RESEARCH REPORT
SWINE HEALTH

Title: Prevention of PRRS by antibody administration – **NPB #05-157**

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Industry Summary:

The objectives were to determine if a practical method of producing antibodies to PRRSV could be found which would neutralize all strains of the virus. The naturally occurring ambient strains of PRRSV were inoculated into pigs and induced virus neutralizing antibodies to all North American strains of the virus tested. These cross reacting (heterologous) antibodies were protective against the PRRSV when passively injected into pigs. These results confirm previous studies by Osorio and suggest that passive immunization of swine may be a method for reducing PRRSV infection.

Scientific Abstract:

The goal of our research was to produce polyvalent hyperimmune plasma with high-titers of virus neutralizing (VN) antibody to both homologous and heterologous PRRSv strains, and to evaluate protection provided by passive immunization of 3-week old piglets with the hyperimmune plasma in a live PRRS virus challenge model. The hyperimmunization process was conducted via infection of gilts or horses with mutant strains of live PRRSv. Hyperimmune plasma had VN titers ranging from 1:64 to 1:1024 by fluorescent focus neutralization when tested against strains used in hyperimmunization. VN titers for North American strains ranged from 1:64 to 1:512. Hyperimmune plasma, as well as normal swine plasma, was harvested at necropsy and used in subcutaneous injections of 3-week old piglets. Pigs were challenged with various PRRSv strains 24 hours after receiving plasma. Viremia was either prevented or reduced in pigs receiving hyperimmune plasma as compared to non-immunized challenge controls. These results confirm the previous report by Osorio et al regarding the protective role of neutralizing antibody in reduction and prevention of viremia due to PRRSv.

Introduction: Porcine reproductive and respiratory syndrome virus (PRRSV) has been a detriment to the pork industry ever since it first emerged in the late 1980s and early 1990s. As a respiratory disease, the virus causes inappetance, lethargy, and increased susceptibility to secondary respiratory infections. Nearly half of the annual economic loss caused by PRRSV infection is due to decreased weights of pigs that reach market. As a reproductive disorder, PRRSV causes stillbirths, mummified piglets, and delay in return to estrus.

Some debate still remains as to the importance of cellular versus humoral immune response post PRRSV infection. Research has shown that virus neutralizing (VN) antibodies may play an important role in increased viability at birth and decreased virus titers and dissemination within infected pigs and decreased transmission to naïve animals. But other researchers have shown that vaccination against PRRSV leading to protection involves a cellular immune response, as monitored by IFN-gamma-producing cells.

The objectives of the work were to produce high titers of VN antibodies to PRRSV and subsequently to passively administer these antibodies to provide protection against challenge with both homologous and heterologous strains of the PRRSV.

Objectives:

1.To determine if antibodies to PRRSV lacking glycans at epitope A which are produced in pigs or horses may passively protect pigs against PRRS.

2. To determine if passive administration of antibodies to PRRSV will decrease mortality rate due to PRRS in suckling and/or weaned pigs in an on-the-farm study.

Materials & Methods: This section should include experimental design, methods and procedures used, number of animals, etc.

Viral propagation. Three field strains of PRRSV were propagated for the production of immune plasma: HLV013, HLV093, and HLV096. Each strain was grown on MARC-145 cells in a 75 cm^2 flask, containing Dulbecco's Minimal Essential Media (DMEM) with 10% Fetal Calf Serum, 1% L-glutamine, and 0.2mg/ml gentimicin. Cells were allowed to grow for 3 days until a confluent monolayer was present. Media was removed from the confluent monolayer and 1 ml of a stock virus was added to the monolayer. The virus and monolayer were incubated at 37°C for 1 hour. Twenty ml of fresh, cold DMEM was added to the flask and incubated until 90-95% cytopathic effect (CPE) was reached. At desired CPE, the neck of the flask was wrapped in parafilm and frozen at -80°C overnight. The flask was thawed in a 37°C water bath. Once thawed, the virus-cell suspension was transferred to a 50 ml tube and centrifuged at 4°C for 15 minutes at 377 x g. The supernatant was removed and aliquoted appropriately for use as live virus in the hyperimmunization process.

Virus titration. Each virus grown for infection was titrated by virus isolation as described previously. Marc-145 cells were grown in 96-well flat-bottom plates for 3 days until monolayers were confluent. A 100 μl aliquot of each virus was serially diluted in 10-fold dilutions out to 10^{-6} in 900 μl of DMEM with 4% fetal calf serum, 200 μg/ml gentimicin, and 2.5 μg/ml Fungizone. Four replicates of each dilution were set up and incubated at 37°C with 5% CO_2 for 8 days and examined for cytopathic effect (CPE). A direct fluorescent antibody assay was also used, in which virus was incubated on cells for 3 days, fixed with cold 70% acetone/30% methanol. A FITC-conjugated monoclonal antibody against the PRRSV nucleocapsid protein, SDOW-17F, was diluted 1:250 in 1xPBS and 60 μl was aliquoted to each well, and incubated for 45 minutes at 37°C. Antibody was removed and wells were washed 3 times with 200 μl 1xPBS. Using an inverted fluorescent microscope, wells were examined for fluorescence. Viral titers were calculated using the Reed-Muench calculation.

Hyperimmunization of naïve gilts. Thirty-six PRRSV negative gilts were received from a specific-pathogen free (SPF) herd and randomized into 5 groups using SAS statistical randomization program. All animals were tested by IDEXX HerdChek® ELISA for antibodies against PRRSV nucleocapsid. Groups 1-4 were maintained in 2 rooms, 4 pens, while group 5 was maintained in 2 separate rooms. The pigs in groups 1-4 were hyperimmunized via infection with different strains of live PRRSV (titers ranging from 7.26×10^2 $TCID_{50}$/ml to 2.28×10^5 $TCID_{50}$/ml). Group 5 did not receive any live PRRSV at any time. Plasma was harvested at different time points from all groups, described in days post first infection (dpi 1), days post second infection (dpi 2), and days post third infection (dpi 3). Experimental groups were divided as shown in Table 1.

Treatment group	Number of pigs	PRRSV strain and number of administrations	PRRSV titer ($CCID_{50}$/ml)	Necropsy
1	4	HLV013 x 1	2.28×10^6	52 dpi 1
2	8	HLV013 x 2	4.74×10^5	29 dpi 2, 86 dpi 1
3	4	HLV013 x 1 + HLV093 x1	7.26×10^2	32 dpi 2, 112 dpi 1
4	4	HLV013 x 1 + HLV093 x 1 + HLV096 x 1	1.39×10^6	22 dpi 3, 55 dpi 2, 135 dpi 1
5	4	None	None	22 dpi 1
	1			70 dpi 1
	3			73 dpi 1
	6			78 dpi 1
	4			100 dpi 1

Table 1. Experimental design for production of immune plasma. Gilts were divided into 5 treatment groups, each group receiving a different hyperimmunization schedule, number, and sequence of PRRSV strains.
*Animals from group 5 were necropsied at different time points post first immunization of groups 1-4 due to housing restrictions.

All pigs in groups 1-4 were infected with HLV013 at 62 days of age (day 0). Live virus was administered intramuscularly (IM) with 2 ml of virus at a titer of 2.28×10^6 $TCID_{50}$/ml. Antibody response was monitored by bleeding all pigs and submitting sera for IDEXX ELISA. All pigs were bled at 22 and 42 dpi 1, after which each group was bled intermittently at different times (Table 2). Group 1 was only infected with HLV013 at day 0.

Trt. group	Action performed at each time point Days post infection 1																									
	17	22	29	42	50	52	59	64	66	71	73	78	80	83	86	87	90	94	100	101	112	113	116	120	127	135
1	-	B	-	B	-	N	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	-	B	-	B	-	-	B	B	B	B	-	-	B	-	N	-	-	-	-	-	-	-	-	-	-	-
3	-	B	-	B	-	-	-	B	-	-	-	-	B	B	-	B	B	B	-	B	N	-	-	-	-	-
4	-	B	-	B	-	-	-	B	-	-	-	-	B	B	-	B	B	B	-	B	-	B	B	B	B	N
5	B	-	N	-	B	-	-	-	-	B	N	N	-	-	-	-	-	-	N	-	-	-	-	-	-	-

Table 2. Pigs were either bled, necropsied, or not handled at each time point. Groups 1-4 were infected with HLV013 at day 0, and subsequently re-infected at different time points (refer to experimental design). Group 5 was not infected at any time, and only a few pigs from group 5 were necropsied at each listed time.
B=Bled
N=Necropsy
- = Pigs not handled

Group 2 was infected with HLV013 at 0 dpi 1 and infected with 2 ml of HLV013 (4.74×10^5 $TCID_{50}$/ml) at 57 dpi 1. Group 3 was infected with HLV013 at day 0, and infected with 2 ml of HLV093 (7.26×10^2 $TCID_{50}$/ml) at 80 dpi 1. Group 4 was infected with HLV013 at day 0, infected with 2 ml of HLV093 (7.26×10^2 $TCID_{50}$/ml) at 80 dpi 1, and infected with 2 ml of HLV096 (1.39×10^6 $TCID_{50}$/ml) at 113 dpi (33 dpi 2).

At 52 days post infection one (dpi 1), 4 pigs were necropsied and plasma harvested, terminating treatment group 1. The remaining three treatment groups were infected and necropsied as follows: group 2 was infected with HLV013 at 57 dpi 1, and necropsied 86 dpi 1(29 dpi 2); group 3 was infected with HLV093 80 dpi 1, and necropsied 112 dpi 1 (32 dpi 2); group 4 was infected with HLV093 80 dpi 1, infected with HLV096 113 dpi 1 (33 dpi 2), and necropsied at 135 dpi 1 (55 dpi 2, 22 dpi 3).
Plasma was submitted to the Iowa State University Veterinary diagnostic laboratory where the PRRS IDEXX HerdChek® ELISA was performed. Fluorescent focus neutralization (FFN) assay was performed by the South Dakota State University Animal Disease Research and Diagnostic Laboratory to determine the level of VN antibodies present in serum as described previously. Briefly, serum was diluted in Eagle's minimal essential medium supplemented with 2% horse serum. Virus was added to each sample and allowed to incubate at 37°C for 1 hour. Each dilution was then transferred to a 96-well plate in which confluent monolayers of MARC-145 cells were grown. Plates were fixed with 80% acetone after 24 hours. FITC conjugated SDOW-17 was incubated in wells for 1 hour to detect infected cells. A dilution was considered positive when there was a 90% or greater reduction in the number of fluorescent foci. Samples were titrated to extinction unless the sample volume was not sufficient. Strains used in FFN tests were SDSU23983, VR-2332, HLV013, HLV092, HLV096, ISU-P, Prime Pac, SD01-08 (European-like), and Lelystad Virus (LV) (see Appendix for sequence homology).

Plasma Harvest. In order to harvest the largest volume of plasma, pigs were intravenously administered Telazol 100 mg/ml(Tiletamine HCl and Zolazepam HCl), with Ketamine (100 mg/ml) and Xylazine (100 mg/ml) and exsanguinated to collect all blood possible. Blood was collected in sterile glass beakers with sodium citrate to prevent clotting. Blood was centrifuged at 2054 x g and plasma was pipetted off and frozen at -20°C. An aliquot of plasma collected at selected intervals and necropsy was submitted to South Dakota State University Animal Disease Research and Diagnostic Laboratory for fluorescent focus neutralization (FFN) assay.

Antibody rate of decay study. To determine the rate at which passively administered antibody would decay post injection, a non-challenge study was conducted in which pigs were passively immunized with immune plasma into the peritoneal cavity and bled frequently. Two pigs were passively immunized with

10 ml per kilogram (kg) of body weight of monovalent immune plasma from pig 370 of treatment group 2 (see above) with an FFN titer of 1:128 against HLV013 (50 ml total administered). Each pig was bled at 0, 4, 8, 12, 24, 48, 72, 96, 120, 168, 240, and 312 hours post immunization (hpi). FFN was run on serum from each time point to show the antibody rate of decay (excluding pig 168 at 120 and 168 hpi). In order to calculate the decay curve, the change in antibody titer over time was divided by the duration of the study[38], i.e. ((peak titer)-(titer at 13 days))/(13 days-(days at peak titer)). The resulting rate of antibody decay is described in VN titer units per day.

