RESEARCH REPORT
SWINE HEALTH

Title: Impact of host immunity and genetics on persistence of PRRS virus in tonsils – **NPB #14-223**

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Industry Summary

Despite extensive efforts to eliminate PRRS from US production facilities, it remains a key disease issue and poses a continued economic threat to the industry, particularly in pig dense areas. A major factor that complicates PRRS control is viral persistence. This project proposed to identify pigs which have persistent PRRSV infections. Pigs with persistent PRRSV infections, or carrier pigs, are a continuing threat to each production unit. Viral survival is maintained because a proportion of the herd have virus which persists in lymphoid tissues (tonsil, lymph nodes) and can be shed occasionally (due likely to stress, diseases or other factors). This shed virus then infects the remaining herd pigs many of which are naïve and thus susceptible. Transmission studies have verified that pigs can harbor the virus for >160 days, and likely longer. Closing a herd for 200 days was thought to be effective before new "clean pigs" could be reintroduced; some would argue for longer times before the PRRS virus becomes extinct.

Currently there is no good technology to accurately identify PRRSV carrier pigs, nor are there procedures to treat pigs to eliminate persistent virus from their tissues. This proposal determined the frequency of pigs with persistent PRRSV by quantitating viral RNA levels in tonsil as a surrogate measure of viral persistence. To perform this, we took advantage of the repository of samples that were collected through the NPB funded PRRS Host Genetics Consortium (PHGC). Each PHGC pig, provided at weaning from current commercial breeding stocks, was infected with PRRSV and followed for 42 days post infection (dpi). Every pig that survived to 42 dpi had tonsil tissue archived. All data is preserved in the PHGC database (www.animalgenome.org/lunney/index.php) including the pig's pedigree, response to PRRSV infection (serum viral and antibody levels and weight gain data), along with extensive genotypic information.

Our work with tonsils from pigs infected with type 2 PRRSV (virulent NVSL or moderately virulent KS-06) showed limited relationship between serum viral load (VL; 0-21 dpi) with tonsil viral RNA levels (TV). This limited correlation is not unexpected since PRRSV is known to persist in tissues, particularly the tonsil, well past the time when serum viremia has cleared. When comparing the two viruses our data indicated that there is much lower cumulative virus with KS-06 as compared to NVSL infected pigs in serum and in tonsil. This grant affirmed that pigs infected with the moderately virulent PRRSV (KS-06) exhibited similar tonsil persistence characteristics to the virulent NVSL infection.

Our statistical analysis (Hess et al. 2018) verified that pigs identified as having persistent serum viremia levels for NVSL had significantly higher TV than pigs that had already cleared serum viremia ($P<0.001$). This trend was similar for KS-06 infection but not as significant. Tonsil virus level was estimated to be lowly heritable for both type 2 PRRSV isolates. Data indicated that an earlier and faster serum virus clearance was associated with lower TV for both NVSL and KS-06 infected pigs. Analysis of weekly weight gain did not reveal any associations with TV (Hess et al. 2018).

Mapping studies revealed several genomic regions that explained a proportion of genetic variance for tonsil viral levels. Several strong candidate genes were identified and may be involved with the host's ability to control viral infiltration/replication and the ability to clear infected cells from tissue and are useful targets for gene expression analyses on tonsil tissue that are underway. These data may provide insight into alternative genes involved in host genetic control of tonsil virus levels and viral persistence (Hess et al., 2018).

