RESEARCH REPORT
SWINE HEALTH

Title: Preweaning surveillance: Finger on the pulse of PRRSV epidemiology, transmission and spread.
NPB #11-113

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Industry Summary: We believe that achieving control of PRRSV will require the industry to develop the capacity to easily, efficiently, and continuously surveil herds for PRRSV. The objective of the research was to explore one possible surveillance option.

In four PRRSV vaccinated commercial swine herds, oral fluid samples were collected from 600 litters (150 samples from each of the 4 herds) 24 hours prior to weaning and serum samples from their dams two days post weaning. All sows had received at least 4 doses of PRRSV vaccine.

Once collected, samples were completely randomized and tested for PRRSV (RT-qPCR and sequencing) and PRRSV antibodies. In addition, PRRSV ORF5 sequencing was attempted on RT-qPCR-positive samples. Virus and antibody assay results were analyzed for associations with farm, sow parity, litter size, time, and infection status.

Testing of pre-weaning oral fluid samples (n = 600) and sow serum samples (n = 600) by PRRSV RT-qPCR resulted in 9 positive oral fluid samples. No PRRSV RT-qPCR-positive serum samples were observed. The positive results were confirmed by blind re-testing at a second laboratory.

Conclusions and observations:

1. PRRSV can be present in litters of clinically normal pigs in well-vaccinated herds at very low levels. That is, the incidence of infection in sampled litters was 1.5%. A striking feature was the highly sporadic nature of the infection. That is, infected litters were "hidden" among a majority of PRRSV-negative litters.

2. The obvious question is, "Where did the virus come from?" The sequence analyses showed that the virus was wild-type virus (not vaccine virus).

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No viremic sows were detected, but sow serum samples from RT-qPCR-positive litters showed significantly higher ($p < 0.05$) mean serum IgG (1.73 vs. 0.98) and commercial kit (1.97 vs. 0.98) S/P ratios, but no difference in IgM or IgA responses. These data fit the previous observation that re-exposure of vaccinated or PRRSV-infected animals did not produce anamnestic IgM or IgA responses, but did produce an anamnestic IgG response.

Although the antibody responses indicated that both the sows and their litters had been infected, the source(s) of the virus was not revealed by these data. Nor is it possible to determine whether the sows were infected before or after the piglets.

3. Collection and testing of oral fluids from litters immediately prior to weaning was useful for the detection of PRRSV. The utility and limitation of this approach should be evaluated in other herds, particularly in herds that are moving toward PRRSV elimination.

Keywords: PRRSV, surveillance, antibody, PCR, oral fluid

Scientific Abstract: Achieving control of PRRSV will require the industry to develop the capacity to easily, efficiently, and continuously surveil herds for PRRSV. The objective of the research was to explore one possible surveillance option.

In four PRRSV vaccinated commercial swine herds, oral fluid samples were collected from 600 litters (150 samples from each of the 4 herds) 24 hours prior to weaning and serum samples from their dams two days post weaning. All sows had received at least 4 doses of commercial PRRSV modified live virus vaccines.

Once collected, samples were completely randomized and tested for PRRSV (RT-qPCR and sequencing) and PRRSV antibodies. In addition, PRRSV ORF5 sequencing was attempted on RT-qPCR-positive samples. Virus and antibody assay results were analyzed for associations with farm, sow parity, litter size, time, and infection status.

Testing of pre-weaning oral fluid samples (n = 600) and sow serum samples (n = 600) by PRRSV RT-qPCR resulted in 9 positive oral fluid samples. No PRRSV RT-qPCR-positive serum samples were observed. The positive results were confirmed by blind re-testing at a second laboratory.

