RESEARCH REPORT
SWINE HEALTH

Title: Characterization of neutralizing antibody responses to PRRSV and association with host - **NPB#12-120**

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Date Submitted: February 18, 2014

Industry Summary:
Virus neutralization (VN) activity following vaccination or infection with porcine reproductive respiratory syndrome virus (PRRSV) is generally considered to be weak and primarily directed against the virus used for infection; i.e. homologous neutralizing (nAb). However, under some circumstances, virus infection/vaccination can elicit cross-protection against a different isolate, in which case the VN response is called heterologous. For this project we took advantage of the samples generated through the PRRS Host Genetics Consortium (PHGC) to identify pigs that possess unique VN properties. Performing over 1,200 virus neutralization assays on samples from pigs experimentally infected with PRRSV, we identified a small percentage of pigs that produced antibodies capable of neutralizing a wide range of genetically diverse PRRSV isolates, which we termed broadly neutralizing antibody (bnAb). We identified several epitopes in PRRSV structural proteins, M, GP5 and GP3 that are associated with the bnAb response. The Identification of antigens that induce a broadly neutralizing response creates the opportunity to develop the next generation of vaccines. Another aspect of vaccine immunity is host genetics. The second objective of the project was to identify pig genomic markers related to the antibody response to PRRSV. The goal is to identify genes that enhance immunity to PRRS vaccines as a means to breed a "vaccine ready" pig. The VN response of pigs was only weakly heritable. One challenge was the wide variation between VN assays, partly a consequence of the subjective nature of the test. As a result, we developed a new VN assay for experimental samples that incorporates fluorescent viruses and eliminates the subjective nature of the VN assay. We also measured non-neutralizing total antibody (tAb), using a Luminex. Analysis of over 1,400 PHGC samples from the PHGC. A genome wide association study linked tAb with two regions on chromosome 7, both of which map to genes that are directly involved in the host immune response. Overall, the results of this study have direct applications for the next generation of PRRSV vaccines. The identification of regions within PRRSV linked with nAb groups creates the opportunity to design vaccines tailored to induce the production of bnAb. Moreover, identification of genomic markers linked with tAb creates the opportunity to produce pig lines which produce a more favorable immune response to the virus.

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Keywords:
PRRSV
Broadly neutralizing antibody
Neutralizing antibody epitopes
Total antibody
Host Genetics
Genome wide association study (GWAS)

Scientific Abstract:
Virus neutralization (VN) activity during infection with porcine reproductive respiratory syndrome virus (PRRSV) is generally considered to be weak and primarily directed against the virus used for infection; i.e. homologous neutralizing antibody (nAb). Based on results from other viruses, we hypothesize that there are pigs that produce neutralizing antibodies to a broad range of genetically diverse PRRSV isolates, and that the production of nAb is linked to host genetics. Over 1,200 serum samples from experimentally infected pigs were analyzed for VN activity against four different PRRSV isolates. Overall, VN titer was negatively correlated with virus load, and positively correlated with weight gain. VN was only weakly heritibale. Based on the breadth of VN titer, samples were placed into one of the following groups: Group 1, no VN; Group 2, homologous VN against only the isolate used for infection; Group 3, heterologous VN with reactivity against 1 or 2 additional isolates; and Group 4, broad VN with activity against all four isolates. VN escape mutants were prepared by selecting for viruses that were resistant to homologous antibody (Group 2) or bnAb (Group 4). Viruses made resistant to homologous serum *in vitro* remained sensitive to broadly neutralizing serum; and conversely, viruses made resistant to broadly neutralizing serum retained sensitivity to neutralization with homologous sera. Sequencing identified mutations in a short hypervariable domain of GP5 associated with escape from homologous neutralization. For viruses resistant to broadly neutralizing serum, non-conserved amino acid changes were identified in GP3 and GP5, as well as an amino acid deletion in the ectodomain region of M. The results demonstrate that homologous and broadly neutralizing responses are distinct and recognize different epitopes. Furthermore, mutations in GP3, GP5 and M, suggest that these proteins may interact to form a conformational broadly neutralizing epitope. Sera were assayed for total antibody (tAb) using an N-specific fluorescent microsphere immunoassay (FMIA). A genome wide association study identified two regions on SSC 7 linked with tAb. Both markers located to MHC. These regions mapped to genes that are directly involved in the host immune response. The identification of regions within PRRSV linked with nAb groups creates the opportunity to design vaccines tailored to induce the production of bnAb. Another outcome of the study was the development of an improved VN assay for the analysis of experimental samples. The assay involves reacting a single dilution of serum with a standard amount of EGFP-PRRSV, in triplicate. Following one hour incubation, contents are transferred to confluent MARC-145 cells in a 96-well tissue culture plate. Cells are then incubated for 48 hours. The plate is then analyzed for EGFP fluorescence using a FLUOstar Omega plate reader (BMG Labtech). To standardize the assay to traditional methods, a set of samples, with known VN used to make a standard curve. Overall, the EGFP based assay provides a quicker, and more accurate method for measuring PRRSV neutralizing activity compared to traditional methods.

