RESEARCH REPORT
SWINE HEALTH

Title: Sampling of adult boars during early infection using a new serum collection technique for PRRS PCR testing prior to semen collection. – **NPB #04-111**

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Abstract: This study evaluated the feasibility of using a new approach for blood collection in boars called the blood swab method. The blood swab method involves puncturing a vein (normally in the ear) with a needle and swabbing the blood with a polyester swab. The swab is then put into saline solution and finally tested by PCR.

The Results of the study showed that 59/60 boars were detected positive using the blood swab method compared with 60/60 with traditional serum collection methods. Only 27 semen samples were positive. There was no statistical difference between likelihood of detecting a positive boar with blood swab method compared to serum. There was less quantity of virus detected by the blood swab method when compared with serum, which can be explained by the dilution effect of the saline and using whole blood rather than serum. The procedure can be implemented as part of the routine monitoring program to detect PRRSV infection in boar studs. The blood swab method is much more convenient than collecting serum by the traditional snaring method and will detect virus much sooner and with greater sensitivity than semen PCR. The blood swab method is being implemented in studs as a result of this study.

Introduction: Previous studies have shown that PRRS virus can shed in semen and this is a well-accepted fact. Serum PCR is more sensitive than semen PCR (Reicks 2004), and will pick up virus by PCR testing within 24-48 hours after an animal is infected. Thus to prevent infecting sow farms (a boar stud can supply as many as 50-100 sow farms), boars should be sampled in the serum (blood) rather than semen. Because taking a blood sample by venipuncture each time a boar is collected (1-2x/week) is dangerous and difficult, semen sampling is currently being used to screen boars for PRRSV infection. Because semen PCR testing is the current industry method of testing boars due to the easily available sample, there could be considerable lag between when a boar stud is infected and when it is closed for semen distribution. An easy sampling technique is needed (other than snare and jugular or vena cava sampling with needle) so that boars could be easily sampled by boar stud staff at each semen collection (1-2 times per week per boar).

Project objectives

1. To evaluate the effectiveness of a skin puncture technique (blood swab method) to collect blood for detection of PRRSV by PCR in experimentally infected boars.
2. To examine the effect of sampling method (skin puncture/blood swab, venipuncture, semen collection, or mouth swab) and viremia level on the ability to detect PRRSV in boars during the first 6 days after experimental infection.

Materials and Methods

1. Animals and sampling.
   A total of 21 boars, between 10 and 12 months of age will be used in the study. All boars were be tested by PCR and ELISA before the beginning of the study to verify their PRRS-free. In order to ensure comparability between groups, the 20 boars were assigned randomly into one of two groups (A or B), inoculated with PRRSV, and sampled according to the following schedule:

   Table 1. Representation of the sampling and testing schedule of boars during the first 6 days after experimental infection with PRRS virus.

Group	Boars Sampled	Hours Post Inoculation
A	1-10 + control	24
B	11-20 + control	48
A	1-10 + control	72
B	11-20 + control	96
A	1-10 + control	120
B	11-20 + control	144

   An additional boar was inoculated at the same time and served as a backup in the event of a boar death or severe health problem. All 21 boars were experimentally infected at day 0. (The virus, a field isolate MNBO4, was provided by Dr. Kurt Rossow from the University of Minnesota Veterinary Diagnostic Laboratory. Because of the logistic and animal handling difficulties associated with sampling all boars once every day, each group of 10 boars (A or B) were sampled every other day during the first 6 days after inoculation. One boar became ill by 72 hours post-inoculation and died. He was replaced in the sampling chart with the extra boar that was inoculated at time 0.

   The following samples were collected at each time:

   a. Semen: collection was done using standard procedures (dummy). Aliquots will be made and stored until testing.
   b. Blood by traditional venipucture: boars will be bled from the anterior vena cava using a 3 inch needle and syringe. Samples will be processed on site to obtain serum. Aliquots will be made and stored until testing.
   c. Blood by skin puncture: a 20 gauge x ½ inch needle was used to puncture through the skin and into a visible vein. This was done either beside the tail head or in the ear. The blood drops were collected using a Rayon polyester swab (to

the point of saturation) that was subsequently immersed into a tube containing 1 ml of 0.9% saline solution.

d. Mouth swab was taken and placed into 1.0 ml of 0.9% saline solution.