Among the 9 PRRSV RT-qPCR-positive samples, 3 oral fluid samples had been used up. The remaining 6 oral fluid samples were submitted for PRRSV ORF5 sequencing and sequences were obtained on 2 of the 6. Pairwise comparisons based on ORF5 nucleotide percent identity showed that, although detected from litters not showing clinical signs, the viruses identified in the study (Farm 2 07/2011 and Farm 3 08/2011) matched viruses detected in serum samples collected from sows in association with abortions. (See Table 1)

A statistical analysis based on PRRSV RT-qPCR quantitative results (Ct values) detected no statistically significant associations with farm, sow parity, or their interactions. However, an analysis of oral fluid antibody responses showed significantly higher mean antibody isotype S/P ratios in RT-qPCR-positive versus negative oral fluid samples ($p < 0.05$; IgM = 0.03 vs. 0.00, IgA = 0.16 vs. 0.04, and IgG = 3.46 vs. 2.36), but no difference in commercial kit S/P responses. Sow serum samples from RT-qPCR-positive litters showed significantly higher ($p < 0.05$) mean serum IgG (1.73 vs. 0.98) and commercial kit (1.97 vs. 0.98) S/P ratios, but no difference in IgM or IgA responses.

Introduction: Cost-effective methods of disease surveillance are needed to support animal health and business decisions at the herd level, improve the quality of field research, and provide timely information on endemic and foreign animal diseases in the national swine herd. The National Pork Board (NPB) has recognized the importance of the problem and stated that their goal for U.S. pork producers is "*a comprehensive and integrated swine health surveillance system*" (Anon, 2010). The long-term goal of this project was to provide the means to implement NPB's "comprehensive and integrated swine health surveillance system". The primary long-standing roadblock to this goal is the inconvenience and cost of acquiring the information, i.e., the expense of collecting and testing statistically appropriate numbers of specimens from individual pigs. A cost-effective solution to the current health information vacuum is vital.

Objectives: The objective of the proposed research was to evaluate an easier, cheaper method to detect and monitor PRRSV circulation in the breeding herd and growing pig populations based on collection and testing of oral fluid samples prior to weaning.

Materials & Methods: The study was performed in four ~12,500 sow breeding herds. Animal housing, feeding, handling, and veterinary care were under the supervision of Seaboard L.L.C. Health Assurance and Welfare personnel. All four herds were considered to be endemically infected with PRRSV on the basis of diagnostic history and on-going surveillance. Commercial PRRSV vaccines were used to control clinical losses, with some differences in the vaccination protocols among sites. In all four herds, replacement gilts were vaccinated twice with a commercial modified-live (MLV) PRRSV vaccine during quarantine. Once entered into the breeding herd, sows were re-vaccinated with a PRRSV vaccine at least twice per year.

Oral fluid specimens were collected from 600 litters of pigs prior to weaning and serum from their dams. All serum samples were assayed for PRRSV antibodies using a commercial indirect ELISA (PRRS X3 Ab Test, IDEXX Laboratories, Inc., Westbrook, ME) performed according to the manufacturer's instruction. In addition, the commercial indirect ELISA (PRRS X3 Ab Test) was modified to detect PRRSV-specific IgM, IgA, and IgG antibody isotypes in serum. All oral fluid samples were assayed for PRRSV antibodies using a commercial indirect ELISA (PRRS Oral Fluids Ab Test) performed according to the manufacturer's instruction. In addition, a commercial PRRSV ELISA was modified for the detection of PRRSV-specific IgM, IgA, and IgG antibody in swine oral fluid, as has previously been described (Kittawornrat et al., 2012).

All samples (600 serum samples, 600 oral fluid samples) were tested for the presence of PRRSV by RT-qPCR. Samples initially identified as RT-qPCR-positive were submitted for confirmatory testing at a second laboratory. The confirmatory sample set included 21 blindly selected RT-qPCR-negative oral fluids and 30 blindly selected RT-qPCR-negative sow serum samples.

Statistical analyses were performed using SAS® 9.2 (SAS® Institute Inc., Cary, NC USA) and MedCalc® 9.2.1.0 (MedCalc Software, Mariakerke, Belgium). Serum and oral fluid S/P ratios were analyzed for significant associations with sow parity, litter size, herd, sampling time point, and their pairwise interactions by analysis of variance (ANOVA). Descriptive and comparative analyses were used to evaluate the quantitative (Ct) and qualitative (pos/neg) RT-qPCR results in serum and oral fluid samples. PRRSV ORF5 nucleotide percent identity of wild type sequences recovered during the study were compared to wild type isolates recovered from the farms either before or after the study, the North American prototype PRRSV (VR-2332), and modified live vaccines.

