Transmission of porcine reproductive and respiratory syndrome virus from persistently infected sows to contact controls

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Abstract

The objective of this study was to determine if porcine reproductive and respiratory syndrome virus (PRRSV) could persist in non-pregnant sows and if persistently infected sows could transmit virus to naive contact controls. Twelve PRRSV-naive, non-pregnant sows (index sows) were infected with a field isolate of PRRSV and housed in individual isolation rooms for 42 to 56 days postinfection. Following this period, 1 naive contact sow was placed in each room divided by a gate allowing nose-to-nose contact with a single index sow. Index sows were not viremic at the time of contact sow entry. Virus nucleic acid was detected by polymerase chain reaction, and infectious virus was detected by virus isolation in sera from 3 of the 12 contact sows at 49, 56, and 86 days postinfection. All 3 infected contacts developed PRRSV antibodies. Virus nucleic acid was detected in tissues of all of the 12 index sows at 72 or 86 days postinfection. Nucleic acid sequencing indicated that representative samples from index and infected contacts were homologous (> 99%) to the PRRSV used to infect index sows at the onset of the study. This study demonstrates that PRRSV can persist in sows and that persistently infected sows can transmit virus to naive contact animals.

Résumé

Cette étude fut entreprise afin de déterminer si le virus du syndrome reproducteur et respiratoire du porc (VSRRP) pouvait persister chez des truies non-gestantes et si des truies infectées de manière persistante pouvaient transmettre le virus à des truies immunologiquement naïves. Pour ce faire, 12 truies non-gestantes et immunologiquement naïves envers le VSPRR (truies index) furent infectées avec un isolat de champ du VSPRR et gardées dans des pièces fermées durant 42 à 56 jours post-infection (PI). Suite à cette période, 1 truie contact naïve fut placée dans chacune des pièces séparées par une barrière permettant ainsi un contact nez à nez avec une seule truie index. Les truies index n'étaient pas virémiques au moment de l'entrée des truies contacts. De l'acide nucléique du VSPRR fut détecté par réaction d'amplification en chaîne par la polymérase, et du virus infectieux détecté par isolement viral à partir du sérum de 3 des 12 truies contacts aux jours 49, 56 et 86 PI. Ces trois truies contacts développèrent des anticorps anti-VSRRP. De l'acide nucléique du VSRRP fut détecté dans les tissus des 12 truies contacts aux jours 72 ou 86 PI. Le séquençage de l'acide nucléique d'échantillons représentatifs provenant des truies index et des truies contacts démontra une homologie (> 99%) au virus SRRP utilisé pour infecter les truies index. Cette étude démontre que le VSRRP peut persister chez des truies et que des truies infectées de manière persistante peuvent transmettre par contact le virus à des animaux naïfs.

(Traduit par Docteur Serge Messier)

Introduction

Persistent infection is defined as the presence of a pathogen in a host following cessation of the acute symptomatic phase of infection (1,2). One form of persistence is latency, where the viral genome is present in the host, but infectious virus is not continuously produced (1,2). Following perturbations of the host cell environment, such as injury or cellular differentiation, reactivation of latent virus and production of infectious virus can occur. An example of a viral group capable of producing latent infections is in the family *Herpesviridae*. Another form of persistence is chronic (productive) infection or "smoldering infection," and is typical of the family *Arteriviridae* (3–5). Chronic (productive) infections replicate continuously and infect low numbers of permissive cells over time. Following cessation of the viremic period, infectious virus can persist in the host and is detectable by conventional diagnostic methods (1–3).

Porcine reproductive and respiratory syndrome virus (PRRSV) is a member of the order *Nidovirales*, family *Arteriviridae*, and genus *Arterivirus*, along with lactate dehydrogenase elevating virus, equine arteritis virus, and simian hemorrhagic fever virus (4). Characteristics of this group include replication in macrophages, a prolonged viremia in the face of circulating antibodies, and persistent infection (5). Clinical signs of acute PRRSV infection in pregnant sows include fever (> 40°C), anorexia, lethargy, and third trimester abortion, along with elevated occurrence of stillbirths, mummies, and preweaning mortality (6,7). By contrast, PRRSV infection in

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non-pregnant sows is characterized by viremia, transient fever (> 40°C), mild anorexia, and lethargy (6,7).

The pathogenesis of PRRSV infection has been described (8). Following inhalation, ingestion, or coitus, exposure to PRRSV results in the infection of mucosal, pulmonary, or regional macrophages. Infected macrophages and free virus are distributed to regional lymph nodes where viral replication ensues. Some virus may escape lymph node processing and replicate in monocytes or other macrophage populations throughout the body. The virus persists in macrophages, resulting in further distribution throughout the body, emigration to mucosal surfaces, contamination of body fluids, and subsequent transmission to naive swine. Infection by PRRSV induces a prolonged viremia post infection; however, the virus can persist in sites throughout the body for extended periods in the absence of detectable viremia (3,9–12). Infectious PRRSV has been isolated from tonsil scrapings from experimentally infected nursery pigs for up to 157 d postinfection (9). Virus nucleic acid has been detected in tonsil biopsies using polymerase chain reaction (PCR) for up to 225 d postinfection in 4-week-old pigs (10). In situ hybridization and PCR techniques have detected PRRSV in lymphoid tissues, lung, and brain from 7 to 57 d postinfection, and in the semen of adult boars for up to 92 d postinfection (11,12).

