RESEARCH REPORT
SWINE HEALTH

Title: Project Cross-Neutralizing Antibodies as Predictors of Protection - **NPB #14-213**

Investigators: Sally Robinson/Michael Murtaugh

Institution: University of Minnesota

Date Submitted: 15 December 2017

Industry Summary:
Neutralizing antibodies are useful predictors of immunity to viral infections, with many examples in animals and humans. In pigs and PRRSV, presence of neutralizing antibodies was shown to predict protection only for re-exposure to the same virus. Since PRRSV is enormously diverse, immunity needs to be cross-protective, but cross-neutralizing antibodies were not widely known to occur. Here, we showed widespread presence of high titers of antibodies with broad cross-neutralizing activity against PRRSV in sow herds. Passive transfer of neutralizing antibodies specifically provided cross-protection, with the amount of protection proportional to the level of neutralizing activity transferred. Herd-level prediction of protection in the field based on broadly neutralizing activity in serum was confounded by high variability among animals in the quality and characteristics of the neutralizing activity. An alternative approach to predict protection by assessing immune memory was initiated by development of a PRRSV-specific B cell tetramer. The reagent is designed to identify memory B cells that respond to PRRSV infection by producing antibodies, and is the technical basis by which vaccines work. Production of the tetramer will facilitate studies to assess its potential for predicting the protection status of pigs in a blood sample without a need for challenge studies.

Contact information: Michael Murtaugh, Department of Veterinary and Biomedical Sciences, University of Minnesota, 1971 Commonwealth Avenue, St. Paul, MN 55108; office phone 612 625 6735; email murta001@umn.edu.

Keywords: PRRS, neutralizing antibodies, swine, cross-protection, immunology, animal health, swine health, virology, B cells

Scientific Abstract:
Vaccine control and prevention of porcine reproductive and respiratory syndrome virus (PRRSV) is difficult to achieve. Neutralizing antibodies, which are useful predictors of immunity against many viral infections, have an uncertain role against PRRSV. Presence of neutralizing antibodies was shown to predict protection only for re-exposure to the same virus. But, since PRRSV is enormously diverse, immunity needs to be cross-protective. In this project, we showed widespread presence of high titers of antibodies with broad cross-neutralizing activity against PRRSV in sow herds. Passive administration of purified immunoglobulins with neutralizing antibodies reduced PRRSV2 infection by up to 96%, and PRRSV1 infection by up to 87%. The amount of protection was proportional to the level of neutralizing activity

transferred. The immune competence of passive immunoglobulin transfer was associated specifically with antibody neutralizing activity since PRRSV-immune serum lacking neutralizing activity was not protective. Herd-level prediction of protection in the field based on broadly neutralizing activity in serum was confounded by high variability among animals in the quality and characteristics of the neutralizing activity. Alternative approaches to predict protection by assessing immune memory were investigated by development of fluorescently labeled PRRSV virions and a PRRSV-specific memory B cell tetramer. Memory B cells are an immunological basis by which vaccines work. The B cell reagent is designed to identify memory B cells that would respond to PRRSV infection by producing protective antibodies. Production of the tetramer will facilitate studies to assess its potential for predicting the protection status of pigs in a blood sample without a need for challenge studies. The results show that immunological approaches aimed at induction of broadly neutralizing antibodies may substantially enhance immune protection against PRRSV. The findings further show that naturally occurring viral isolates are able to induce protective humoral immunity against unrelated PRRSV challenge, thus removing a major conceptual barrier to vaccine development.

Introduction:

PRRSV is incredibly challenging to comprehend and control immunologically. There are inconsistencies in our knowledge of PRRSV immunity and a lack of understanding of what constitutes a protective immune response (reviewed in (1)). Factors such as extensive viral genetic and antigenic diversity, large populations of intensively managed animals and swine-dense regions which facilitate spread of virus, persistence of virus for extended periods in pigs where it can be transmitted to susceptible animals, and inability of current vaccines to prevent or control infection in populations all contribute to the problem of PRRSV spread and persistence. Since PRRSV is rapidly changing and enormously diverse, immunity needs to be cross-protective for effective control or elimination of viruses in the field. In reality, failure to achieve broad cross-protection is the root cause of herd immunity failure, and the lack of effective predictors of cross-protection is a critical weakness in assessing immune intervention and prevention strategies.

