An evaluation of test and removal for the elimination of porcine reproductive and respiratory syndrome virus from 5 swine farms

Scott A. Dee, Michael D. Bierk, John Deen, Thomas W. Molitor

Abstract

The objective of this field study was to evaluate the protocol of test and removal (T&R) for the elimination of porcine reproductive and respiratory syndrome virus (PRRSV) from 5 chronically infected breeding herds. The T&R protocol involved sampling the entire breeding herd in one day, testing sera by polymerase chain reaction and ELISA to detect previously exposed and/or infected animals, and subsequently removing them from the herd. Following completion of T&R, breeding herds were monitored for 12 consecutive months, using ELISA, for the presence of antibodies to PRRSV. In order to be classified as a PRRSV-negative herd, all samples collected over the 12-month monitoring period were required to be negative by ELISA (s/p ratio < 0.4). At the conclusion of the monitoring period, all 5 farms were PRRSV-negative, according to the defined testing criteria. Approximately 2.2% (74/3408) ELISA false positive samples were detected across all 5 farms during the monitoring period. The diagnostic cost required during the T&R protocol was approximately US $10.66 per animal tested. Limitations of the study were a lack of herds with large (> 2000 sows) breeding herd inventories, and herds with a history of PRRSV vaccination.

Résumé

Cette étude avait pour objectif d'évaluer un protocole basé sur l'Épreuve et le Retrait (E&R) afin d'éliminer le virus du syndrome respiratoire et reproducteur porcin (VSRRP) de 5 troupeaux reproducteurs infectés chroniquement. Le protocole E&R consistait à échantillonner le troupeau au complet en une journée, à soumettre les échantillons de sérum à une épreuve d'amplification en chaîne par la polymérase (ACP) et par ELISA pour détecter les animaux préalablement exposés et/ou infectés, et de les retirer du troupeau. Une fois le programme E&R complété, les animaux reproducteurs furent testés pendant une période de 12 mois consécutifs à l'aide d'une épreuve ELISA pour détecter l'apparition d'anticorps dirigés contre le VSRRP. Afin de se qualifier comme troupeau VSRRP-négatif, tous les échantillons prélevés durant la période de surveillance de 12 mois devaient être négatifs à l'épreuve ELISA (ratio s/p < 0,4). À la fin de la période de surveillance, les 5 fermes étaient considérées négatives pour le VSRRP, selon les critères définis pour l'étude. Environ 2,2 % (74/3408) des échantillons obtenus des 5 fermes durant la période de l'étude donnèrent un résultat faussement négatif à l'épreuve ELISA. Les coûts associés au diagnostic durant la période du protocole E&R était de 10.66$US par animal éprouvé. Les limitations de cette étude proviennent du manque de troupeaux avec un grand nombre (> 2000 truies) d'animaux reproducteurs en inventaire, et de troupeaux avec une historique de vaccination contre le VSRRP.

(Traduit par docteur Serge Messier)

Introduction

Porcine reproductive and respiratory syndrome virus (PRRSV) is a significant pathogen to the global swine industry. Economic analyses have documented losses averaging US $228 per sow over a 12-month period due to elevated mortality rates, reduced growth, and excessive medication and vaccination costs (1,2). Previous attempts to eliminate PRRSV using the combination of segregated production, nursery depopulation, and vaccination (3,4) have failed, due to the ability of PRRSV to produce persistent infection and its ability to be transmitted both vertically and horizontally (5–7). A recently completed study indicated that PRRSV persistence can occur in chronically infected swine breeding herds, and that detectable virus is both infectious and virulent (8). Therefore, removing animals previously exposed to PRRSV was proposed as a possible means to eliminate the pathogen from infected farms. This strategy is known as "test and removal" (T&R), and has been used to eliminate Aujeszky's disease virus and *Actinobacillus pleuropneumoniae* from infected farms (9,10). Field investigations on the use of T&R for the elimination of PRRSV have produced promising results (11,12). This process consisted of blood-testing the entire breeding herd in a single day, identifying PRRSV-infected animals using both an antibody and a PRRSV nucleic acid test, and immediately removing positive animals from the farm. Therefore, it is important a thorough understanding of PRRSV diagnostics prior to the initiation of a T&R project.

