RESEARCH REPORT
SWINE HEALTH

Title: Evaluation of a Test & Removal Protocol for the Elimination of PRRS Virus

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Objectives: The objective of this project was to evaluate the protocol of Test & Removal and Wean & Removal protocols for the elimination of PRRS virus from commercial swine herds.

Materials and methods:
Farm selection
Two study groups, a Test and Removal group (T&R) and a Wean and Removal group (W&R) were established. Members of the American Association of Swine Practitioners were contacted by telephone to recruit candidate farms. For inclusion in the study, all farms had to fulfill the following criteria:

1.A period of greater than or equal to 2 years must have elapsed following the original PRRSV infection and the initiation of the study.
2. A period of greater than or equal to 1 year must have elapsed since the initiation of the study and the last recorded clinical episode of PRRS in the breeding herd.
3. The PRRSV seroprevalence in the breeding herd prior to the study must be $\leq$ 25 %.
4. The farm must be located a minimum of 3.2 km from other PRRSV infected farms.
5. The farm must not have used PRRSV vaccine $\geq$2 years prior to initiation of the study, and could not vaccinate at anytime during the study period.

The decision to place a farm within the T&R or W&R group was based on the desire of the farm owner and/or the recommendation of the practicing veterinarian. Along with each group of 5 farms, 2 PRRSV positive farms and 2 PRRSV negative farms were included as controls. Positive control farms had to fulfill all 5 criteria, while negative controls needed to fulfill criteria 4 and 5. It was not planned to attempt PRRSV-elimination activities in the positive control farms. It was planned to monitor any changes in their PRRSV serostatus throughout the study period, and determine whether PRRSV elimination could occur independently of T&R or W&R.

Data analysis

The relationship between the use of a protocol and the elimination of PRRSV from farms in each study group was analyzed for statistical significance by Fisher's exact test.

Seroprevalence estimation

Prior to initiation of the T&R or W&R protocol, the seroprevalence of the breeding herd was calculated. A random sample of 29-34 breeding animals was collected, according to herd size. This sample size was required to estimate prevalence in farms with breeding herd inventories ranging from 200-1500 sows, where the expected true prevalence of PRRSV antibodies was $\leq$ 10% or $\geq$ 90% at an accuracy of +/- 10%. Differences in the initial mean breeding herd seroprevalence level detected between the T&R and W&R groups were analyzed for significance by 2 sample t-test. In addition, 30 random samples were collected from 10-week old nursery pigs and 5-6 month old finishing pigs and tested by ELISA to determine if PRRSV infection was taking place after weaning. This sample size was capable of detecting at least 1 infected pig in each age group at an estimated prevalence of 10 % at a 95 % confidence. If PRRSV infection was detected post-weaning, infected nursery and/or finishing facilities were depopulated immediately prior to T&R or W&R.

Protocol of Test & Removal

The T&R protocol involved the use of the IDEXX ELISA test for the detection of PRRSV antibodies, in combination with the polymerase chain reaction (PCR) assay for the detection of PRRSV nucleic acid. The entire breeding herd (sows, boars and replacement gilts) was tested in a single day. Animals that were both ELISA and PCR positive were considered to be viremic on the day of sampling. Animals that were ELISA positive but serum PCR negative were considered previously exposed to PRRSV, but were not actively viremic on the day of sampling. Animals that were ELISA negative and PCR positive were considered to be acutely infected. Animals with any one of these diagnostic profiles were removed from the farm within 1-2 days upon receipt of the results. Finally, animals that were negative on both tests were determined to be non-infected, and remained in the herd.

Protocol of Wean & Removal

Wean and Removal (W&R) consisted of testing weekly groups of weaned sows using only the IDEXX ELISA test. Upon receipt of the results, seropositive weaned sows were removed from the herd on a weekly basis, and replaced by seronegative gilts. All boars were tested on the same day at the start of the protocol, and seropositive boars were immediately removed. This process was repeated weekly until the entire breeding herd had been tested.