Pigs generate a well-documented humoral immune response against PRRSV (2-4), however the role of neutralizing antibodies in protection is not clear. Homologous protection against PRRSV from passive transfer experiments demonstrates proof of principle that serum neutralizing antibodies can protect against PRRSV infection under optimal, controlled conditions (5, 6). However the slow kinetics, small magnitude and limited breadth of protection observed from the neutralizing antibody response suggested that it is not an important predictor, nor provides a realistic approach for achieving widespread protection against PRRSV (reviewed in (1, 7)).

Nevertheless, it has long been recognized that neutralizing antibodies are a critical part of the immune armory for defense against viruses, and are the mechanism by which many effective vaccines work to protect against viral infections (8). Over the last 5 years, significant breakthroughs have been made in the knowledge and understanding of naturally-occurring, potent and broadly-neutralizing antibodies against other genetically diverse RNA viruses such as HIV and influenza. Factors attributed as being most critical for such breakthroughs are tools such as high-throughput serum neutralization assays and techniques for identification of memory B cells (reviewed in (9)). We have begun to apply similar unbiased approaches to re-evaluate neutralizing antibodies to PRRSV in pigs. We discovered that high-titer cross-neutralizing antibodies exist to PRRSV in the field. We determined the role of cross-neutralizing antibodies in sow herd protection against virulent field isolates, and developed a B cell tetramer reagent with the potential to predict protection status with a simple blood sample.

Objectives:

1. Determine if cross-neutralizing antibodies protect pigs against diverse PRRSV strains
2. Evaluate the level of cross-neutralizing antibodies necessary for protection
3. Examine if cross-neutralizing antibody titers predict protection in field settings

4. Develop reagents and assays for prediction of PRRS immune memory

Materials & Methods:

Sow blood collection

Serum samples from sows of mixed parity were collected at farms in serum separator tubes, and shipped to the University of Minnesota. Serum was collected and stored at -20°C. Herd history was obtained as it pertained to PRRSV outbreaks, along with health records for the individual animals tested. For large serum volumes, sows were transported to the University of Minnesota and euthanized. The study was approved by and conducted under the guidelines of the University of Minnesota Institutional Animal Care and Use Committee, protocol #1402-31319A.

Cells and PRRSV isolates

MARC 145 cells were cultured in MEM medium (Gibco, Grand Island NY) supplemented with sodium bicarbonate, non-essential amino acids, HEPES buffer (Sigma, St. Louis MO), gentamycin sulfate (Cellgro Mediatech, Manassas VA) and 10% FBS (Sigma, St. Louis MO). PRRSV isolates VR2332 (GenBank U87392), MN184 (EF442777), and SD 01-08 (AY395080) (also called SDEU) were propagated in MARC 145 cells. Infectious titers ($TCID_{50}$) were determined by cytopathic effect on MARC 145 cells and calculated by the Reed & Muench method. Field isolates were isolated in culture and used as described in the Results.

PRRSV ELISA

Microtiter plates were coated with 100 ng PRRSV nucleocapsid (N) per well, and serum samples were diluted 1:50 in 5% non-fat dry milk (NFDM) in phosphate-buffered saline containing 0.05% Tween 20 (PBST). Detection antibody, horseradish peroxidase-conjugated goat anti-pig IgG (Bethyl Laboratories Inc. Montgomery TX), was used at a 1:100,000 dilution in 5% NFDM in PBST. Immune complexes were revealed by peroxidation of 3,3',5,5'-tetramethylbenzidine (TMB) substrate (KPL, Gaithersburg MD) and stopped with 1 M phosphoric acid. Absorbance was read at 450 nm. Controls for the assay included known PRRSV-positive and negative sera.

ELISA-based PRRSV serum neutralization (SN) assay

SN assays were performed as described by Robinson et al. (18) with the following modifications. Two percent FBS was added to MEM culture media for serum sample and virus dilutions to improve adherence of the MARC 145 cell monolayer. All wells in each plate were scored by light microscopy to evaluate the condition of the monolayer at the conclusion of the SN assay.

SN assays were performed across a range of multiplicity of infection (MOI) from 4 (128,000 viral $TCID_{50}$/well) with 2 fold dilutions down to an MOI of 0.008 (250 $TCID_{50}$/well). Subsequent assays were performed at an MOI of 0.5, equivalent to 16,000 viral $TCID_{50}$/well. To detect neutralizing activity against the genotype 1 SD 01-08 (SDEU) strain, cells were incubated for 48 h after infection since this strain replicated more slowly in MARC 145 cells than did genotype 2 viruses. 50% SN titer was determined by the reciprocal of the serum dilution when the inhibition of infection reached 50%.

