RESEARCH REPORT
SWINE HEALTH

Title: Assesment Of Vertical Transmission From Parity One Sows Infected With A Low Dose And Mild Pathogenic Prrsv Isolate – **NPB #04-191**

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II. Abstract

In order to generate a protocol to sample lactating piglets to evaluate PRRSV chronically infected herds twelve PRRSV naïve pregnant sows were individually housed and assigned to three different groups. Sows in group A were injected with 3 mL of sterile MEM, group B with 10^1 $TCID_{50}$ total dose of PRRSV isolate MN 30-100 and sows in group C were inoculated with 10^2 $TCID_{50}$ total dose. All sows were intramuscularly injected at 90 days of gestation. Piglets were intensively observed and sampled during all lactation period. PRRSV real time PCR was used to identify and quantify the virus.

Under the conditions of this study, where completely susceptible sows were inoculated at 90 days of gestation, dose of inoculated virus did not affect the proportion of viremic piglets at birth or the viral load in serum (RNAc/mL). Four days of age seems to be best sampling age compared to birth (pre-colostrum intake) or weaning. There is no evidence that sampling should be concentrated on early farrowings because the number and viral load of positive pigs is not different; however in a chronically infected farm, those sows that have not been exposed to the virus are more likely to early farrow compared to previously infected sows. It could be suggested that stillbirths, mummies and "very sick" pigs are a better sample than all other pigs in order to catch potential positive pigs that means that the PRRS virus is still actively being transmitted in the sow herd. There is no reason to sample lighter litters or piglets at birth but as expected affected litters will have a lower growing performance during lactation. Real time PCR on blood swab could be used as an alternative tool for herd monitoring in the case that a very often evaluation is recommended and when for some reason a veterinarian can not be present.

III. Introduction

The first and most important step to control and eradicate PRRSV from infected populations is to "stabilize" the immunity in the sow herd, which can be reached by different approaches like herd closure, intentional mass exposure or gilt acclimatization. After applying these procedures, the challenge for swine practitioners is to evaluate the virus "activity" in those chronically infected herds. Nowadays the most common used strategy to perform this evaluation consists on testing sera from piglets for PRRSV PCR. Questions about number of litters to be sampled, age and clinical signs of the piglets or the effect of pooling on PCR results do not have clear answers today.

IV. Objectives:

- To determine the incidence of transplacental PRRSV infection and the proportion of infected piglets within infected litters within parity one litters following infection of the dam with a low dose of a virulence isolate; and to identify clinical signs in individuals and litters detected as PRRSV PCR positive.
- To evaluate the concentration of PRRS virus in serum of piglets infected transplacentally during late gestation.
- To validate the PCR technique on blood samples collected using filter disks to detect PRRSV US isolates and the impact of pooling.
- To determine if PRRSV transmission occurs within 24 hours of birth within litters.

V. Materials and Methods

Twelve PRRSV naïve pregnant sows were individually housed in isolation rooms at the College of Veterinary Medicine of the University of Minnesota. Sows were identified and then assigned to three different groups (4 sows per group). Sows in group A were injected with 3 mL of sterile MEM, group B with 10^1 $TCID_{50}$ total dose of PRRSV isolate MN 30-100 and sows in group C were inoculated with 10^2 $TCID_{50}$ total dose. All sows were intramuscularly injected at 90 days of gestation. In order to confirm infection sows were sampled 5 and 14 DPI (days post inoculation) and PRRSV PCR and ELISA were performed. At 113 days of gestation, farrows were synchronized by injecting 10 mg (total dose) of prostaglandin (Lutalyse ®) IM. Piglets were dried, identified, weighted, tail docked and serum sampled before colostrum intake. Serum and milk samples were also taken from every sow. Immediately after that piglets were encouraged to ingest colostrum and a heat source was provided. At four days of age serum samples were taken from the piglets. Twice a day clinical signs were recorded in every litter taking special care in the Biosecurity protocol to avoid transmission between litters. At 17 days of age serum samples were taken, piglets were weighted and humanely euthanized using 100 mg/Kg of

sodium pentobarbital IV. Tonsil samples were individually collected and stored at -80C. Serum and milk samples were also taken from the sows.

To determine the number and viral load of viremic piglets at birth, 96 hours and at weaning age, PRRSV real time PCR was performed based on the detection of the ORF 7 region of the PRRS virus sequence in individual serum samples. Proportions among groups and age were compared using Fisher's Exact Test (Statistix 8 ®, Tallahassee FL). To identify clinical signs (fuzziness, light weight, dome head pigs, dyspnea, diarrhea) associated with viremic piglets individual information was recorded from 1 to 18 days of age. Sensitivity of blood swab technique for PRRSV PCR was compared to serum collection technique by diagnostic test agreement analysis (*WinEpiscope 2.0* ®, UK). Effect of pooling serum samples for PRRSV PCR was studied by obtaining the *maximum dilution still positive* from the relationship between the quantitative PCR result and the threshold used by the real time technique.