Introduction:
The neutralizing antibody response during PRRSV infection is weak and delayed, and often directed towards only genetically related (homologous) isolates. However, within a population of infected pigs, there is a small subpopulation of pigs that clear virus and are protected. We predict that these pigs produce a unique response that is related to host genes. The current research is an extension of work conducted by the PRRS Host Genetics Consortium (PHGC), an international effort to locate genomic markers and characterize host response pathways during PRRSV infection. Each study involves experimental infection of pigs with PRRSV isolate NVSL 97-7895 (NVSL; PHGC1-9), or KS 06-72109 (KS06; PHGC10-14). For each study, ~200 three week old pigs. As part of the PHGC, several PRRS-related phenotypes are measured until the termination of the study at 42 days after infection. Samples, including serum and oral fluid, are collected and stored for future studies. DNA from each pig is genotyped using a 60 K SNP chip. The objectives of the current project are to measure nAb responses in sera from PHGC pigs and locate the nAb response to markers on the host genome. The project takes advantage of the tremendous amount of leveraging from the PHGC, which has in storage approximately 2,000 samples available for analysis. The overall hypothesis pursued in this project is that the characteristics of nAb produced in response to PRRSV infection are partly determined by host genes

Objectives from original proposal
Objective 1. *Phenotype PHGC pigs according to serum neutralizing antibody (nAb) response*.
The purpose of objective 1 is to screen serum samples from the PHGC for virus neutralizing activity against the homologous virus (challenge isolate), as well as a panel of three genetically diverse PRRSV isolates.
Objective 2. *Locate genomic markers linked to the different nAb responses.* As part of the PHGC, the pigs incorporated in this study have already been genotyped using the Illumina Porcine 60k Beadchip. In addition, other phenotypic data such as average daily gain and virus load have already been measured. The purpose of objective 2 is to determine factors, such as host genetics, associated with the production of nAb.

Materials and Methods:
Sera Sample Source. Sera samples were obtained from experimental challenge studies conducted by the PHGC and partly funded by previous NPB funding. The experimental challenge model is described in Boddicker et al. (7). Serum samples, collected 42 days post-infection, were incorporated into virus neutralization (VN) assays.
Virus neutralization (VN) assay. Isolation and propagation of PRRSV was performed on MARC-145 cells. PRRSV stocks were prepared and tittered end-point titration. Briefly, serial 1:10 dilutions of virus were added to confluent MARC-145 cells in a 96-well disuse culture plate, and the endpoint determined as the last well showing signs of PRRSV induced cytopathic effects (CPE). The final TCID50 was determined by the method of Spearman and Karber (2).
The measurement of virus neutralizing activity in serum (VN) was performed similar to the method described in Rowland et al., (8). Briefly, approximately 200 $TCID_{50}$ of PRRSV, diluted in 100 µl of tissue culture medium, was incubated with 100 µl of 1:2 serial dilutions of pig serum (with an initial dilution of 1:8) in a 96-well tissue culture plate. Following a 1 hr incubation at 37ºC 5%CO_2, the samples were transferred to a 96-well tissue culture plate containing confluent MARC-145 cells. Cells were incubated for 4 days, then examined for CPE. Results were reported as the VN titer. As controls, each assay plate included an internal positive control PRRSV isolate was titrated to confirm the concentration of infectious virus used in the assay. The assay results were accepted if the internal positive control and PRRSV isolate stock concentration was within one dilution of their respective values. For the initial analysis, VR-2332, KS-06, P129 and NVSL were the isolates used in the neutralization assays. Based on the breadth of VN titer, samples were placed into one of the following groups: Group 1, no VN; Group 2, homologous VN against only the isolate used for infection; Group 3, heterologous VN with reactivity against 1 or 2 additional isolates; and Group 4, broad VN with activity against all four isolates.