Immunoglobulin (Ig) isolation from serum

Antibodies were isolated from PRRSV-immune serum of a sow with high cross-neutralizing activity and a PRRSV-immune sow negative for cross-neutralizing activity. PRRSV-negative control Ig was isolated from serum pooled from three naïve pigs. Total immunoglobulins were isolated by sequential precipitation with caprylic (octanoic) acid and ammonium sulfate. Briefly, serum was centrifuged, filtered and heat-treated at 56°C for 30 min. An equal volume of acetate buffer (60 mM, pH 4.0) was added and pH adjusted to 4.5. Caprylic acid (Sigma-Aldrich, St. Louis, MO) was added 1:15 w/w and stirred for 30 min at room temperature. The mixture was then centrifuged at 10,000 x g for 30 min and the supernatant containing the Ig

fraction was poured off and passed through a 70 μm mesh filter. Tris-HCl was used to adjust the pH to 7.4.
Saturated ammonium sulfate (Sigma-Aldrich, St. Louis, MO) was slowly added to a final concentration of 40%, stirred at 4°C overnight, and centrifuged. Pellets were resuspended in PBS. Purified Ig were dialyzed in PBS in Spectra/Por 2 membranes (Spectrum Laboratories, Rancho Dominquez, CA).

Immunoglobulin concentrations were measured using a porcine IgG ELISA quantitation kit (Bethyl Laboratories, Inc., Montgomery TX). Antibody, standards and sample dilutions were in 5% NFDM in PBST and the stop solution was 1M phosphoric acid. Protein samples with and without 5% β-mercaptoethanol to reduce disulfide bonds were subjected to denaturing sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) in 4 to 20% gradient Mini-Protean Precast Gels (Bio-Rad Laboratories, Hercules CA) to evaluate purity. Bands were visualized with Imperial protein stain (Thermo Scientific, Rockford, IL), and destained in deionized water.

Passive transfer experimental design
Thirty-two weaned pigs approximately 3 weeks of age were sourced from a PRRSV-negative breeding herd. Pigs were weighed and randomly assigned to seven groups upon arrival at the University of Minnesota College of Veterinary Medicine animal isolation facility. On the day prior to viral challenge pigs were anesthetized with Telazol in xylazine by intramuscular injection. Blood was collected into serum separator tubes to test for PRRSV by RT-PCR and for SN. Purified neutralizing or non-neutralizing PRRSV immune Ig or PRRSV-negative Ig was injected via a 14 gauge catheter into the peritoneal cavity.

Twenty-four hours later, blood was collected from all pigs to evaluate the PRRSV-neutralizing activity in serum at time of viral challenge (day 0 with respect to challenge). At this time, pigs in groups 2-7 were infected by intramuscular inoculation in the neck of 1×10^5 $TCID_{50}$ of PRRSV MN184 or SDEU. Uninfected animals were sham-inoculated with the same volume of tissue culture media intramuscularly. Animals were evaluated daily thereafter for clinical signs of illness, including fever, coughing/sneezing, lethargy, and anorexia. Blood samples were collected from all animals on days 3, 7, 10, and 14 after viral challenge. At 21 days afte by intramuscular injection of Telazol/xylazine as described above. Blood was collected and euthanasia performed by intravenous barbiturate overdose. Necropsies were performed on all animals for evaluation of gross lung morphology and collection of lung, spleen, tonsil and lymph nodes into RNA stabilization reagent (RNAlater, Qiagen). Tissues were kept at 4 °C overnight and frozen to -20°C. Blood samples were collected in serum separator tubes, and were centrifuged to collect serum which was frozen at -20°C for further analysis.

Viremia was compared between the groups of interest to determine whether immune Igs were protective against infection, and whether they provided cross-protection against different virus strains compared to animals receiving control Igs. The number of pigs needed per group was estimated by a power analysis based on mean level of viremia and variation between individuals estimated from previous studies. Calculations were based on a 2 log reduction in viremia, (from 10^6 RNA copies/ml of serum to 10^4 with $\pm 5 \times 10^3$ standard deviation in the control group, and $\pm 5 \times 10^5$ in the Ig groups). To achieve 95% power at 1% alpha level, we calculated that each group would require a minimum of 3 animals. To ensure that valid results would be obtained in case of animals requiring early endpoints, or wider variation between individuals, the study was conducted with 4 or 5 animals per group. In addition, the design allowed for combining groups for statistical analysis based on Ig status. Details of treatment groups is shown in Table 1.