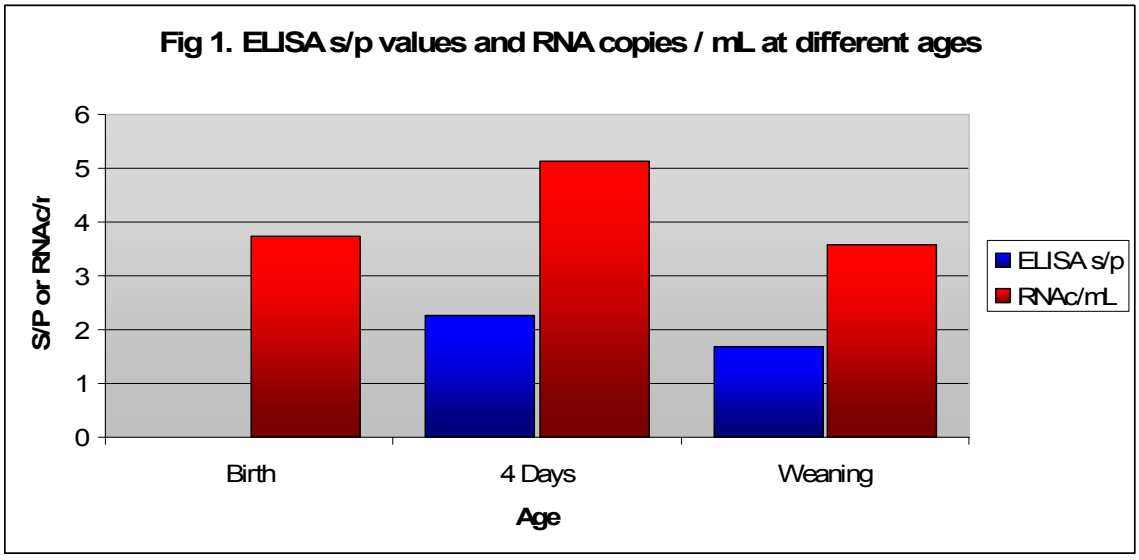
VI. Results and Discussion

- Proportion and viral load of viremic pigs:

There was no difference between proportion of viremic piglets of group B (10^1 $TCID_{50}$) and C (10^2 $TCID_{50}$) at birth (Fisher's Exact Test p value = 0.8593). Total proportion of viremic pigs (PCR +) at birth was 53 out of 65 = 81.54%. All litters had positive pigs at birth. No one negative out of 40 pigs was detected at 4 days of age coming from infected sows (B and C) and only two negative out of 18 piglets from these groups at weaning age. In order to "normalize" the number of RNA copies per mL (RNAc/mL) in piglet sera born from infected sows it was necessary to transform the data to Logarithm (Log). When the Log RNA c / mL of the piglets at birth were compared between group B and C, no difference was observed (T test p value = 0.9954). Under the conditions of this study, where completely susceptible sows were inoculated at 90 days of gestation, dose of inoculated virus did not affect the proportion of viremic piglets at birth or the viral load in serum (RNAc/mL). This high proportion of viremic pigs at birth could be directly related with the fact that all inoculated sows were completely susceptible to PRRSV infection; however in a chronically infected farm a percentage of the sows have been previously exposed to PRRSV having some level of immunity which could reduce the proportion of viremic piglets at birth.

Serum RNAc/mL Log values of piglets coming from infected sows were compared among three different ages (birth, 4 days and weaning) in order to evaluate under this experiment conditions the better sampling age to catch the potential positive pigs. One-Way ANOVA was used and significant difference was found among groups (One-Way ANOVA p value = 0.0000). T test mean comparison was used to evaluate specific differences between groups finding that RNAc/mL were higher at 4 days of age than at birth (T test p value = 0.00002) or at weaning (T test p value = 0.000005) but RNAc/mL at birth and at weaning were not different (T test p value = 0.6163). IDEX ELISA test was performed on piglets at birth, 4 days and at weaning. S/p values look higher at

4 days of age (s/p average = 2.25) than at weaning (s/p average = 1.68) however when means are compared with the T test there is no significant difference (T test p value = 0.1009). Under the conditions of this experiment 4 days of age seems to be best sampling age compared to birth (pre-colostrum intake) or weaning (figure 1).