Selection for VN escape mutants. Viruses that could escape VN escape were prepared by selecting for viruses that were resistant to homologous neutralizing antibody (hnAb) or bnAb. The bnAb serum sample, 16-45, was from a PHGC pig immunized with MLV, then challenged with PRRSV isolate KS 06-062742 (KS62); whereas, the hnAb samples, 16-21 was derived from a pig challenged with only the virus. At the end of the study (71 days past vaccination, 42 days past challenge), large volumes of sera were collected from pigs. Samples were assayed against a total of 10 Type II isolates, as well as a single Type I PRRSV isolate. Selection for mutants was accomplished using a two-step process. The first step was to perform a checkerboard VN titration of virus versus antibody on a 24 well plate of MARC-145 cells. Plates were incubated for 96 hours at 37ºC and 5% CO2, and then examined for the presence of PRRSV induced CPE. The last well in each row showing CPE were pooled. In a second step, the pooled virus was expanded on a T-25 flask of MARC cells and the titration repeated on a 24 well plate until VN activity was no longer present.

Sequencing PRRSV structural genes. RNA was extracted from tissue culture supernatants RT-PCR performed using a One Step RT-PCR kit. The entire 3.1kb region containing the PRRSV structural genes was amplified using conserved forward and reverse primers. Additional reactions were performed, which split the structural gene region into two overlapping fragments. PCR products were gel purified, cloned into the pCR 2.1, and sequenced (ACGT Inc). Sequences were analyzed using CLC Main Workbench 6 software.

Measurement of total antibody response. PRRSV N protein is often incorporated for measuring total PRRSV specific antibody (tAb). Measurement of tAb wasperformed using fluorescent microsphere immunoassay(FMIA) or Luminex. Briefly, approximately 2500 beads, coupled with PRRSSV N protein, were mixed with a 1:400 dilution of serum, in duplicate, on a 96-well plate. The plate was incubated for 30 min with gentle shaking, placed on a magnet, then washed three times with PBS-10% goat serum (PBS-GS). Pig antibody was detected by the addition of biotin-SP-conjugated goat anti-swine secondary antibody and incubated for 30 min. After washing, streptavidin-conjugated phycoerythrin (2 µg/ml in PBS-GS) was added. After a 30 min incubation, the plates were washed and microspheres were resuspended in PBS-GS. Immunoreactivity of samples with microspheres was detected using MAGPIX with Luminex® xPONENT 4.2 software. Each plate included internal positive and negative controls. The results were reported as the mean fluorescence intensity (MFI) of the sample minus MFI of negative control, divided by MFI of the standard positive control minus MFI of negative control.

Statistical and Genome Wide Association Study (GWAS). Analyses were carried out using an animal model in ASReml in order to utilize pedigree information. Variance components were estimated in order to establish the presence of a heritable genetic component to the nAb respone and tAb response. For tAb, the negative control MFI and median sample MFI within each plate

were fitted as covariates to control for plate-to-plate variation. Trial and parity of the sow nested within trial were fitted as additional fixed effects. Pen nested within trial, litter, plate, and the genetic effect of pig were included in the model as random effects. To assess the presence of an association with tAb response, VL, REB, WG, and nAb activity were then each separately added to the model. After identification of a SNP associated with tAb in the GWAS (see below), the genotypes at this SNP were added as a fixed effect to the animal model to assess its association with tAb response, nAb activity, VL, REB, and WG. A genome-wide association study using 60k SNP data was conducted using the BayesB option in GenSel, with the proportion of SNPs with no effect (π) assumed to be 0.99. For this analysis, trial, parity nested within trial, pen nested within trial, and plate were fitted as fixed effects and negative control MFI and median sample MFI were fitted as covariates.