Table 1. Treatment groups for passive transfer of immunoglobulins.

Group	Category	Viral Challenge	Treatment (number of pigs)
1	Uninfected negative control	None	Neutralizing Ig (n=2) Non-neutralizing Ig (n=2)
2	PRRSV immune neutralizing Ig	SDEU	Neutralizing Ig (n=5)
3	PRRS immune non-neutralizing Ig	SDEU	Non-neutralizing (n=5)
4	Infection positive control	SDEU	Negative Ig (n=2) No Ig (n=2)
5	PRRSV immune neutralizing Ig	MN184	Neutralizing Ig (n=5)
6	PRRS immune non-neutralizing Ig	MN184	Non-neutralizing (n=5)
7	Infection positive control	MN184	Negative Ig (n=2) No Ig (n=2)

Viral RNA isolation and reverse transcription quantitative PCR (RT-qPCR)
Viral RNA was isolated from serum using a QIAmp Viral RNA Mini kit (Qiagen, Valencia CA), eluted into 50 µl of RNase-free water and stored at -80 °C. Complementary cDNA was synthesized with High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Carlsbad CA) with random hexamer primers. Primer sequences TCA ACT GTC CCA GTT GCT GG (forward) and AAA TGT GGC TTC TCA GGC TTT T (reverse) amplified a 96 bp ORF7 fragment for genotype 1 PRRSV. Primer sequences AAC CAC GCA TTT GTC GTC (forward) and TGG CAC AGC TGA TTG ACT GG (reverse) amplified a 198 bp ORF6-7 fragment for genotype 2 PRRSV. PCR reactions in 20 µl, contained 5 µl of cDNA, 10 µl of SYBR Green PCR Master Mix (PerfeCTa SYBR Green FastMix, Quanta Biosciences, Gaithersburg MD) and 200 ng of each primer. Reactions were run in a Stratagene Mx3000P thermal cycler (Agilent Technologies, Inc. Santa Clara CA), with activation at 95°C for 1 minute, 35 cycles of denaturation at 95°C for 3 seconds and annealing/extension at 60°C for 25 seconds, followed by a dissociation step. All samples were run in duplicate.

PRRSV was quantified using the standard curve method with gel purified PCR products from MN184 and SDEU prepared as described above. The cDNA concentration was determined by spectrophotometry and number of copies of the template was calculated. Serial 10-fold dilutions were used to construct a standard curve, ranging from $>10^6$ to <1 copy per reaction. Identification of positive samples was determined by the presence of a C_t value and thermal denaturation analysis. Negative controls without cDNA were used in all PCR plates. Statistical analysis for differences in mean levels of viremia between groups at each time point was conducted by the Wilcoxon Rank Sum Test (Mann-Whitney U Test). Conservative p values of ≤0.05 were considered significant. Area under the curve analysis to determine percent reduction in total viremia was performed in GraphPad Prism (GraphPad Software, La Jolla, CA).

Identification and synthesis of conserved ectodomain peptides
Whole genome sequences from 58 unique PRRSV strains, including genotypes 1 and 2 were obtained from GenBank. Sequences of ORF2a, ORF2b, ORF3 and ORF4, representing GP2, E, GP3, and GP4, respectively, were concatenated for each PRRSV strain, aligned using Geneious R6 version 6.1.7 (Biomatters Ltd., Auckland, New Zealand), translated to identify regions of amino acid conservation, and predicted ectodomain regions were identified within the ectodomain fragments. Six linear peptides, one each from GP2 and E, and two each from GP3 and GP4, were synthesized by GenScript (Piscataway, NJ). Dilutions from 1 mg/ml peptide stocks were made in MEM containing 2% FBS.

Cellular receptor competition assay
Each of the 6 peptides, a mixture of the 6 peptides, and vehicle only controls were diluted to 10 µg/ml in MEM containing 2% FBS. Five-fold serial dilutions of the peptides were made in

MEM 2% FBS to 0.016 μg/ml. Dilutions and control samples were added to duplicate wells of MARC 145 cells and incubated at 37°C for 1 hour. PRRSV strain VR2332 was added at an MOI of 0.5 in the presence of peptides and inc ubated at 37°C for an hour. The peptide/virus mixture was decanted, cells were washed once with warmed PBS, and complete MEM with 10% FBS was added for an additional 23 hour incubation. Cells were washed with PBS and processed for the PRRSV neutralization assay.

