RESEARCH REPORT
SWINE HEALTH

Title: Mechanisms of Failed Protection Against PRRS in Sow Herds - **NPB #06-171**

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Date Submitted: 29 September 2010

Industry Summary

Serum inoculation of gilts with on-farm isolates is used to protect endemically infected sow herds against reproductive PRRS. However, outbreaks still occur in which the offending virus is genetically similar to the inoculation virus across the entire genome, raising the possibility that "homologous" protection is not complete. Here, we showed that prebreeding gilts inoculated with a virulent virus and challenged in late gestation had incomplete immune protection against challenge with the identical (homologous), immunizing virus as well as a 98% similar heterologous virus. Late gestation challenge resulted in 15% abortions of challenged sows in both groups. Approximately 55% of conceived pigs were weaned in each test group, and viremic pigs at weaning were identified at a rate of 27% in heterologous challenge and 10% in homologous challenge, with at least 1 viremic pig in every litter (n=14, homologous; n=15, heterologous). In contrast, 100% of piglets born to uninoculated, challenged gilts were viremic and failed to thrive. We conclude that live virus inoculation provides immunological protection against reproductive PRRS, but protection is not complete, even against homologous challenge.

Keywords
Swine, porcine reproductive and respiratory syndrome virus, serum inoculation, immunity, PCR, antibody

Scientific Abstract

For many swine producers the critical point in control of endemic PRRS is prevention of virus transmission from pregnant sows to piglets, i.e. weaning negative pigs. However, common-sense practices involving whole herd exposure to on-farm isolates that is expected to provide complete, homologous immunity are not completely successful and rebreaks with significant reproductive disease and transmission of PRRSV to the nursery still occur. Initial studies showed that outbreak viruses were genetically similar to the immunizing virus, suggesting that serum inoculation was not completely effective. Controlled experiments replicating field conditions of gilt exposure to virulent virus, followed by late gestation challenge with the identical virus (homologous challenge) or genetically related virus (heterologous virus >98% similar) were performed to evaluate the level of protection. In both situations, sows were protected against acute, reproductive PRRS compared to non-immune, challenged controls. However, acute abortions were not prevented and occurred in about 15% of challenged sows. Fifty-five percent of conceived pigs were weaned in each test group. Viremic

pigs were weaned at a rate of 27% in heterologous challenge and 10% in homologous challenge, with a least 1 viremic pig in every litter (n=14, homologous; n=15, heterologous). We conclude that live virus inoculation provides immunological protection against reproductive PRRS, but protection is not complete, even in the case of homologous challenge.

Introduction

Porcine reproductive and respiratory syndrome virus (PRRSV) is a member of the Arteriviridae family in the order Nidovirales that emerged in the late 1980's almost simultaneously in North America and Europe and quickly became endemic in pig populations around the World (Hill, 1990; Wensvoort, et al, 1991). The disease is characterized by a respiratory disease in growing pigs (pneumonia and respiratory difficulties) and reproductive failure in pregnant sows, characterized by late-term abortions, premature farrowing, stillbirths, mummified and weak-born piglets. Economic losses can be catastrophic, sometimes reaching 100% deaths in farrowing/weaning units with an estimated annually costs of $560 million in losses in the United States (Neumann, et al, 2005).

Given the rapid mutational rate of PRRSV, estimated to be the highest of any known virus (one percent per year, Prieto, 2009), protection of a herd is sometimes achieved by immunizing the gilts with the circulating strain to provide protection during the reproductive phase. However, despite these intensive efforts, PRRSV outbreaks still occur putting in doubt serum exposure efficacy, not to mention the cost and labor of the process. Licensed, attenuated vaccines are perceived to provide partial or no protection against reproductive disease in sow herds. Thus, inoculation of gilts and sows with virulent viruses isolated on the farm is believed to provide complete protection. Since PRRS outbreaks occur on farms using serum inoculation, but the recovered virus appears to be derived from the inoculating virus, we determined if virulent virus inoculation provided protection against PRRS.