Antibody maintenance curve. In addition to the decay curve, another passive immunization study was done to determine the level at which virus-neutralizing antibody could be maintained by repeated immunizations. Two pigs were passively immunized by subcutaneous injections of 1.25 ml of immune plasma per kg of body weight. The immunization was repeated at 48 hours and again at 96 hours, for a total of 3 injections. Each pig was bled at 0, 12, 24, 48, 96, and 144 hpi. FFN was run on serum from each time point to demonstrate the maintenance of antibody (excluding pig 166 at 12 hpi).

Source of animals. All swine were received from a known PRRSV negative herd. Pigs were randomized using SAS statistical randomization (SAS institute, 1998).

Production of neutralizing antibodies in horses. Two horses were used to evaluate the ability of virulent PRRSV to induce high neutralizing antibody titers in equines. Each horse received a total of five intramuscular injections of virulent PRRSV. The same strain (HLV013) was used in the first two injections, while three different strains were used for subsequent injections. Each inoculation consisted of a 10 ml volume split between three injection sites. All strains were sequenced and titrated at 10^5 TCID-50 per ml. Horses were inoculated on Days 0, 21, 41, 84, and 126, and study termination occurred on Day 137. Large volumes of blood were collected with added sodium citrate. Whole blood was then centrifuged to collect plasma.

Concentration of equine plasma. Plasma collected at study termination was put into lengths of dialysis tubing (12-14,000 MWCO) and covered with 40,000 MW polyethylene glycol. After 3.75 hours the concentrated plasma was collected and combined with raw plasma, resulting in a final concentration of 3.8x. The same procedure was used on normal horse plasma. All plasma was sterilized through .45 µm filters.

Passive immunization of piglets using equine plasma. Study I utilized filter-sterilized equine plasma (normal and ά-PRRSV). Eight pigs were each injected

(sub-Q) with 80 ml of ά-PRRSV equine plasma, and four pigs were each injected (sub-Q) with 80 ml of normal equine plasma. Blood was collected at various times following immunization and tested to determine circulating virus-neutralizing antibody (FFN) titers.

Study II utilized the concentrated equine plasma (normal and ά-PRRSV). Six pigs received concentrated ά-PRRSV plasma at a rate of 10 ml per kg bodyweight. Two pigs received a dose of 5 ml per kg bodyweight. Four pigs received concentrated normal equine plasma at a rate of 7.5 ml per kg bodyweight. Blood collection and testing methods were the same as Study I.

Experiments to determine if pigs could be passively immunized against challenge by PRRSV. In each of 4 experiments, 4 pigs received immune plasma and 4 pigs received normal plasma (see Table 3) and were challenged with either a homologous or heterologous strain of PRRSV. In addition, 2 sentinel pigs were added to each group subsequent to challenge in order to determine if passive immunization decreased the transmission of the virus to susceptible pigs. In experiments 1 and 3 an additional group of 4 pigs were included that received immune plasma but were not challenged with PRRSV in order to determine the level of antibody passively transferred in the absence of challenge virus.

Table 3. Experimental design for challenge experiments of passively immunized piglets.

Treatment group	Number of pigs (n)	Plasma received	Live virus administered	Exposure to challenged pigs	Experiments including each group
1	4	α-PRRSV	+	-	1, 2, 3, 4
2	4	NSP	+	-	1, 2, 3, 4
3	2	-	-	+ (to trt* 1)	1, 2, 3, 4
4	2	-	-	+ (to trt 2)	1, 2, 3, 4
5	4	α-PRRSV	-	-	1, 3

*trt = Treatment

The polyvalent immune plasma used was from one gilt that was infected with 3 PRRSV strains at 3 separate times (HLV013, HLV093, HLV096, kindly provided by Dr. Kay Faaberg, University of Minnesota). This immune plasma (α-PRRSV) had an FFN titer of 1:1024 against HLV013 and an IDEXX HerdCheck® ELISA S/P ratio of 2.833 (nucleocapsid antibody) as determined by Iowa State University Veterinary diagnostic laboratory. FFN titers of this plasma against HLV096 and HLV092 were 1:512 and 1:256, respectively. Pigs were weighed at -2 days post infection (dpi). Pigs were immunized at day -1 and challenged with 2 ml of live PRRS virus intranasally, 1 ml/nare at day 0. At day 3, sentinel pigs were moved to the room with the appropriate principle challenge group. Pigs were bled at 0, 3, 7, 10, 14, and 21 days post exposure (dpe) to the challenged group. Blood was collected in serum-separator vacutainer tube and centrifuged at 3500 RPM for 25 minutes. Serum was aliquoted and stored at -80°C for virus titration, and at -20°C for serology. FFN assay was run on serum from 24 hours post immunization (hpi) and 96 hpi (table).

Challenged pigs were necropsied at 14 dpc and sentinel pigs were necropsied at 21 dpe. All pigs were sedated with Telazol/Xylazine/Ketamine, 1ml per 100 pounds bodyweight. Each pig was administered 1 ml plus 1 ml per 10 pounds bodyweight of Sleepaway by intravenous injection. Blood was collected from each pig and processed as described above. Bronchial alveolar lavage (BAL) fluid was collected at necropsy. Lungs were removed from each carcass and were severed from the trachea. Dulbecco's Modified Eagle's Medium (DMEM) containing 0.5 mg/ml gentamicin was used to lavage the lungs. 25 ml of DMEM was pipetted into the lungs. The lungs were massaged and fluid was poured into a conical tube. The BAL fluid was kept on ice and processed immediately after necropsy was completed. BAL fluid was vortexed and dispensed into three, 1 ml aliquots and stored at -80^{o}C.

At necropsy, macroscopic lung lesions were scored as previously described. Each lung lobe was given a number to represent the percentage of each lobe affected by pneumonia. Ten points, five dorsal and five ventral, were possible for each of the following: right cranial (anterior) lobe, right middle lobe, cranial portion of the left cranial lobe and caudal portion of the left cranial lobe. Five points were possible for the accessory lobe. Fifteen points were assigned to the dorsal portion of each caudal lobe and 12.5 points were assigned to the ventral portion of each caudal lobe. A total of 100 points was possible to estimate the severity of visible pneumonia. Lung scores were statistically analyzed using the Kruskal-Wallis step-wise comparison via SAS statistical analysis.

Tissues sampled for histopathology were as follows: tonsil, heart, lymph nodes—deep cervical, tracheobronchial, iliac and external inguinal, and lung—one section from each lung lobe. Each tissue was blindly scored as previously described. Lung lesions were assigned a numerical value between 0 and 5 as follows: 0 – No lesions, 1 – mild multifocal interstitial pneumonia (IP), 2 – mild diffuse IP, 3 – moderate multifocal IP, 4 – moderate diffuse IP, 5 – severe multifocal IP and 6 – severe diffuse IP. Heart samples were given a value

between 0 and 3 as follows: 0 - normal, 1 – mild multifocal lymphohistiocytic myocarditis (LM), 2 – moderate LM, 3 – severe LM. Tonsil and lymph nodes were each give a score between 0 and 3 as follows: 0 – normal, 1 – mild lymphoid hyperplasia (LH), 2 - moderate LH, 3 – severe LH. Scores were statistically analyzed using the Kruskal-Wallis step-wise comparison using SAS statistical analysis.

Experiment 1. Homologous challenge with HLVO13: intraperitoneal immunization with 0.6ml/kg of immune plasma. The first challenge experiment was conducted by passively immunizing pigs with 1 intraperitoneal immunization. Pigs were to be administered 1.0 ml/kg by injection of the intraperitoneal cavity with the appropriate plasma (NSP or α-PRRSV), but the weight of the pigs was underestimated, and the actual dosage was 0.6 ml/kg. Pigs were challenged intranasally with 2 ml of HLV013 at 1.16×10^6 $CCID_{50}$/ml, yielding a total innoculum dosage of 2.32×10^6 CCID. A group of non-challenged pigs receiving hyperimmune plasma were included in this study.

Experiment 2. Homologous challenge with HLVO13: subcutaneous immunization with 2 ml/kg of immune plasma. The first homologous challenge experiment was repeated with a higher dosage of immune plasma administered by a different route of administration. In this experiment, 2 ml/kg of the appropriate plasma (NSP or α-PRRSV) was administered by subcutaneous injection. Pigs were challenged intranasally with 2 ml of HLV013 at 1.16×10^6 CCID/ml, yielding a total innoculum dosage of 2.32×10^6 CCID. Non-challenged pigs receiving immune plasma were not included in this experiment.

Experiment 3. Homologous challenge with HLVO96: subcutaneous immunization with 2ml/kg of immune plasma. In this experiment, 2 ml/kg of the appropriate plasma (NSP or α-PRRSV) was administered by subcutaneous injection. Pigs were challenged intranasally with 2 ml of HLV096 at 7.91×10^6 $CCID_{50}$/ml, yielding a total innoculum dosage of 1.58×10^7 CCID. A group of non-challenged pigs receiving immune plasma were included in this study.

Experiment 4. Heterologous challenge with HLVO92: subcutaneous ummunization with 2ml/kg of immune plasma. In this experiment, 2 ml/kg of the appropriate plasma (NSP or α-PRRSV) was administered by subcutaneous injection. Pigs were challenged intranasally with 5 ml of HLV092 at 7.91×10^3 $CCID_{50}$/ml, yielding a total innoculum dosage of 4×10^4 CCID. Non-challenged pigs receiving immune plasma were not included in this experiment

Virus titration. Serum samples from each pig were assayed for the presence of live virus by virus isolation as described previously. Marc-145 cells were grown in 96-well flat-bottom plates for 3 days until monolayers were confluent. 100 ul of serum was serially diluted in 10-fold dilutions out to 10^{-6} in tubes containing 900 ul of DMEM with 4% fetal calf serum, 200 ug/ml gentimicin, and 2.5 ug/ml

Fungizone. Four replicates of each dilution were set up and incubated at 37°C with 5% CO_2 for 8 days and examined for cytopathic effect (CPE). A direct fluorescent antibody assay was also used, in which serum was incubated on cells for 3 days, fixed with cold 70% acetone/30% methanol. A fluorescein-conjugated anti-PRRSV monoclonal antibody, SDOW-17F, was diluted 1:250 in 1xPBS and 60 ul was aliquoted to each well, and incubated for 45 minutes at 37°C. Antibody was removed and wells were washed 3 times with 200 ul 1xPBS. Using an inverted fluorescent microscope, wells were examined for fluorescence. Viral titers were calculated using the Reed-Muench calculation and log 10 conversions of each titer were performed prior to mean calculations. Samples with no detectable virus were assigned a value of 1. Mean titers were analyzed by t test using JMP.

Serology. Serum was submitted to the Iowa State University Veterinary diagnostic laboratory where the PRRS IDEXX HerdChek® PRRS ELISA was performed. Fluorescent focus neutralization (FFN) assay was performed by the South Dakota State University Animal Disease Research and Diagnostic Laboratory to determine the level of VN antibodies against the PRRSV strain used in challenge present in serum as described previously. Briefly, serum was diluted in Eagle's minimal essential medium supplemented with 2% horse serum. Virus was added to each sample and allowed to incubate at 37°C for 1 hour. Each dilution was then transferred to a 96-well plate in which confluent monolayers of MARC-145 cells were grown. Plates were fixed with 80% acetone after 24 hours. FITC conjugated SDOW-17 was incubated in wells for 1 hour to detect infected cells. A dilution was considered positive when there was a 90% or greater reduction in the number of fluorescent foci.