Neutralizing serum competition assay
Peptides (each alone and a mixture of all 6, at a final concentration of 10 μg/ml and 1 μg/ml) were mixed with serum (1/32 dilution of PRRSV neutralizing serum, the same Ig concentration of negative serum, or no serum control) for 1 hour at 37°C. PRRSV VR2332 was added to an MOI of 0.5 and incubated for one hour, and the mixtures were transferred to cells. Plates, also containing untreated control monolayers, were incubated for 1 hour, and washed with PBS. After 23 hour, cells were washed with PBS, fixed, and permeabilized and processed as described to quantify PRRSV infection in cells.

Results:
1. Determine if cross-neutralizing antibodies protect pigs against diverse PRRSV strains
A passive transfer study was designed to address the hypothesis that cross-neutralizing PRRSV immunoglobulins protect pigs against infection from diverse strains of PRRSV. Purified immunoglobulins were passively transferred to naïve pigs, subsequently challenged with either a genotype 1 or 2 PRRSV, and level of viremia monitored compared with positive and negative controls.

Antibodies were purified from serum of a sow with high cross-neutralizing activity and one with low neutralizing activity. In addition, antibodies were purified from a PRRSV-negative animal (from an unrelated herd). After peritoneal administration of the antibodies, animals were monitored clinically following viral challenge. Infection resulted in subclinical infection with both SDEU and MN184 virus strains, as pigs had no clinical signs of respiratory disease such as increased respiratory rate, coughing, sneezing, fever or lethargy. There were no differences in average daily weight gain between groups over the study duration.

Neutralizing activity was present in the serum of pigs that received neutralizing Igs at the time of viral challenge (24 hours post Ig administration), but not in those receiving non-neutralizing, PRRSV negative or no Igs. Pigs that received neutralizing Igs had significantly lower levels of SDEU and MN184 viremia compared with pigs receiving PRRSV negative Ig or no Ig. Overall levels of viremia were higher for MN184 infected pigs than SDEU. There was no difference in levels of viremia between pigs receiving non-neutralizing antibodies and negative or no antibodies for either virus. Reduction in total viral load attributable to neutralizing Ig administration, determined by area under the curve, was 87% for SDEU and 96% for MN184. The in vitro neutralizing activity of sow 7 serum from which the neutralizing antibodies were isolated was not appreciably different between that against SDEU and MN184.

The results showed that cross-neutralizing antibodies from commercial sows mediated cross-protection against diverse PRRSV strains, including across genotypes.

2. Evaluate the level of cross-neutralizing antibodies necessary for protection
In the passive transfer experiment, recipient pigs were infused with 242 mg IgG to achieve an estimated circulating concentration of 0.52 mg/ml of blood. The calculated PRRSV infection inhibition activity, taken from tests on concentrated Ig, was 80% for neutralizing Ig. *In vivo* viral neutralizing activity 24 hours after Ig administration was present only in pigs that received neutralizing Ig. On day 1 when viral challenge occurred, an average 28% inhibitory activity was present in serum diluted ¼ that decreased with further dilution. There was no detectable neutralizing activity present in pigs receiving immune Ig without neutralizing activity, non-immune Ig, or no Ig.

Administration of MN184 resulted in viremic infection that peaked at day 7, remained high through day 14, and showed equivalent kinetics in the control and non-neutralizing Ig-

treated animals. Treatment with neutralizing Ig reduced peak viral loads 95 and 96% at days 7 and 14 compared to pigs not receiving neutralizing Ig. Significant reduction was present at the first sampling time and was maintained through day 14. Administration of SDEU resulted in viremic infection with identical kinetics in the control and non-neutralizing Ig-treated animals that peaked at day 14. Pigs treated with neutralizing Ig had viral loads reduced by up to 87% on day 10. Thus, the results of this experiment showed that passively administered immunoglobulins that has 20 to 40% neutralizing activity against PRRSV strain VR2332 gave significant protection against virulent challenge with PRRSV2 strain MN184 and PRRSV1 strain SD 01-08.

3. Examine if cross-neutralizing antibody titers predict protection in field settings

Figure 1 shows a small sample of our analyses of PRRSV broadly neutralizing activity in serum of commercial sow herds. Serum samples from five sows, of various parities, in a herd in North Carolina with endemic PRRSV were obtained following a PRRSV 1-7-4 outbreak. Neutralizing activity against five lab and field virus isolates was analyzed as shown below. Serum samples were serially diluted to a limit of 1:256 and the 50% titer was determined. Results shown as >256 mean that neutralizing activity was greater than 50% at this final dilution. It is apparent from the data in Figure 1 that there is individual animal variation in the neutralizing response to the same virus, and that neutralizing activity is highly variable against different viruses. As a result, the neutralizing activity in one serum cannot be used to predict the neutralizing activity in another serum. Likewise, the neutralizing activity against one virus cannot be used to predict the neutralizing activity against another virus.