Protocol of monitoring

Following the completion of the respective protocols, breeding herds were monitored monthly by ELISA for 12 consecutive months. A random sample of 52-58 breeding animals was collected, according to herd size. This sample size was capable of detecting at least 1 positive pig at an estimated prevalence of $\geq$ 5 % at a 95 % confidence in farms with breeding herd inventories of 200-1500 sows. Following the depopulation of the nurseries or finishers, 10 samples were collected from 8-10 week and 5-6 month old pigs on a monthly basis. This sample size was capable of detecting at least 1 positive pig at an estimated prevalence of $\geq$ 30 % at a 95 % confidence. The control farms were tested in a similar manner.

Endpoint measure of success

In order for a study herd to be considered PRRSV-negative, 12 consecutive months of ELISA negative (s/p ratio < .4) test results were required following completion of the selected protocol, and no evidence of PRRSV infection was to be detected post-weaning. If a breeding herd PRRSV seroprevalence of $\geq$5 % was detected for 3 consecutive months, or if PRRSV seroconversion was detected post-weaning, the monitoring protocol for that specific farm was terminated and the farm was classified as PRRSV-positive. If an individual animal was found to be ELISA positive during a monthly sample, the animal was considered a "singleton reactor" and was re-tested by ELISA and PCR. If either test was positive, the singleton reactor was removed from the herd and necropsied. Tonsils and lymph nodes (sternal, lateral retopharyngeal, internal iliac, and superficial inguinal) from the singleton reactor were collected and tested for PRRSV by PCR, VI and immunohistochemistry (IHC).

Results

The study was conducted from October 1999 to July 2000. Ten farms fulfilled all criteria and were selected for inclusion in the study. Five farms were placed in each study group. All farms were located in the Minnesota. The mean breeding herd inventory in the T&R group was 769 sows (range = 318-1095), with a mean of 669 (range = 210-1295) in the W&R group. Each group contained 3 farms that used segregated production and 2 that used single site production. Eight of the farms were closed herd multipliers, raising all replacement females internally. Two farms in the T&R group purchased replacement stock from a PRRSV-negative source. All farms used artificial insemination, with on-farm AI laboratories for collection and dilution of semen. The breeding herd inventories of the positive control farms in the T&R group were 1605 and 550. The negative control farms in this group consisted of 418 and 2998 sows, respectively. Positive control farms in the W&R group consisted of 1492 sows, and 652 sows. The negative controls in this group had breeding herd inventories of 185 and 952. All of the positive and negative control farms used segregated production and had on-farm AI centers.

Diagnostic data: T&R

Application of Test and Removal resulted in successful elimination of PRRSV for 12 consecutive months from all 5 farms in the study group. The initial breeding herd seroprevalence at the start of the study ranged from 5-15 % (mean = 10 %) across all 5 farms. The percentage of sows removed following the whole herd test ranged from 2.1-10.7 %. The majority (77-100 %) of removed animals were ELISA positive: PCR negative; however, a percentage of ELISA positive: PCR positive (1.1-18 %) or ELISA negative: PCR positive (0-4.5 %). Of this latter group, ELISA s/p ratios ranged from .25-.39. Partial depopulation of nurseries and/or finishers occurred in farms 1, 2, 3, and 5,

depending on the point of PRRSV infection post-weaning. Seroconversion to PRRSV as determined by ELISA was not detected post-weaning in any of the 5 farms during the monitoring phase. During the 12-month monitoring period, a total of 3408 ELISA samples were collected across the 5-breeding herds. Of these, 74 ELISA positive samples were detected (2.1%), with approximately 1-2 ELISA positive samples detected per 60 animals tested each month. All 74 were re-tested by ELISA and PCR. All were individually PCR negative; however, 9 remained ELISA positive. These 9 sows were removed, necropsied, and tested according to the defined protocol. Four of these sows were removed from farm 1, 1 from farm 2, and 4 from farm 3. All tissue and serum samples tested were negative for PRRSV by PCR, VI and IHC. The diagnostic cost/breeding animal tested was approximately $10.66 US. This included the cost of the ELISA ($4.00 US/sample) and $6.60 US for each sample tested by PCR. Although the laboratory cost to run the PCR was $20.00 US/sample submitted, sera were pooled 3:1 in order to reduce cost. The time required to complete a T&R was approximately 7-10 working days, including sample collection, processing, testing, interpretation of results, and removal of animals. The initial and final seroprevalence of the positive control farms were 20 % and 15 %, and 25 % and 35 %. A significant relationship ($p = .0079$) was detected between the use of T&R and the successful elimination of PRRSV from farms within this group, as compared to the status of the positive control farms at the end of the monitoring period. Negative control farms remained seronegative throughout the study.