VII. Results: Report your research results by objective.

Upon arrival, all pigs were PRRSV negative, as determined by assay for nucleocapsid antibody (IDEXX ELISA) with Sample/Positive (S/P) ratios ranging from 0.000 to 0.112. All pigs were tested again immediately prior to the first infection and again were negative for PRRSV with ELISA S/P ratios ranging from 0.000 to 0.046. By 22 dpi, 90% pigs in groups 1-4 had seroconverted as assayed on the IDEXX ELISA with S/P ratios ranging from 0.407 to 2.206 (data not shown) whereas group 5 pigs were negative when tested at 17 dpi of groups 1-4 (S/P ratios ranging from 0.00 to 0.04). At 22 dpi, all 4 pigs in treatment group 1 had positive FFN titers ranging from 1:32 to 1:512 against the homologous strain HLV013, but only 1 pig had a titer, which was very low, against heterologous strains (Table 4). By 42 dpi, homologous titers ranged between 1:128 and 1:512. Each pig had very low positive titers to at least 1 heterologous strain. FFN titer to HLV013 did not increase by necropsy at 52 dpi.
Treatment group 2 had FFN titers of at least 1:16 by 22 dpi 1 against the infection strain, but no positive FFN titers against heterologous strains (Table 5). At 42 dpi 1, FFN titers were between 1:32 and 1:128 against HLV013. There was no increase in FFN titer between 64 and 71 dpi 1 (7 and 14 dpi 2).
At 22 dpi, treatment group 3 had FFN titers ranging from 1:64 to >1: 512 against HLV013, but no positive titers against heterologous strains until 42 dpi (Table 6). At 94 dpi 1 (14 dpi 2), titers to heterologous strains increased. For example, titers against SD23983 at 42 dpi ranged from 1:4 to 1:32 and at 94 dpi ranged from 1:128 to 1:512. By necropsy at 112 dpi 1 (32 dpi 2), all heterologous strain titers either remained the same or decreased from those at 94 dpi 1.
Treatment group 4 had no positive titers against heterologous strains at 22 dpi 1, but homologous titers ranged from 1:256 to >1:512 (Table 7). By 42 dpi 1, two pigs had positive titers against heterologous strains. By 94 dpi 1 (14 dpi 2), homologous titers ranged from 1:512 to 1:4096. By necropsy at 135 dpi 1 (55 dpi 2, 22 dpi 3), titers against all strains were either the same or decreased from that at 94 dpi 1.
The literature states that VN antibody is produced approximately two weeks after nucleocapsid antibody, which usually is detected between 7 and 9 dpi. Since group 5 did not have nucleocapsid antibody at any time as determined by ELISA (data not shown), it was assumed that they did not have VN antibodies. Sera from 4 pigs in group 5 were assayed by FFN to confirm the pigs were negative by another assay, and were negative for VN antibodies. At each necropsy of group 5 pigs, all S/P ratios for nucleocapsid antibody remained below the cutoff of 0.4, indicating all of the group 5 pigs remained negative throughout the study.
A summary of VN antibody response to selected PRRSV strains from all groups is presented in Table 8.

In the antibody rate of decay study, VN titers of immunized pigs were detected by 4 hours post immunization (hpi) (Figure 1). For pig 1099, the peak VN titer was 1:64 at 48 hpi and the final VN titer was 1:16 at 312 hpi (13 days). The rate of VN antibody decay for pig 1099 was calculated to be -4.36 VN titer units/day, calculated from peak to end of study. For pig 168, the peak VN titer was 1:32 at 12 hpi and the final titer was 1:16 at 312 hpi. The rate of VN antibody decay for pig 168 was -1.45 VN titer units/day, calculated from peak to end of study. VN antibody titers did not drop below 1:16 at any time point during the study for either pig.

In the antibody maintenance study, pig 165 had a positive titer at 12 hpi (Figure 2). A blood sample could not be taken from pig 166 at 12 hpi. By 24 hpi, both pigs had positive FFN titers and maintained positive titers for the duration of the study, 144 hpi. Pig 165 had a peak titer of 1:32 at 144 hpi. Pig 166 had a peak titer of 1:16 at 48 hpi that was maintained until 144 hpi.

Table 4. FFN titers for treatment group 1 were determined against different PRRSV strains at each time point for each pig. This group was infected with HLV013 at day 0. ND= Not determined

Days post infection	Pig numbers	FFN PRRSV strains									
		HLV013	HLV096	PrimePac	VR2332	SDSU23983	ISU-P	HLV093	HLV092	SD01-08 (EU-like)	LV
22	358	1:256	ND	ND	<1:4	<1:4	ND	ND	<1:4	ND	ND
	393	>1:512	ND	ND	<1:4	<1:4	ND	ND	<1:4	ND	ND
	395	1:32	ND	ND	<1:4	<1:4	ND	ND	<1:4	ND	ND
	400	>1:512	ND	ND	1:4	<1:4	ND	ND	1:8	ND	ND
42	358	1:256	ND	ND	1:8	1:8	ND	ND	ND	ND	ND
	393	1:256	ND	ND	<1:4	1:4	ND	ND	ND	ND	ND
	395	1:128	ND	ND	1:4	1:4	ND	ND	ND	ND	ND
	400	>1:256	ND	ND	1:16	1:16	ND	ND	ND	ND	ND
52	358	1:128	1:32	1:16	1:4	1:4	1:64	1:16	1:4	< 1:4	< 1:4
	393	1:64	1:128	1:32	1:8	1:4	1:256	1:16	1:16	< 1:4	< 1:4
	395	1:128	1:64	1:4	1:4	1:2	1:128	1:16	1:4	< 1:4	< 1:4
	400	1:256	1:128	1:64	1:16	1:8	1:256	1:8	1:8	< 1:4	< 1:4

Table 5. FFN titers for treatment group 2 were determined against different PRRSV strains at each time point for each pig. This group was infected with HLV013 at day 0 and HLV013 at 57 dpi 1. ND= Not determined.

Days post infection	Pig numbers	FFN PRRSV strains									
		HLV013	HLV096	PrimePac	VR2332	SDSU23983	ISU-P	HLV093	HLV092	SD01-08 (EU-like)	LV
22	353	>1:512	ND	ND	1:2	1:2	ND	ND	1:2	ND	ND
	355	1:64	ND	ND	1:2	1:2	ND	ND	1:2	ND	ND
	357	>1:512	ND	ND	1:2	1:2	ND	ND	1:2	ND	ND
	361	1:16	ND	ND	1:2	1:2	ND	ND	1:2	ND	ND
	370	1:128	ND	ND	1:2	1:2	ND	ND	1:2	ND	ND
	382	>1:512	ND	ND	1:2	1:2	ND	ND	1:2	ND	ND
	386	>1:512	ND	ND	1:2	1:2	ND	ND	1:2	ND	ND
	398	>1:512	ND	ND	1:2	1:2	ND	ND	1:2	ND	ND
42	353	1:32	ND	ND	1:4	1:4	ND	ND	ND	ND	ND
	355	1:64	ND	ND	1:8	1:64	ND	ND	ND	ND	ND
	357	1:64	ND	ND	1:8	1:8	ND	ND	ND	ND	ND
	361	1:64	ND	ND	1:4	1:8	ND	ND	ND	ND	ND
	370	1:128	ND	ND	1:8	1:16	ND	ND	ND	ND	ND
	382	1:64	ND	ND	1:2	1:8	ND	ND	ND	ND	ND
	386	1:64	ND	ND	1:4	1:4	ND	ND	ND	ND	ND
	398	1:64	ND	ND	1:4	1:32	ND	ND	ND	ND	ND
64	353	1:128	ND	ND	ND	ND	ND	ND	ND	ND	ND
	355	1:256	ND	ND	ND	ND	ND	ND	ND	ND	ND
	357	1:128	ND	ND	ND	ND	ND	ND	ND	ND	ND
	361	1:128	ND	ND	ND	ND	ND	ND	ND	ND	ND
	370	1:256	ND	ND	ND	ND	ND	ND	ND	ND	ND
	382	1:64	ND	ND	ND	ND	ND	ND	ND	ND	ND
	386	1:256	ND	ND	ND	ND	ND	ND	ND	ND	ND
	398	1:32	ND	ND	ND	ND	ND	ND	ND	ND	ND
71	353	1:128	ND	ND	ND	ND	ND	ND	ND	ND	ND
	355	1:128	ND	ND	ND	ND	ND	ND	ND	ND	ND
	357	1:64	ND	ND	ND	ND	ND	ND	ND	ND	ND
	361	1:128	ND	ND	ND	ND	ND	ND	ND	ND	ND
	370	1:256	ND	ND	ND	ND	ND	ND	ND	ND	ND
	382	1:64	ND	ND	ND	ND	ND	ND	ND	ND	ND
	386	1:256	ND	ND	ND	ND	ND	ND	ND	ND	ND
	398	1:32	ND	ND	ND	ND	ND	ND	ND	ND	ND
86	353	1:128	1:32	1:4	<1:4	1:4	1:64	<1:4	<1:4	<1:4	<1:4
	355	1:256	1:64	1:32	1:4	1:16	1:128	1:8	<1:4	<1:4	<1:4
	357	1:128	1:32	1:16	1:4	1:16	1:64	<1:4	1:8	<1:4	<1:4
	361	1:256	1:64	1:4	1:4	1:16	1:64	<1:4	<1:4	<1:4	<1:4
	370	1:128	1:64	1:64	1:16	1:32	1:256	<1:4	1:8	1:4	1:4
	382	1:128	1:64	1:8	1:4	1:32	1:64	<1:4	1:4	<1:4	<1:4
	386	1:128	1:128	1:64	1:8	1:16	1:256	<1:4	1:8	<1:4	<1:4
	398	64	1:32	1:8	1:4	1:16	1:32	<1:4	<1:4	<1:4	<1:4

Table 6. FFN titers for treatment group 3 were determined against different PRRSV strains at each time point for each pig. This group was infected with HLV013 at day 0 and HLV093 at 80 dpi 1. ND= Not determined.

Days post infection	Pig numbers	FFN PRRSV strains									
		HLV013	HLV096	PrimePac	VR2332	SDSU23983	ISU-P	HLV093	HLV092	SD01-08 (EU-like)	LV
22	352	1:64	ND	ND	<1:4	<1:4	ND	ND	<1:4	ND	ND
	354	>1:512	ND	ND	<1:4	<1:4	ND	ND	<1:4	ND	ND
	366	>1:512	ND	ND	<1:4	<1:4	ND	ND	<1:4	ND	ND
	381	1:64	ND	ND	<1:4	<1:4	ND	ND	<1:4	ND	ND
42	352	1:64	ND	ND	1:8	1:16	ND	ND	ND	ND	ND
	354	1:16	ND	ND	1:4	1:32	ND	ND	ND	ND	ND
	366	1:16	ND	ND	<1:4	1:4	ND	ND	ND	ND	ND
	381	1:64	ND	ND	<1:4	1:8	ND	ND	ND	ND	ND
94	352	1:1024	1:1024	1:1024	1:256	1:256	1:4096	1:32	1:256	1:4	1:4
	354	1:1024	1:1024	1:1024	1:256	1:256	1:1024	1:16	1:64	1:4	1:4
	366	1:1024	1:1024	1:512	1:128	1:128	1:2048	1:16	1:128	1:4	1:4
	381	1:4096	1:2048	1:4096	1:256	1:512	1:1024	1:16	1:128	1:4	1:4
112	352	1:2048	1:512	1:2048	1:256	1:32	1:1024	1:8	1:256	< 1:4	< 1:4
	354	1:1024	1:256	1:256	1:128	1:64	1:512	1:8	1:64	< 1:4	< 1:4
	366	1:1024	1:256	1:256	1:64	1:64	1:1024	1:8	1:64	< 1:4	< 1:4
	381	1:1024	1:512	1:512	1:128	1:128	1:1024	1:8	1:128	1:4	1:4