Currently available diagnostic tests for the detection of PRRSV include virus isolation (VI) and polymerase chain reaction (PCR).

Department of Clinical and Population Sciences, University of Minnesota College of Veterinary Medicine, 385 Animal Science/Veterinary Medicine Building, 1988 Fitch Avenue St. Paul, Minnesota 55108 USA.

Address correspondence and reprint requests to Dr. Scott Dee, telephone: 612-625-4786, fax: 612-625-1210, e-mail: deexx004@tc.umn.edu.

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Virus isolation is labor-intensive, has a lower level of sensitivity compared to PCR, and is dependent on viable virus in the sample (13). Polymerase chain reaction detects viral nucleic acid in tissues and body fluids, and is both highly sensitive and specific (14). The IDEXX ELISA (IDEXX Laboratories, Westbrook, Maine, USA) test is a serologic test used routinely in diagnostic laboratories worldwide. The ELISA test detects the formation of PRRSV antibodies 9 to 13 d after virus exposure (15). Results are reported in the form of sample-to-positive (s/p) ratios, and ratios of 0.4 or higher are considered positive.

Limitations of T&R have been documented (11,12), and include a high degree of labor involved in testing a whole herd, and diagnostic costs that approach US $10.00/tested sow. Furthermore, a low seroprevalence (< 15%) of sows found to be positive by ELISA (ELISA+) is required to reduce the impact of animal removal on the productivity and profitability of the farm (11,12). The purpose of this paper is to describe the results of an observational pilot study conducted to evaluate the ability of T&R to eliminate PRRSV from 5 breeding stock farms.

Materials and methods

Farm selection

For inclusion in the study, all farms had to fulfil the following criteria: 1) A period of ≥ 24 mo must have elapsed following the original PRRSV infection and the initiation of the study; 2) A period of ≥ 12 mo must have elapsed since the initiation of the study and the last recorded clinical episode of PRRS in the breeding herd, as previously defined by Loula (16); 3) The estimated seroprevalence of PRRSV in the breeding herd prior to the initiation of the study must be ≤ 25%; 4) The farm must be located a minimum of 3.2 km from other PRRSV-infected farms; and 5) The farm must not have used PRRSV vaccine prior to initiation of the study, and could not vaccinate at anytime during the study period.

Along with the study farms, 2 PRRSV-positive farms and 2 PRRSV-negative farms were included as controls. Positive control farms had to fulfil all 5 criteria, while negative controls needed to fulfil criteria 4 and 5. The status of both the positive and negative control farms had been verified by a minimum of 12 mo of serologic monitoring (IDEXX ELISA) and clinical histories (16). Instead of attempting PRRSV elimination activities in the positive control farms, the PRRSV serostatus was monitored for any changes throughout the study period to determine whether PRRSV elimination could occur independently of T&R. The relationship between the use of T&R and the final PRRSV status of farms in the study group versus that of the positive control farms was analyzed for statistical significance by Fisher's exact test (17). This test was selected due to the small number of farms in the study, and the fact that it is an exact test frequently used to evaluate the success or failure of treatments (farms that used T&R) versus positive controls (farms that did not use T&R).

Serologic profiling and prevalence estimation

Thirty days prior to initiation of the T&R protocol, the seroprevalence of the breeding herd was estimated (18). A simple random sample of 29 to 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 to 1500 sows, where the expected true prevalence of PRRSV antibodies was ≤ 10% or ≥ 90% at an accuracy of ± 10%. In addition, 30 random samples were collected from 10-week-old nursery piglets and 5- to 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 seroprevalence of 10% at a 95% confidence. If PRRSV infection was detected post-weaning, infected nursery and/or finishing facilities were depopulated 24 to 48 h prior to T&R (19).

