUSSION

When considering host response to infection, a number of factors can influence how the host responds to infection. These include, but are not limited to, host genetics, the genetics of the pathogen, and how these interact. While the previous chapter focused on how the information from the studies of the PHGC can be utilized to make selection decisions, the focus of this chapter will be to discuss how we can use these various quantitative genetics and genomics approaches to better understand the biology driving the phenotypes that are observed during infection.

When considering a genetically diverse, rapidly mutating pathogen such as PRRSV, the genetic makeup of the pathogen is of particular importance when looking at disease phenotypes (Darwich et al., 2010). In order to better understand what might be common themes during infection, genetically distinct isolates of PRRSV isolates were used in the studies of this dissertation were isolated ~10 years apart and had an 11% difference in GP5 nucleotide sequence (Ladinig et al., 2015), which is similar to the average level of divergence in GP5 that is found in North American PRRSV isolates (Shi et al., 2010). Given the genetic, as well as temporal and geographical, differences between these two isolates, similarities in host response to these two virus isolates may be indicative of host response to North American PRRSV variants.

The Wood’s curve has previously been shown to appropriately model PRRS serum viremia data during the course of infection and objectively identify pigs that experienced a rebound in viremia (Islam et al., 2013). In Chapter 3, the Wood’s curve was used to
estimate different aspects of the serum viremia curve of individual animals, such as peak viremia (PV), time to peak viremia (TP), maximal rate of decay (Vmax), time to maximal decay rate (Tmax), as well as viral load (VL), defined as the area under the Wood’s curve from 0-21 dpi. Pigs infected with NVSL had 16±2% higher VL than pigs infected with KS06. Analysis of the Wood’s curve characteristics revealed important differences in the shape of the viremia curve under infection with NVSL and KS06, in that pigs infected with NVSL had 14±2% higher PV and reached PV 2.5±0.6 days earlier than pigs infected with KS06. Maximal decay rate was reached 3.9±0.7 days earlier and was 36±14% faster under infection with NVSL than KS06 (Chapter 3). The profile for KS06 was consistent with what would be expected from a less virulent isolate of PRRSV (Beyer et al., 2000), which was further supported by the 3.7±1.5 kg higher weight gain that was observed for the KS06 infected pigs (Chapter 3). Thus, utilizing the Wood’s curve functions provided a means of characterizing differences in how different isolates of PRRSV behaves over the course of infection, in order to assess differences in virulence.

In chapters 3-5, Legendre polynomials were fitted to the collected weekly weights. This approach has similar benefits to viremia, in the way that fitting a weight curve to each animal can reduce the amount of noise involved with a single measurement, but, more importantly, can be used to get a fitted weight value for each individual throughout the course of the trial. Modeling the dynamics of serum viremia and weight also allowed for the interpolation of daily viremia and weight values, providing a more in-depth analysis of the changing relationships between response traits throughout infection. These daily measurements would be difficult to collect because they are labor intensive and daily serum viremia measurements would be very stressful for the pigs.
The Genetics of Host Response to PRRS

Organisms have evolved to make the best use of the (often limited) resources they have available. Food and nutrient resources must be divided between maintenance, health, growth and reproduction aspects. These are prioritized based upon age, environment and availability of energy resources. In the context of health, immune response can be considered expendable under normal conditions, requiring fewer resources (Rauw, 2008). However, in a disease context, the host’s ability to mount an immune response and successfully clear the pathogen is related to the host’s ability to mitigate the impact the disease has on the animal. In the most extreme case, this will be the difference between life and death (Rauw, 2012). The cost of mounting an immune response depends on the resources available, whereby the costs of mounting an immune response is greater if there are fewer resources (Doeschl-Wilson et al., 2009). The allocation of resources between different areas differs between individuals and may lead to differences in their ability to respond to and perform under infection.

Antibody response, viremia, and weight gain following infection are all complex processes. The relationship between immune response and growth is, however, expected to change over time, as the animal places different levels of emphasis on these traits throughout the course of infection (Coop and Kyriazakis, 1999; Doeschl-Wilson et al., 2009). Genetic correlations between response traits across different time points showed dynamic relationships, which revealed different relationships between S:P, viremia and weight gain pre-peak viremia versus post-peak viremia (Chapters 3 and 4). For example, genetic correlations of antibody response at 42 dpi with 3-day weight gain were negative
early after infection but turned positive later. These results suggest that animals that placed
more emphasis on immune response early in infection and therefore suffered with lower
early weight gain, reaped the benefits of this later in infection by more effectively clearing
the virus, which is consistent with the findings of previous simulation studies investigating
this phenomenon (Doeschl-Wilson et al., 2009). These results emphasize that the
interpretation of the genetic correlations between traits in a disease is context dependent
and that the time frame that is considered is crucial for interpretation, as the overall
correlations of antibody response with VL and WG did not show these dynamic
relationships. Interestingly, genetic correlations between antibody response at 42 dpi and
daily serum viremia were most negative during the period when antibodies are expected to
be produced, which correspond to the time of maximal decay of viremia.