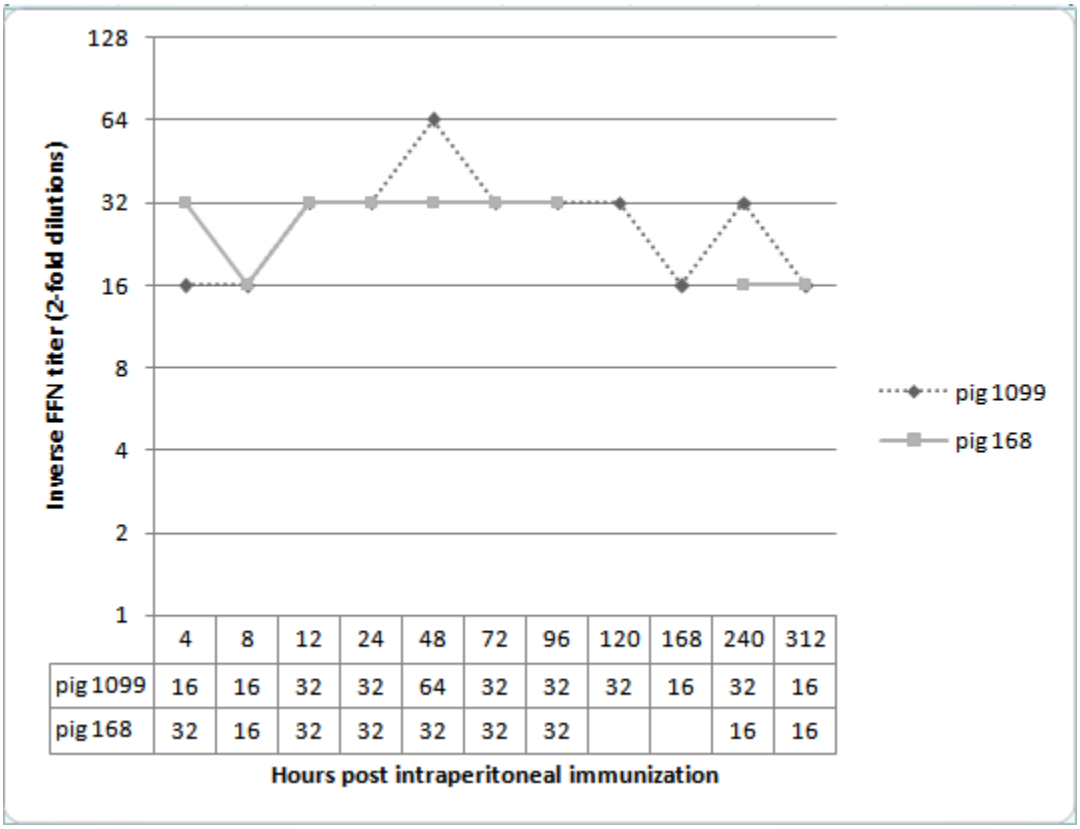
Table 7. FFN titers for treatment group 4 were determined against different PRRSV strains at each time point for each pig. This group was infected with HLV013 at day 0, HLV093 at 80 dpi 1, and HLV096 at 113 dpi 1 (33 dpi 2). ND= Not determined.

Days post infection	Pig numbers	FFN PRRSV strains									
		HLV013	HLV096	PrimePac	VR2332	SDSU23983	ISU-P	HLV093	HLV092	SD01-08 (EU-like)	LV
22	373	>1:512	ND	ND	<1:4	<1:4	ND	ND	<1:4	ND	ND
	375	1:256	ND	ND	<1:4	<1:4	ND	ND	<1:4	ND	ND
	376	>1:512	ND	ND	<1:4	<1:4	ND	ND	<1:4	ND	ND
	379	>1:512	ND	ND	<1:4	<1:4	ND	ND	<1:4	ND	ND
42	373	1:64	ND	ND	1:4	1:8	ND	ND	ND	ND	ND
	375	1:16	ND	ND	1:2	1:2	ND	ND	ND	ND	ND
	376	1:32	ND	ND	1:2	1:2	ND	ND	ND	ND	ND
	379	1:64	ND	ND	1:8	1:32	ND	ND	ND	ND	ND
94	373	1:2048	1:1024	1:512	1:128	1:256	1:1024	1:8	1:64	1:4	1:4
	375	1:4096	1:1024	>1:512	1:512	1:512	1:4096	1:16	1:512	<1:4	<1:4
	376	1:512	1:512	1:512	1:512	1:256	1:1024	1:32	1:128	<1:4	<1:4
	379	1:1024	1:1024	1:1024	1:256	1:64	1:512	1:16	1:64	1:4	1:4
135	373	1:1024	1:256	1:128	1:64	1:64	1:256	1:8	1:32	1:8	1:8
	375	1:1024	1:512	1:512	1:512	1:256	1:2048	1:8	1:256	1:4	1:4
	376	1:64	1:128	1:128	1:32	1:64	1:256	1:16	1:64	< 1:4	< 1:4
	379	1:256	1:256	1:256	1:64	1:64	1:512	1:8	1:64	1:4	1:4

Table 8. Range and geometric mean VN titers in serum were determined against different PRRSV strains at necropsy.

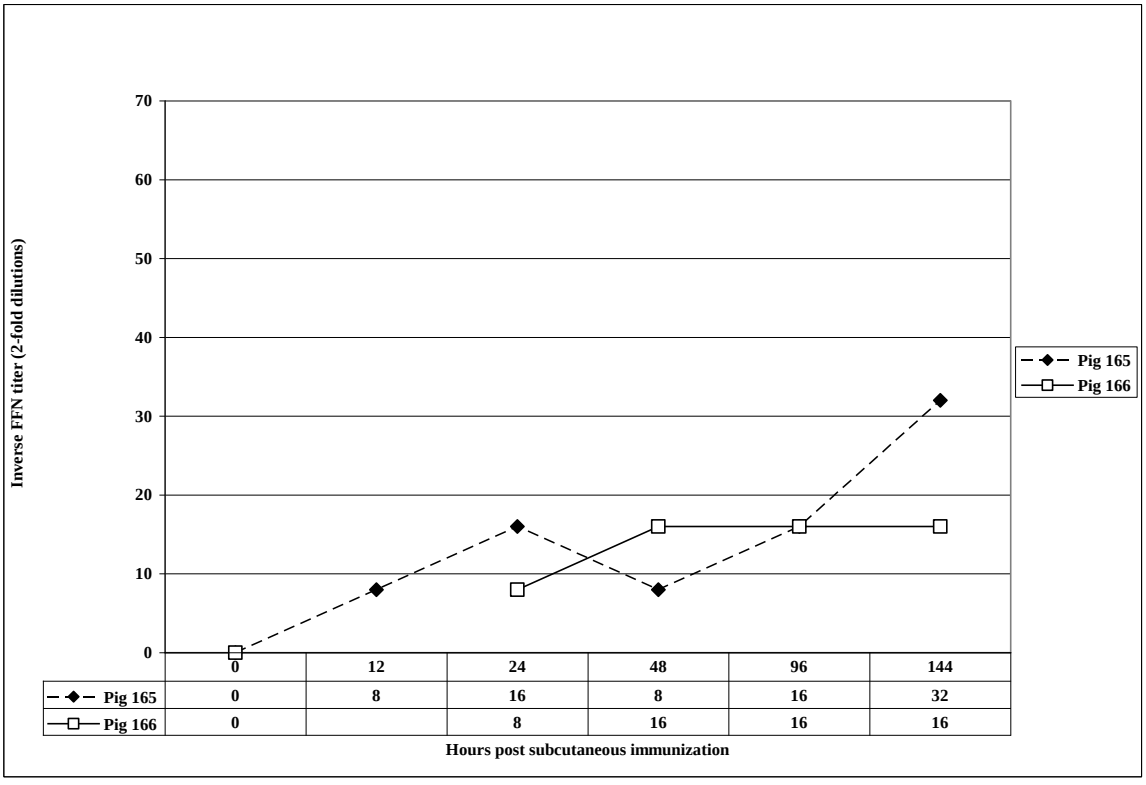
Treatment group	Range of VN titers against HLV013	Geometric mean VN titer against HLV013	Range of VN titers against ISU-P	Geometric mean VN titer against ISU-P
1	1:64-1:256	1:128	1:64-1:256	1:152
2	1: 64-1:256	1:140	1:32-1:256	1:90
3	1:1024-1:2048	1:1218	1:512-1:1024	1:861
4	1:64-1:1024	1:362	1:256-1:2048	1:512
5	<1:4	<1:4	ND*	ND*

*Not Determined



	4	8	12	24	48	72	96	120	168	240	312
pig 1099	16	16	32	32	64	32	32	32	16	32	16
pig 168	32	16	32	32	32	32	32			16	16

Figure 1. Antibody rate of decay. Neutralizing antibody titers in serum were determined by FFN against HLV013. Each pig was immunized once with 10 ml/kg immune plasma.



	0	12	24	48	96	144
Pig 165	0	8	16	8	16	32
Pig 166	0		8	16	16	16

Figure 2. Antibody maintenance curve. Neutralizing antibody titers in serum were determined by FFN against HLV013. Both pigs each received 3 intraperitoneal injections of 1.25ml/kg immune plasma.

Production of neutralizing antibodies in horses. Horses did not become febrile or show any clinical signs of disease throughout the study. No injection site reactions were observed. No detectable virus was present in serum when tested on MARC-145 cells.
Both horses had an initial FFN titer of <1:4 against HLV013. At study termination on Day 137, the horses had a geometric mean FFN titer of 1:181 against HLV013.

Concentration of equine plasma. The ά-PRRSV equine plasma used in the concentration procedure had a beginning FFN titer of 1:256. Following concentration to 3.8x the FFN titer increased to 1:1024. The normal equine plasma used in the concentration procedure had a beginning titer of <1:4. Following concentration to 3.4x the FFN titer increased to 1:8.

Passive immunization of piglets using equine plasma. In Study I, piglets (n=8) that received ά-PRRSV equine plasma had a geometric mean FFN titer of 1:6 at 24 hours post immunization. Piglets (n=8) that received normal equine plasma had a geometric mean FFN titer of <1:4 at 24 hours post immunization.

In Study II, piglets (n=2) that received concentrated ά-PRRSV equine plasma at a rate of 5 ml/kg had a geometric mean FFN titer of 1:16 at 24 hours post immunization. Piglets (n=6) that received the same plasma at a rate of 10 ml/kg had a geometric mean FFN titer of 1:25 at 24 hours post immunization. Piglets (n=4) that received concentrated normal equine plasma at a rate of 7.5 ml/kg had a geometric mean FFN titer of <1:4 at 24 hours post immunization.

Experiment 1. All pigs administered α-PRRSV had positive VN antibody titers (≥1:4) at 24 hours post immunization (hpi), 0 dpc (Figure 3), but none of the titers were greater than 1:8. At 3 dpc, titers in the α-PRRSV group ranged from <1:4 to 1:16.
Although all challenged pigs had detectable virus at all time points, there was a delay in immune response in the α-PRRSV group, seroconverting for nucleocapsid antibody by 14 dpc while the NSP group seroconverted by 10 dpc (Table 9). Viremia levels in the α-PRRSV group had a significantly lower mean titer than the NSP group at 3 dpc (p=0.018) and 6 dpc (p=0.012), but not at dpc 10 (p=0.203) and 14 (p=0.857) (Table 10).
Macroscopic lung lesions were evaluated and recorded at necropsy (Table 11). The α-PRRSV group had a mean lung score of 17.8 which was not a statistically significant difference from the NSP group mean lung score of 35.7 (p=0.0571). Histopathologic lesions also were examined by Dr. Halbur and scored (Table 12). The α-PRRSV group did not have any significant difference in histopathologic lesions than the NSP group in lung, LN, tonsil, or heart (all p values ≥0.3).

The sentinel pigs exposed to the challenged pigs were also evaluated for antibody, virus, macroscopic and hisopathologic lesions caused by PRRSV. One of the sentinel pigs exposed to the α-PRRSV group never became viremic and never seroconverted for nucleocapsid antibody (Table 13). The second pig of this group was viremic by 3 dpe and was positive for nucleocapsid antibody by 14 dpe as compared to 10 dpe for both of the sentinels exposed to NSP group. Both sentinels in the latter group were also viremic beginning at 3 dpe. Macroscopic lung lesions for the sentinels exposed to the α-PRRSV group had a mean of 2.0, compared with a 7.0 of the sentinels exposed to the NSP group (no statistical significance, p value=0.667) (Table 14). Histopathologic lesions did not have significant differences between the two groups of sentinels (all p values=1) (Table 15). As indicated by the data, transmission occurred in both groups of sentinel pigs, although transmission may have been delayed or viral exposure decreased.