Finally, we probed for host genes that might be involved in persistence by analyzing pig gene expression in tonsil RNA. Overall, 12,597 genes were determined to be expressed in the tonsil with 1646 and 336 differentially expressed genes (DE genes, $q\leq0.2$) identified between PRRSV isolate and TVclass, respectively (Dong et al., 2018, in preparation). Pathway analysis results showed that both KS-06 and high TV were associated with DE genes predicted to increase the quantity, proliferation, differentiation, cell movement and adhesion of immune cells, especially T cells, compared to NVSL and low TV. Preliminary results indicate that there were 4 DE genes that were significantly up-regulated in tonsils from pigs from high versus low tonsil viral level: CXCL10, TBX21, CCL5 and CCL19. Overall, nursery pigs infected with a less pathogenic PRRSV isolate, or that have higher tonsil viral level, have a stronger tonsil immune response. Collectively, these DE gene results suggest that KS-06 infection may result in less tonsil tissue damage by regulating genes related to cell and tissue morphology. High tonsil virus levels may activate the expression of genes that trigger cellular immune responses to clear virus that persists in tonsils and inhibits virus replication.

These findings contribute to our understanding of the mechanisms involved in tonsil pathology induced by PRRSV infection in pigs. Based on this, efforts can be planned to selectively breed for pigs with lower tissue persistence or, alternately, to identify means of stimulating anti-viral responses in pigs with persistent PRRSV infections.

Keywords: PRRS, persistence, tonsil, viral RNA, database, host genetics

Scientific Abstract:

Despite extensive efforts to eliminate PRRS from US production facilities, it remains a key disease issue and poses a continued economic threat to the industry, particularly in pig dense areas. A major factor that complicates PRRS control is viral persistence. Viral survival is maintained because a proportion of the herd has persistent virus which is shed occasionally (due likely to other diseases or stress). This shed virus then infects the remaining herd pigs which are naïve and thus susceptible. This project proposed to identify pigs which have persistent PRRSV infections and to determine if there are immune or genetic correlates of PRRSV persistence.

Currently there is no good technology to accurately identify PRRSV carrier pigs. This proposal determined the frequency of pigs with persistent PRRSV using viral RNA levels in tonsil as a surrogate measure of persistence. To perform this, we took advantage of the repository of samples that were collected through the NPB funded PRRS Host Genetics Consortium (PHGC). Each PHGC pig, provided at weaning from current commercial breeding stocks, was infected with a type 2 PRRSV isolate and followed for 42 days post infection (dpi). Every pig that survived to 42 dpi had tonsil tissue archived. Moreover, the PHGC database (www.animalgenome.org/lunney/index.php) contains extensive data on each PHGC pig, including its pedigree, response to PRRSV infection (viral and antibody levels and weight gain data), and extensive genotypic information (60K SNP chip).

Our work with tonsils from pigs infected with virulent NVSL97 or moderately virulent KS-06 type 2 PRRSV isolates showed limited relationship between serum viral load (VL; 0-21 dpi) with tonsil viral RNA levels. This limited correlation is not unexpected since PRRSV is known to persist in tissues, particularly the tonsil, well past the time when serum viremia has cleared. When comparing the two viruses our data indicated that there is much lower cumulative virus with KS-06 as compared to NVSL97 infected pigs. This grant

affirmed that pigs infected with a moderately virulent PRRSV (KS-06) exhibited similar tonsil persistence characteristics to the virulent NVSL infection.

Hess et al. (2018) verified that pigs identified as having persistent serum viremia levels for NVSL had significantly higher TV than pigs that had cleared serum viremia ($P<0.001$). There was a similar trend for KS-06 but differences were not as significant. Tonsil virus level was estimated to be lowly heritable for both type 2 PRRSV isolates (Hess et al. 2018). An earlier and faster virus clearance was associated with lower TV for both NVSL and KS-06 infected pigs. Analysis of weekly weight gain did not reveal any associations with TV. When anti-PRRS antibody responses were analyzed there were significant associations with TV in KS-06, but not NVSL, infected pigs (Hess et al. 2018). 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.

More detailed genome wide association studies (GWAS) revealed several genomic regions that explained a proportion of genetic variance and several strong candidate genes. Genes involved with the host's ability to control viral infiltration/replication and the ability to clear infected cells from tissue may be useful targets for gene expression analyses on tonsil tissue that are underway (Hess et al., 2018).