Objectives

Objective. To assess in pregnant sows the biological difference in viruses reisolated following rebreaks in "protected" herds.

Research Question 1. Do viruses recovered from rebreaks change in virulence?

Research Question 2. Do viruses recovered from rebreaks escape homologous immunological protection?

Materials & Methods

Four control groups were utilized in this study. The heterologous group consisted of gilts inoculated with the BB strain (genebank EF532803) and challenged at 210 days later, in late gestation with the BA strain (genbank EF532802). The homologous group consisted of gilts inoculated with the BA strain and challenged at 210 days later, in late gestation with the same strain (BA). The negative group consisted of gilts inoculated with the BA strain and not challenged in late gestation. The positive group consisted of gilts not vaccinated and challenged at 210 days, in late gestation, with the BA strain. Inoculations and challenges were performed by intramuscular injections of 1ml of 1.0 X 10^5 copies of the corresponding viral strain.

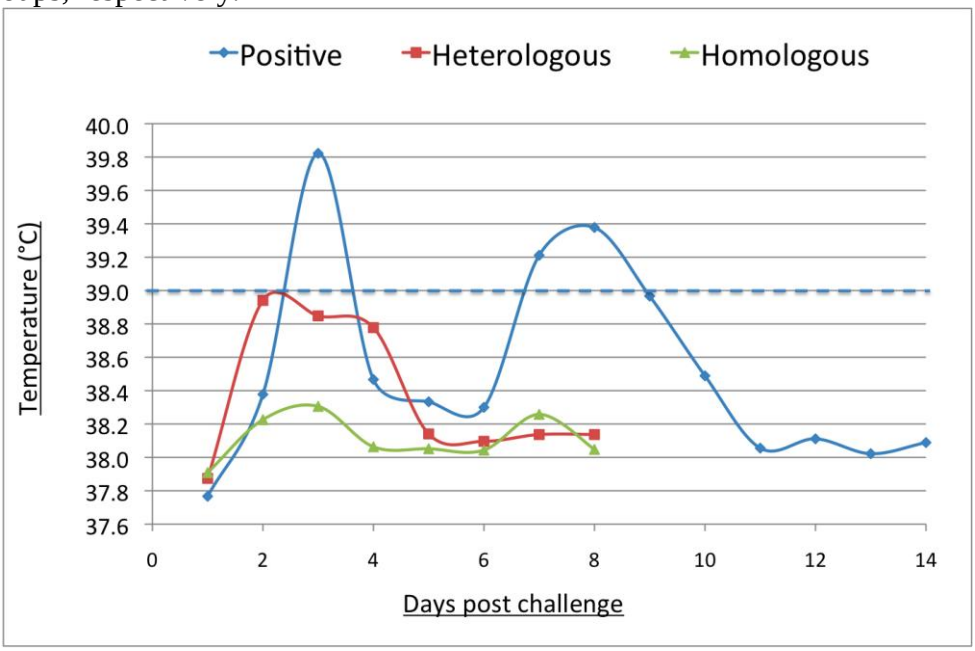
Serum was obtained from vaccinated sows at Days 0, 3, 7 and 21days post infection to test for seroconversion via ELISA and viremia levels via qRT-PCR. ELISA was performed at the University of Minnesota Veterinary Diagnostic lab. For qRT-PCR, 140ul of serum were used to extract RNA using the Qiagen Viral Extraction Mini Kit. Protocols are based on manufacturer's manual and eluting with 50 ul of RNAse Free water. Complementary DNA (cDNA) synthesis was performed with 10 ul of eluted RNA using the High Capacity cDNA synthesis kit from Applied Biosystems. This kit includes all the materials needed for synthesis (dNTP's, RT enzyme and random hexamers). Protocols are based on manufacturer's manual and the resulting 20 ul of cDNA are diluted 1/10X by adding 180 ul of water. Parameters for the Mx3000p thermal cycler were: 95° for 10 minutes, 50 cycles at 95° for 15 seconds and 60° for 1 minute and a dissociation step. The reaction mix consisted of 2.5 ul of 2.0 uM Forward primer (aaccacgcatttgtcgtc), 2.5 ul of 2.0 uM Reverse primer (tggcacagctgattgactgg), 5.0 ul of cDNA and 10.0 ul of 2X FastStart SYBR-green Quanta mix.

