PONENCIAS MAGISTRALES

STRATEGIES FOR THE CONTROL AND ERADICATION OF PRRS

Scott A. Dee DVM MS PhD Dipl; ACVM
Associate Professor, College of Veterinary Medicine
University of Minnesota, St. Paul, MN

INTRODUCTION

Porcine reproductive and respiratory syndrome virus (PRRSV) is an economically significant pathogen of the global swine industry. Economic analyses have documented losses averaging $252 per sow over a one year period due to elevated mortality rates, reduced growth and excessive medication and vaccination costs (Dee and others 1997a). While a number of control strategies have been developed, they are inconsistent and cannot be applied to all farms. Due to the propensity of PRRSV to undergo mutation, commercially available vaccines have limited efficacy. Like other RNA viruses, PRRSV mutates to escape environmental and host immunological pressure. Therefore, the ability to successfully control the disease for extended periods using traditional methods appears unlikely. At this time, many practitioners and producers feel the long-term viability of the US swine industry will be better served by PRRS eradication, rather than by coexistence.

Early attempts to eradicate the disease using segregated production (Dee and others 1993) or management and vaccination (Dee and others 1998) have failed, due to the ability of PRRSV to produce persistent infection, and to be transmitted both vertically and horizontally (Wills and others 1997, Wagstrom and others 1998). These reports emphasize the importance of detecting and removing persistently infected breeding animals (Test and Removal), in order to prevent transmission of virus to offspring prior to weaning. The following paper will review published control strategies and provide a review of preliminary eradication efforts using the principle of Test and Removal.

THE VIRUS

PRRSV is an RNA virus classified in the order *Nidovirales*, family *Arteriviridae* and genus *Arterivirus*_(Cavanaugh 1997). Other members include Lactate dehydrogenase elevating virus of_mice, Equine viral arteritis virus and Simian hemmorrhagic fever virus. These viruses are capable of inducing a prolonged viremia in the presence of antibodies, replicating in macrophages and producing persistent infections. Persistent infection is defined as "the continued presence of a virus in a host for extended periods of time post infection" (Ahmed 1996). RNA viruses such as PRRSV do not revert to inactive states post infection, but rather continue to replicate at some level within certain sites in the body. Controlled studies have isolated PRRSV for up to 157 days post infection from tonsil tissue (Wills and others 1997) and identified the presence of PRRSV nucleic acid in boar semen 93 days post infection (Christopher-Hennings and others 1995); however, infection is not life long. Transmission of PRRSV has been documented through semen, saliva, milk, colostrum, urine, feces, as well as

hematogenously (Wagstrom and others 1998). Spread of the virus from persistently infected animals to susceptible swine has been reported to occur 17 weeks post infection (Albina and others 1994), yet little information regarding the mechanism of enhanced replication with subsequent shedding is available at this time.

PRRS DIAGNOSTICS

Current diagnostic tests for PRRSV detection can identify viable virus (virus isolation [VI]), viral antigen (immunohistochemistry, fluorescent antibody test), viral nucleic acid (polymerase chain reaction [PCR], in-situ hybridization) and circulating IgG antibodies (IDEXX ELISA[R] test). (Collins and others 1996) PRRSV can be isolated in porcine alveolar macrophages or in a continuous cell line derived from African green monkey kidneys known as MA-104 cells; however, VI is labor-intensive, has a lower level of sensitivity compared to PCR, and is dependent on viable virus in the sample. Immunohistochemistry (IHC) detects PRRSV antigen in formalin fixed tissues using a variety different labeling and detection methods. PCR detects viral nucleic acid in tissues and body fluids and is both highly sensitive and specific. Finally, in-situ hybridization (ISH) detects PRRSV-RNA in formalin-fixed tissue and may posses the highest degree of sensitivity of all the aforementioned assays, but is not routinely available in most diagnostic laboratories. All of these tests can be used to detect PRRSV in antemortem and postmortem samples. Since PRRSV has been isolated from a vast number of tissue sites following cessation of viremia, attempts to detect persistent carriers through the testing of sera by PCR alone are unlikely to succeed. The collection of tonsil biopsies has been proposed as an alternative measure for detecting PRRSV in the live animal (Wills and others 1997), but the issues of test sensitivity and specificity in chronically infected animals collected from field-based populations has not been addressed at this time. Also, PRRSV may persist in adult animals at sites other than the tonsil.