As expected, a major QTL was identified for S:P in the MHC in pigs infected with
either NVSL or KS06. Interestingly, despite the strong correlations observed of S:P with
viremia or weight gain, the MHC appeared to play a minor, if any, role in these observed
correlations (Chapter 4). The same observation was made by Serao et al. (in preparation),
concerning the genetic correlation of antibody response with sow reproductive
performance during a PRRS outbreak in a sow herd. These findings suggest that, rather
than being governed by a small number of loci with pleitropic effects, the responses that
are influencing these traits are largely polygenic, which is what one might expect if the
genetic correlations between these traits are picking up shifts in the allocation of energetic
resources, and the costs/benefits of such a task (Roff, 2007).

When evaluating the genetic correlations between viremia and weight gain
(Chapter 3), for pigs infected with NVSL, high pre-peak viremia was associated with lower
early WG but with higher late WG. For pigs infected with KS06 there was a moderate negative association between pre-peak viremia and early WG, and a weaker negative correlation with late WG. Overall, the correlations observed for KS06 were weaker than those observed for NVSL, consistent with the conclusion that KS06 is less virulent than NVSL. A strong positive genetic correlation between antibody response at 42 dpi and early viremia was observed for NVSL infected animals, which was not the case for KS06 infected animals. The results from these analyses suggest that animals that have a strong early response to PRRSV infection, typically due to high early viremia levels, tend to have an early decrease in weight gain but higher antibody levels at 42 dpi and lower viremia later in infection. These findings are consistent with resource allocation theory (Rauw, 2012) which proposes that animals that divert their energy resources to fighting the infection early on tend to reap the benefits of that in the later stages of the trials through lower serum viremia and higher weight gain. These findings are also consistent with results obtained from a mathematical modeling approach to assess the relationship between nutrient availability, immune response, and pathogen load by Doeschl-Wilson et al. (2009). The strong positive genetic correlation of antibody response at 42 dpi with early viremia that was observed for NVSL but not for KS06 may be due to differences in virulence (Doeschl-Wilson et al., 2009; Rauw, 2012). Animals infected with NVSL had a stronger and longer lasting negative correlation of antibody response with weight gain than KS06, suggesting that the antibody response to PRRSV infection was costlier for infection with NVSL (Chapter 4). This is consistent with the observed difference in weight gain between animals infected with these two isolates and supports the notion that NVSL is more virulent than KS06 (Chapter 3).
The biological factors influencing PRRSV persistence are poorly understood. In chapter 4, phenotypic associations between the ability to clear the virus from serum was favorably associated with tonsil virus levels, suggesting that there is a relationship between serum viremia and the persistence of PRRSV in the tonsils. The heritability of tonsil virus levels was estimated to be low; however, this estimate might improve if the microenvironment (i.e. the environment of the tonsil) is better capture (e.g. the size or weight of the tonsil is known, the prevalence of macrophages is known, etc.). Despite the low heritability estimates, genomic analysis of tonsil virus levels was able to identify genomic regions that, although they explained only a small percentage of the genetic variance, may be biologically relevant. Biological processes of genes that were in regions that were identified as being associated with tonsil virus levels included heparin sulfate processing, T-cell activation/signaling, the complement protein C1q, a cluster of CCL chemokines, and modification of the Golgi apparatus. Heparin sulfate is a known PRRSV receptor (Vanderheijden et al., 2001). T-cells play a vital role in the adaptive immune response and persistence of PRRSV in tissue has been shown to be the consequence of immune response failure (Gomez-Laguna et al., 2013). PRRSV is thought to manipulate the Golgi apparatus during replication through the establishment of a replication complex (Suarez, 2000). Therefore, the pig’s ability to control viral replication in tonsils may depend on the animal’s ability to develop an effective adaptive immune response in order to control viral replication. PRRSV N-protein specific antibody response may play a role in this ability that is independent of neutralizing antibodies via C1q, because IgG is known to interact with this protein complex. Being able to adjust tonsil virus level for tonsil size and the number of macrophages in the tonsil may lead to a deeper understanding of the role
that these processes play in host response to PRRSV tonsil virus levels or identify additional QTL associated with tonsil virus levels.

**Biological Insights into WUR10000125**

PV was the only curve characteristic with which WUR genotype had a consistent and highly significant association across the two PRRSV isolates evaluated in this thesis (Chapter 3). The association between PV and WUR genotype is consistent with the role of GBP5 in the innate immune response, which plays an important role early in infection. GBP5 is involved in the formation of the inflammasome via NLRP3 (Shenoy et al., 2012), and animals with the AA genotype do not produce functional GBP5 (Koltes et al., 2015). As a consequence, these animals have higher serum viremia levels, compared to animals with the AB or BB genotype (Boddicker et al., 2012). This information may be of particular importance because understanding the mechanisms by which GBP5 acts and the time during it has the strongest effect gives us a better understanding of host response to infection and allows us to delve deeper into the inflammasome pathway that GBP5 is involved in and potentially identify other useful targets of selection of pigs for improved response to PRRSV infection.