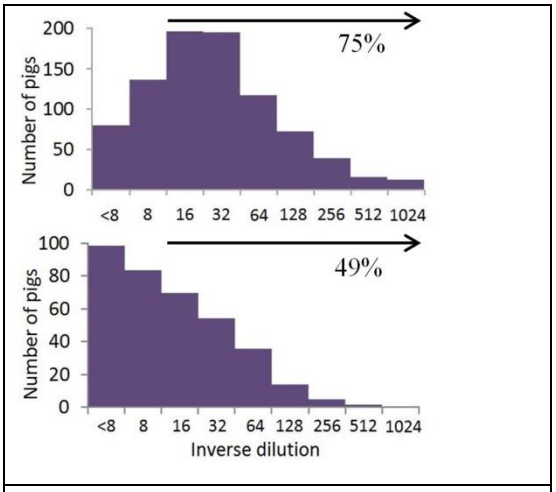

Fig. 1 Distribution of homologous nAb for pigs challenged with NVSL (upper panel) ; n=854) or KS06 (lower panel; n=358). The arrow represents nAb titers which The proportion of each population with biologically relevant titers are given under each arrow.

Results:

Objective 1. Phenotypic classification of PHGC pigs according to serum neutralizing antibody (nAb) response. One goal of objective 1 was to screen serum samples for VN activity against the homologous virus (challenge isolate). Homologous neutralizing assay results are presented in Fig. 1. The distributions are separated for pigs challenged with NVSL and pigs challenged with KS06. VN activity ranged from no detectable activity to VN titers greater than 512. There was also a marked difference between the distributions of the two viruses, with a greater percentage of negative samples in pigs infected with K-S06.

Serum samples were assayed against the homologous isolate as well as a panel of three genetically diverse PRRSV isolates. The results showed that serum samples could be placed into one of four distinct VN categories: Group 1, no measureable VN activity; Group 2, homologous VN against only the isolate used for infection; Group 3, heterologous VN with reactivity against 1 or 2 additional isolates; and Group 4, broad VN with activity against all four isolates. Distributions for pigs in each category are presented in Fig. 2. Similar to the results for homologous nAb, there were obvious differences in the distributions for pigs challenged with NVSL versus KS06. However, there were similar proportions of pigs in the bnAb category for both challenge groups (4.3% for NVSL: 4.5% for KS06).

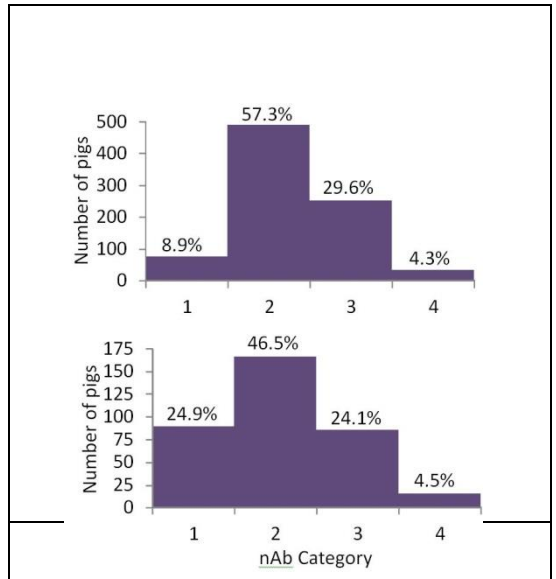

Fig. 2. Distribution of nAb category for pigs challenged with NVSL (panel A; n=854) or KS06 (panel B; n=368). The proportion (%) of the total population is indicated.

Selection for hnAb and bnAb escape mutants. A bnAb serum sample from Group 4, 16-45, showed neutralizing activity against all viruses assayed, including a panel of 10 Type II PRRSV isolates and a Type I isolate. A serum sample from Group 2, 16-2, neutralized only homologous isolate, KS62. For the selection of isolate KS62 with serum 16-45, resistance to neutralization was achieved after 6 iterations of the selection protocol and the new VN resistant virus called K62-4R. In the case of K62 incubated in the presence of 16-21, a single well with CPE surrounded by CPE-negative wells appeared on the first 24 well plate. Medium from this well was collected and the virus expanded on a T-25 flask. Resistance to neutralization with 16-21 was confirmed. The new virus was named KS62-2R.