Finally, tonsil RNA was probed for host genes that might be involved in persistence. Overall, 12,597 genes were determined to be expressed in the tonsil with 1646 and 336 differentially expressed genes (DE genes, $q\leq0.2$) identified between PRRSV isolate and TVclass, respectively (Dong et al. 2018 in preparation). Pathway analysis results showed that both KS-06 and high TV were associated with DE genes predicted to increase the quantity, proliferation, differentiation, cell movement and adhesion of immune cells, especially T cells, compared to NVSL and low TV. Preliminary results indicate that there were 4 DE genes that were significantly up-regulated in tonsils from pigs from high versus low tonsil viral level: CXCL10, TBX21, CCL5 and CCL19. Overall, nursery pigs infected with a less pathogenic PRRSV isolate, or that have higher tonsil viral level, have a stronger tonsil immune response.

Collectively, these DE gene results suggest that KS-06 infection may result in less tonsil tissue damage by regulating genes related to cell and tissue morphology. High tonsil virus levels may activate the expression of genes that trigger cellular immune responses to clear virus that persists in tonsils and inhibits virus replication. These findings contribute to our understanding the mechanisms involved in tonsil pathology induced by PRRSV infection in pigs. Based on this, efforts can be planned to selectively breed for pigs with lower tissue persistence or, alternately, to identify means of stimulating anti-viral responses in pigs with persistent PRRSV infections.

Introduction:

A major obstacle to the control and prevention of PRRSV infection in pigs is the capacity of the virus to become persistent within a production system (>167 days) (Wills et al., 1997; 2003; Rowland et al., 2003). As noted in an Extension update, "Persistence is the single most significant epidemiological feature of PRRS virus. Carrier animals represent the constant threat of transmission to susceptible herd mates and the initiation of a PRRS outbreak. At present, we do not have the technology to accurately, rapidly, and cheaply identify carriers. Neither the absence of viremia nor serum antibody levels is an indicator of carrier status. In fact, some carrier animals have low serum antibody levels, e.g., <0.40 S/P on the commercial ELISA. Thus, the existence of carrier animals profoundly complicates all aspects of PRRS prevention and control (Zimmerman et al., 2015)."

Moreover, because PRRSV is an RNA virus, its sequence randomly changes as it replicates, which results in viral genetic variants that can escape immunity in previously vaccinated pigs. Pigs with persistent PRRSV can experience a resurgence of circulating virus, triggering a secondary outbreak (Rowland and Morrison, 2012). This is a major concern to naïve pigs introduced into the herd after the initial PRRSV outbreak. 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, hampering efforts to identify them despite high levels of PRRSV in tissues (Wills et al., 2003).

Over time, viral loads decrease by >1,000-fold in tonsil and lymph nodes, the primary sites of viral persistence (Xiao et al., 2004). Evans et al. (2010) developed a stochastic model of within-herd PRRSV transmission dynamics, which indicated a balance between "fade-out" and persistence. Molecular analyses revealed that viral RNA was present in tissues of infected pigs until 202 dpi (Molina et al., 2008). The mechanism of persistence is not completely understood, but likely includes the emergence of viral variants that

can escape host defenses (Rowland et al., 1999). Horter et al. (2002; 2003) found that infectious PRRSV is present in most pigs for the first 105 dpi, and suggested that RT-PCR assays of tonsil scrapings was the most effective combination of assay and sample for detecting PRRSV carriers.

Horter et al. (2002) noted that antibody response levels did not distinguish PRRSV carrier from non-carrier animals. Mulupuri et al. (2008) suggested that the delayed response against the GP5 protein of PRRSV early in infection may contribute to the prolonged acute infection and the establishment of persistence. Upregulation of serum cytokines [interleukin-1β (IL-1β), IL-8 and interferon-γ (IFNγ)] early after infection was significantly correlated with lower lymph node or tonsil tissue viral RNA levels (Lunney et al., 2010). Rodríguez-Gómez et al. (2013) suggested persistence was due to impairment of the immune function of antigen presenting cells. This may explain the negative role PRRS has on co-infections, contributing to polymicrobial diseases such as Porcine Respiratory Disease Complex (PRDC) and porcine circovirus associated disease (PCVAD) (Opriessnig et al., 2011; Gómez-Laguna et al; 2013a,b).