Results: Among 9 PRRSV RT-qPCR-positive oral fluid samples, 3 samples had been used up. The remaining 6 oral fluid samples were submitted for PRRSV ORF5 sequencing and sequences were obtained on 2 of the 6. Pairwise

comparisons based on ORF5 nucleotide percent identity showed that, although detected from litters not showing clinical signs, the viruses identified in the study (Farm 2 07/2011 and Farm 3 08/2011) matched viruses detected in serum samples collected from sows in association with abortions. (See Table 1)

A statistical analysis based on PRRSV RT-qPCR quantitative results (Ct values) detected no statistically significant associations with farm, sow parity, or their interactions. However, an analysis of oral fluid antibody responses showed significantly higher mean antibody isotype S/P ratios in RT-qPCR-positive versus negative oral fluid samples ($p < 0.05$; IgM = 0.03 vs. 0.00, IgA = 0.16 vs. 0.04, and IgG = 3.46 vs. 2.36), but no difference in commercial kit S/P responses. Sow serum samples from RT-qPCR-positive litters showed significantly higher ($p < 0.05$) mean serum IgG (1.73 vs. 0.98) and commercial kit (1.97 vs. 0.98) S/P ratios, but no difference in IgM or IgA responses.

Table 1. Pair-wise comparison of PRRSV isolates from the study farms based on ORF5 nucleotide percent identity

Isolate	Farm 2 11/2010	Farm 2[1] 07/2011	Farm 2 11/2011	Farm 2 03/2012	Farm 3 12/2010	Farm 3[1] 08/2011	ATP[2]	MLV[3]	VR2332[4]
Farm 2 11/2010		99.0	98.6	99.3	86.5	86.0	89.0	87.3	87.3
Farm 2 07/2011[1]	99.0		98.1	98.6	86.2	85.7	88.3	86.7	86.7
Farm 2 11/2011	98.6	98.1		99.3	86.4	86.2	88.3	86.4	86.4
Farm 2 03/2012	99.3	98.6	99.3		86.5	86.4	88.7	86.7	86.7
Farm 3 12/2010	86.5	86.2	86.4	86.5		99.0	89.3	86.0	86.0
Farm 3 08/2011[1]	86.0	85.7	86.2	86.4	99.0		88.8	85.5	85.5
ATP	89.0	88.3	88.3	88.7	89.3	88.8		90.2	90.5
MLV	87.3	86.7	86.4	86.7	86.0	85.5	90.2		99.3
VR2332	87.3	86.7	86.4	86.7	86.0	85.5	90.5	99.3	

[1] *Sequences from Farm 2 isolate 07/2011 and Farm 3 isolate 08/2011 were derived from litter oral fluid samples collected in the study. Other Farm 2 and Farm 3 viruses were derived from sow serum samples collected in association with abortions.* ***NOTE*** *that sow serum samples either preceded (Farm 2 11/2010 and Farm 3 12/2010) or followed (Farm 2 11/2011 and 03/2012) the viruses detected in the study.*

Discussion: Explain your research results and include a summary of the results that is of immediate or future benefit to pork producers.

1. PRRSV can be present in litters of clinically normal pigs in well-vaccinated herds at very low levels. That is, the incidence of infection in sampled litters was 1.5%. The presence of PRRSV in these litters explains subsequent PRRSV circulation in nursery or wean-to-finish populations. A striking feature was the highly sporadic nature of the infection. That is, infected litters were "hidden" among a majority of PRRSV-negative litters.

2. The obvious question is, "Where did the virus come from?" The sequence analyses showed that the virus was wild-type virus (not vaccine virus).

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Although the antibody responses indicated that both the sows and their litters had been infected, the source(s) of the virus was not revealed by these data. Nor is it possible to determine whether the sows were infected before or after the piglets.

3. Collection and testing of oral fluids from litters immediately prior to weaning was useful for the detection of PRRSV. The extensive use of PRRSV vaccines in these four herds undoubtedly played a major role in the outcomes observed. It would be useful to evaluate this approach in other herds, particularly in herds that are attempting PRRSV elimination.