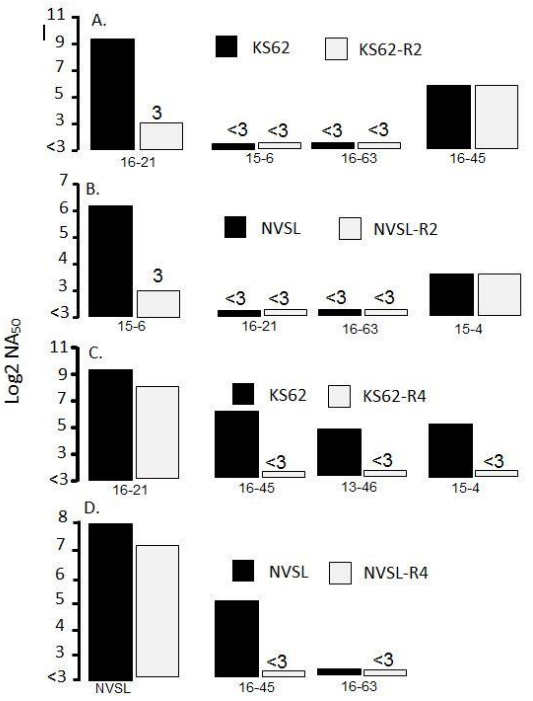

Fig. 4. Neutralizing activity in viruses made resistant to homologous or broadly neutralizing sera.

The neutralization properties of parent and resistant viruses are described in Figure 3. For KS62-2R, the Log2 NA50 against serum 16-21 was reduced by six log2s relative to the parent, KS62 (see panel A). However, KS-62R retained the capacity to be neutralized by broadly neutralizing serum 16-45. As controls, K62 and K62-2R were not neutralized by serum, 16-16, which lacked neutralizing activity, or serum 15-6, a serum which showed homologous activity against another virus, NVSL. Results for KS62-4R are shown in Figure 4C. KS62-4R virus was not neutralized by 16-45 or other sera showing broadly neutralizing sera, such as serum 15-4 and 13-46, which were derived from pigs infected with NVSL and KS06, respectively. K62-4R retained sensitivity to homologous serum, 16-21.

The selection protocol was repeated using a second virus, NVSL. Serum 15-6, with a homologous VN titer of 256, was chosen to select for resistance to homologous VN activity Selection for resistance to broadly neutralizing serum was performed using serum 16-45. VN resistance was achieved after 6 rounds of selection. Compared to NVSL, the new virus, NVSL-2R, showed a 3 log reduction in NA_{50} against serum 15-6, but retained the capacity to be neutralized by broadly neutralizing sera (see Figure 3B). NVSL-4R, obtained after seven rounds of selection with serum 16-45, lost the capacity to be neutralized by 16-45, but retained the capacity to be neutralized by homologous serum, 15-6 (Figure 3D). Together, these results show that viruses can be made resistant to sera possessing homologous and broadly neutralizing activities. Furthermore, these data suggest that homologous and broad VN activities represent separate and distinct properties.

Amino acid changes related to nAb escape. Amino acid changes associated with resistance to serum neutralization were identified by comparing parent and mutant virus peptide sequences within in the structural genes ORFs 2-6. Amino acid changes are summarized in Table 1.

Table 1: Location of amino acid changes in the structural proteins of VN-resistant viruses

	KS62-R2		NVSL-R2		KS62-R4		NVSL-R4		NVSL-P10	
AA	**Nt**	**AA**	**Nt**	**AA**	**Nt**	**AA**	**Nt**	**AA**	**Nt**	**AA**
ORF2a-GP2 (256 amino acids)										
53							T159A	D53E	T159A	D53E
80	T238C	S80P								
91									G291A	M97I
189			C567G	H189Q			C567G	H189Q	C567G	H189Q
237			A711G	I237M						
ORF3-GP3 (254 amino acids)										
30			A88G	T30A						
85			C255A	D85E			C255A	D85E	C255A	D85E
93									T278C	M93T
96							C286T	P96S		
143									T427C	F143L
ORF4-GP4 (178 amino acids)										
129			G385A	V129I			G385A	V129I	G385A	V129I
ORF5b-GP5 (200 amino acids)										
32									A95G	N32S
34									A101T	N34I