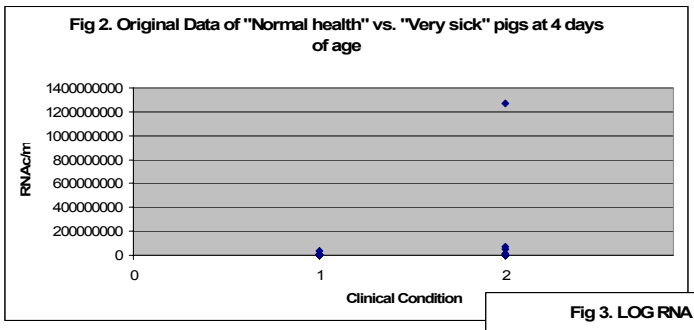
- Clinical signs in PRRSV PCR positive litters and individuals:

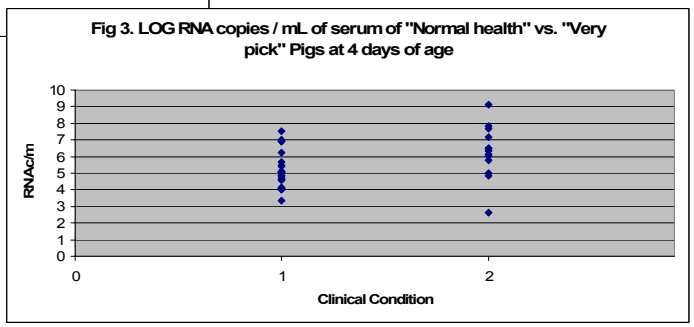
A number of clinical signs as fuzziness, dome head pigs, dyspnea and diarrhea were recorded during lactation period however given the high number of positive pigs and the low prevalence of those clinical signs no correlation was found between any of those clinical signs and PRRSV detection.

Four out of 8 inoculated sows farrowed between 3 to 4 days earlier than the expected farrowing date based on the herd average. Three of them were part of group C (10^2 $TCID_{50}$) and one of group B (10^1 $TCID_{50}$). Sample size did not allow finding a significant difference between proportions of early farrowing; however the RNAc/mL of piglets born from early farrowings were compare with piglets born also from infected sows but at a normal farrowing date. Results show that there is no difference in terms of RNAc/mL in serum (T test value = 0.7151) or proportion of viremic piglets at birth (Fisher's Exact Test p value = 0.3839) between early and normal farrowing. Under the conditions of this study there has not found evidence that sampling should be concentrated on early farrowings because the number and viral load of positive pigs is not different; however in a chronically infected farm, those sows that have not been exposed are more likely to early farrow compared to previously infected sows. In that case those early farrowing sows might have a higher probability of produce viremic piglets at birth.

When the RNAc/mL data from piglets at 4 days of age was analyzed it became interesting that some piglets (13) that were classified as "very sick" for showing hyperthermia, fuzziness, high depression and anorexia and were sampled just about to die or even postmortem (recently died) exposed a very high viral load. Using a mean

comparison T test it was found that the RNAc/mL of those "very sick" pigs were higher than the RNAc/mL in the rest of the pigs (T test value = 0.0297). Figures 2 and 3 show why the data have to be transformed in logarithms.





In some cases was possible to sample stillbirths and mummies and RNAc/mL in serum of those piglets was compare with the born alive piglets. The Log RNAc/mL of stillbirth or mummies were higher than the RNAc/mL in the born alive piglets (T test value = 0.0439). Under the specific conditions of this trial it could be suggested that stillbirths, mummies and "very sick" pigs are a better sample than all other pigs in order to catch potential positive pigs that means that the PRRS virus is still actively being transmitted in the sow herd.

Born alive piglets were weighted at birth and One-Way ANOVA was applied to analyze potential difference among the 3 groups (A, B and C). No significant difference was found (One-Way ANOVA p value = 0.0657). Those that survived were weighted again at weaning. Weaning piglet weights were different among groups (One-Way ANOVA T test value = 0.0000). Piglets in group A (negative control) were heavier than pigs in groups B (T test value = 0.0123) and pigs in group C (T test value = 0.0001). Pigs in group B were heavier than pigs in group C (T test value = 0.00003). Weights of PRRSV PCR negative piglets at birth were not different than weight of PRRSV PCR positive pigs at birth (T test value = 0.9485). In this trial the dose of inoculated virus did not affect the weight at birth but infected litters had lower weights at weaning than negative litters. PRRSV PCR negative and positive piglets had no different weight. Under the conditions of this study there is no reason to sample lighter litters or piglets at birth but as expected affected litters will have a lower growing performance during lactation.

- Evaluation of PRRSV real time PCR on blood swabs:

As an alternative blood sampling tool blood swab from tail after docking was tested. Forty blood swab samples were analyzed with PRRSV real time PCR and compared against matched samples of serum collected by the

traditional venipuncture using the Vaccutainer System ®. Sensitivity and specificity of the blood swab technique using the serum from venipuncture collection as a "gold-standard" were calculated. Results are shown in table 1.