In addition to PRRSV detection, viral isolates can be characterized by molecular sequencing of open reading frame (ORF) 5 (virus envelope) and 6 (virus matrix) regions. (Collins and others 1998) Open reading frame 5 codes for the PRRSV envelope and is the portion of the virus that initially interacts with the pig immune system. Open reading frame 5 is a variable portion of the virus prone to mutation and is therefore useful for characterizing PRRSV isolates within a farm or animal and is also important for monitoring virus mutations. Sequencing PRRSV isolates is important for understanding the evolution or mutation of PRRSV within pig populations and may help in determining the rate of change in the virus over time and factors associated with virus persistence.

The IDEXX ELISA[R] test is a serologic test used routinely in diagnostic laboratories worldwide (Collins and others 1996). The ELISA test detects the formation of PRRSV-antibodies 9-13 days after virus exposure. Results are reported in the form of a sample to positive (s/p) ratios and levels of .4 or higher are considered positive. PRRSV persistently infected animals can be serologically positive by ELISA for 56 to 225 days after infection (Wills and others 1999). Therefore, the presence of an antibody in a live non -vaccinated animal, in the absence of detectable viremia, is indicative of one of the following situations:

1.The animal is persistently infected with PRRSV present in a tissue site.

2.The animal has cleared the virus and is no longer infected.
3.A false positive result has occurred due an error in test specificity.

PRRSV can infect immune- privileged sites, such as the male and female reproductive tract, cornea and brain (Shin and others 1996). These sites are protected from the immune system and infection does not result in a detectable serologic response. Unfortunately, these studies were not conducted for a sufficient length of time to determine whether PRRSV could persist in such sites for extended periods. Hypothetically, if persistent infection of immune privileged sites were to occur, an animal that was negative on both serum PCR and ELISA could potentially fall into one of the following categories:

1.The animal is persistently infected with virus in an immune-privileged site.
2.The animal has cleared the virus; antibody decay has occurred and is no longer detectable.
3.The animal has undergone peracute infection and virus or antibody cannot be detected, due to the issue of test sensitivity.

These issues form the basis of the need to further explore the persistently infected breeding animal, and will be covered later in the lecture.

The most important aspect of utilizing PRRS diagnostics is to accurately determine the point of infection within the life of the pig. This information enhances the decision-making process regarding selection of the control strategy. New techniques, such as Polymerase Chain Reaction (PCR) are especially helpful for achieving this goal. Positive PCR signals can be sequenced and samples of field virus can be differentiated from modified-live virus vaccine isolates. The use of PCR is valuable, particularly when investigating PRRS problems in lactation or nursery. This test is much more sensitive than virus isolation, and results are available in a shorter period of time (24 to 48 hours). Recent data have shown that shedding of PRRS virus can be limited to gilt litters during lactation, and that shedding can occur at any time during the suckling period, resulting in infected piglets at one to two days of age (Dee and Philips 1999). An example of a protocol to detect transmission of field virus from sows to pigs during the lactation period is as follows:

1. **Collect sera from 5-7 day old piglets, collecting 1-2 piglets/ litter**
2. **Sample across parities (6 piglets/parity)**
3. **Pool sera 3:1 within each parity**
   Example: Six parities present in lactation on a given sampling day
   6 parities x 6 piglets sampled/parity = 36 samples
   Samples pooled 3:1 = 12 samples submitted

If virus shedding is occurring during lactation, the five to seven day old piglet has been shown to be a sensitive sample for the detection of PRRSV prior to weaning (Rossow KD, personal communication 1999). During the sampling process, samples should be kept cool at all times, and should be shipped to the diagnostic laboratory over night on ice. It is better to refrigerate samples than to freeze them at 20^0 C.