56	T167C	L57P			
57			G172A	N57D	
ORF6-M (174 amino acids)					
10					Y10 deletion
70			A208C	K70Q	

Within the 3.1 Kb region there were only two mutations in KS-62 that resulted in changes in amino acids. The first was a serine to proline change at position 80 of GP2, and the second was an asparagine to aspartic acid change at amino acid position 57 of GP5. For NVSL-2R, seven mutations resulted in amino acid changes. In addition to the amino acid changes that occurred during selection with serum, there was the possibility that mutations would appear during the serial passage of virus on MARC-145 cells. Therefore, NVSL was passaged 10 times in MARC-145 cells in the absence of serum and the structural genes sequenced. The results for NVSL-P10 showed three changes, highlighted in grey, that were also found in NVSL-2R; a histidine to phenylalanine at position 189 in GP2; an aspartic acid to glutamic acid at position 85 in GP3, and a valine to isoleucine at position 129. Of the remaining four amino acid changes, the leucine to proline at position 56 in GP5 is non-conserved. This change is immediately adjacent to the asparagine to aspartic acid change at position 56 in KS62-2R. Amino changes for KS62-R2 and NVSL-R2 map to hypervariable region 2 in the ectodomain of GP5 (see illustration in Figure 3).

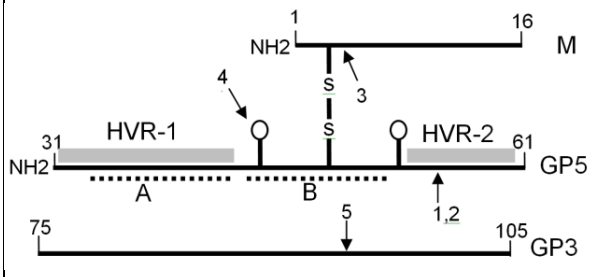

Figure 4. Location of mutations in viruses made resistant to sera with homologous and broad VN activities. The regions cover the predicted ectodomain regions for GP5 and M, as well, as amino acids 75-105 for GP3. Hypervariable regions (HVR) 1 and 2 are identified with grey rectangles. The conserved N-glycosylation sites are identified with circles. The locations of the decoy epitope, A, and conserved epitope, B (Ostrowski et al., 2002) are identified by dotted lines. 1 and 2 identify locations of changes in KS62-R2 and NVSL-R2. 3-6 identify location of changes in bnAb escape mutants.

For KS62-4R, a single amino acid change was observed. In M, the tyrosine at position 10 was completely deleted. The deletion is located near the base of the ectodomain region and immediately adjacent to the conserved cysteine at position 9, which forms the disulfide bond with cysteine at position 48 in GP5 (see Figure 4). Considering that M is the most conserved structural protein, an amino acids deletion was unexpected. For NVSL-R4, there was only a single amino acid change that did not appear in the MARC-145 cell-adapted virus; a non-conserved proline to serine change at amino acid 96 in GP3. We also performed the serum selection protocol for the Lelystad virus. After seven rounds of selection in the presence of broad VN serum, 16-45, a resistant virus was recovered. After sequencing the structural genes of the new virus, LV-4R, only one amino acid change was observed, an A to G at nucleotide 136, which resulted in a asparagine to aspartic acid at amino acid 46. The result was the loss of one of the conserved asparagines, and loss of the corresponding N glycosylation site.

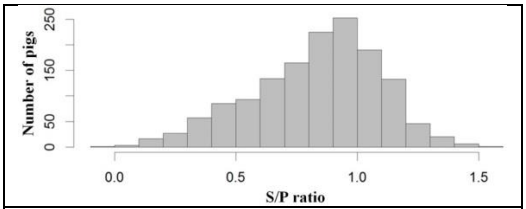

Figure 5. Distribution of Total Antibody Response (S/P ratio).

Measurement of total antibody (tAb). As described in methods, FMIA incorporating the N-protein of PRRSV was used to measure the total antibody response. A distribution of the tAb response of 1,444 sera samples is shown in Figure 6. TheS/P ratios ranged from <0.1 to 1.6. The mean tAb S/P ratio was 0.84+/-0.26.

Objective 2. Locate genomic markers linked to the different antibody responses.

Table 2. Factors associated with variation in the homologous nAb response.