Diagnostic data: W&R

Consecutive PRRSV seroprevalence levels of $\geq$ 5 % were detected during the first 3 months of the monitoring process in all 5 farms in the W&R group. Therefore, based on definition of a PRRSV-negative farm used in this study it was concluded that these breeding herds were still infected. Breeding herd seroprevalence prior to the start of the W&R protocol ranged from 12-25% with a mean of 16 % across the 5 farms in the group. This difference was determined to be significant ($p = .0075$) when compared to the mean seroprevalence of the T&R group (10 %) at the same point in time. Prevalence levels detected during the third month of monitoring ranged from 7-10 %. Two farms were not able to obtain a source of negative gilts for the third phase of the protocol of replacement stock. Therefore, all potential replacements were serially tested a minimum of 2 times to document a negative or declining PRRSV-serostatus prior to entry into the breeding herd. Partial depopulation of nurseries and/or finishers occurred in all 5 farms, depending on the point of PRRSV infection post-weaning. The time required for completion of a W&R protocol on a study farm ranged from 6-7 months, and the diagnostic cost (ELISA only) was $4.00 US/sample. Throughout the course of the study, the PRRSV seroprevalence one of positive control farm increased from 15 to 100%, while the seroprevalence of the other remained relatively unchanged (25 to 35 %). No difference was detected ($p = 1$) between the final PRRSV status of the farms that used W&R and the positive controls. Negative control farms remained seronegative throughout the study.

Discussion

Potential reasons for the failure of the Wean and Removal protocol are as follows:

1. The PRRSV seroprevalence was $\geq$ 15 % in 3 W&R farms in contrast to 1 T&R farm, indicating a greater degree of exposure to PRRSV in the former group.
2. The protocol required $\geq$ 6 months before the entire breeding herd was tested.

3. Improper removal of seropositive animals, due the high genetic value of specific animals, production issues, the loss of ear tag identification, and the lack of compliance.
4. Recording errors at the time of testing, such as the incorrect labeling of serum tubes, or improper reading of animal identification.
5. The use of PRRS positive replacement gilts in 2 of the farms.

As expected, the primary limitations of T&R were the labor requirements on testing day, diagnostic costs, and the removal of productive sows from the herd. To minimize the impact of animal removal on herd productivity, the majority of removed sows were taken to off-site facilities to gestate and farrow, and were replaced by PRRSV-negative pregnant gilts. The difference in the diagnostic costs between the 2 protocols was due primarily to the PCR. The PCR was important for 2 reasons: to detect animals that were acutely infected but had not had sufficient time to seroconvert, and to eliminate the need to conduct multiple whole herd tests.

This study was designed to be an observational pilot study. Therefore, its limitations included the small sample size of each group; the size of the breeding herds of the study farms, and the exclusion of PRRSV vaccinated farms from the project. Plans to assess the efficacy of T&R in farms with inventories of $\geq$ 2000 sows that vaccinate against PRRSV are currently underway.

While Test and Removal requires further evaluation under a broader range of commercial settings, the results of this study indicate that it is a method capable of consistently eliminating PRRSV from farms that have similar characteristics to those defined in the study. While W&R did not appear to be as effective, there were prevailing circumstances that may have affected the outcome of farms in this group. The identification of potential pitfalls, such as improper recording, or certain personnel issues described above is helpful information to avoid repetition of these mistakes, and enhance the success rate of future W&R projects.