Cross-sectional sampling by ELISA according to stage of production can be useful to determine the point of infection in the life of the pig. Approximately 10 samples should be collected from the breeding herd, recently weaned pigs, eight to 10 week old nursery pigs and five to six month old finishers (Dee and Joo 1994a). Larger sow herds, such as 1000 head or more frequently require larger sample sizes (n = 30 to 60), allowing for sampling across parities. Testing by parity (n = five samples/parity) may indicate whether programs need to focus on a specific subpopulation within the herd, such as gilts. Unfortunately, diagnostic tests that differentiate antibodies due to natural infection from those induced following vaccination are not available at this time. Furthermore, it is also important to realize that PRRSV infection, without the presence of opportunistic pathogens such as *Mycoplasma hyopnuemoniae* or *Streptococcus suis* may have little impact on the health and performance of pigs. Therefore, it is important to use clinical observations and production records in conjunction with diagnostic data to properly understand the status of the sampled population. Once the pattern of virus spread and the age in which the pig is infected is determined, intervention strategies can be initiated.

VACCINATION

Due to the necessity of cell-mediated immunity to control PRRS, modified-live virus vaccines appear to be more efficacious than killed preparations (Dee personal experience 1994-1999). Duration of immunity studies indicates the modified-live preparations are protective for up to at least 4 months (Polson, DD personal communication 1994). While MLV products do shed in naïve populations (Torrison and others 1996), transmission has not been detected following re-vaccination (Dee and Philips 1999). Diagnostic profiling is especially critical when vaccination is employed to avoid immunizing pigs concurrently infected with field virus. It is important to develop farm-specific vaccination programs, based on individual farm diagnostic data, rather than promote standardized protocols. Proper handling of modified live virus vaccines is important to enhance efficacy. An adequate needle length will insure that intramuscular penetration is obtained. A 3.81 cm needle is required for adult breeding animals, while 2.54 cm is required for finishers and 1.27cm for weaners. The product must be rehydrated using the proper diluent, and administered within 24 hours following hydration. Finally, using a designated syringe, rinsed with only with hot water, not disinfectant, is important as well (Dee personal experience 1994 to1999).

GILT DEVELOPMENT

Gilt development is very important component of a PRRS control program. Direct introduction of naïve or actively infected replacement stock will enhance viral shedding and exacerbate PRRS problems (Dee and Joo 1994b, Dee and others 1994). Therefore, it is critical to establish a gilt development program, consisting of quarantine and an acclimatization period, to properly develop replacement stock prior to entry to the herd (Dee 1997). Changes in gilt development strategies in the US include the introduction of gilts as weaners or 25 kg piglets, housing them in designated developer facilities, and establishing vaccination and acclimation protocols in an attempt to equalize the health level of this population to the existing breeding herd. Despite the use of cull

sows and/or infected nursery pigs as sources of farm-specific viral strains, consistent induction of natural exposure can be very frustrating, particularly in chronically infected herds, due to lack of active circulation of field virus. Therefore, it is important to maintain the use of vaccination in gilt developer protocols to reduce the risk of developing naïve subpopulations. The purchase of gilts from PRRSV-negative sources enhances the importance of proper natural exposure to farm-specific strains prior to herd entry. If PRRSV positive sources are employed, it is important to insure that a change in source does not occur, and that actively infected animals do not directly enter the recipient herd. For this reason, quarantine periods of 60 days or more are employed, and that quarantine facilities function on an all in-all out basis.