Two known PRRS-free boars from the original source herd were used to obtain negative samples for quality control purposes and to monitor potential cross-contamination problems during sample handling. These boars, one for each group, were sampled at the same time periods and tested by PCR. Additional information collected at the time of sampling included rectal temperature.

2. Sample testing.
   All samples were tested by PCR performed at the University of Minnesota Veterinary Diagnostic Laboratory. Results will be reported as PCR positive or negative for PRRSV. In addition, viremia (measured as viral particles/ml) was obtained using a quantitative PCR for each sample that was initially PCR positive. Serum samples were tested for PRRS antibodies using IDEXX ELISA at the Minnesota Veterinary Diagnostic Laboratory to verify the antibody-free status of the boars before inoculation and to verify infection (boars will be sampled approximately 23 days post-inoculation).
3. Statistical Analysis
   Descriptive statistics were initially performed to summarize and describe the data on diagnostic testing, pooling, and temperature. Results of PCR testing on the various sample types at different times after inoculation were compared based on the proportion of agreement and 90% confidence interval. Proportion agreement was calculated for each time after inoculation as (Number of boars positive in both tests + Number of boars negative in both tests)/ Total number of boars tested (reference). The 90% confidence interval for each proportion was calculated using exact methods (Statistica® 6.0, StatSoft, Tulsa, OK). Survival analysis methods, specifically the interval-censored Cox proportional hazards regression model, were used to model the time-to-first detection of PRRS virus by PCR in each sample type (Therneau, Grambsch). Results calculated included median time, and 90% confidence interval, to the first PCR positive test (time when 50% of the pigs were expected to test PCR positive for first time). Association between fever and a PCR positive test was evaluated in the survival model. Fever (yes or no) was included in the model as a time-dependent variable. Assumption of proportional hazards was evaluated based on the plot of the log (-log ($\hat{S}$)) and of the Schoenfeld residuals. Plots of the Cox-Snell residuals were used to assess overall goodness-of-fit for the final model (Therneau, Grambsch). All statistical analyses considered a P-value < 0.1 as statistically significant and were performed using statistical software (S-Plus 6.2, Insightful Corp. Seattle, WA).

Results: Blood swab samples were successfully obtained for all samples. Sampling from the ear using the blood swab method during collection was successful on each attempt (52/52). Sampling from a vein beside the tail head was less successful (9 successful samples taken out of 20 attempted).

Serum PCR samples were positive at all sample times post-inoculation. Blood samples were positive on all but 1 sample post-inoculation. Semen samples were positive 27 out of 60 samples and mouth swabs were positive on 19 out of 61 samples (see table 2).

Virus was detected by PCR on serum and blood swab samples 24 hours prior to any detection in semen or by mouth swabs. All boars sampled from 48 hours on were detected positive on serum and blood swab, whereas, only 4 of 10 and 3 of 10 were detected positive at 48 hours by semen and mouth swab respectively. In addition, there were no sampling times in which all boars tested were detected positive on semen or mouth swab.

A larger amount of virus was detected by serum PCR when compared to blood swab PCR. A smaller amount of virus was detected by semen and mouth swab PCR when compared to either blood swab or serum PCR (table 3).

Evaluation of the proportion of agreement showed nearly perfect agreement between PCR testing in serum and PCR testing in blood swabs, with values ranging from 90% (90% c.i.: 61% - 99.5%) at 24 hrs. to 100% (90% c.i.: 74% - 100%) thereafter (Figure 1). PCR testing in semen or mouth showed less agreement with serum PCR testing throughout the study period (proportion of agreement between 0% and 80% depending on the sampling time) (Figure 1). Results of the McNemar's test for paired samples showed a significant difference between the results of serum and semen PCR at 24 hrs. (P-value = 0.004) and 48 hrs. (P-value = 0.041). Results between serum and mouth PCR were significantly different at 24 hrs. (P-value = 0.004), 48 hrs. (P-value = 0.023), 96 hrs. (P-value = 0.023), and 120 hrs. (P-value = 0.041).

