RESEARCH REPORT
SWINE HEALTH

Title: Determining the duration of PRRSV persistence in breeding age swine -**NPB # 01-123**

Investigator: Scott Dee

Institution: University of Minnesota

Date Received: 6/3/2002

Abstract:
The purpose of this study was to determine if PRRSV persists in a large population of breeding age female swine housed under commercial conditions for 120 to 180 days post-infection (pi) and to evaluate if experimentally infected animals shed virus to naïve sentinels beyond 90 days pi. One hundred and twenty PRRSV naïve gilts, 4 months of age, were infected by the intranasal route with 5-ml ($10^{2.4}$ $TCID_{50}$ total dose) of a field isolate of PRRSV. Following experimental infection, index animals were organized into 1 of 3 groups (A, B, and C), 40 animals per group. To assess the dynamics of the experimental infection, a monitoring group of 30 index pigs was blood-tested on days 1, 3, 14, 30, 60, 90, 120, 150, and 180 pi. To assess shedding, 30 PRRSV-naïve sentinels were commingled with index animals on day 90 pi, were tested every 14 days throughout the period of 90 to 180 days pi. To assess persistence, 40 index and 10 sentinel animals were slaughtered at 120 (group A), 150 (group B), and 180 (group C) days pi. Lymphoid tissues were collected, pooled, and tested for PRRSV by PCR and VI. Results indicated that PRRSV was not detected in any tissue pools from each of the 3 groups and all sentinels remained PRRSV-negative throughout the study.

Introduction:

Porcine reproductive and respiratory syndrome virus (PRRSV) infection constitutes one of the major disease problems in the swine industry today (1). Estimated losses of $228 per inventoried sow per year have been reported due to increased mortality, reduced growth rates, and elevated medication and vaccination costs (2). PRRSV is an RNA virus classified in the order *Nidovirales,* family *Arteriviridae*, and genus *Arterivirus* (3). Other members of the *Arterivirus* group include lactate dehydrogenase elevating virus of mice, equine viral arteritis virus and simian hemorrhagic fever virus (4). These viruses can induce a prolonged viremia in the presence of antibodies, replicate in macrophages and produce persistent infections (5). Persistent infection is defined as “the continued presence of a virus in a host for extended periods of time post infection” (6,7). RNA viruses such as PRRSV do not revert to inactive states post infection (pi), but rather continue to replicate at some level within certain sites in the body (5-7). Experimental studies have recovered viable

For more information contact:
National Pork Board, P.O. Box 9114, Des Moines, Iowa USA
800-456-7675, **Fax:** 515-223-2646, **E-Mail:** porkboard@porkboard.org, **Web:** http://www.porkboard.org/

PRRSV at 157 days post infection from tonsil tissue of growing pigs, and PRRSV nucleic acid has been detected in boar semen at 92 days pi (8,9).

In chronically infected farms, transmission of PRRSV may occur in the breeding herd, resulting in recurrent episodes of PRRS-related reproductive disease, and infection of weaned pig populations, secondary to vertical or horizontal transmission from sow to pig prior to weaning (10,11). Evidence of PRRSV transmission in endemically infected breeding herds has been reported (11,12). Within such populations, PRRSV-infected and naïve subpopulations of sows co-exist, infected animals appear to cluster in small groups or exist as singletons, and naïve sows can develop PRRSV-antibodies following exposure to virus (12,13).

However, little data are available regarding the duration of PRRSV persistence within a large experimental population of breeding age swine housed under commercial conditions. Bierk and others recently conducted a diagnostic investigation of chronic PRRSV infection in an endemically infected field population (14). Diagnostic data from 60 adult breeding swine (45 sows and 15 boars) indicated that approximately 2% of the sampled population harbored PRRSV. No conclusions could be drawn regarding whether the PRRSV-positive animals were persistently infected, nor could the investigators determine the duration of persistence due to the inability to identify the exact time that individual animal infection took place. Another publication from the same group demonstrated persistent infection and shedding of PRRSV from experimentally infected sows to contact controls in non-pregnant sows from 49 to 86 days pi. Limitations of this study included the use of small groups of animals, the use of facilities that were not representative of commercial swine systems, and the inability to assess PRRSV persistence beyond 90 days pi. Therefore, the purpose of this study was to determine if PRRSV could persist in a large population of breeding age female swine housed under commercial conditions from 120 to 180 days pi, and to evaluate if experimentally infected animals could shed virus beyond 90 days pi.

Objectives:

1. To determine the duration of PRRSV persistence in a group of breeding age swine following experimental infection.

2.To determine if experimentally infected breeding age female swine can transmit PRRSV to age-matched sentinels > 90 days post-infection.