NURSERY DEPOPULATION

Nursery depopulation (ND) is a cost-effective means to interrupt horizontal transmission of PRRSV from older, infected pigs to those recently weaned. Significant improvements in nursery pig daily gain, mortality, treatment cost and profitability have been published, following implementation of ND (Dee and others 1997a, Dee and others 1997b). Despite such improvement, re-infection of nurseries with PRRSV is a frequent event, often taking place 12 to18 months following completion of the depopulation protocol. Therefore, it has been speculated that the primary benefit of this strategy may be for the control of opportunistic pathogens, rather than PRRSV. Nursery depopulation consists of emptying all nursery rooms, washing and disinfecting, and allowing the facility to remain empty for a minimum of two days. Due to the poor viability of PRRSV outside of the pig, extended periods of downtime can be avoided, enhancing facility utilization and eliminating the virus from the nursery pig population. Disinfectants frequently recommended include phenol or formaldehyde-based products to reduce survivability of the virus in the environment. This strategy can also be applied to PRRSV-infected finishing populations. Changes in production and financial data over a 24-month period from 34 nurseries in the US are summarized in table 1.

THE ROLE OF THE PERSISTENTLY INFECTED BREEDING ANIMAL

The goal of the following study was to determine if PRRSV persistence could occur in chronically infected breeding swine, and to determine the prevalence of persistently infected animals in a field population. The study is based on the central hypothesis that the frequency of PRRSV persistence within a field-based breeding population is low; however, this sub-population is capable of harboring virus which is infectious and virulent. To address these issues, the study was divided into two phases, each with a set of specific aims:

Phase 1:

1. To calculate the prevalence of PRRSV persistence within infected breeding herds.
2. To assess the ability of PRRSV to persist in immune privileged sites.

Phase 2:

1.   To determine if persistently infected animals harbor virus which is infectious and virulent.

Materials and methods
Phase 1: Experimental Design

In order to measure PRRSV persistence at the herd level, specific farm and animal criteria were established. It was hypothesized that fulfillment of such criteria at both the population and individual animal level would enhance the ability to select animals that were persistently infected, and avoid those that had recently been exposed to virus. The criteria for farm selection were as follows:

1. A period of greater than or equal to two years must have elapsed following the original PRRSV infection and the initiation of the study.
2. A period of greater than or equal to one year must have elapsed since the initiation of the study and the last recorded clinical episode of PRRS in the breeding herd.
3. At the initiation of the study, the seroprevalence of the breeding herd must be less than or equal to 25 percent, as determined by the ELISA test.
4. The farm must employ multiple-site production.
5. The breeding herd population must be located a minimum of 3.2 km from other PRRSV infected farms.
6. The farm must have had no previous history of PRRS modified-live virus vaccine usage.

The requirement for multiple site production was included to reduce the risk of horizontal transmission of PRRSV from the other production segments, i.e. nursery and finishing, of the selected farm. The distance of 3.2 km from other PRRSV infected farms was designed to minimize the risk of infection from an outside source. Finally, the lack of PRRSV modified live vaccine usage was important to avoid confusion regarding interpretation of diagnostic data.

Specific diagnostic criteria were also established to enhance selection of animals that were previously exposed to PRRSV, but not recently infected. Animals selected for removal from the herd due to poor reproductive performance or chronic arthritis were purchased at weaning, tested by ELISA and serum PCR and organized into one of three groups:

Group#	# pigs	#sows	#boars	ELISA range	ELISA result	serum PCR
1	20	15	5	.1-.39	negative	negative
2	20	15	5	.4-1.0	positive	negative
3	20	15	5	>1.0	positive	negative

Sixty animals (45 sows and 15 boars) were included in the study set. This sample size was capable of detecting at least one positive pig at an estimated prevalence of greater than or equal to 5% at a 95% level of confidence. Selected animals were then transported to the University of Minnesota Veterinary Diagnostic Laboratory for euthanasia and necropsy.