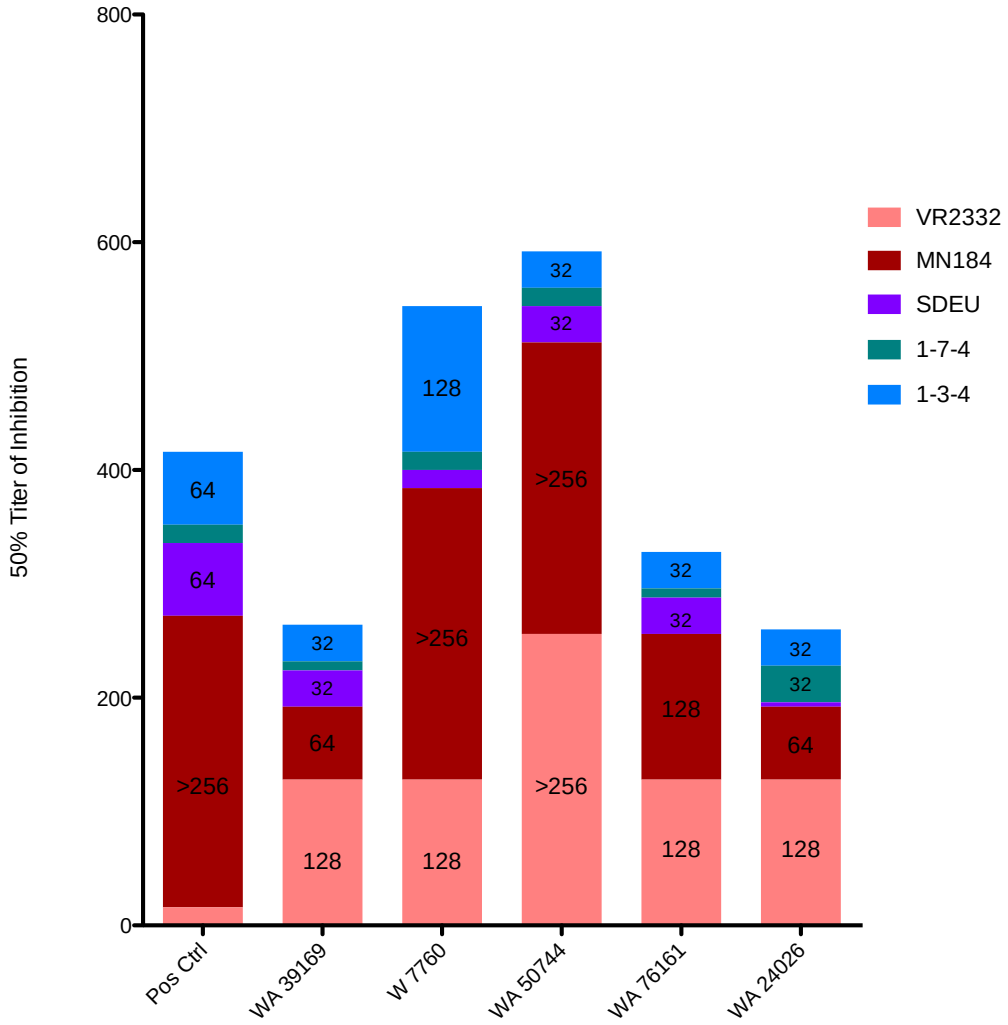
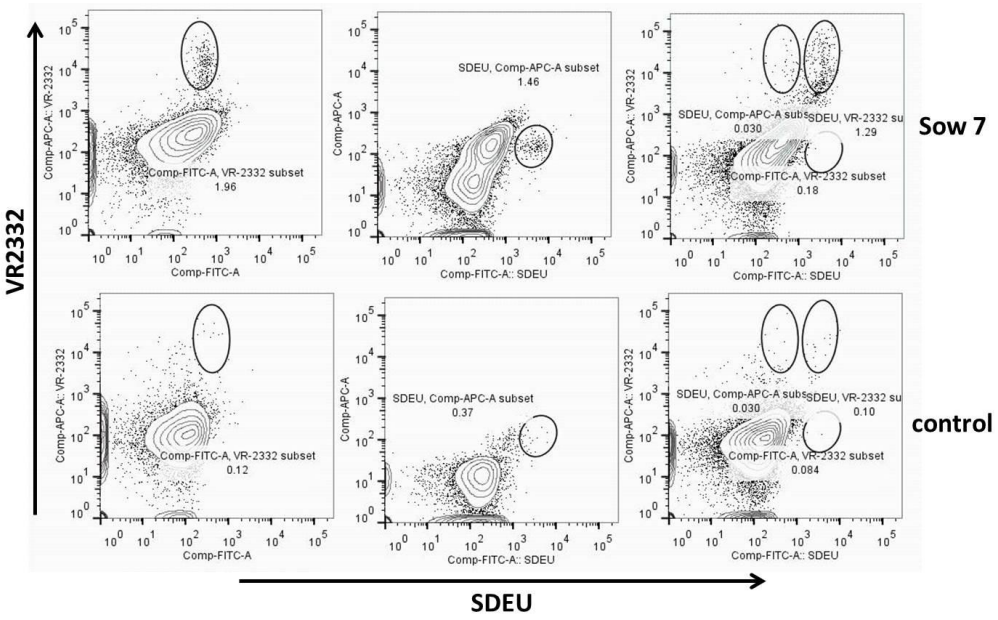