Table 1. Serum from venipuncture vs. blood swab PCR results

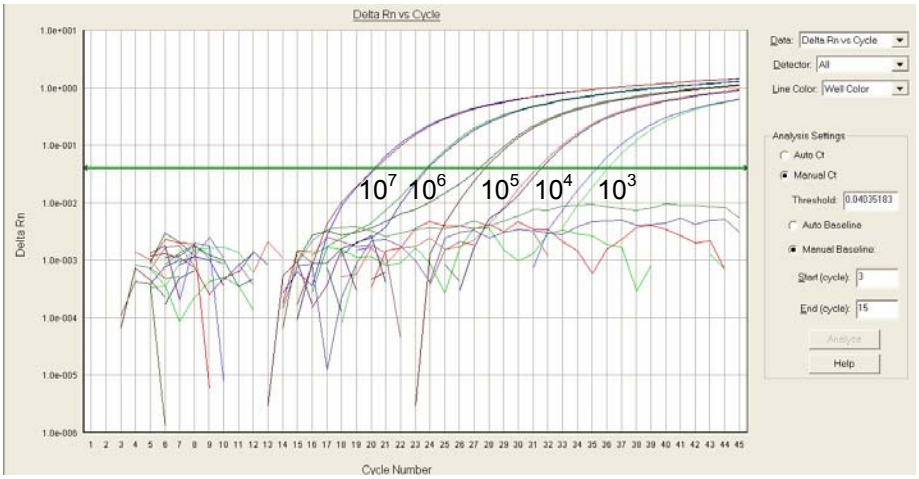
		Serum		
		Positive	Negative	Total
Blood Swab	Positive	25	1	26
	Negative	8	6	14
	Total	33	7	**40**

A diagnostic test of agreement was applied to the results to determine the percentage of agreement (*Kappa* value = 0.441*)* between both techniques. This *Kappa* value can be interpreted as a moderate agreement between both techniques. Sensitivity was calculated as 0.76 and specificity as 0.86 for this data. Real time PCR on blood swab could be used as an alternative tool for herd monitoring in the case that a very often evaluation is recommended and for when some reason a veterinarian can not be present; however, the clinical evaluation in order to select the best samples, given our results, is important to increase the chances to catch those potentially positive pigs.

- Evaluation of the pooling effect:

It is the interest of our group to keep working with pooling strategies in order to increase the chances to catch potentially PRRSV positive piglets. At this point, even that it was not part of this grant; a model is being developed to calculate the most appropriate number of samples to be pooled in terms of sensitivity, cost and flexibility. The PRRSV real time system that was used is based on ORF 7. After a validation process it has been established that 37 Ct (cycles) is the cut value where negative and positive samples could be discriminated (see amplification plot of the PRRSV standard curve in Table 2). Since real time PCR generates quantitative data and a threshold value a "maximal dilution still positive" for the study group of pigs could be determined. The standard curve is a 10 fold dilution of a known amount of virus harvested from cell culture that has to be included in every plate with the samples. When a Ct value of 37 is transformed into RNAc per well an average value of 0.4067 is obtained. This value is converted then from the 2 µL per well into 1 mL of sample (5.0838) which will be the "detection limit of the test". Using this "detection limit" samples were theoretically diluted 1/2, 1/3, 1/5 and 1/10 (more than 10 dilutions might not be practical). Given the high RNAc/mL of most of the samples, only when samples were diluted 10 times a 4.68% of reduction in number of positive was detected. Before that dilution the same number of positive were deceted.

Table 2. PRRSV MN 30 100 Standard Curve from 10^7 $TCID_{50}$ to 10^3 $TCID_{50}$



Under the conditions of this experiment, maybe because the sows were completely susceptible to the virus, most of the RNAc/mL values obtained were very high and pooling does not seem to affect much the ability of detecting at least one positive sample. However, in chronically infected farms, were a percentage of sows have been exposed the viral load of positive pigs might or might not be lower depending on the assumptions of partial or complete immunity.

VII. Lay Interpretation

In order to generate a protocol to sample lactating piglets to evaluate PRRSV chronically infected herds twelve PRRSV naïve pregnant sows were individually housed and assigned to three different groups. Sows in group A were injected with 3 mL of sterile MEM, group B with 10^1 $TCID_{50}$ total dose of PRRSV isolate MN 30-100 and sows in group C were inoculated with 10^2 $TCID_{50}$ total dose. All sows were intramuscularly injected at 90 days of gestation. Piglets were intensively observed and sampled during all lactation period. PRRSV real time PCR was used to identify and quantify the virus.

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