Gene expression profiling analyses that compared animals with the AA and AB WUR genotypes have reported that AA animals have a reduced capacity to turn off their immune responses to PRRSV infection (Schroyen et al., 2015). AA animals place greater emphasis on a humoral immune response than AB animals, presumably due to the lack of an effective immune response in the inflammasome pathway involving GBP5 (Schroyen et al., 2015). Estimates of the genetic correlation estimates of antibody response at 42 dpi
with viremia and weight gain across the course of infection painted an interesting picture when estimated separately for AA and AB/BB animals. AA animals showed a strong negative genetic correlation between antibody response and viremia, nearly reaching -1 between 2 and 3 weeks post infection. Estimates for AB/BB animals showed a low positive genetic correlation between antibody response and viremia at all time points. AA animals showed a more constant negative genetic correlation between antibody response and weight gain during the course of infection than AB/BB animals, with a negative correlation observed between these traits of about -0.6 from 0 to 21 dpi. Conversely, AB/BB animals showed a positive genetic correlation of antibody response with weight gain after 7 dpi, with a maximum genetic correlation of ~0.6 at 25 dpi (Chapter 4). These results support the findings from the gene expression studies, which suggest that AA animals are less effective at combating PRRSV infection, therefore combating PRRS comes at greater cost to them.

**Future Directions**

**Antibody response at multiple time points**

The trials of the PHGC focused on only one time point for measurement of antibody levels, 42 dpi, so an experiment involving collection of antibody levels at multiple time points will help to elucidate the mechanisms driving antibody response to PRRSV infection and aid in understanding the disease and vaccine development. Important phenotypes that may improve our understanding of response to PRRSV infection are the time it takes for PRRSV-specific antibodies (of different isotypes, including both neutralizing and non-
neutralizing) to be detected in serum, rate of antibody production and the timing and level of the plateau of antibody level.

**Vaccination as a method of phenotype collection**

Vaccines are designed to confer resistance to natural infection with disease through the by priming the immune response. Currently, the most effective commercially available PRRS vaccine is a modified live vaccine, which has limited success, presumably due to the high level of variation observed between PRRSV isolates (Huang and Meng, 2010). Estimating the genetic correlations between response to vaccination and response to common field variants of PRRSV, as well as conducting GWAS with functional annotation, may provide insight into the biological mechanisms that influence host response to vaccination and infection, and provide valuable information for the design of more effective vaccines by creating vaccines that will elicit stronger, longer lasting immune response.

**The similarity in host response between subtypes of PRRSV**

Identifying approaches of containment that are effective across different PRRSV variants is of major concern to the swine industry. Keeping in mind that PRRS is a global swine disease, it would be useful to explore a suite of PRRSV isolates that are representative of different geographical regions. There are two major genotypes of PRRSV with substantial differences in both their genome and pathology: North American and European (Wensvoort et al., 1992). Both of the isolates used in the studies of this dissertation were North American PRRSV isolates. Therefore, a study involving isolates of the European genotype would be useful for a more comprehensive understanding of the biological underpinnings influencing the host’s response to PRRSV infection. Studies
investigating host response to a number of different isolates will also provide insight into variable virulence factors between virus isolates, e.g. our studies were able to identify that NVSL is a more virulent isolate than KS06, the addition of a greater number of isolates could reveal more complex relationships between isolates. This information may prove useful in management of PRRS outbreaks.

**Additional genome wide association studies**

GWAS have been conducted and have successfully identified a major QTL for VL. In the context of viremia, expanding the GWAS to use the daily predicted viremia values warrants attention. It would be of particular interest to track how the genetic variance explained by particular windows changes over the course of infection. Identification of the changing associations between particular genomic regions and viremia levels will aid in elucidating the biological mechanisms involved with response to PRRSV infection and may help to identify additional genomic regions associated with response to PRRSV infection that could be used in selection programs. Additional phenotypes to explore as a function of time include weight gain, antibody response, and cytokine response to PRRSV infection. Measuring these phenotypes across time may provide the means of taking an integrative approach to understanding how the host responds to PRRSV infection. This would be beneficial, not only from the standpoint of genetic selection but also in understanding the biological mechanisms behind PRRSV infection, which has the potential to aid in development of management practices and vaccines.
Conclusions

The studies in this dissertation have assessed a collection of PRRSV response phenotypes under experimental infection with one of two distinct PRRSV and evaluated their potential for selection for improved PRRS response that is effective across North American PRRSV variants. High genetic correlations were observed between response to infections with the NVSL and KS06 isolates for most PRRSV response traits. Dynamic relationships were observed between serum viremia, weight gain, and antibody response, thus emphasizing that the relationships between traits is context dependent. Delving further into how these dynamic processes are related can provide insight into how the host is responding to infection, and may aid in the development of more powerful phenotypes to identify animals that have a more favorable response to PRRSV infection, which is likely to reduce the economic and animal welfare costs associated with PRRS and help with containment. A better understanding of how the host is responding to infection and how this differs between PRRSV isolates may provide a more directed approach to understanding the underlying biological mechanisms driving response to infection, which may aid in the development of more effective methods for protecting the animals from disease. Selection using a PRRS selection index including the traits PV, WG, and S:P, as well as the WUR genotype would be successful at selecting animals for improved response to PRRSV infection.

References