Survival analysis results indicated that median time to a PCR positive test (time when 50% of the pigs are expected to be PCR positive) in serum samples and in blood swab samples was 24 hrs. (90% c.i.: 24 – 48 hrs.). Median time to a PCR positive test was 72 hrs. (90% c.i.: 48 – 96 hrs.) for semen samples and 72 hrs. (90% c.i.: 72 – 120 hrs.) for mouth samples. Fever was not associated with testing PCR positive regardless of the sample type (lowest P-value: 0.62).

Table 2. Summary of PCR and ELISA test results and occurrence of fever at various times after experimental infection with PRRS virus.

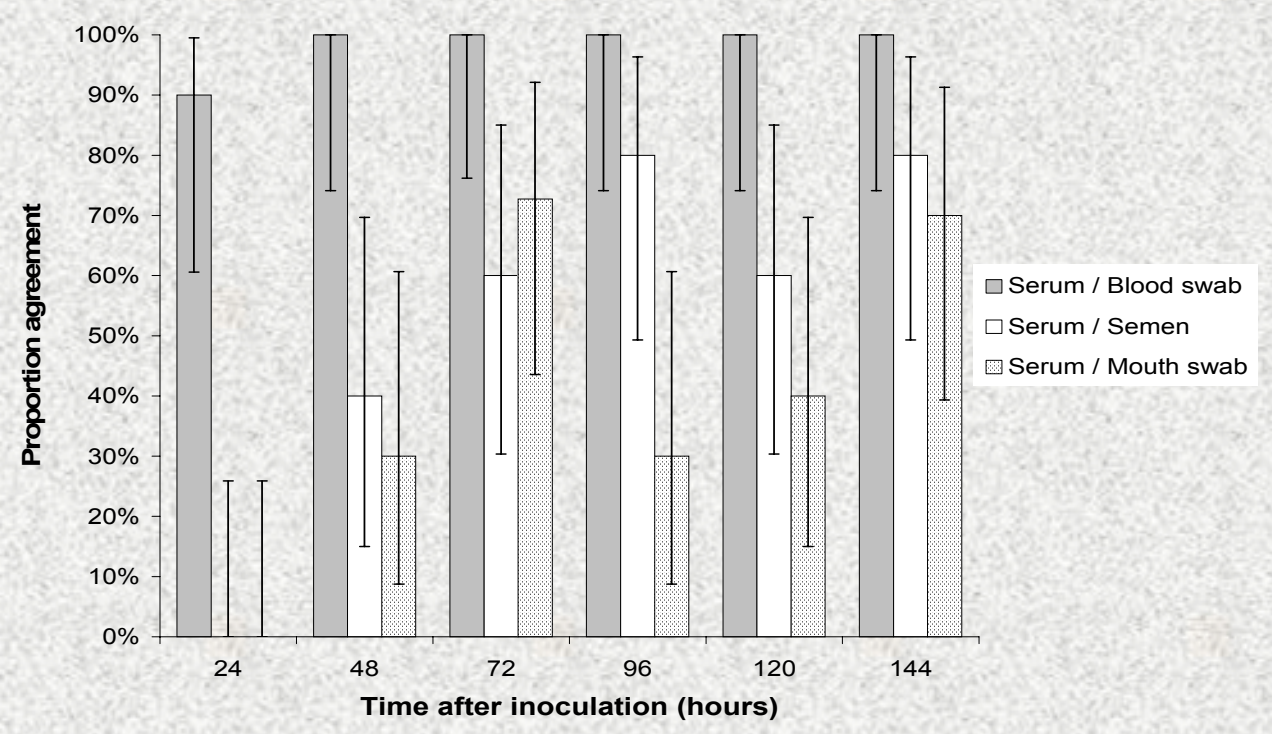
Sampling time (hours) [1]	Fever	Serum PCR positive	Semen PCR positive	Blood swab PCR positive	Mouth swab PCR positive	ELISA positive
0	0/21	0/21	N.A.	N.A.	N.A.	0/21
24	0/21	10/10	0/10	9/10	0/10	N.A.
48	3/21	10/10	4/10	10/10	3/10	N.A.
72	2/21	11/11	6/10	11/11	8/11	N.A.
96	3/20	10/10	8/10	10/10	3/10	N.A.
120	2/20	10/10	6/10	10/10	4/10	N.A.
144	4/20	10/10	8/10	10/10	7/10	N.A.
528	N.A.[2]	N.A.	N.A.	N.A.	N.A.	20/20