	Variance (%)	Standard Error (%)
Animal (h^2)	2.8	5.0
Plate	9.3	4.4
Pen	3.3	1.9
Litter	0.13	0.03

Estimation of heritability (h^2) and other factors associated with homologous nAb. The goal of objective 2 was to determine whether genetic factors of the host are linked with the nAb response. As summarized in Table 2, heritability explains 2.8+/-5.0% of variation in the nAb response. The greatest factor for variation was assay plate variation (9.3+/-4.4%). Overall, results indicate that there is too much variation in the assay to determine whether the homologous nAb response is heritable. However, VN titer was negatively associated with virus load and positively associated with weight gain (0-21 dpi).

Estimation of heritability and other factors associated with the tAb response. Even total antibody (tAb) is non-neutralizing, there was a negative associated between tAb with viral load and weight gain, and a positive association with the VN titer. In a genome-wide association study using 60k SNP data, the proportion of markers with no effect (π) was estimated to be 0.99 using BayesCPi in GenSel. The GWAS discovered two 60k windows on chromosome 7 associated with tAb response (see Figure 5). The first marker mapped to a region linked with MHC I. The second mapped to a region associated with MHC class II.

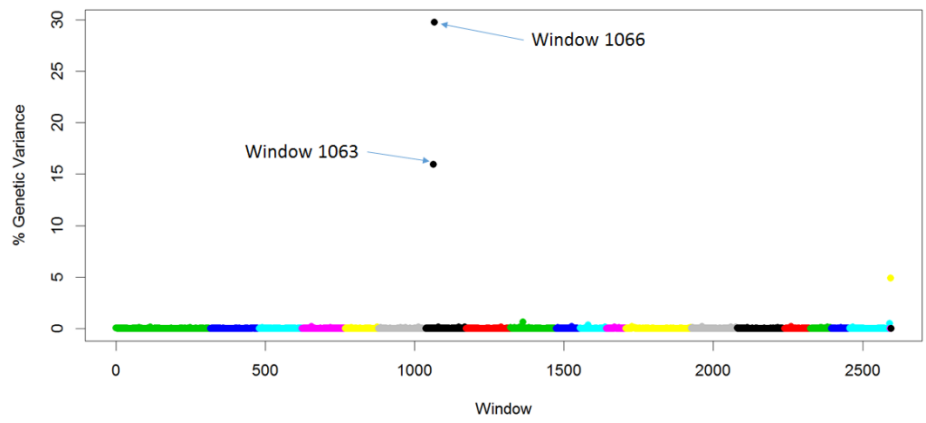

Figure 5. Manhattan plot of genome wide association study (GWAS) of the total antibody response . GWAS was performed using method Bayes-B with =0.99, showing the proportion of variance in genomic estimated breeding values that is explained by each window.

IX.

Discussion:

The incorporation of sera samples that were previously collected as part of the PHGC allowed for the analysis of the role of host genetics in the antibody response. The results provide strong evidence for the significance of nAb during early infection with PRRSV, including negative associations with viremia and weight gain. The results for homologous nAb demonstrate that the PRRSV challenge isolate plays a role in the nAb response. Even though we could not correlate VN activity with genomic markers, the variation within the classical VN assay prompted the development of a novel VN assay that incorporates EGFP-tagged PRRSV. The assay

involves reacting a single dilution of serum with a standard amount of EGFP-PRRSV, in triplicate. Following one hour incubation, contents are transferred to confluent MARC-145 cells in a 96-well tissue culture plate. Cells are then incubated for 48 hours. The plate is then analyzed for EGFP fluorescence using a FLUOstar Omega plate reader (BMG Labtech). To standardize the assay to traditional methods, a set of samples, with known VN used to make a standard curve. The unique aspect of this assay is that it incorporates a single dilution of serum, which reduces variation caused by performing serial dilutions in traditional assays. Preliminary assay results indicate the novel assay is a faster, more accurate means for measuring the nAb response.

Pigs were placed into four distinct groups based on their neutralizing activity to four genetically diverse Type II isolates. While the majority of pigs with VN activity were placed into Group 2 (homologous nAb) we identified a small population of pigs that show neutralizing activity to all four isolates (group 4; bnAb). There was a similar proportion of Ggroup 4 pigs regardless of challenge isolate (NVSL or KS06). At this time the role of host genetics in the production of bnAb is unknown. Surprisingly, the *in vitro* neutralizing activity of certain Group 4 (bnAb) samples was extended to Type I PRRSV isolates, which only share ~65% nucleotide identity at the ORF5 nucleotide level with Type II isolates. Overall, the different groups of nAb responders suggested the presence of group specific epitopes within the PRRSV proteome.