Research has shown that there are host genetic components involved in determining how pigs respond to PRRSV infection (Halbur et al 1998; Vincent et al 2005; 2006; Petry et al 2005; 2007; Ait-Ali et al 2007; Lewis et al 2007; Lunney and Chen, 2010). To elucidate clearly the role of host genetics in the control of PRRS requires a large number of animals. This recognition formed the basis for the creation of the PRRS Host Genetics Consortium (PHGC); which would 1) support and organize the infection of thousands of pigs, 2) collect, catalog and distribute tens of thousands of samples for analysis by PHGC participants, and 3) develop and maintain a consortium database (Lunney et al., 2011; Rowland et al., 2012; Dekkers et al., 2017). NPB provided critical early support of this effort (NPB grants #07-233, 09-208, 10-033).

Analyses of the first 8 PHGC trials of commercial pigs from 6 different genetic sources affirmed that serum viremia and weight gain after infection with a virulent type 2 PRRSV isolate (NVSL, NVSL 97-7985) were moderately heritable at 0.39 and 0.34, respectively (Boddicker et al., 2014b). State-of-the-art genome wide association studies (GWAS) were performed and identified a region on swine chromosome 4 (SSC4), marked by the SNP WUR10000125 (WUR), that is associated with PRRS resistance/susceptibility and explained 15.7% of the genetic variance for VL and 11.2% for WG42 (Boddicker et al., 2012; 2014a,b). Further analyses of pigs infected with a moderately virulent type 2 PRRS isolate (KS-06; KS-2006-72109; PHGC trials 10-14) affirmed the role of the SSC4 region and WUR on PRRSV infection (Hess et al., 2016). Dunkelberger et al. (2017) affirmed that SSC4 also regulates pig responses to PRRSV vaccination.

This NPB grant used sophisticated genome mapping techniques to determine whether there are phenotypic traits, genomic regions and host genes that regulate tonsil virus persistence. We hypothesized that animals that clear serum viremia levels faster, and thus have lower serum viremia at 35 or 42 dpi, will also have lower tonsil virus levels (TV) at that time, and that TV would have a heritable genetic component. We also probed for mechanisms controlling tonsil PRRS viral levels using deep sequencing of tonsil RNA gene expression (RNA-seq) and statistical analyses. By comparing data from pigs with high versus low TV and using bioinformatic tools we aimed to identify molecular pathways and genes involved in anti-persistence responses. With this knowledge, efforts can be planned to selectively breed for these pigs or, preferably, to identify means of stimulating these responses in pigs with persistent PRRSV infections.

Objectives from original proposal

1. Determine levels of PRRS viral RNA in porcine tonsils of pigs infected with different PRRSV isolates and compare these to serum viral levels as indicators of PRRSV persistence
2. Estimate heritability of PRRS persistence and its genetic correlations with serum viremia, growth, and antibody response following PRRS infection; use genome wide association studies (GWAS) to identify genomic regions associated with PRRSV persistence
3. Use RNA-seq analyses to evaluate genes and pathways involved in maintaining and regulating tonsil tissue PRRS viral RNA levels

Materials & Methods:

Study design

As part of the original NPB funded PHGC grants (#07-233, 09-208, 10-033) tonsil samples were collected from every pig that survived until the end of the PRRSV infection trial, typically to 42 dpi. Pigs were brought into the Kansas State University (KSU) biosafety level 2 (BSL-2) facility at weaning (~21 days age), infected a week later with either of 2 well characterized type II PRRSV isolates [NVSL 97-7985 (NVSL) or KS-2006-72109 (KS-06)] and followed for 35 or 42 dpi. Whole blood and serum samples were collected on 0, 4, 7, 11, 14, 21, 28, 35 and 42 dpi and body weight of the animals were measured weekly. Tonsil, ears and other samples were collected at the end of the study. All samples were catalogued, shared between Kansas State University and BARC and stored for use in later studies or distributed to appropriate testing labs. Data on every pig was entered into the PHGC database (www.animalgenome.org/lunney/index.php). Genomic DNA samples from every PHGC pig and available parent was genotyped using the Illumina Porcine SNP60 BeadChip.