Sequencing of positive samples after challenge was performed using a set of primers spanning the ORF5 of PRRSV. Primer used were ATGTTGGGGAAATGCTTGAC (Forward) in pair with either GACTCACCTTTAGGGCATATATCATCA (Reverse 1) or GCAAGCACAAACGGCATCT (Reverse 2). Molecular identification of ORF5 sequences was done via local NCBI blastn of obtained chromatograms against a database of 12,000 ORF5 sequences that included both strains used in the study.

Results

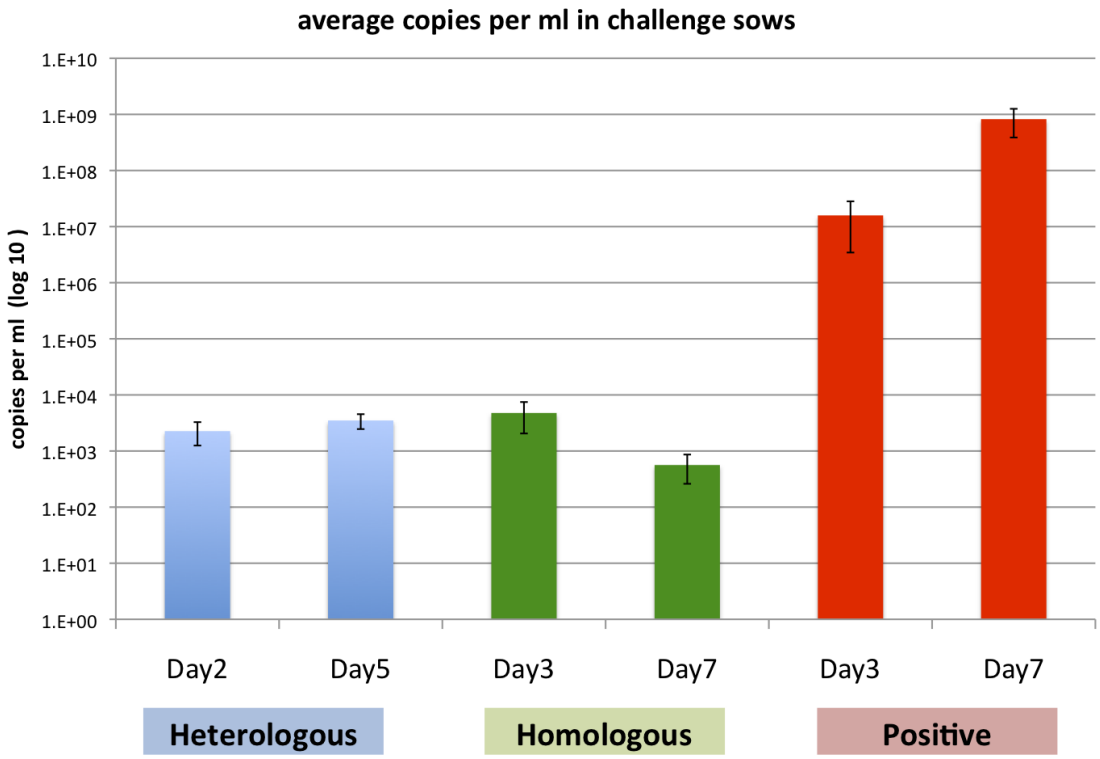
Fever, defined as temperatures over 39°C degrees, were observed in challenged sows from the homologous, heterologous and naïve challenge (positive) groups, with homologous challenge being least severe (Figure 1). This trend correlates with viremia levels observed in sows and their litters (Figures 2 and 3). However, fever did not correlated with abortions. Abortions were observed in homologous and heterologous groups at 13.3% and 14.3% respectively, but were absent from the positive challenge group.