Experiment 2. At 1 dpi, VN antibody titers in the α-PRRSV group were higher than those achieved in experiment 1, and titers of the NSP group were negative (Figure 4). The VN titers reached are of levels that have been shown to be protective in passive immunization. VN titers of the α-PRRSV group were maintained at 1:16 or higher and the NSP group remained negative. With VN titers of at least 1:16, we would expect to see a decrease in or prevention of viremia in α-PRRSV immunized pigs.
Two of the four α-PRRSV pigs were positive for nuclecapsid antibody at 10 dpc and all 4 had serocnverted by 14 dpc. One of the 4 NSP pigs had seroconverted for nuclecapsid antibody by 6 dpc, 3 seroconverted by 10 dpc and all 4 seroconverted by 14 dpc (Table 16). None of the α-PRRSV group were viremic at 3 dpc, as opposed to the NSP group in which all 4 pigs were viremic at 3 dpc, with a mean viremia level of 4.89×10^5 $CCID_{50}$/ml (p value=0.000125) (Table 17). By 6 dpc, 3 of the 4 α-PRRSV pigs became viremic with a mean titer of 1.02×10^2 $CCID_{50}$/ml, which was significantly different from the mean titer of the NSP pigs at 6 dpc, 3.67×10^5 $CCID_{50}$/ml (p value=0.010). By 10 dpc, the difference between the two groups was insignificant (p value=0.065) but by 14 dpc, there was a difference (p value=0.009) between the two groups. The delay in viremia and significant difference in viremia levels at 3, 6, and 14 dpc all correlate with the results of Lopez et al., which showed 1:8 VN titers as preventing/decreasing viremia.
Macroscopic gross lung lesions scored for the α-PRRSV group were significantly different (p value=0.0286) from the NSP group with a mean score of 6.0 while the NSP group had a mean score of 26 (Table 18), indicating that some protection was provided by VN antibodies at levels that would be predictive of protection. Histopathological lesions of tissues were scored but did not differ significantly (all p values ≥ 0.0857) between the α-PRRSV and the NSP groups (Table 19). Sentinel pigs exposed to α-PRRSV had a delay in viremia and immune response (Table 20). This group was ELISA negative until 14 dpe when both pigs seroconverted, showing delay in transmission. Both pigs became viremic at 7

dpe while sentinel pigs exposed to NSP pigs were viremic by 3 dpe. The latter group of pigs was seropositive for nucleocapsid antibody by 10 dpe.
Macroscopic lung lesions for the sentinels exposed to the α-PRRSV group had a mean score of 29 while the sentinels exposed to the NSP group had a mean of 10 (p value=0.333) (Table 21). Histopathology scores did not differ significantly (all p values ≥0.667) between the groups of sentinels (Table 22).

Experiment 3. VN antibody titers for the pigs administered α-PRRSV were present at 1 dpi, peaked with a mean inverse titer of 13.45 at 4 dpi and were maintained at an inverse titer of 8.00 (Figure 5), which is the VN titer shown to consistently block/delay viremia. Of the 4 pigs in this group, 3 never became viremic and only 1 had seroconverted for nucleocapsid antibody by 10 dpc, all 4 having seroconverted by 14 dpc (Table 23). At 3 dpc the NSP group had a mean titer of 1.55×10^4 $CCID_{50}$/ml while the α-PRRSV group had no detectable virus (p value=5.85×10^{-5}) (Table 24). By 6 dpc, the NSP group had a mean titer of 8.70×10^3 while the α-PRRSV group was still negative (p value=0.004). At 10 and 14 dpc, the NSP group had mean titers of approximately 10^4 and 10^2, while the α-PRRSV group was not viremic at 10 dpc and only 1 pig was viremic at 14 dpc (both p values ≤ 0.015), indicating that the VN levels reached were effective at preventing viremia.
Gross lung lesions were scored at necropsy, means of 11.00 and 17.25 for the α-PRRSV group and the NSP group (p value= 0.971), respectively, indicating that, although viremia was prevented, dissemination and pathology was not prevented by immunization (Table 25). Tissues were submitted for histopathology but could not be determined for this experiment due to misplacement of the slides and tissues.
Sentinel pigs exposed to α-PRRSV pigs did not become viremic throughout the study (Table 26). One of the sentinel pigs exposed to the NSP group became viremic by 3 dpe and remained viremic, while the other pig was only viremic at 10 dpe. Both pigs in this group were seropositive for nucleocapsid antibody by 14 dpe.
Macroscopic lung lesions were different between the two groups with a mean score of 15.00 for the sentinels exposed to α-PRRSV pigs and a mean score of 63.00 for the sentinels exposed to NSP pigs (p value=0.333), which is not a statistically significant difference, due to the small sample size, but is an observed difference (Table 27). Unfortunately there is no histopathology data to support these results due to misplacement of these tissues as well.
Experiment 4. By 1 dpi, the group immunized with α-PRRSV had a mean inverse VN titer of 9.51 (Figure 6) and none of the pigs had a VN level below 1:8 through 6 dpc. NSP administered pigs did not have inverse FFN titers greater than 4 throughout the study. At 3 dpc, 1 of 4 α-PRRSV pigs was viremic giving the group a mean titer of 1.26×10^1 $CCID_{50}$/ml as compared to the 4 of 4 NSP pigs that were viremic with a mean titer of 1.07×10^5 $CCID_{50}$/ml (Table 28) (p value=5.99×10^{-4}). By 6 dpc, all of the challenged pigs were viremic and maintained viremia until necropsy. At 6 dpc, the α-PRRSV pigs had mean viremia levels of 5.37×10^3 and the NSP pigs had a mean titer of 5.62×10^5

$CCID_{50}$/ml (Table 29) (p value=0.064). At 10 dpc, the NSP pigs had mean viremia titer of 2.82 x 10^4 $CCID_{50}$/ml while the α-PRRSV group had a mean titer of 1 x 10^3 $CCID_{50}$/ml (p value=0.010). α-PRRSV pigs did not seroconvert for nucleocapsid antibody until necropsy at 14 dpc while 3 of the NSP pigs had seroconverted by 10 dpc. At necropsy, mean viremia levels for the α-PRRSV group and the NSP group were 1.26 x 10^4 and 6.76 x 10^4 $CCID_{50}$/ml, respectively (p value=0.021).

Macroscopic lung lesions assessed at necropsy averaged 52.5 for the α-PRRSV group and 48.5 for the NSP group (p value=0.343) (Table 30). Histopathological lesions did not differ greatly between the two groups (all p values≥0.486) (Table 31), but all of the pigs in both groups did have severe multifocal interstitial pneumonia.

Sentinel pigs exposed to the NSP group became viremic by 3 dpe, while only 1 pig exposed to the α-PRRSV group became viremic at 10 dpe and both pigs were only viremic at 21 dpe (Table 32). Seroconversion for nucleocapsid antibody occurred by 10 dpe in the sentinels exposed to the NSP group and only occurred in 1 of the sentinels exposed to the α-PRRSV group at 21 dpe, with 1 pig did not seroconvert at any time.

Macroscopic lung lesions in sentinel pigs had a mean score of 23.5 for the group exposed to the α-PRRSV pigs and 38 for the group exposed to the NSP pigs (p value=0.667) (Table 33). Histopathology showed an observed difference in interstitial pneumonia and myocarditis between the two groups, but due to small sample size, the statistical difference is not significant (all p values=0.333) (Table 34).

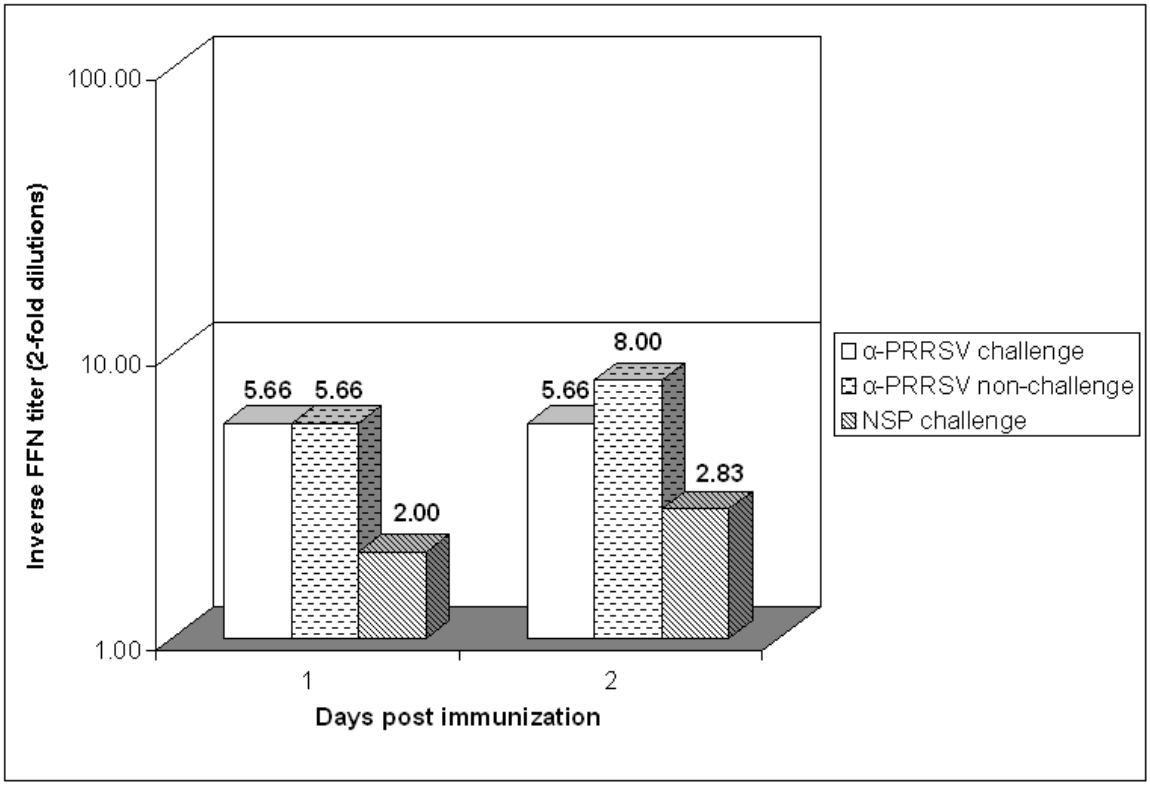


Figure 3. Experiment 1. Quantification of virus neutralizing antibody titers in either pigs passively immunized with antibody (α-PRRSV) or pigs administered normal plasma (NSP) and challenged with HLV013.

Table 9. Experiment 1. Presence of nucleocapsid antibody and PRRS virus determined in principle pigs either passively immunized with antibody to PRRSV (α-PRRSV) or administered normal swine plasma (NSP) and challenged with HLV013.

	Number of pigs positive (4 pigs per group)							
	ELISA*	VI**	ELISA	VI	ELISA	VI	ELISA	VI
Days post challenge	3		6		10		14	
α-PRRSV	0	4	0	4	0	4	4	4
NSP	0	4	0	4	4	4	3+	3+

*Antibody in serum against nucleocapsid protein
**PRRSV isolation from serum
+One pig died on day 11 due to complications with bleeding unrelated to PRRSV

Table 10. Experiment 1. Titration of live virus ($CCID_{50}$/ml) in serum of pigs administered either plasma with antibody (α-PRRSV) or normal plasma (NSP) and challenged with HLV013.