[1] Time in hours after experimental inoculation.
[2] Not available (sample was not tested).

Table 3. Summary of PRRS virus concentration (and standard deviation) by sample type and sampling time measured using quantitative PCR (IVP/ml) in boars after experimental infection.

Sampling time (hours)	Serum	Semen	Blood swab	Mouth swab
24	13.0 (23.6)	N.A.	1.1 (1.2)	N.A.
48	12.7 (18.8)	0.0 (0.0)	0.5 (0.5)	0.1 (N.A.)
72	102.6 (306.3)	0.1 (0.1)	15.0 (47.4)	0.1 (0.1)
96	68.8 (52.0)	0.2 (0.2)	2.0 (2.1)	0.1 (N.A.)
120	108.5 (105.9)	0.0 (0.1)	2.6 (2.9)	0.1 (0.0)
144	475.0 (383.1)	0.1 (0.1)	17.6 (13.3)	0.0 (0.0)

Figure 1. Proportion of agreement, and 90% confidence interval, between PCR tests run on serum samples and blood swabs, between serum and semen samples, and between serum and mouth swabs from pigs at various times after experimental infection with PRRS virus.



Discussion: Typically, boar studs have used semen PCR as a monitoring tool for PRRS. Due to cost constraints, boar studs have typically only tested a percentage of boar ejaculates by semen PCR. Based on this study and a prior study by this author, the likelihood of detecting virus in a boar stud before an individual boar could be shedding virus in semen is low. The quantity of virus in the semen in this study was often at the minimum detection level, which would mean pooling of boar ejaculates further reduces the chances of detecting virus in a boar stud during the early stages of infection in the population. The results of this study indicate that the blood swab

sampling method would detect virus sooner and in larger quantity than semen PCR. The blood swab method would seem to be a more appropriate method for monitoring of PRRS negative boar studs to detect virus sooner when compared to the current industry practice of PCR testing of semen.

While convenient, mouth swab PCR does not appear to be an appropriate method for detecting PRRS virus in boar studs. Virus was found less frequently than any of the other methods. The quantity of virus in the positive mouth swab samples was also typically at the minimum detection level, so pooling of mouth swab samples could not be justified.

All but one sample was positive using the blood swab method for this study. Virus was found in higher quantity in serum when compared to blood swab samples which is to be expected. There is a dilution effect of the blood swab when compared to serum. First, red blood cells remain in the sample when using the blood swab method and therefore dilute out any PRRS virus that would be present. Second, placing the saturated swab into 1 ml of saline diluted out any virus that would be present. Strategies to reduce this dilution effect are being explored. Taking into account convenience, safety, and sensitivity of detecting virus, the blood swab method seems to be the most appropriate monitoring method for PRRS negative boar studs.

References

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Lay Interpretation: In the study, serum had a higher quantity of virus than the blood swab samples as expected.

The blood swab method can be done on boars each collection day, thus is user-friendly when compared with snaring and collection of blood from the neck. The blood swab samples detected virus earlier and had more virus quantity than semen or mouth swab samples, so finding virus in pooled samples would be much more likely. This study verifies results of prior work by this author which suggested that semen PCR was not effective at detecting virus from boars during the first 6 days after infection. Blood swab sampling should replace semen sampling for boar studs.

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