Procedures:

Source of animals and housing

One hundred and twenty 4-month old PRRSV naïve gilts were obtained from a source known to be negative for PRRSV based on 5 years of diagnostic data and the absence of clinical signs of PRRS in all phases of production (2,10,11). The gilts were housed at the University of Minnesota Center for Swine Disease Eradication research farm in a mechanically ventilated finishing building consisting of 10 pens, the pens being 10m by 2.5m in size with partially slatted floors. Animals were placed 12 per pen, and provided $1m^2$ space.

Infection model

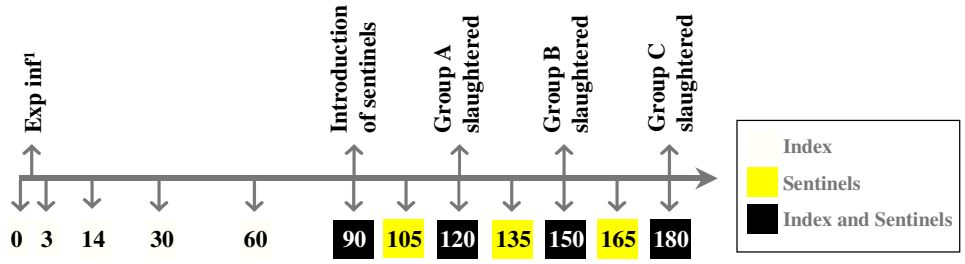
Upon arrival to the experimental farm, all gilts were individually identified using numbered ear tags. On day 0, gilts were infected by the intranasal route with 5 ml of a $10^{2.4}$ $TCID/_{50}$ (total dose) of a field isolate of PRRSV (14). To assess the PRRSV status of the population over the course of the study, a monitoring group of 30 animals was organized by randomly selecting 3 animals from each pen. This sample size was sufficient to estimate prevalence where the true expected prevalence is $\leq$ 10% or $\geq$ 90% at a 95% confidence with a $\pm$ 10% accuracy. Ten 8-week old PRRSV naïve pigs

originating from the same source were housed in a separate facility, 30m from the experimental facility and served as negative controls.

Assessment of persistence and shedding

Following experimental infection, the 120 index animals were organized into 1 of 3 groups, (A, B, or C), 40 animals per group. Each group also included 10 animals from the monitoring group, and groups were represented in each pen. To evaluate whether PRRSV could persist within the experimentally infected population from 120 to 180 days pi, it was planned to market group A at 120 days pi, group B at 150 days pi, and group C at 180 days pi, collect selected tissues at slaughter, and test for PRRSV. The sample size of 40 animals per slaughter group was capable of detecting at least 1 PRRSV-infected animal assuming an estimated prevalence of 2% at a 95% confidence (14). Based on an initial infection at 4 months of age, the respective ages of each group at slaughter was projected to be Group A: 240 days, Group B: 270 days, and Group C: 300 days. In order to evaluate whether the experimentally infected population could shed PRRSV during the period of 90 to 180 days pi of the index animals, 30 PRRSV naïve age-matched sentinel gilts were introduced to the experimental facility at day 90 pi of the index population. Sentinels were individually tagged, mixed directly with the index animals (3 sentinels per pen) and bled every 14 days to document a PRRSV negative status. Figure 1 provides a timeline that summarizes the events that occurred during the study period.

Fig. 1: Timetable for specific actions taken during the experiment and the schedule sampling of index and sentinel animals.



[1] = experimental infection of index pigs

Sampling methods and diagnostic testing

The PRRSV-infected monitoring group was blood-tested on days 0, 1, 3, 14, 30, 60, 90, 120, 150, and 180 pi. All 30 sentinels were blood-tested upon arrival to the facility (day 90 pi of the index animals), and were re-sampled every 14 days throughout the period of 90 to 180 days pi for a total of 6 sampling periods. Negative controls were tested on arrival to the farm and at the end of the study. Sera were tested for the presence of PRRSV antibodies using the IDEXX ELISA, for PRRSV nucleic acid using polymerase chain reaction (PCR), and for viable PRRSV using virus isolation (VI). Specifically, the IDEXX ELISA (IDEXX Laboratories Westbrook, ME) and the Taqman™ PCR were used (Perkin-Elmer Applied Biosystems, Foster City, CA). Samples were

assessed for viable virus using MARC-145 continuous cell lines and porcine alveolar macrophages (16-18).