Tonsil virus levels

The PHGC tonsils were shipped from Kansas State University (KSU) to BARC and stored in -80 freezers until processed for RNA. Specifically, samples were kept on ice as a piece of tonsil (~30mg, pea size) was removed and weighed. This grant proposed to determine whether pigs infected with a different PRRSV isolate [heterologous KS-06] would exhibit similar tonsil persistence characteristics to pigs infected with the NVSL PRRSV isolate. To improve RNA quality, we added a pretreatment protocol wherein tonsil was treated with pre-chilled (-80^{0}C) RNAlater®-ICE for >16 hours at -20^{0}C. The tonsil sample was then placed into a FastPrep tube containing a grinding ball; homogenized using -20^{0}C pre-chilled plates for 2 x 2 min at 30 Hz for 4 minutes total on Mixer Mill Model MM301 tissue grinder. The lysate was then extracted using the Norgen Animal Tissue RNA Purification Kit, Proteinase K, and on-column DNAse treatment. The resulting RNA was aliquoted and stored in the -80 freezer. RNA quality and quantity was affirmed using an Agilent 2200 Tape station. The average total RNA yield was ~103 ug/ 90 uL (range 1.4 – 394 ug) with an average RNA quality score, known as RIN#, = 6.3 +/- 0.7 (range 4.4 - 8.2).

Serum viremia and tonsil virus levels (TV) were measured using a semi-quantitative TaqMan PCR assay for PRRSV RNA, as described in Boddicker et al. (2012), with samples run in duplicate using 96-well plates. Serum and tonsil assay results were reported as the log10 of PRRSV RNA copies per ml of serum and per mg of tissue, respectively. For the NVSL trials (3, 5 and 7) the Applied Biosystems AgPath ID NA & EU PRRSV reagents (AB assay) were used to measure TV, while the Tetracore U.S. and EURO PRRSV Master Mix reagents (Tetracore assay) were used for the KS-06 trials (11 and 14) because the AB primers failed to amplify cDNA of the KS-06 isolate. Assessment of tonsil RNA quality and viral levels, and viremia profile modelling using the Wood's function, are reported in detail in Hess et al (2018).

Host gene expression analyses

Tonsil RNAs were used to identify pig genes that were differentially expressed (DE genes) using RNA sequencing analyses (RNA-seq). Tonsil samples were chosen for RNA-seq based on high or low levels of PRRSV and used to assess abundance of mRNA for each gene expressed. As a result, 15 NVSL-high, 13 NVSL-low, 12 KS-06-high, and 10 KS-06-low samples were selected for RNA-seq analyses. Paired-end RNA-seq (forward and reverse) was performed at the ISU DNA facility. The program RSEM was used to calculate gene expression levels with reference to the *Sus scrofa* 10.2 genome sequences (and updated to 11.1 build) and gene annotation from Ensembl to obtain TPM (transcripts per million) of 22811 genes. The statistical analysis software QuasiSeq was used to identify DE genes ($q<0.1$) between pigs infected with NVSL versus KS-06, pigs with high versus low TV, and their interaction. Ingenuity Pathway Analysis (IPA) software was used to identify causal relationships and biological functions of the DEGs (Dong et al., 2017; Dong et al., in preparation).