Phase 1: Laboratory Procedures

For the purpose of virus detection, three general groups of tissues were established, and a total of 19 tissues were collected from each animal. These groups included **lymphoid tissues** (tonsil, spleen, thymus, medial iliac, superficial inguinal, sternal and lateral retropharyngeal lymph nodes), **immune-privileged tissues** (testis, accessory sex

glands, adrenal gland, ovaries, uterus, cornea, and brain) and **non lymphoid/non-immune-privileged tissues** (liver, kidney, lung, heart, and mammary gland). All samples were analyzed by polymerase chain reaction (Taqman PCR), virus isolation (VI), immunohistochemistry (IHC), and examined microscopically for lesions of PRRSV infection. Open reading frame (ORF) 5 and a portion of ORF 6 of all isolates recovered were sequenced as previously described.

Phase 2: Experimental Design

In order to determine if isolates detected during the initial phase were infectious and/or virulent, a late gestation pregnant sow model was employed. Isolates from the initial phase of this study were administered to PRRSV-naive sows during late gestation, and clinical and diagnostic observations of dams and fetuses recorded over a 14 day period. Two pregnant sows were selected from a PRRSV negative source and were housed in separate rooms within an isolation facility at the University of Minnesota College of Veterinary Medicine. ELISA serology and clinical history verified the PRRSV negative status of the source farm over a two-year period. Prior to inoculation, animals were tested for the presence of PRRSV antibodies using ELISA and for the presence of PRRSV using PCR and VI. Sows were inoculated intra nasally with cell culture medium containing 10^2 TCID$_{50}$ per ml of a PRRSV isolate recovered from Phase 1, at day 95 of gestation. A negative control sow was included in the study, housed in a separate room and inoculated intra nasally with virus-free cell culture media.

Phase 2: Laboratory Procedures

All animals were observed twice daily throughout the 14 day study period for clinical signs, including inappetance, fever, and lethargy. Serum was collected on day 0, 1, 3, 5, 7, 10, 12, 14 post-infection (pi) and analyzed by ELISA, PCR, and VI. Animals were then necropsied on day 109 of gestation (day 14 pi). Maternal tissues were collected and analyzed for the presence of PRRSV as described in Phase 1. Fetal tissues, including lung, heart, spleen, kidney, brain, superficial inguinal lymph node, and tonsil that were analyzed for the presence of PRRSV by PCR. Fetal thoracic fluid was tested for the presence of fetal IgG and IgM by agar gel immunodiffusion (AGID) and stomach contents were submitted for aerobic bacteriologic culture. All tissues were screened for other pathogens as well.

RESULTS

Phase 1:

A 1500 sow breeding herd was selected for the study following fulfillment of all six-farm criteria. Sixty animals were selected from this population according to the individual animal diagnostic criteria. The ELISA data from these animals can be summarized as follows: Group one animals had a mean ELISA s/p ratio of .310 (SD = .05, range = .22-.37), group two had a mean s/p ratio of .639 (SD = .15, range = .49-.98), and group 3 had a mean of 1.37 (SD = .38, range = 1.05-2.25). All 60 animals were serum PCR and VI negative. Following the aforementioned protocol of sample collection and analysis, PRRSV was isolated from a sample of lateral retropharyngeal lymph node from one sow. The lymph node was found to be positive to PRRSV antigen by immunohistochemistry, but negative by PCR. Microscopic lesions of PRRSV infection were not detected. This animal had an ELISA s/p ratio of 1.20 at

necropsy, and its serum was negative for PRRSV by PCR and virus isolation. Sequencing data indicated that the isolate possessed a predicted cut pattern of 1-4-2. All other samples from this animal, and the other 59 animals sampled were negative on all tests for the presence of PRRSV, resulting in a prevalence of PRRSV persistence in the representative sample tested of approximately 1.7%.

Phase 2:

Following inoculation of the naïve sows at 95 days of gestation, PRRSV antigen was detected in sera of both sows by PCR by day three post-infection (pi) with positive ELISA readings detected by day 10 pi. Clinical signs of PRRSV infection such as lethargy, inappetance, and fever were observed in both sows.