Test and removal protocol

The T&R protocol involved the use of the IDEXX ELISA test for the detection of PRRSV antibodies, in combination with the Taqman (Perkin-Elmer Applied Biosystems, Foster City, California, USA) PCR assay for the detection of PRRSV nucleic acid (20). An s/p ratio of 0.4 or higher was considered to be positive by ELISA (ELISA+). The entire breeding herd (sows, boars and replacement gilts) was blood-tested in a single day. Sera were collected using a different syringe (12 mL) and needle (18-gauge, 3.81 cm) for each animal. In order to minimize degradation of PRRSV nucleic acid, collection kits were lined with ice packs to ensure that samples were kept cool during the collection process. Samples were centrifuged (1500 × *g*) for 15 min and the sera were separated, refrigerated at 4°C, and delivered to the University of Minnesota Veterinary Diagnostic Laboratory on ice. Three samples were pooled together for PCR testing and 1-mL aliquots from each sow were stored at −70°C. If a positive serum pool was detected, the 3 individual aliquots were immediately tested to identify the specific animal(s) responsible for the positive reading.

The decision to remove or retain animals was based on a previously published diagnostic protocol (12). Animals that were both ELISA+ and positive by PCR (PCR+) were considered to be viremic on the day of sampling. Animals that were ELISA+ but negative by PCR (PCR−) were considered previously exposed to PRRSV but not actively viremic on the day of sampling. It was not known whether these animals harbored PRRSV on the day of sampling or if they had cleared virus and were in the process of antibody decay. Animals that were negative by ELISA (ELISA−) and PCR+ were considered to be acutely infected, but had not had sufficient time to generate an ELISA s/p ratio of ≥ 0.4. Animals with any one of these 3 diagnostic profiles were removed from the farm within 24 to 48 h of 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 serologic monitoring

Following the completion of the T&R protocol, all breeding herds were monitored monthly by ELISA. A simple random sample of 52 to 58 breeding animals was collected, according to herd size (18). This sample size was capable of detecting at least 1 positive pig at an estimated prevalence of ≥ 5% at a 95% confidence in farms with breeding herd inventories of 200 to 1500 sows. Following the depopulation of the nurseries or finishers, 10 samples were collected

Table I. Characteristics of farms in the Test and Removal study group

Farm #	Herd size	# Sites	Initial prevalence[a]	Detection of last singleton reactor	Date of last monthly test	Final prevalence[b]
1	792	3	10%	December 1998	September 1999	0%
2	812	3	10%	May 1999	December 1999	0%
3	825	3	15%	August 1999	January 2000	0%
4	1095	1	5%	April 2000	May 2000	0%
5	318	1	10%	June 1999	September 1999	0%
Mean	769		10%			0%
(+) controls						
farm A	1605	3	20%	May 2000	May 2000	15%
farm B	550	3	25%	May 2000	May 2000	35%
(−) controls						
farm C	418	3	0%	NA	May 2000	0%
farm D	2998	3	0%		May 2000	0%

[a] ELISA PRRSV estimated seroprevalence of breeding herd prior to T&R

[b] ELISA PRRSV estimated seroprevalence following completion of T&R (month 12)

from 8- to 10-week-old and 5- to 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 ≥ 30% at a 95% confidence. The control farms were tested in a similar manner.

In order for a study farm to be considered PRRSV-negative, 12 consecutive months of ELISA− (s/p ratio < 0.4) test results were required following completion of the T&R protocol, and no evidence of PRRSV infection was to be detected post-weaning. If a breeding herd PRRSV seroprevalence of ≥ 5 % was detected for 3 consecutive months, or if PRRSV seroconversion was detected post-weaning, the monitoring protocol was terminated and the farm was classified as PRRSV-positive. If an individual ELISA+ animal was detected during a sampling period, the animal was considered a "singleton reactor." A second serum sample was collected from these individuals within 24 h and was tested by ELISA and PCR (20). If one or more of these tests was positive, the singleton reactor was removed from the herd, slaughtered, and necropsied. Tonsils and lymph nodes (sternal, lateral retropharyngeal, internal iliac, and superficial inguinal) were collected and tested for PRRSV by PCR, VI, and immunohistochemistry (IHC) (21). If evidence of PRRSV was detected in serum or tissue, the animal was considered a true positive. If the animal was ELISA+, serum PCR−, and all tissues were determined to be PRRSV-negative, and the animal was considered a false positive.