Selection criteria for collection of tissues at slaughter included tonsil and a minimum of 2 lymph node sites. Specifically, the superficial inguinal and sternal lymph nodes (LN) were required, based on ease of accessing these sites at slaughter, and data from a previous study that demonstrated frequent detection of PRRSV nucleic acid by PCR in these sites (15). It was also planned to collect extra samples of other lymphoid sites (tracheobronchial LN, medial iliac LN, and/or lateral retropharyngeal LN) if positive visual confirmation of the site could be made. Along with each group of 40 index pigs, 10 sentinels were processed in a similar manner. Samples were collected during the evisceration process on the kill floor, or as carcasses were chilling at 4^{0} C. Samples were pooled by individual animal, transported on ice to the University of Minnesota Veterinary Diagnostic Laboratory, and tested for PRRSV using PCR and VI. Prior to pooling, all tissues were confirmed to be of lymphoid origin by microscopic examination, and tissues were evaluated for the presence of lesions suggestive of PRRSV infection (17-19). Representative PRRSV isolates from index pigs or sentinels were nucleic acid sequenced to determine the degree of homology with the isolate used for the experimental infection (20). Negative control sera were tested by ELISA and these animals were not included in any of the 3 slaughter group

Results:

All 30 index pigs from the monitoring group were PRRSV-negative on arrival, as verified by PCR, VI, and ELISA (table 1). Serial testing of the monitoring group indicated successful experimental infection. On day 3 pi, 24/30 of the tested animals were PCR positive and 6/30 were VI positive. Molecular sequencing of a randomly selected PRRSV isolate recovered from an index pig indicated 100% homology with the isolate used for the experimental infection. On day 7 pi, 29/30 of the pigs in the monitoring group were PCR positive and 19/30 were VI positive; however, all were ELISA negative (sample-to-positive ratio < 0.4). On day 15 pi, 30/30 pigs in the monitoring group were ELISA positive, while 27/30 and 8/30 were PCR and VI positive, respectively. The number of ELISA positive animals detected from day 30 to 180 pi are as follows: 30/30 (days 30 and 60 pi), 29/30 (days 90 and 120 pi), 19/20 (day 150 pi) and 9/10 (day 180 pi). All serum samples collected on days 30 to 180 were PCR and VI negative. All sentinel pigs were PRRSV-negative by PCR, VI, and ELISA on arrival (day 90 pi of the population), and remained PCR, VI, and ELISA-negative throughout days 90 to 180 pi. The serologic data is summarized in Figure 2.

At each slaughter date (120, 150, and 180 pi), 50 pools of tissues (40 index animals and 10 sentinels) were collected, for a total of 150 tissue pools across all 3 of the slaughter collection dates. Tonsil and sternal and superficial inguinal LN were collected from all index and sentinel female swine. In addition, samples of tracheobronchial LN, lateral retropharyngeal LN, and/or medial iliac LN sites were collected from 66 of the 120 index animals. Of the 66 animals where supplemental sampling was possible, tonsil and 5 LN sites were collected from 10 animals, tonsil and 4 LN sites were collected from 23 animals, and tonsil and 3 LN sites from 33 animals. The presence of lymphoid tissue in all samples was confirmed microscopically, prior to pooling. Microscopic evidence of PRRSV infection were not detected (19). All tissue pools were negative by PCR and VI. The negative control pigs remained ELISA-negative throughout the study.

Figure 2: PRRSV status of sera collected from index gilts and sentinels during days 90-180 of the study

	Index Sows			Sentinels		
	PCR (+)	**VI (+)**	**ELISA (+)**	**PCR (+)**	**VI (+)**	**ELISA (+)**
Day 1	0[a]/30[b]	0/30	0/30	na	na	na
Day 3	23/30	0/30	0/30	na	na	na
Day 15	27/30	8/30	30/30	na	na	na
Day 30	0/30	0/30	30/30	na	na	na
Day 60	0/30	0/30	30/30	na	na	na
Day 90	0/30	0/30	29/30	0/30	0/30	0/30
Day 120	0/30	0/30	29/30	0/30	0/30	0/30
Day 150	0/30	0/30	19/20	0/20	0/20	0/20
Day 180	0/30	0/30	9/10	0/10	0/10	0/10

na = not available (sentinels not present until 90 days pi of index pigs)
a/b = number animals positive/number animals tested

To conclude, our results indicate that PRRSV persistence and transmission in large populations of breeding age female swine may to be limited to < 120 days pi. As of this writing, this is the largest PRRSV persistence study that has ever been conducted in breeding age female swine. Other strengths of this study included the use of a field isolate previously recovered from an aviremic sow that harbored PRRSV in lymphoid tissues, the use of commercial facilities to mimic field conditions, and the introduction of PRRSV-naïve sentinels directly commingled with index animals, maximizing animal-to-animal interaction. These results demonstrate the value of eliminating PRRSV-naïve subpopulations and enhancing viral clearance through the exposure of an isolated, static population of non-pregnant breeding swine to a defined concentration of PRRSV.