Treatment Group	Pig #	VI*	Group mean	VI	Group mean	VI	Group mean	VI	Group mean
Days post challenge		3		6		10		14	
α-PRRSV	172	2.50E+04		7.91E+04		1.16E+05		7.91E+04	
	176	1.39E+04		2.50E+03		2.50E+02		7.91E+02	
	182	7.90E+02		2.50E+02		1.39E+03		2.50E+01	
	183	2.50E+03	5.12E+03	2.50E+03	3.33E+03	5.39E+03	3.84E+03	2.50E+05	4.45E+03
NSP	169	5.39E+05		5.39E+06		7.91E+05		1.16E+04	
	174	2.50E+06		7.91E+05		2.50E+04		NA	
	180	5.34E+06		7.91E+05		1.16E+05		2.50E+04	
	181	2.50E+04	6.51E+05	7.91E+04	7.18E+05	2.50E+03	4.89E+04	1.16E+03	6.95E+03
p value			0.018		0.012		0.203		0.857

*PRRSV isolation

A t-test was performed to calculate p values

Table 11. Experiment 1. Gross lung lesions at necropsy of challenged pigs passively immunized with antibody to PRRSV (α-PRRSV) or pigs receiving normal swine plasma (NSP) and challenged with HLV013. Total possible score is 100 per pig.

Treatment Group	Pig #	Total gross lung score	Group Mean [a]
α-PRRSV	172	27	17.8
	176	11	
	182	9	
	183	24	
NSP	169	49	35.7
	174	NA	
	180	29	
	181	29	

Kruskal-Wallis step-wise comparison was performed ([a] p value=0.0571)

Table 12. Experiment 1. Histopathological evaluation and score of interstitial pneumonia by microscopic lesions of challenged pigs immunized with either antibody to PRRSV (α-PRRSV) or pigs receiving normal swine plasma (NSP) and challenged with HLV013.

Treatment Group	Pig #	Lung (0-6)	Mean	LN* (0-3)	Mean	Tonsil (0-3)	Mean	Heart (0-3)	Mean
α-PRRSV	172	4	3.50	2	1.75	2	2.00	0	0.50
	176	3		1		2		0	
	182	3		2		2		1	
	183	4		2		2		1	
NSP	169	5	4.33	2	2.00	2	2.00	1	1.00
	174	NA		NA		NA		NA	
	180	4		2		2		1	
	181	4		2		2		1	
p value		0.286		1		1		0.429	

*Lymph Nodes

Kruskal-Wallis step-wise comparison was performed to calculate p values

Table 13. Experiment 1. Detection of antibody and PRRSV in serum of sentinel pigs exposed to either pigs passively immunized with antibody to PRRSV (α-PRRSV) or exposed to pigs receiving normal swine plasma (NSP) and challenged with HLV013.

	Number of pigs positive (2 sentinel pigs per group)									
	ELISA*	VI**	ELISA	VI	ELISA	VI	ELISA	VI	ELISA	VI
Days post exposure	3		7		10		14		21	
α-PRRSV	0	1	0	1	0	1	1	1	1	0
NSP	0	2	0	2	2	2	2	2	2	2

*Antibody detected to nucleocapsid protein
**PRRSV isolation

Table 14. Experiment 1. Gross lung lesions at necropsy of sentinel pigs exposed to either pigs passively immunized with antibody to PRRSV (α-PRRSV) or exposed to pigs receiving normal swine plasma (NSP) and challenged with HLV013. Total possible score is 100 per pig.

Treatment Group	Pig #	Total gross lung score	Group Mean [a]
α-PRRSV	178	0	2.0
	179	4	
NSP	170	11	7.0
	175	3	

Kruskal-Wallis step-wise comparison was performed ([a] p value=0.667)

Table 15. Experiment 1. Histopathological evaluation and score of interstitial pneumonia by microscopic lesions of sentinel pigs exposed to pigs immunized with either antibody to PRRSV (α-PRRSV) or pigs receiving normal swine plasma (NSP) and challenged with HLV013.

Treatment Group	Pig #	Lung (0-6)	Mean	LN* (0-3)	Mean	Tonsil (0-3)	Mean	Heart (0-3)	Mean
α-PRRSV	178	1	1.50	1	1.50	1	1.50	0	0
	179	2		2		2		0	
NSP	170	3	2.00	2	2.00	2	2.00	1	0.50
	175	1		2		2		0	
p value		1		1		1		1	

*Lymph Nodes
Kruskal-Wallis step-wise comparison was performed to calculate p values

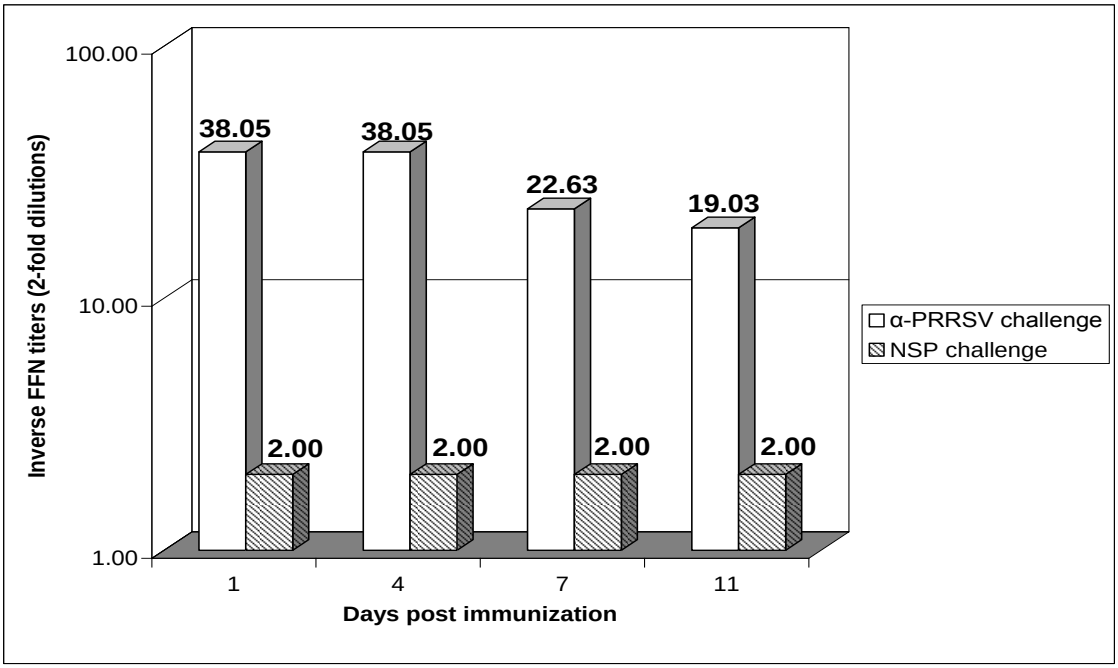


Figure 4. Experiment 2. Quantification of virus neutralizing antibody titers in either pigs passively immunized with antibody (α-PRRSV) or pigs administered normal plasma (NSP) and challenged with HLV013.

Table 16. Experiment 2. Antibody and live virus determined in principle pigs either passively immunized with antibody to PRRSV (α-PRRSV) or administered normal swine plasma (NSP) and challenged with HLV013.

	Number of pigs positive (4 pigs per group)							
	ELISA*	VI**	ELISA	VI	ELISA	VI	ELISA	VI
Days post challenge	3		6		10		14	
α-PRRSV	0	0	0	3	2	3	4	4
NSP	0	4	1	4	3	4	4	4

*Antibody detected to nucleocapsid protein
**PRRSV isolation

Table 17. Experiment 2. Titration of live virus ($CCID_{50}$/ml) in serum of pigs administered either plasma with antibody (α-PRRSV) or normal plasma (NSP) and challenged with HLV013.

Treatment Group	Pig #	VI*	Group mean	VI	Group mean	VI	Group mean	VI	Group mean
Days post challenge		3		6		10		14	
α-PRRSV	1252	<2.5E+01		5.39E+02		<2.5E+01		2.50E+01	
	1257	<2.5E+01		7.91E+02		2.50E+04		7.91E+02	
	1267	<2.5E+01		2.50E+02		7.91E+02		7.91E+02	
	1274	<2.5E+01	1.00E+00	<2.5E+01	1.02E+02	5.39E+03	5.71E+02	2.50E+03	4.45E+02
NSP	1251	5.39E+05		7.91E+05		7.91E+04		1.16E+05	
	1255	1.16E+06		7.91E+05		7.91E+05		1.16E+05	
	1264	7.91E+05		2.50E+05		7.91E+05		7.91E+03	
	1273	1.16E+05	4.89E+05	1.16E+05	3.67E+05	1.16E+05	2.75E+05	1.16E+05	5.93E+04
p value			1.25×10^{-4}		0.01		0.065		0.009

*PRRSV isolation

A t-test was performed to calculate p values

Table 18. Experiment 2. Gross lung lesions at necropsy of challenged pigs passively immunized with antibody to PRRSV (α-PRRSV) or pigs receiving normal swine plasma (NSP) and challenged with HLV013.Total possible score is 100 per pig.

Treatment Group	Pig #	Total gross lung score	Group Mean[a]
α-PRRSV	1252	6	6.0
	1257	11	
	1267	6	
	1274	5	
NSP	1251	30	26.0
	1255	34	
	1264	22	
	1273	17	

Kruskal-Wallis step-wise comparison was performed ([a] p value=0.029)

Table 19. Experiment 2. Histopathological evaluation and score of interstitial pneumonia by microscopic lesions of challenged pigs immunized with either antibody to PRRSV (α-PRRSV) or pigs receiving normal swine plasma (NSP) and challenged with HLV013.

Treatment Group	Pig #	Lung (0-6)	Mean	LN* (0-3)	Mean	Tonsil (0-3)	Mean	Heart (0-3)	Mean
α-PRRSV	1252	1	2.50	2	1.50	2	2.50	0	0.75
	1257	4		2		2		1	
	1267	4		1		3		1	
	1274	1		1		3		1	
NSP	1251	5	4.50	1	1.25	1	1.25	3	2.00
	1255	5		1		1		2	
	1264	4		2		2		2	
	1273	4		1		2		1	
p value			0.130		1		0.089		0.114

*Lymph Nodes

A Kruskal-Wallis step-wise comparison was performed to calculate p values

Table 20. Experiment 2. Detection of antibody and PRRSV in serum of sentinel pigs exposed to either pigs passively immunized with antibody to PRRSV (α-PRRSV) or exposed to pigs receiving normal swine plasma (NSP) and challenged with HLV013.

	Number of pigs positive (2 sentinel pigs per group)									
	ELISA*	VI**	ELISA	VI	ELISA	VI	ELISA	VI	ELISA	VI
Days post exposure	3		7		10		14		21	
α-PRRSV	0	0	0	2	0	2	2	2	2	2
NSP	0	2	0	2	2	2	2	2	2	1

*Antibody detected to nucleocapsid protein
**PRRSV isolation

Table 21. Experiment 2. Gross lung lesions at necropsy of sentinel pigs exposed to either pigs passively immunized with antibody to PRRSV (α-PRRSV) or exposed to pigs receiving normal swine plasma (NSP) and challenged with HLV013.Total possible score is 100 per pig.

Treatment Group	Pig #	Total gross lung score	Group Mean [a]
α-PRRSV	1258	42	29
	1265	16	
NSP	1253	8	10
	1256	12	

A Kruskal-Wallis step-wise comparison was performed ([a] p value=0.333)

Table 22. Experiment 2. Histopathological evaluation and score of interstitial pneumonia by microscopic lesions of sentinel pigs exposed to pigs immunized with either antibody to PRRSV (α-PRRSV) or pigs receiving normal swine plasma (NSP) and challenged with HLV013.