Figure 1. Rectal sow temperatures after challenge. In blue, red and green lines homologous, heterologous and naïve challenge groups, respectively.



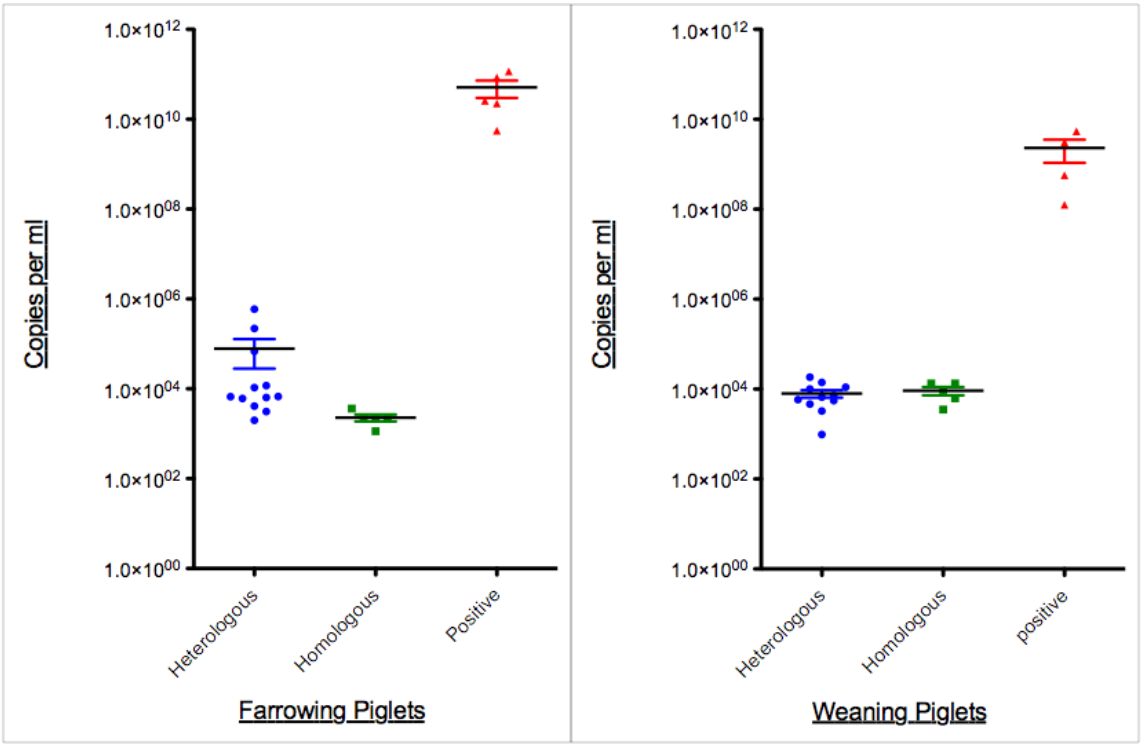
Virus levels in challenge sows was measured via quantitative reverse transcriptase-PCR and reported in copies per ml. The negative control (non-challenge) group tested negative for all reactions. Comparison of heterologous, homologous and positive controls clearly shows differences between uninoculated and inoculated groups (Figure 2).

Figure 2. Sow viremia levels after challenge. In blue, green and red the mean copies per ml in each sow in presented for heterologous, homologous and positive control groups respectively. Error bars are the standard error for each group as calculated in GraphPad Prism 5.0.