Future studies should focus on repeating the study using larger sample sizes and tissue sets, as well as assessing the key immunological responses that bring about elimination of virus from the persistently infected adult pig between days 90 to 120 pi. Answers to these issues could prove to be very helpful in controlling PRRS throughout the global swine industry.

Acknowledgements
We would like to recognize and thank the National Pork Board for the financial support of the study.

References

1. Loula TJ. Mystery pig disease. Agri Prac 1991;12:23-34.

2. Dee SA, Joo HS, Polson DD, Marsh WE. Evaluation of the effects of nursery depopulation on the profitability of 34 pig farms. Vet Rec 1997;140:498-500.

3.Cavanaugh D. Nidovirales: A new order compromising *Coronoviridae* and *Artiriviridae*. Arch Virol 1997;142:629-633.

4. Plagemann PGW, Moennig V. Lactate dehydrogenase elevating virus, equine arteritis virus and simian hemorrhagic fever virus: a new group of positive-strand RNA viruses. Adv Virus Res 1992;41:99-92.

5. Collins, JE, Benfield, DA, Christianson WT, et al. Isolation of swine infertility and respiratory syndrome virus (isolate ATCC VR-2332) in North America and experimental reproduction of the disease in gnotibiotic pigs. J Vet Diag Invest 1992; 4:117-126.

6. Ahmed R, Morrison LA, Knipe DM. Persistence of viruses. In: Fields BN, Knipe DM, Howley PM, eds. Field's Virology. Philadelphia: Lippencott-Raven, 1996: 219-249.

7. Ahmed R, Morrison LA, Knipe DM. Viral persistence. In: Nathanson N, Ahmed R, Gonzalez-Scarano F, Griffin D, Holmes K, Murphy F, Robinson H, eds. Viral Pathogenesis. Philadelphia: Lippencott-Raven, 1997;181-206.

8. Wills RW, Zimmerman JJ, Yoon KJ, et al. Porcine reproductive and respiratory syndrome virus: a persistent infection. Vet Micro 1997;55:231-240.

9. Christopher-Hennings J, Benfield DA, Nelson EA. Persistence of porcine reproductive and respiratory syndrome virus in serum and semen of adult boars. J Vet Diag Invest 1995;7:456-464.

10. Dee SA, Joo HS. Clinical investigation of recurrent reproductive failure with PRRS virus in a swineherd. JAVMA 1994;204:1017-1018.

11. Dee SA, Philips RE. Use of polymerase chain reaction to detect vertical transmission of PRRS virus in piglets from gilt litters. Swine Health and Production 1999;7:237-239.

12. Dee SA, Joo HS, Henry S, et al. Detecting subpopulations after porcine reproductive and respiratory syndrome virus infection in large breeding herds using multiple serologic tests. Swine Health and Production 1996; 4:181-184.

13.Dee SA, Molitor TW, Rossow KD. Epidemiological and diagnostic observations following elimination of PRRS virus from a breeding herd of pigs by the test and removal protocol. Vet Rec 2000;146:211-213.

14. Bierk MD, Dee SA, Rossow KD, et al. A diagnostic investigation of chronic PRRS virus infection in a swine breeding herd. Vet Rec 2001 148, 687-690.

15. Bierk MD, Dee SA, Rossow KD, Collins JE, Otake S, Molitor TW. Transmission of PRRS virus from persistently infected sows to contact controls. Can J Vet Res 2001;65: 261-266.

16.Snyder ML, Mermer B, Anderson PR, Wensvoort G, Hill HT. Evaluative data for an immunodiagnostic ELISA for PRRS. Proc 2nd Int Symp on PRRS 1995:15.

17.Molitor TW, Tune KA, Shin J, Collins J, Kapur V. Applications of TaqMan ™ PCR in the detection of PRRS virus. Proc Allen D. Leman Swine Conf 1997:173-175.

18. Bautista EM, Goyal S, Yoon IJ, Joo HS, Collins J. Comparison of porcine alveolar macrophages and CL 2621 for the detection of porcine reproductive and respiratory syndrome virus and anti-PRRS antibody. J Vet Diagn Invest 1993;5:163-165.

19.Rossow KD, Morrison RB, Goyal SM, Singh GS, Collins JE. Lymph node lesions in neonatal pigs congenitally exposed to porcine reproductive and respiratory syndrome virus. J vet Diagn Invest 1994;6:368-371.

20.Murtaugh, MP, Elam, MR, Kakach, LT. Comparison of the structural protein coding sequences of the VR-2332 and Lelystad virus strains of the porcine reproductive and respiratory syndrome virus. Arch Virol 1998;140:1451-1460.