Statistical analyses

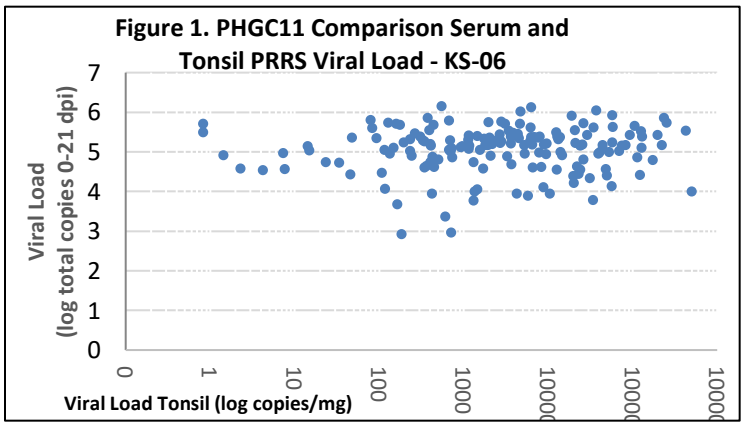
Estimates of the heritability of TV and of phenotypic associations of TV with other PRRS response traits were obtained from the model described in Hess et al (2018). The relationships of TV with serum viremia characteristics, weekly weight gains, and antibody response were estimated by separately including each of

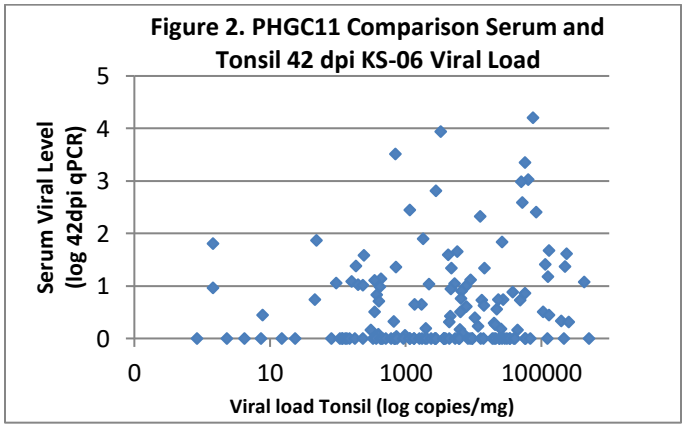
these traits as a fixed covariate in the model. When estimating the association between TV and WUR genotype, WUR genotype was added as a fixed effect. The significance threshold was set to P≤0.05, and the coefficient of determination of the independent variable was used to assess the proportion of TV explained by the covariate. For Genome Wide Association Studies (GWAS) the Bayes C method implemented in GenSel 4.73 (Fernando and Garrick, 2012) was used to test associations of 60k SNPs with TV as detailed in Hess et al (2018).

Results:

Obj.1 Determine levels of PRRS viral RNA in porcine tonsils of pigs infected with different PRRSV isolates and compare these to serum viral levels as indicators of PRRSV persistence

For this grant tonsil RNA was prepared from another 3 PHGC trials: PHGC7 for NVSL and PHGC11 and PHGC14 for KS-06 PRRSV infections. All samples were stored at -80 ^{0}C. We verified RNA quality based on RNA Integrity Number (RIN) then quantified tonsil viral load (TV). Data for PHGC11 (KS-06 infection) are shown in Figures 1,2.





Our previous work with tonsils from pigs infected with NVSL PRRSV showed limited relationship between serum viral load (VL; 0-21 dpi) with tonsil viral RNA levels (copies/mg tissue). The data in Figure 1 affirm this result for tonsils collected from KS-06 PRRSV infected pigs. Similarly, Figure 2 shows that there is only a limited relationship between the final, 42 dpi serum KS-06 viremia levels with tonsil viral RNA levels as was found for NVSL infected pig tonsil RNAs. This limited correlation is not unexpected since PRRSV is known to persist in tissues, particularly the tonsil, well past the time when serum viremia has cleared.