Figure 1. Cumulative 50% neutralizing titers against 5 diverse PRRSV strains for 5 sows from a commercial herd with endemic PRRS, and one positive control.

In terms of predicting protection at a herd level, the challenge is further exacerbated by the inability to know what kind of virus will be entering the herd. We conclude, therefore, that cross-neutralizing antibody titers are unlikely to serve as reliable predictors of protection.

4. Develop reagents and assays for prediction of PRRS immune memory

Two sets of reagents that facilitate analysis and understanding of PRRS immune memory were developed: purified and fluorescently labeled PRRSV virions, and PRRSV antigen-specific memory B cell tetramers. Figure 2 shows that memory B cells from a sow with broadly neutralizing antibodies (sow 7) bind to both Alexa 488-labeled PRRSV1 (SDEU) and Alexa 647-labeled PRRSV2 (VR2332). Fluorescently labeled viruses have the potential to identify memory B cells with neutralizing antibodies since the antibodies must bind viruses in order to effect neutralization.



% CD21+ cells	VR2332+	SDEU+	cross-reactive
Sow 7	1.96	1.46	1.29
PRRSV-neg	0.12	0.37	0.10

Figure 2. Flow cytometry of splenocytes isolated from PRRSV immune, broadly neutralizing sow 7 compared with a PRRSV-naïve control animal. Cells were stained with a porcine anti-CD21 antibody and incubated with fluorescently labeled VR2332-Alexa 647 and/or SDEU-Alexa 488. The left panel has VR2332 only, middle SDEU only, and right both viruses. All panels were gated on CD21+ B cell events. The table shows the % of CD21+ cells that bind each virus, and those that are cross-reactive.

A memory B cell tetramer for specific characterization of PRRSV B cell and antibody immunity was produced by targeting nsp7. Nsp7 is a nonstructural protein that has distinct advantages due to its conserved protein sequence. Nsp7 from one PRRSV2 reacts broadly with antibodies produced in all pigs against all PRRSV2, enabling extensive investigations in commercial herds. Also, in contrast to many PRRSV purified, recombinant proteins, nsp7 is readily soluble in physiological buffers at neutral pH, which is a requirement for tetramer construction. Briefly, we expressed nsp7 protein in E. coli and biotinylated it at a 1:1 ratio. Western blotting confirmed appropriate biotinylation and tetramer formation between nsp7 and fluorescent streptavidin. The detailed methods for PRRSV nsp7-tetramer production, characterization, and validation are published: Rahe MC, Gustafson K, Murtaugh MP. 2017. B cell tetramer development for veterinary vaccinology. Viral Immunol. doi: 10.1089/vim.2017.0073.

Discussion:

The results of this project show that broadly neutralizing antibodies against PRRSV are frequently produced in sows and that they provide cross-protection against heterologous viral infection. These findings provide a rational basis for further investigation of the nature of broadly neutralizing antibodies and the viral target or targets of neutralization. Structural understanding of antibody neutralization of PRRSV infection can inform vaccine design, help explain the variation in protection afforded by previous vaccine or virulent field virus exposure, and ultimately increase the ability of producers to control and prevent PRRS disease in pigs.