Sows were necropsied at day 109 of gestation (14 days pi). Sow A had a mean rectal temperature of 39° C (SD = .822, range = $37.2-39.6^{\circ}$ C) throughout the 14 day observation period, while Sow B had a mean rectal temperature of 38.2° C (SD = 1.32, range = $37.2-40.1^{\circ}$ C). The negative control animal, Sow C, had a mean rectal temperature of 38.3° C, (SD = .685, range = $37.7-39.2^{\circ}$ C). Sow A presented with a litter that included 10 fresh, six partially autolyzed, and two younger mummified fetuses. Pooled fetal tissues from Sow A (fetuses #1-5, 6-9, 10-14, and 15-16) were all positive for PRRSV nucleic acid by PCR. Thoracic fluids from nine of the 16 fetuses analyzed from Sow A were positive for IgG and IgM by AGID techniques.

Tissues collected from Sow A including mandibular, lateral retropharyngeal medial iliac lymph nodes, tonsil, heart, uterus, kidney, and umbilical cord were positive by PCR. Superficial inguinal and ovarian lymph nodes were weakly positive. In contrast Sow B presented with a litter of six fetuses that appeared fresh and viable. Pooled fetal tissues from fetuses #1-3, and 4-6 were positive for PRRSV nucleic acid by PCR. Fetuses # 4, 5, and 6 were positive for IgG and IgM by AGID. Maternal tissues that were PCR positive included uterus, mandibular, sternal, lateral retropharyngeal and superficial inguinal lymph nodes. All tissue and serum samples from Sow C were all negative for PRRSV-antigen and antibodies. A representative viral isolate, recovered from a sample of tonsil from Sow A was sequenced as described above, and found to be identical to the original isolate recovered in Phase 1. All maternal and fetal tissues were screened for the presence of other pathogens, including parvovirus, circovirus types 1 and 2, Aujeszkey's disease virus, Encephalomyocarditis virus, Enterovirus, and Leptospirosis sp. All samples were determined to be negative.

AN UPDATE ON TEST AND REMOVAL FOR PRRS ERADICATION

INTRODUCTION

While a number of PRRS control strategies have been developed, they are inconsistent and cannot be applied to all farms (Zimmerman and others 1998). Previous attempts to eliminate PRRSV using segregated production (Dee and others 1993), management and vaccination (Dee and others 1998) have failed, due to the ability of PRRSV to produce persistent infection and to be transmitted both vertically and horizontally (Wills and others 1997, Christianson and others 1993, Wagstrom and others 1997). A recently completed study indicated that PRRSV persistence can occur in breeding swine, and that persistent virus is infectious and virulent (Bierk and others 2000). Therefore, detecting and removing persistently infected breeding animals was proposed as a

possible means to eliminate PRRSV from infected breeding herds. This strategy is known as "Test and Removal" (T&R), and has been used to eliminate Aujeszky's Disease virus (ADV) and *Actinobacillus pleuropneumoniae* from infected farms (Thawley and others 1982, Nielsen and others 1976). Field investigations on the use of T&R for the elimination of PRRSV have produced promising results (Dee and Molitor 1998, Dee and others 2000). Elimination of PRRSV by T&R consists of blood-testing the entire breeding herd in a single day, identifying PRRSV-infected animals using both an antibody and a virus test, and immediately removing positive animals from the farm. Data from these studies indicate that removed animals tend to cluster or exist as singletons throughout the gestating population (Figure 1). ELISA s/p ratios of these clusters appear to be normally distributed (Figure 2).

Limitations of T&R have been well documented, and include a high degree of labor involved in a whole herd test, and diagnostic costs that approach $10.00 US/tested sow (Dee and Molitor 1998, Dee and others 2000). Furthermore, a low seroprevalence (< 15 %) of ELISA positive sows is required to reduce the impact of animal removal of the productivity and profitability of the farm. A modification of T&R that has been successfully applied for the elimination of ADV is the protocol of Wean and Removal (W&R). This procedure consists of the testing of weaned sows using an ELISA test for the detection of anti-ADV antibodies. Seropositive animals are removed on a weekly basis until the entire breeding herd has been tested. This method requires less labor; reduced diagnostic cost, and does not negatively impact the productivity of the farm through the removal of pregnant sows. Recently, an observational pilot study was conducted to evaluate the ability of T&R and W&R to eliminate PRRSV from an expanded number of commercial farms.