Viremia levels in piglets were different between heterologous-positive and homologous-positive groups (Table 1, $p<0.001$, Tukey-Kramer ANOVA). These differences were observed both at farrowing and at weaning (Figure 3).

Figure 3. Viremia levels in piglets at farrowing and weaning.

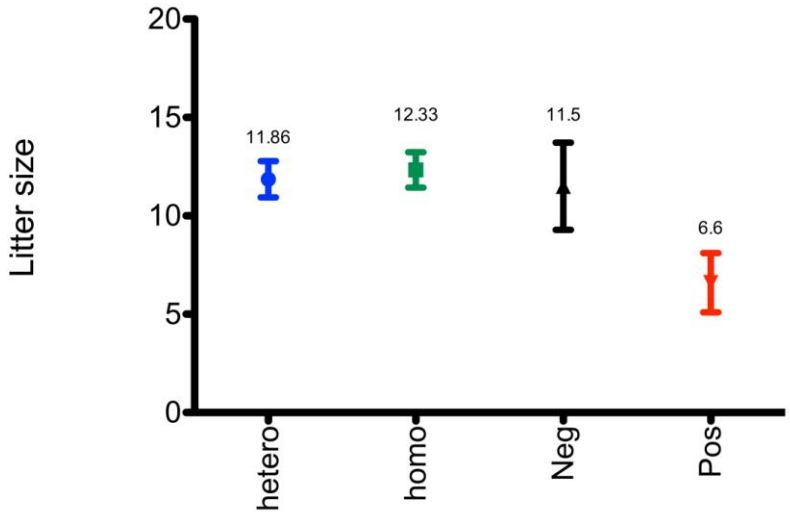


Failure to thrive analysis (pigs suitable for delivery to nursery divided by total conceived pigs) showed that heterologous and homologous groups had a 45% mortality rate in contrast to 100% mortality in the positive group. These findings indicated that inoculation did not prevent PRRS reproductive disease and transmission but it was better than without intervention. Litter size was also measured by averaging the number of born piglets per sow (Figure 4).

Table 1. Summary of viremic piglets percentages, mortality rates and litter size among groups.

	Heterologous	Homologous	Negative	Positive
Infected at farrowing	36/185	3/166	1/46	38/40
	16.3%	**1.8%**	**2.2%**	**95.0%**
Infected at weaning	33/121	11/112	2/44	12/12
	27.2%	**9.8%**	**4.5%**	**100.0%**
Failure to wean	83/185	74/166	2/46	40/40
	44.9%	**44.6%**	**4.3%**	**100.0%**
Average live-born litter size	11.86	12.33	11.5	6.6

Figure 4. Average litter size in control groups. Data are mean ± SEM.



Discussion

The purpose of the study was to investigate the level of protection in pregnant gilts provided by a live virus against a homologous or heterologous strain exposure. Strain selection for the study was based on historical data indicating that the BA strain was recovered from an outbreak that occurred in a BB immunized herd (Mark Wagner, pers. communication). When the outbreak strain was sequenced and compared to the inoculation virus, both strains shared 98.3% sequence identity across the whole genome. Similar observations have been reported among swine producers leading to the subjective perception that inoculation with on-farm isolates are ineffective in controlling infections by heterologous strains. Alternatively, it was possible that field observations were due to other factors such as lack of uniform inoculation, inadvertent inactivation of the viral inoculum, and so forth. Our findings indicate that serum inoculation provided strong protection to animals against challenge with either heterologous or homologous strains. Clinical signs, percentage of PRRSV infected pigs at weaning, and viral loads in piglets were significantly lower in inoculated groups than in the unprotected positive control group. In summary, while PRRSV transmission was not prevented, serum inoculation greatly diminished the severity of the disease in what otherwise would have been 100% losses in weaned pigs.

Acknowledgements. Mark Wagner, DVM, Fairmont Veterinary Clinic, was a key collaborator and study participant without which the experiments could not have been performed.

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