Treatment Group	Pig #	Lung (0-6)	Mean	LN* (0-3)	Mean	Tonsil (0-3)	Mean	Heart (0-3)	Mean
α-PRRSV	1258	5	3.50	2	1.33	2	2.00	1	0.50
	1265	2		1		2		0	
NSP	1253	4	3.00	3	2.50	NA	2.00	1	0.50
	1256	2		2		2		0	
p value		1		0.667		1		1	

*Lymph Nodes

A Kruskal-Wallis step-wise comparison was performed to calculate p values

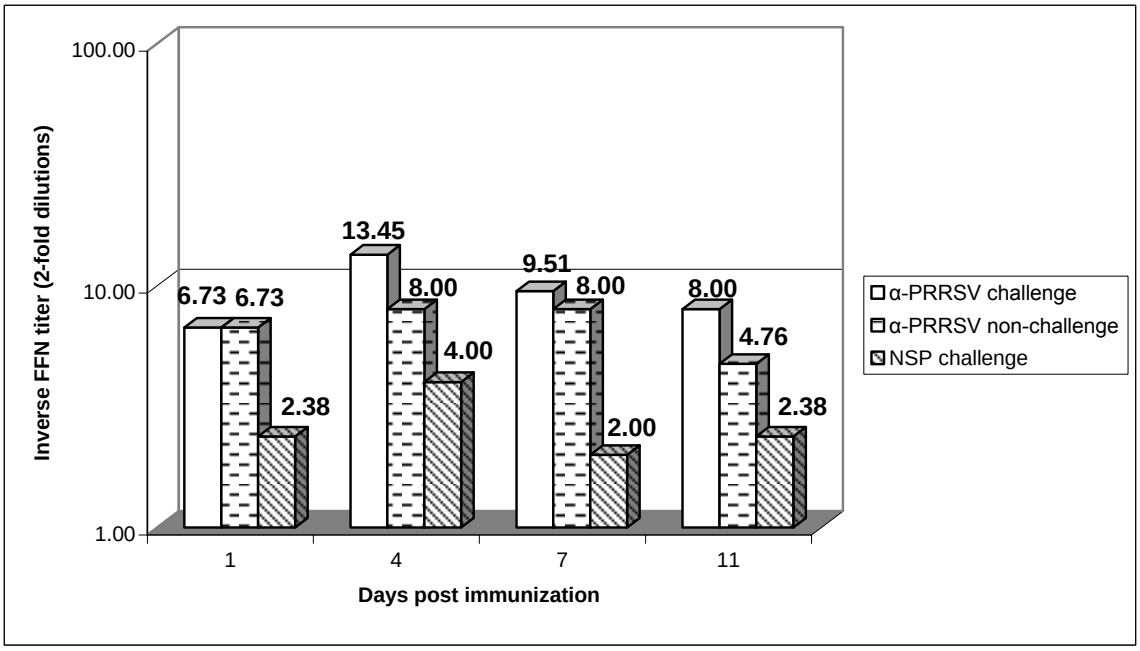


Figure 5. Experiment 3. Quantification of virus neutralizing antibody titers in either pigs passively immunized with antibody (α-PRRSV) or pigs administered normal plasma (NSP) and challenged with HLV096.

Table 23. Experiment 3. Antibody and live virus determined in principle pigs either passively immunized with antibody to PRRSV (α-PRRSV) or administered normal swine plasma (NSP) and challenged with HLV096.

	Number of pigs positive (4 pigs per group)							
	ELISA*	VI**	ELISA	VI	ELISA	VI	ELISA	VI
Days post challenge	3		6		10		14	
α-PRRSV	0	0	0	0	1	0	4	1
NSP	0	4	0	4	1	4	3	4

*Antibody detected to nucleocapsid protein
**PRRSV isolation

Table 24. Experiment 3. Titration of live virus ($CCID_{50}$/ml) in serum of pigs administered either plasma with antibody (α-PRRSV) or normal plasma (NSP) and challenged with HLV096.

Treatment Group	Pig #	VI*	Group mean	VI	Group mean	VI	Group mean	VI	Group mean
Days post challenge		3		6		10		14	
α-PRRSV	189	<2.5E+01		<2.5E+01		<2.5E+01		<2.5E+01	
	194	<2.5E+01		<2.5E+01		<2.5E+01		<2.5E+01	
	196	<2.5E+01		<2.5E+01		<2.5E+01		7.90E+01	
	197	<2.5E+01	1.00E+00	<2.5E+01	1.00E+00	<2.5E+01	1.00E+00	<2.5E+01	2.98E+00
NSP	186	7.90E+03		7.91E+02		2.50E+04		7.90E+01	
	190	2.50E+04		2.50E+03		1.16E+06		5.39E+03	
	191	1.16E+04		1.16E+05		1.16E+05		7.90E+03	
	193	2.50E+04	1.55E+04	2.50E+04	8.70E+03	5.39E+03	6.53E+04	1.16E+02	7.90E+02
p value			5.85×10^{-5}		0.004		0.002		0.015

*PRRSV isolation

A t-test was performed to calculate the p values

Table 25. Experiment 3. Gross lung lesions at necropsy of challenged pigs passively immunized with antibody to PRRSV (α-PRRSV) or pigs receiving normal swine plasma (NSP) and challenged with HLV096. Total possible score is 100 per pig.

Treatment Group	Pig #	Total gross lung score	Group Mean [a]
α-PRRSV	189	9	11.00
	194	24	
	196	1	
	197	10	
NSP	186	21	17.25
	190	1	
	191	42	
	193	5	

A Kruskal-Wallis step-wise comparison was performed ([a] p value=0.971)

Table 26. Experiment 3. Detection of antibody and PRRSV in serum of sentinel pigs exposed to either pigs passively immunized with antibody to PRRSV (α-PRRSV) or exposed to pigs receiving normal swine plasma (NSP) and challenged with HLV096.

	Number of pigs positive (2 sentinel pigs per group)									
	ELISA*	VI**	ELISA	VI	ELISA	VI	ELISA	VI	ELISA	VI
Days post exposure	3		7		10		14		21	
α-PRRSV	0	0	0	0	0	0	0	0	0	0
NSP	0	1	0	1	0	1	2	2	2	1

*Antibody detected to nucleocapsid protein
**PRRSV isolation

Table 27. Experiment 3. Gross lung lesions at necropsy of sentinel pigs exposed to either pigs passively immunized with antibody to PRRSV (α-PRRSV) or exposed to pigs receiving normal swine plasma (NSP) and challenged with HLV096. Total possible score is 100 per pig.

Treatment Group	Pig #	Total gross lung score	Group Mean [a]
α-PRRSV	199	13	15.00
	200	17	
NSP	187	60	63.00
	198	66	

A Kruskal-Wallis step-wise comparison was performed ([a] p value=0.333)

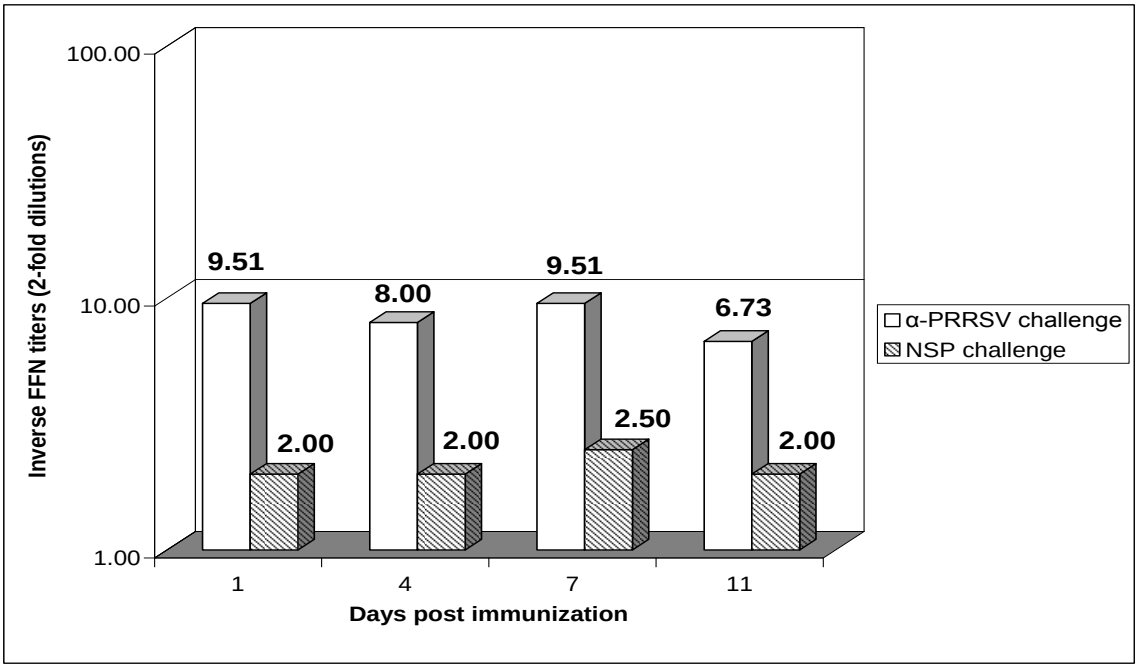


Figure 6. Experiment 4. Quantification of virus neutralizing antibody titers in either pigs passively immunized with antibody (α-PRRSV) or pigs administered normal plasma (NSP) and challenged with HLV092.

Table 28. Experiment 4. Antibody and live virus determined in principle pigs either passively immunized with antibody to PRRSV (α-PRRSV) or administered normal swine plasma (NSP) and challenged with HLV092.

	Number of pigs positive (4 pigs per group)							
	ELISA*	VI**	ELISA	VI	ELISA	VI	ELISA	VI
Days post challenge	3		6		10		14	
α-PRRSV	0	1	0	4	0	4	4	4
NSP	0	4	0	4	3	4	4	4

*Antibody detected to nucleocapsid protein
**PRRSV isolation

Table 29. Experiment 4. Titration of live virus ($CCID_{50}$/ml) in serum of pigs administered either plasma with antibody (α-PRRSV) or normal plasma (NSP) and challenged with HLV092.

Treatment Group	Pig #	VI*	Group mean	VI	Group mean	VI	Group mean	VI	Group mean
Days post challenge		3		6		10		14	
α-PRRSV	1915	<2.5E+01		5.39E+05		7.91E+02		7.91E+03	
	1926	<2.5E+01		7.91E+02		7.91E+02		5.39E+03	
	1937	<2.5E+01		2.50E+02		1.16E+03		2.50E+04	
	1942	2.50E+01	1.26E+01	7.91E+03	5.37E+03	1.16E+03	1.00E+03	2.50E+04	1.26E+04
NSP	1902	5.39E+04		7.91E+05		7.91E+04		2.50E+04	
	1913	2.50E+04		7.91E+05		1.16E+04		7.91E+04	
	1935	1.16E+05		1.16E+06		7.91E+03		1.16E+05	
	1936	7.91E+05	1.07E+05	1.16E+05	5.62E+05	7.91E+04	2.82E+04	7.91E+04	6.76E+04
p value			5.99×10^{-4}		0.064		0.010		0.021

*PRRSV isolation

A t-test was performed to calculate p values

Table 30. Experiment 4. Gross lung lesions at necropsy of challenged pigs passively immunized with antibody to PRRSV (α-PRRSV) or pigs receiving normal swine plasma (NSP) and challenged with HLV092. Total possible score is 100 per pig.

Treatment Group	Pig #	Total gross lung score	Group Mean [a]
α-PRRSV	1915	59	52.5
	1926	52	
	1937	48	
	1942	51	
NSP	1902	49	48.5
	1913	47	
	1935	45	
	1936	53	

A Kruskal-Wallis step-wise comparison was performed ([a] p value=0.343)

Table 31. Experiment 4. Histopathological evaluation and score of interstitial pneumonia by microscopic lesions of challenged pigs immunized with either antibody to PRRSV (α-PRRSV) or pigs receiving normal swine plasma (NSP) and challenged with HLV092.

Treatment Group	Pig #	Lung (0-6)	Mean	LN* (0-3)	Mean	Tonsil (0-3)	Mean	Heart (0-3)	Mean
α-PRRSV	1915	5	5.00	1	1.75	1	1.75	2	1.75
	1926	5		2		2		2	
	1937	5		2		2		2	
	1942	5		2		2		1	
NSP	1902	5	5.00	1	1.75	2	2.00	2	1.25
	1913	5		2		2		1	
	1935	5		2		2		1	
	1936	5		2		2		1	
p value		1		1		1		0.486	

*Lymph nodes

A Kruskal-Wallis step-wise comparison was performed to calculate p values

Table 32. Experiment 4. Detection of antibody and PRRSV in serum of sentinel pigs exposed to either pigs passively immunized with antibody to PRRSV (α-PRRSV) or exposed to pigs receiving normal swine plasma (NSP) and challenged with HLV092.