The study was conducted from October 1998 to May 2000. Ten farms fulfilled all criteria and were selected for inclusion in the study. Five farms (1-5) were placed in the T&R group, while farms 6-10 were placed in the W&R group. All farms were located in the Midwestern US. 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: TEST AND REMOVAL GROUP

According to the definition of a PRRSV-negative farm, 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. 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 negative sows. 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 by Fisher's exact test. Negative control farms remained seronegative throughout the study.

DIAGNOSTIC DATA: WEAN AND REMOVAL GROUP

Consecutive PRRSV seroprevalence levels of ≥ 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) by 2 sample t-test 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 of one positive control farm increased from 15 to 100%, while the seroprevalence of the second control farm 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 by Fisher's exact test. Negative control farms remained seronegative throughout the study.

DISCUSSION

PRRSV was eliminated from all farms in the T&R study group. Twelve consecutive months of seronegative results were obtained from the breeding herds of all 5 farms that used T&R. Similar results were not obtained from the farms using the W&R protocol. 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. Furthermore, the difference in the mean breeding herd seroprevalence of the T&R group (10 %) versus the W&R group (16 %) at the beginning of the study was found to be significant at a p value of .0075.

2. The protocol required $\geq$ 6 months before the entire breeding herd was tested. It has been documented that PRRSV persistence occurs in breeding swine (Bierk and others 2000), therefore, it was speculated that this protocol of testing allowed these animals to remain in the breeding herd for an extended period.

3. In certain cases, ELISA positive animals were not removed as directed. Reasons for improper removal were the high genetic value of specific animals, production issues, the loss of ear tag identification, and the lack of compliance by certain members of the farm staff.

4. Recording errors at the time of testing, such as the incorrect labeling of serum tubes, or improper reading of animal identification, resulted in improper animal retention or removal.

5. The use of replacement gilts that had been previously infected with PRRSV at 10-12 weeks of age in 2 of the farms in this group may have led to the development of persistently infected gilts or boars. Besides the inherent risk involved in this procedure, it also required serially testing of seropositive replacements multiple times prior to entry into the breeding herd, thereby raising diagnostic costs to $6.00-7.00 US/animal tested in these farms.