Assaying such a large population of pigs allowed for the selection of serum samples which represent extremes in terms of PRRSV nAb responders; i.e., serum samples used for selection of homologous nAb escape mutants (Group 2 sera) had unusually high titers (≥256) that are specific for a single isolate. The sample used for selection of the bnAb escape mutants (Group 4 sera) was capable of neutralizing at least 10 Type II isolates and a Type I isolate. The patterns of amino acid changes for escape mutants further confirm the differences between homologous and broadly neutralizing activities. The 2R mutants both possessed mutations in GP5 HV2 region (see Figure 4). The 4R mutants showed a more complex pattern. Non-conserved amino acid changes in GP3 were found for both NVSL-4R and KS62-4R viruses. The results provide strong evidence of group-specific epitopes within the PRRSV proteome. These results may provide an explanation for discrepancies in the literature describing a variety of neutralizing epitopes and stress the important of characterizing the specific nature of the neutralizing response for antibody reagents utilized in experimental studies of PRRSV immunity.

Interestingly, the tAb response was linked with several desirable factors related to PRRS. Increased tAb was negatively correlated with viremia and positively correlated with weight gain and the production of nAb. GWAS identified two regions linked with the production of tAb. Both markers mapped to regions that play a crucial role in the host's ability to fight infection and harbors many genes associated with immune response.

In conclusion, the results of this study have direct applications for the next generation of PRRSV vaccines. The identification of regions within the PRRSV proteome linked with nAb groups creates the opportunity to design vaccines tailored to induce the production of bnAb. Moreover, identification of genomic markers linked with tAb creates the opportunity to produce pig lines which produce a more favorable immune response to vaccination.

Presentations referencing this work:

Trible, B.R., Popescu, L.N., Wang, Y., Kerrigan, M.A., Rowland, R.R.R. Characterizing the antibody response following experimental PRRSV infection in a large population of pigs. November 29-30, 2012. International PRRS symposium, Kansas City MO.

Hess, A., Trible, B.R., Boddicker, N.J., Rowland, R.R.R., Lunney, J.K., Carpenter, S., Dekkers, J.C.M., Factors influencing neutralizing antibody response to experimental infection of piglets with porcine reproductive and respiratory syndrome virus. November 29-30, 2012. International PRRS symposium, Kansas City MO.

Trible, B.R., Popescu, L.N., Kerrigan, M.A., Rowland, R.R.R. Characterizing the neutralizing antibody (nAb) response following infection with porcine reproductive and respiratory syndrome virus. March 5, 2013. Phi Zeta Research Day, Manhattan KS.

Hess, A., Trible, B.R., Boddicker, N.J., Rowland, R.R.R., Lunney, J.K., Carpenter, S., Dekkers, J.C.M. Factors associated with neutralizing antibody response in piglets after experimental infection with the porcine

reproductive and respiratory syndrome virus. March 11-13, 2013. ASAS Midwestern Section/ADSA Midwestern Branch Annual Meeting, Des Moines IA.

Trible, B.R., Popescu, L.N., Wang, Y., Kerrigan, M.A., Rowland, R.R.R. Evaluating the Antibody Response Following Experimental Infection with Porcine Reproductive and Respiratory Syndrome Virus (PRRSV). April 4-6, 2013. Annual Meeting of the American Society for Microbiology-Missouri Valley Branch, Tulsa OK.

Hess, A., Trible, B.R., Boddicker, N.J., Rowland, R.R.R., Lunney, J.K., Carpenter, S., Dekkers, J.C.M.,Identification of a major QTL associated with total antibody response in piglets experimentally infected with porcine reproductive and respiratory syndrome virus. July 8-12, 2013. ADSA ASAS Joint Annual Meetings, Indianapolis, IN.

Manuscripts in preparation referencing this work:

Trible, B.R., Monday, N., Rowland, RRR., Homologous and broadly neutralizing antibody responses following infection with porcine reproductive and respiratory syndrome virus (PRRSV) recognize distinct epitopes, Manuscript in preparation.

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