	Number of pigs positive (2 sentinel pigs per group)									
	ELISA*	VI**	ELISA	VI	ELISA	VI	ELISA	VI	ELISA	VI
Days post exposure	3		7		10		14		21	
α-PRRSV	0	0	0	0	0	1	0	1	1	2
NSP	0	2	0	2	2	2	2	2	2	2

*Antibody detected to nucleocapsid protein
**PRRSV isolation

Table 33. Experiment 4. Gross lung lesions at necropsy of sentinel pigs exposed to either pigs passively immunized with antibody to PRRSV (α-PRRSV) or exposed to pigs receiving normal swine plasma (NSP) and challenged with HLV096. Total possible score is 100 per pig.

Treatment Group	Pig #	Total gross lung score	Group Mean [a]
α-PRRSV	1938	8	23.5
	1939	39	
NSP	1901	49	38.0
	1934	27	

A Kruskal-Wallis step-wise comparison was performed ([a] p value=0.667)

Table 34. Experiment 4. Histopathological evaluation and score of interstitial pneumonia by microscopic lesions of sentinel pigs exposed to pigs immunized with either antibody to PRRSV (α-PRRSV) or pigs receiving normal swine plasma (NSP) and challenged with HLV092.

Treatment Group	Pig #	Lung (0-6)	Mean	LN* (0-3)	Mean	Tonsil (0-3)	Mean	Heart (0-3)	Mean
α-PRRSV	1938	1	2.0	1	1.0	1	1.0	0	0
	1939	3		1		1		0	
NSP	1901	6	5.5	2	2.0	2	2.0	2	2.0
	1934	5		2		2		2	
p value		0.333		0.333		0.333		0.333	

*Lymph nodes
A Kruskal-Wallis step-wise comparison was performed to calculate p values

VIII. Discussion:

Although pigs in experiment 1 did not have a high level of passively acquired antibody, protection was still provided. Pigs in experiment 2 showed a significant decrease in viremia levels at all time points except for 10 dpc. Protection was also shown in the form of less severe interstitial pneumonia in the immunized pigs and in the sentinels exposed to this group. There was also a delay in transmission to the sentinel pigs exposed to the immunized group as compared with the sentinels exposed to the NSP treated pigs. These results are correlative to that of Lopez et al., supporting the evidence that low levels of VN antibody can delay viremia. Due to the small sample size and the design of the statistical analysis, the difference in lung score lesions of the sentinel pigs was not significant, but is an observed difference.

In experiment 3 the immunized pigs had no detectable viremia, except for 1 pig at necropsy that had an extremely low level of virus. Gross lung lesions did not differ greatly between the two groups but both groups had low macroscopic lung lesion scores, indicating the virus may not have been virulent. The sentinel pigs exposed to the α-PRRSV immunized group did not become viremic nor did they seroconvert at any time point, indicating that transmission did not occur. Macroscopic lung lesions were different between the two groups, although not determined statistically significant due to sample size. This numerical difference in macroscopic lung lesions and the lack of viremia in sentinels indicates that transmission was prevented when sentinels were exposed to α-PRRSV immunized pigs, which was not shown by Lopez et al. The sentinel pigs exposed to the NSP group had a mean macroscopic lung score of 63, indicating their exposure dose was high and the virus may have become more virulent by passage through the NSP pigs.

Experiment 4 showed protection in the form of decreased viremia in the immunized pigs, which was a significant difference at all time points except at 6 dpc, indicating that the VN levels were high enough to delay viremia. Macroscopic lung lesions were very high in both the immunized and non-immunized pigs, possibly due to the virulence of the challenge strain or the increased volume of challenge virus. The sentinel pigs exposed to the α-PRRSV group demonstrated delay in transmission, but minimal protection based on macroscopic and histopathologic lesions. In experiment 4, a greater inoculum dose was utilized due to low titer of the virus.

Each of the challenge studies demonstrated some level of protection provided by passive immunization with immune plasma. Protection was shown in the form of delay in viremia, level of viremia, decrease in lung lesions, or delay and/or lack of transmission to naïve sentinels. The greatest level of protection was demonstrated in homologous challenge with HLV096 (Experiment 3) even though the VN antibody titers were highest in Experiment 2 against HLV013. According to published results by Lopez et al., the high VN levels achieved by passive immunization of pigs in experiment 2 would be expected to result in

“sterilizing immunity” of 50% of the animals, which was not shown, as 75% of the animals became viremic by 6 dpc. But sterilizing immunity was demonstrated in experiment 3 pigs, in which viremia was prevented in the immunized pigs and the sentinels exposed to these pigs, even though the VN levels in the passively immunized pigs were not at sterilizing levels of 1:32 as indicated by Lopez et al. *In vitro* studies have shown VN antibodies against GP5 are able to reduce PRRSV attachment to macrophages and inhibit virus neutralization. But attachment and internalization are only inhibited when homologous PRRSV strains and VN antibodies are used. Future research could include *in vitro* inhibition studies to determine if the immune plasma used in passive studies is capable of completely inhibiting attachment and internalization of both homologous and heterologous PRRSV strains.

It is interesting that the HLV096 challenge strain (experiment 3) and the heterologous challenge strain (HLV092, experiment 4) only differ by 4 amino acids in the 21-60 amino acid range of GP5 and have the same glycan pattern. The VN antibody levels are very similar between experiments 3 and 4 and yet the level of protection was greater in experiment 3. It would be beneficial to repeat the study with the same volumes of HLV096 and HLV092 challenge virus to determine if this difference in protection was due to an increase in volume of challenge virus. It would also be interesting to repeat this experiment with other heterologous strains with varying levels of relatedness to the strains used in immune plasma production. The outcome would aid in determining if the decrease in protection is due to the immune plasma lacking antibody specific to the challenge strain or if it is due to the relatedness of the challenge virus to the virus used in producing the immune plasma.

Although we did demonstrate protection, we were unable to show more protection than previous studies. Future research could include generating immune plasma with the same PRRSV strains, but in a different infection order, as well as generating plasma with different PRRSV strains. It would be interesting to determine if antibodies to less glycosylated strains are more effective when initially produced, or being induced after multiple infections. Additional research including increased dosage of passive antibody would also be beneficial. If we had the opportunity and resources to repeat these studies, it would be interesting to monitor the antibody levels for a longer period of time post-challenge.

Experimental inoculation of horses with virulent PRRSV generated neutralizing antibodies. These antibodies were detectable in passively immunized piglets at 24 hours post immunization when either whole or concentrated equine plasma was used. The fact that equine antibodies are not detectable in the current screening ELISA may be useful for monitoring passively immunized, exposed piglets. Also, the relatively large volumes of plasma that may be obtained from horses could improve the practicality of passive immunization in certain instances.

Maternally derived antibodies against PRRSV have shown to protect against PRRSV exposure in piglets. But there is no data evaluating immune activity in such piglets. Research has shown that maternally derived antibodies against

certain viruses can provide complete protection in offspring, but also completely inhibit an immune response in the offspring when exposed to the virus. This is the case with swine influenza. But maternal antibodies against Bovine Viral Diarrhea virus (BVDV) provide protection while allowing the offspring to develop an immune response. It would be of great value to determine if an immune response in passively immunized piglets is inhibited by passive antibodies. This could be done by including a second challenge in the previously described experimental design to see if an immune response was induced in passively immunized piglets when exposed to live PRRSV a first time. If an immune response was induced during first infection/exposure to the virus while preventing clinical symptoms, this would be an ideal strategy for farmers. This would allow a method to immediately protect piglets against PRRSV infection and induce an immune response to protect them against future PRRSV infection.

Summary of Results. Results from immune plasma production indicated that multiple infections of naïve gilts generated significantly high VN titers. Although it was not determined whether number of infections, number of strains used, or time post infection had the greatest impact on VN antibody production, it was shown that the highest VN levels against heterologous strains were produced by pigs infected with more than 1 PRRSV strain. Although some heterologous VN titers were generated, VN titers against Lelystad Virus and SD01-08 (European-like) were never produced in 19 of 20 pigs (one pig had a VN titer of 1:8 at 135 dpi).

Immune plasma was produced with sufficient VN levels that could be passively administered to naïve piglets. Passive immunization was demonstrated in both the antibody decay study and the antibody maintenance study, indicating VN levels that have previously been shown to significantly reduce viremia in immunized pigs could be reached via subcutaneous or intraperitoneal immunization. Although antibody level endpoints were never determined, we did show that the VN levels remain at levels considered protective for at least 13 days. This could be very beneficial in movement of pigs from farrowing to nursery barns, as not all pigs are weaned at exactly the same time.

The infection of gilts with two different strains (Group 2) or with three different strains (Group 3) of PRRSV resulted in high titer neutralizing antibodies to all North American strains evaluated but no cross reactivity to the European strains in 7 of 8 pigs (Tables 5 and 6). It is interesting to note that strain HLV093 (used as the second infecting strain in both Groups 3 and 4) has a unique glycosylation pattern as compared to all other strains. Strain HLV093 lacks a glycan at AA position 44 which is centered in the neutralizing epitope region of GP5. It is tempting to speculate that the absence of this glycan allows for the inducement of cross reacting antibodies to the other NA strains tested by FFN. However, further work is necessary to confirm this statement.

The research reported herein has shown the ability to generate high VN antibody titers in naïve gilts. Passively administering this immune plasma has provided some level of protection against PRRSV.

Horses were also able to generate VN antibodies following inoculation with virulent PRRSV. These antibodies were detectable in piglets following passive immunization with equine plasma.

Each of the challenge studies demonstrated some level of protection provided by passive immunization with high titer VN antibodies. Protection was shown by delay in viremia, level of viremia, decrease in macroscopic and microscopic lung lesions, or delay and/or lack of transmission to naïve sentinels. The greatest level of protection was demonstrated in homologous challenge with HLV096 (Experiment 3) even though the VN antibody titers were highest in Experiment 2 against HLV013. Recent publication by Lopez et al. would predict that the high VN levels achieved by passive immunization of pigs in experiment 2 would result in "sterilizing immunity" of 50% of the animals, which was not shown (75% of the animals became viremic by 6 dpc). However, sterilizing immunity was demonstrated in experiment 3 pigs, in which viremia was prevented in the immunized pigs and the sentinels exposed to these pigs, even though the VN levels in the passively immunized pigs were not at sterilizing levels of 1:32 as determined by Lopez et al.

Maternally derived antibodies against PRRSV have shown to protect against PRRSV exposure in piglets. But there is no data evaluating immune activity in such piglets. Research has shown that maternally derived antibodies against certain viruses can provide complete protection in offspring, but also completely inhibit an immune response in the offspring when exposed to the virus. This is the case with swine influenza. But maternal antibodies against Bovine Viral Diarrhea virus (BVDV) provide protection while allowing the offspring to develop an immune response. It would be of great value to determine if an immune response in passively immunized piglets is inhibited by passive antibodies. This could be done by including a second challenge in the previously described experimental design to see if an immune response was induced in passively immunized piglets when exposed to live PRRSV a first time. If an immune response was induced during first infection/exposure to the virus while preventing clinical symptoms, this would be an ideal strategy for farmers. This would allow a method to immediately protect piglets against PRRSV infection and induce an immune response to protect them against future PRRSV infection.

Although previous passive immunization studies have shown sterilizing immunity in some young piglets and prevention of reproductive failure, our results were different due to several factors. In relation to Osorio's work with pregnant gilts, the age of the pigs may have an impact on the resulting protection. Because older pigs have a more developed immune response, this in conjunction with passively administered VN antibodies may work together to prevent infection. Future work in our laboratory could include passive immunization studies with pregnant gilts to determine if the immune plasma we produced is as effective. Because the pork industry is so negatively affected by PRRSV, much research is needed to find a solution. Despite a passive immunization strategy only being a temporary solution (since it would not induce a natural immune response in the pigs immunized), if protection against multiple PRRSV strains could be shown,

passive immunization could be an extremely beneficial option for the pork industry.