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 ADV 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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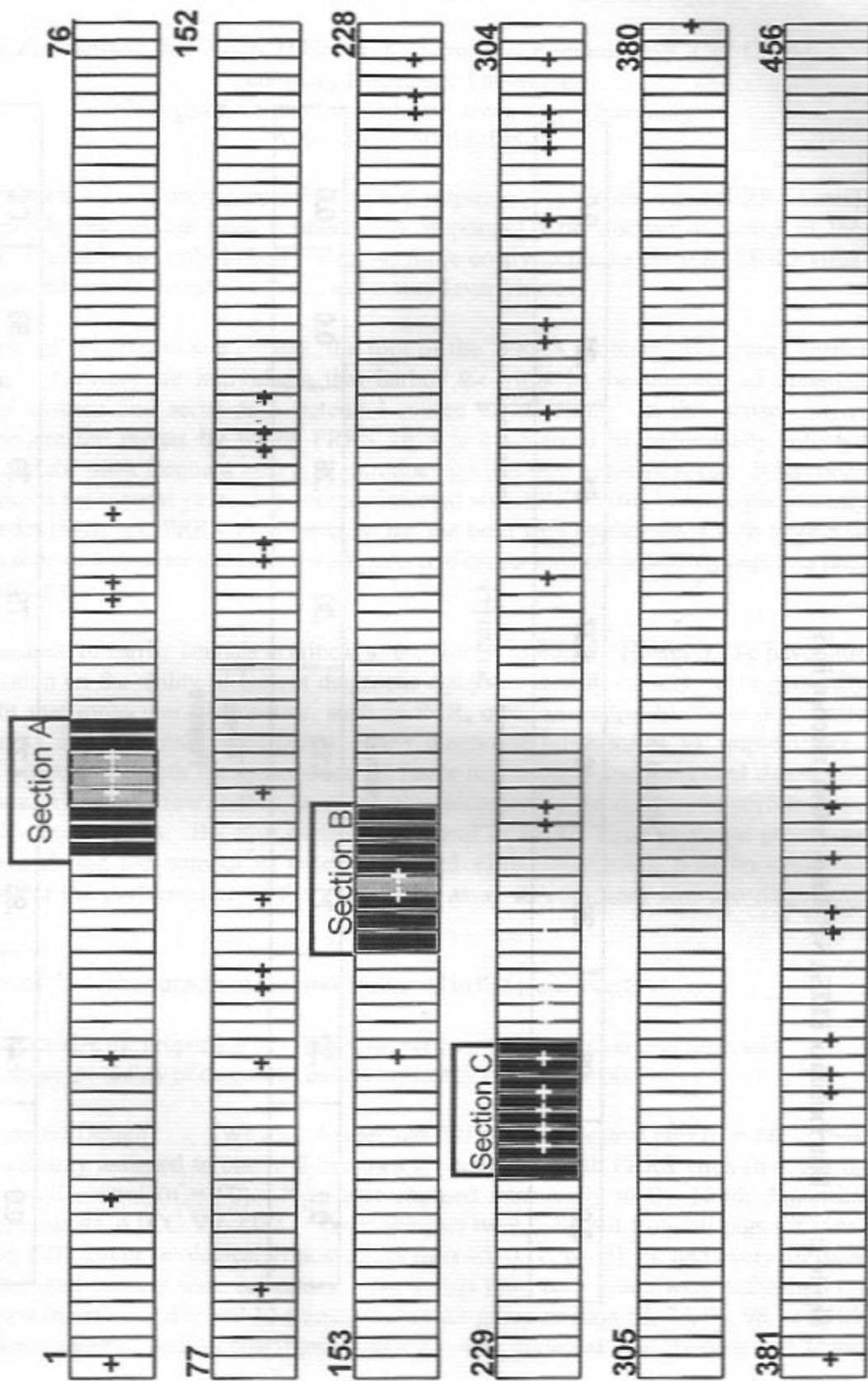## Table 1: Mean differences in the performance of 34 nurseries before and after nursery depopulation

Group	#farms	AGD (kg) before	after	%mortality before	after	feed: gain before	after	medication$ before	after
1	16	0.25	0.38*	9.7	2.3*	1.83	1.78	$1.75	$.96
2	7	0.25	0.34*	14.4	2.0*	2.00	1.85	$2.71	$1.33
3	5	0.29	0.37*	7.0	1.7*	2.06	1.85	$2.36	$1.57
4	6	0.26	0.41*	10.9	1.2*	1.95	1.70	$1.88	$1.06
TOTAL	34	0.26	0.38*	10.2	1.9*	1.91	1.77	$1.88	$1.06

* = significant (p<.0001) by ANOVA

Table 2: Relationship between diagnostic results and animal status

ELISA	PCR	interpretation	decision
+	+	viremic	remove
+	-	exposed/infected?	remove
-	+	viremic	remove
-	-	non-infected	retain

Figure 1: Location of Foci of Infection in Gestation Building

Figure 2: Distribution of ELISA S/P Ratios within Infection Foci

0.0	.08	.26	.49	1.37	.51	.21	0.0

Serum (+)

Section A

0.0	.32	.71	1.15	.30	.26	0.0	0.0

Tissue Positive

Section B

0.0	.41	.95	1.23	.75	.39	.58	0.0

Section C