The results also strongly indicate that broadly neutralizing titers are unlikely to predict protection at the herd level. The variation in neutralizing activity against any given PRRSV among pigs in a herd is tremendous, and the relative neutralizing activity in any given serum

sample against a range of challenge viruses also is tremendous. Given the additional uncertainty of what kind of PRRSV might be introduced into the herd in the future, the ability to make a useful prediction is low or nil.

While neutralizing antibody titers may not be able to predict protection, two tools for analysis of PRRSV-specific memory B cells were developed. Fluorescently labeled PRRSV virions bind to antibodies displayed on memory B cells that are specific for proteins displayed on the surface of the virus. Some of the proteins must by definition be targets of neutralizing antibodies. Since the virions are fluorescent, the bound memory B cells can be isolated by FACS and the recovered cells grown and characterized. In the medium-to-long term, cells producing neutralizing antibodies can be isolated and characterized. The results will facilitate analysis of the characteristics of neutralizing antibodies and the cells making them, and specific antibody tools for unambiguous identification of neutralization targets that range from virus isolate-specific to universal. Development of PRRSV-specific B cell tetramers will facilitate studies to assess PRRSV immune status of pigs with a simple blood test, and studies to assess their potential for predicting the protection status of pigs in a blood sample without a need for challenge studies.

Overall, the results show that immunological approaches aimed at induction of broadly neutralizing antibodies may substantially enhance immune protection against PRRSV. The findings further show that naturally occurring viral isolates are able to induce protective humoral immunity against unrelated PRRSV challenges, thus removing a major conceptual barrier to vaccine development.

Publication Outcomes:

Robinson SR, Li J, Nelson EA, Murtaugh MP. 2015. Broadly neutralizing antibodies against the rapidly evolving porcine reproductive and respiratory syndrome virus. Virus Research 203:56-65.

Robinson SR, Rahe MC, Gray DK, Martins KV, Murtaugh MP. 2017. Porcine reproductive and respiratory syndrome virus neutralizing antibodies provide in vivo cross-protection to PRRSV1 and PRRSV2 viral challenge. PLOS ONE. In review.
Rahe MC, Gustafson K, Murtaugh MP. 2017. B cell tetramer development for veterinary vaccinology. Viral Immunol. doi: 10.1089/vim.2017.0073.

Cited Literature

1. Murtaugh MP, Genzow M. Immunological solutions for treatment and prevention of porcine reproductive and respiratory syndrome (PRRS). Vaccine. 2011;29(46):8192-204.
2. Molina RM, Cha SH, Chittick W, Lawson S, Murtaugh MP, Nelson EA, et al. Immune response against porcine reproductive and respiratory syndrome virus during acute and chronic infection. Veterinary Immunology and Immunopathology. 2008;126:283-92.
3. Mulupuri P, Zimmerman JJ, Hermann J, Johnson CR, Cano JP, Yu W, Murtaugh MP, et al. Antigen-specific B-cell responses to porcine reproductive and respiratory virus infection. Journal of Virology. 2008;82:358-70.
4. Nelson EA, Christopher-Hennings J, Benfield DA. Serum immune responses to the proteins of porcine reproductive and respiratory syndrome (PRRS) virus. J Vet Diagn Invest. 1994 Oct;6(4):410-5.
5. Lopez OJ, Oliveira MF, Garcia EA, Kwon BJ, Doster A, Osorio FA. Protection against porcine reproductive and respiratory syndrome virus (PRRSV) infection through passive transfer of PRRSV-neutralizing antibodies is dose dependent. Clin Vaccine Immunol. 2007 Mar;14(3):269-75.
6. Osorio FA, Galeota JA, Nelson E, Brodersen B, Doster A, Wills R, et al. Passive transfer of virus-specific antibodies confers protection against reproductive failure induced by a virulent strain of porcine reproductive and respiratory syndrome virus and establishes sterilizing immunity. Virology. 2002 Oct 10;302(1):9-20.

7. Darwich L, Diaz I, Mateu E. Certainties, doubts and hypotheses in porcine reproductive and respiratory syndrome virus immunobiology. Virus Res. 2010 Dec;154(1-2):123-32.
8. Burton DR. Antibodies, viruses and vaccines. Nat Rev Immunol. 2002 Sep;2(9):706-13.
9. Burton DR, Poignard P, Stanfield RL, Wilson IA. Broadly neutralizing antibodies present new prospects to counter highly antigenically diverse viruses. Science. 2012 Jul 13;337(6091):183-6.