Results

Characteristics of study farms

The study was conducted from October 1998 to May 2000. All farms were located in the midwestern United States. Individual farm characteristics are summarized in Table I. The mean breeding herd inventory of the farms in the study was 769 sows (range, 318 to 1095). Three of the farms used segregated production and 2 used single-site production. Three of the farms were closed herd multipliers, raising all replacement females internally. Two farms purchased replacement stock from a PRRSV-negative source. All farms used artificial insemination (AI), with on-farm AI laboratories for collection and dilution of semen. The breeding herd inventories of the positive control farms were 1605 (farm A) and 550 (farm B). The negative control farms (C and D) in this group consisted of 418 and 2998 sows, respectively. All of the positive and negative control farms used segregated production and had on-farm AI centers.

Diagnostic data

According to the definition of a PRRSV-negative farm, application of T&R 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 to 15% (mean, 10%) across all 5 farms (Table I). The percentage of sows removed following the whole-herd test ranged from 2.1 to 10.7% (Table II). The majority (77 to 100%) of removed animals were ELISA+: PCR−; however, a percentage of ELISA+: PCR+ (1.1 to 18%) or ELISA−: PCR+ (0 to 4.5%). Of this latter group, ELISA s/p ratios ranged from 0.25 to 0.39. Partial depopulation of nurseries and/or finishers occurred in farms 1, 2, 3, and 5. Seroconversion to PRRSV as determined by ELISA was not detected post-weaning on 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+ samples were detected (2.2%), all with s/p ratios ranging from 0.4 to 0.75. No more than 2 positive samples were present in an individual monthly sample set at one time. All 74 were re-tested by ELISA and PCR within 24 h following receipt of the positive result. While all were serum PCR−, 9 remained ELISA+, with s/p ratios ranging from 0.4 to 0.83. These 9 sows were removed, slaughtered, 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 date that the last ELISA+ sample was detected in the breeding herd as compared with the date of the final month of monitoring is summarized in Table I.

Table II. Diagnostic data of removed animals from Test and Removal study farms

Farm #	# Tested	# Removed	ELISA+ PCR+	ELISA+ PCR−	ELISA− PCR+	ELISA− PCR−
1	792	66 (8.3%)	1	64	1	726
2	812	77 (9.5%)	6	69	2	735
3	825	88 (10.7%)	4	84	0	737
4	1095	23 (2.1%)	0	23	0	1072
5	318	22 (6.9%)	4	17	2	296
Mean	769	55 (7.2%)	3	51	1	713
% of removed			5.5	92.7	1.8	

The diagnostic cost per breeding animal tested was approximately US $10.66. This included the cost of the ELISA (US $4.00/sample) and US $6.60 for each sample tested by PCR. Although the laboratory cost to run the PCR was US $20.00/sample submitted, sera were pooled 3:1 in order to reduce cost. The time required to complete a T&R was approximately 7 to 10 working days, including sample collection, processing, testing, interpretation of results, and removal of animals. While some removed animals were slaughtered, whenever possible, productive animals were moved to off-site facilities to gestate and farrow. The offspring derived from these animals remained segregated from the pigs weaned from ELISA− and PCR− sows.

The initial and final seroprevalence of the positive control farms were 20% and 15% for farm A and 25% and 35% for farm B. A significant relationship ($P = 0.0079$) was detected by Fisher's exact test between the use of T&R and the PRRSV status of the 5 T&R farms, as compared with the PRRSV status of the positive control farms at the end of the monitoring period. A total of 1092 and 1218 samples were collected from negative control farms C and D respectively. Of these samples, 11 (1.0%) from farm C and 15 (0.5%) from farm D were determined to be ELISA+. The s/p ratio range of these samples was 0.4 to 1.05. All positive animals were retested within 24 h by ELISA and serum PCR. Three samples from farm C and 5 from farm D were ELISA+ on the second test. All were serum PCR−. Clinical signs of PRRSV infection (16) were not observed at the time the ELISA+ samples were detected or at anytime throughout the study period. Therefore, these animals were not removed, and the herds were considered PRRSV-negative.