When comparing the two viruses our data indicate that there is much lower cumulative virus with KS-06 as compared to NVSL infected pigs (data not shown). This grant affirmed that pigs infected with a different PRRSV exhibited similar tonsil persistence characteristics. This is important since KS-06 infected pigs show slower post viral peak serum clearance levels and thus potentially will exhibit different anti-viral responses.

Islam et al. (2013) developed mathematical descriptions of PHGC serum viremia profiles wherein 3 viremic categories were established: cleared (uni-modal serum viremia that was below detection within 42 dpi), persistent (transient experimental persistence over 42 dpi) and rebound (biphasic within 42 dpi). Hess et al. (2018) then used those categories for quantitative analysis of TV levels and serum profiles and verified that pigs which were classified as having persistent serum viremia levels for NVSL had significantly higher TV than pigs that had cleared serum viremia (P<0.001). Pigs with rebound serum virus had intermediate average TV, which was not significantly different from the average TV of cleared or persistent animals (P>0.1). For KS-06, the direction of the effect of serum viremia status on TV was the same as for NVSL but differences were not as significant (Hess et al. 2018). An earlier and faster virus clearance was associated with lower TV for both NVSL and KS-06 infected pigs. Animals that had lower levels of serum viremia throughout the trial, or on the day that the tonsils were collected, had lower TV for both NVSL and KS-06 infected pigs (Hess et al. 2018).

Obj.2. Estimate heritability of PRRS persistence and its genetic correlations with serum viremia, growth, and antibody response following PRRS infection; use genome wide association studies (GWAS) to identify genomic regions associated with PRRSV persistence

The deep phenotyping generated for pigs during the PHGC trials has enabled probing host genomic control of many different PRRS response traits. Now that we have quantitated tonsils RNAs for PRRS viral levels, this data can be used to determine whether the differences seen are heritable and whether there are genetic effects that can be mapped to specific chromosomes (and to exact genes or regulatory factors). As recently reported by Hess et al. (2018) tonsil virus level was estimated to be lowly heritable for both type 2 PRRSV isolates, heritabilities were not significantly different from zero (Table 1).

Table 1: Estimates of heritability for the log of tonsil virus level for two PRRSV isolates.

Isolate	Heritability (s.e.)	Phenotypic SD
NVSL	0.05 (0.06)	1.03
KS-06	0.11 (0.10)	0.89
BOTH	0.09 (0.06)	0.98

Litter variance was zero for all analyses. Phenotypic SD includes the effects of trial, pen, animal and residual.

Analysis of weekly weight gain did not reveal any associations with TV (Hess et al. 2018). However, there was a marginally significant (P=0.04) positive association of weight gain (only for NVSL infected pigs) 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. When anti-PRRS antibody responses were analyzed there were significant associations with TV in KS-06, but not NVSL, infected pigs (Hess et al. 2018). The estimates of the regression coefficients suggest 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 revealed several genomic regions that explained a proportion of genetic variance. Several strong candidate genes were identified including genes involved with the host's ability to control viral infiltration/replication and the ability to clear infected cells from tissue (Hess et al., 2018). These genes may be useful targets for future gene expression analyses on tonsil tissue to gain insight into the host genetic control of tonsil virus levels and viral persistence.

Obj. 3 Use RNA-seq analyses to evaluate genes and pathways involved in maintaining and regulating tonsil tissue PRRS viral RNA levels

Tonsil RNA was probed for host genes that might be involved in persistence. Overall, 12,597 genes were determined to be expressed in the tonsil based on the Ensembl 11.1 pig genome. Using the Voom package with a model that included Isolate, TVclass, sex, RIN score, and genotype at WUR, 1646 and 336 differentially expressed genes (DEGs, $q \leq 0.2$) were identified between PRRSV isolate and TVclass, respectively (Dong et al., 2018, in preparation). The effects of WUR and any interactions were not significant. Ingenuity Pathway Analysis (IPA) results showed that both KS-06 and high TV were associated with levels of DE gene expression that were predicted to increase the quantity, proliferation, differentiation, cell movement and adhesion of immune cells, especially T cells, compared to NVSL and low TV (Dong et al. 2018 in preparation).

Preliminary data identified 4 DE genes that were significantly up-regulated in tonsils from pigs with high versus low tonsil viral level: CXCL10, TBX21, CCL5 and CCL19. These genes likely activate the polarization of blood cell functions to trigger cellular immune response. Genes CCL5, RSAD2 and CXCL10, which were up-regulated in pigs with high TV may inhibit virus replication in tonsil tissue (Dong et al. 2018). For validation, NanoString gene expression analyses were performed; they verified the effect of TV level for the KS-06 infected pigs, i.e., 9 of 10 DE genes ($p<0.1$) had the same expression pattern with the mRNA-seq results as with NanoString. Overall, nursery pigs infected with a less pathogenic PRRSV isolate, or that have higher tonsil viral level, have a stronger tonsil immune response.

Discussion:

Our work with tonsils from pigs infected with NVSL97 or KS-06 type 2 PRRSV showed limited relationship between serum viral load (VL; 0-21 dpi) with tonsil viral RNA levels. This limited correlation is not unexpected since PRRSV is known to persist in tissues, particularly the tonsil, well past the time when serum viremia has cleared. This grant affirmed that pigs infected with a moderately virulent PRRSV (KS-06) exhibited similar tonsil persistence characteristics to tonsils from pigs infected with virulent NVSL. This is important since KS-06 infected pigs show slower post viral peak serum clearance levels.

Hess et al. (2018) verified that pigs which were classified as having persistent serum viremia levels for NVSL had significantly higher TV than pigs that had cleared serum viremia ($P<0.001$). For KS-06, the direction of the effect was the same but not as significant. An earlier and faster virus clearance was associated with lower TV for both NVSL and KS-06 infected pigs. Animals that had lower levels of serum viremia throughout the trial, or on the day that the tonsils were collected, had lower TV for both NVSL and KS-06 infected pigs. Thus, serum viremia can be an indicator of pigs that are more likely to have persistent PRRSV. Unfortunately, tonsil virus level was lowly heritable for both type 2 PRRSV isolates so it is a trait for which genetic selection is not an option.

Analysis of weekly weight gain did not reveal any associations with TV as has been found for many traits for which persistent PRRSV is not apparent, affirming that persistence remains a silent problem for producers for herd risk of reinfection. Detailed analyses of anti-PRRS antibody responses revealed significant associations with TV in KS-06, but not NVSL, infected pigs. Our data suggest 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 (Hess et al., 2018).

More detailed GWAS revealed several genomic regions that explained a proportion of genetic variance and several strong candidate genes. Genes involved with the host's ability to control viral infiltration/replication and the ability to clear infected cells from tissue may be useful targets for gene expression analyses on tonsil tissue that are underway. These results may provide insight into alternative genes involved in host genetic control of tonsil virus levels and viral persistence (Hess et al., 2018).

Next we probed tonsil RNA for host genes that might be involved in persistence revealed numerous DE genes based on PRRSV isolate or TVclass. Detailed pathway analysis revealed immune gene pathways and a few DE genes that were significantly up-regulated (CXCL10, TBX21, CCL5 and CCL19). Overall, nursery pigs infected with a less pathogenic PRRSV isolate, or that have higher tonsil viral level, have a stronger tonsil immune response (Dong et al. 2018 in preparation).

Collectively, these genome mapping and DE gene results provide new insight into how the pig's immune system is involved in PRRSV persistence. Specifically KS-06 infection may result in less tonsil tissue damage by regulating genes related to cell and tissue morphology whereas NVSL infection stimulated genes that inhibited movement of immune cells and, thus, this isolate may be better at avoiding immune responses by hiding in tonsil tissue. The genes identified by these studies should be useful targets for future efforts to activate cellular immune responses to clear persistent PRRSV infections. These findings contribute to our understanding the mechanisms involved in tonsil pathology induced by PRRSV infection in pigs.

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