Discussion

Based on the parameters defined in the monitoring program, it appeared that PRRSV was eliminated from all farms that employed T&R. Evidence of PRRSV transmission was not detected in the breeding herd or in the weaned pig populations of the 5 study farms over the 12-month monitoring period. While a small number of sows were ELISA+ on consecutive tests, extensive diagnostic evaluation of these singleton reactors indicated no evidence of PRRSV in selected tissues. To further investigate the serological response of these singletons, an indirect fluorescent antibody test (IFAT) was conducted as an adjunct test (22). All 9 animals that were found to be ELISA+ on consecutive tests were found to be IFAT−. In addition to these findings, ELISA+ samples were detected in the negative control farms sampled throughout the 21-month study period. While removal and necropsy of singleton reactors from the negative control farms was not conducted, all ELISA+ sera were found to be PCR− and IFAT−. Similar observations have been reported, both from swine clinicians and diagnosticians, from samples collected from herds known to be PRRSV-negative (S.A. Dee, personal observation; G. Spronk, G. Kennedy, D. Benfield, J. Collins, K. Rossow, D. Polson, C. Moore, W. Christianson, personal communications). Therefore, it was concluded that the singleton reactors were false positives, resulting in an overall ELISA false positive rate of 2.2% (74 ELISA+ samples/3408 samples collected) during the 12-month monitoring period across all 5 farms.

It was not certain if T&R was necessary on farm 4. Only 2.1% of the tested animals were removed, and all 23 samples were ELISA+: PCR−. The nurseries and finishers from this farm were also seronegative, so while the farm did have a previous history of clinical PRRS, it is likely that active PRRSV infection was non-existent at the time of testing. However, it has been documented that the prevalence of PRRSV-infected breeding animals may be low (1.7%) in chronically infected farms, and antemortem tests cannot distinguish carrier animals from those previously infected that have cleared the virus (8). Unfortunately, animals removed from this farm were not available for necropsy.

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. While the PCR added extra cost to the T&R protocol, it was used 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. Testing the entire breeding herd multiple times is a common practice during T&R procedures targeted for the elimination of Aujeszky's disease virus or *Actinobacillus pleuropneumoniae*, but with the size of today's swine operations, multiple whole-herd tests for the elimination of PRRSV could be very labor intensive and costly. The sensitivity of the PRRSV PCR has recently been questioned (23), but the inability to detect serologic evidence of PRRSV circulation during the 12-month monitoring period indicates that all PRRSV-positive animals were removed through the combined use of virus and antibody tests.

During the T&R procedure, animals that were negative by both tests were allowed to remain in the herd. A recently completed study indicated the absence of detectable PRRSV in tissues collected

from breeding swine with ELISA s/p ratios ranging from 0.1 to 0.39 (8). Based on these findings, and on data from another published work (5), it appears that pigs that harbor PRRSV are likely to remain seropositive until virus has been cleared from the host and the antibodies generated following the initial infection have had sufficient time to decay.

This study was designed to be an observational pilot field study, and, therefore, it possessed some obvious limitations. One such limitation was the small sample size of each group. Unfortunately, budgetary constraints limited the number of farms that could be included. To strengthen the study, positive control farms were included to monitor changes in the PRRSV status of farms where no attempt was made to eliminate PRRSV by either protocol, and a significant association was detected in the PRRSV status of the treatment and positive control farms by Fisher's exact test. The inclusion of the negative control farms demonstrated that ELISA+ animals could be detected in PRRSV-naïve farms. This information was helpful when interpreting the significance of the singleton reactors detected in the 5 T&R farms.

Other limitations included the breeding herd inventories of the study farms and the exclusion of PRRSV-vaccinated farms from the project. In today's industry, breeding herd inventories exceeding 2000 sows are common in the United States. Furthermore, since differential serologic tests for PRRSV are currently not available, farms that vaccinated against PRRSV were not included in this initial study, to enhance interpretation of diagnostic data. Plans to assess the efficacy of T&R in farms with inventories of $\geq$ 2000 sows that vaccinate against PRRSV are currently underway.

While T&R 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. However, it is unlikely that it will maintain this level of success. In conclusion, PRRSV elimination strategies are in the early phases of development and much more information and testing is necessary. It is likely that the results of future studies on this topic will result in the generation of multiple strategies, and present practitioners with a number of options, similar to the history of Aujeszky's disease virus elimination. It will then be up to the veterinarian and farm owner to determine which protocol should be applied to each specific case.

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