Routes of PRRSV excretion from persistently infected swine include serum, semen, milk, colostrum, urine, feces, and saliva (12–17). Following experimental infection, PRRSV has been detected in serum (up to 210 d postinfection) (12), semen (up to 92 d postinfection) (13), saliva (up to 42 d postinfection) (14), feces (up to 38 d postinfection) (15), urine (up to 28 d postinfection) (16), and mammary secretions (up to 9 d postinfection) (17). Prolonged shedding of persistent PRRSV from congenitally infected pigs has been described (12,18). Offspring originating from sows infected with PRRSV at 85 to 90 d of gestation maintained a detectable viremia for 210 d postinfection and shed virus to naive contacts for up to 112 d postinfection (12). In another study, naive contact controls seroconverted following exposure to 22-week-old pigs originating from sows infected at 90 d of gestation (18). Transmission of PRRSV between persistently infected nursery pigs and age-matched sentinels has also been reported for up to 60 to 67 d following experimental inoculation of weaned pigs by the intranasal (IN) route (19).

Factors that enhance renewed replication and shedding of PRRSV from persistently infected pigs are not clear at this time. Attempts to initiate shedding of PRRSV from experimentally infected pigs following the administration of corticosteroids produced contradictory results (18,20). One potential compound that has not been thoroughly studied at this time is lipopolysaccharide (LPS). Lipopolysaccharide is a potent macrophage activator and is ubiquitous in swine environments. A major component of the cell wall of gram-negative bacteria, LPS is present in fecal material and in dust particles from swine facilities (21–24). In an unpublished study, pigs experimentally infected with PRRSV demonstrated a detectable viremia 24 to 48 h after administration of an intramuscular (IM) dose (5 μg/kg) of LPS 8 wk postinfection (Molitor TW, personal communication). These pigs were not viremic at the time of LPS administration.

Despite a great deal of information describing PRRSV persistence in weaned pigs and boars, little published data regarding PRRSV persistence in sows exists at this time. Experimentally infected sows have been reported to transmit PRRSV to naive sentinels for up to 99 d postinfection (25). However, it was never proven that the PRRSV used to infect the index sows and the strain of PRRSV recovered from the contacts was homologous. A diagnostic investigation of a chronically infected field population indicated that 1 of 60 breeding swine harbored infectious and virulent PRRSV; however, the dates the sampled animals were actually infected was not known at the time of necropsy (26). Finally, the co-existence of seropositive and seronegative sows in 10 chronically infected farms has been described (27). This report also documented seroconversion of selected sows to PRRSV over a 6-month testing period (27). While these reports suggest that persistently infected sows may be a source of PRRSV to naive penmates in endemically infected herds, more information is needed. Therefore, the purpose of this experiment was to develop a model to test if PRRSV persistence occurs in non-pregnant sows, and to determine if persistently infected sows could shed PRRSV to naive contact controls.

Materials and methods

Definition of PRRSV persistence in non-pregnant sows

For the purpose of this study, a persistently PRRSV-infected, non-pregnant sow was defined as an animal that had progressed beyond the acute phase of the PRRSV infection yet still harbored detectable virus (1,2). Clinical and diagnostic parameters used to define the acute phase of PRRSV infection included viremia, anorexia, fever (> 40°C), and lethargy (6,7). A non-pregnant sow was considered persistently infected if all 4 parameters were negative at the end of a specified testing period following experimental infection, but in which PRRSV was still detectable at necropsy (3,9–12).

Experimental design

A total of 3 replicates were conducted to fulfil the required level of power (0.80) at a 95% level of significance. Each replicate consisted of an 86-day period and required a total of 10 animals (4 experimentally infected (index) sows, 4 contact control sows, and 2 negative control sows), for a total of 30 sows were used during the entire study. Non-pregnant sows were purchased at weaning from a commercial farm known to be PRRSV-negative. The status of the farm was documented by the absence of PRRS-related clinical signs, along with negative PRRSV serology (IDEXX Herd Check ELISA; IDEXX Laboratories, Westbrook, Maine, USA) collected monthly from all stages of production over a 3-year period. The ELISA test defines a negative animal as one that has a sample to positive ratio (s/p ratio) of < 0.4 (29). Prior to initiating each replicate, 5 sows were delivered to the College of Veterinary Medicine of the University of Minnesota isolation facility and housed in individual rooms. Sows and their respective rooms were numbered 1, 2, 3, 4, or 5. Sows 1 through 4 were designated as index animals, while sow 5 served as the negative control. Two days following their arrival to the isolation facility, index sows 1 to 4 were inoculated IN with 5 mL ($10^{2.4}$ $TCID_{50}$ total dose) of a field isolate of PRRSV (26). The negative control sow was not inoculated. All sows were observed daily and tested repeatedly to document the progression of the infection.

Table I. Summary of PCR results from tissues of index and infected contact sows

Tissue	MLN	LRLN	TBLN	SLN	MILN	SILN	TNSL	LNG	LLVG	SG
Replicate 1										
Index-1	+	+	−	+	−	+	−	−	−	−
Index-2	+	−	−	+	+	−	+	−	−	−
Index-3	−	+	+	+	−	−	−	+	−	−
Index-4	−	−	−	+	+	−	−	−	−	−
Contact	+	+	+	+	+	+	+	+	−	−
Replicate 2										
Index-1	−	+	−	+	+	+	+	−	−	+
Index-2	−	−	−	−	+	−	−	−	−	−
Index-3	+	+	+	+	−	+	+	−	−	−
Index-4	−	−	+	−	+	+	−	+	+	−
Contact	+	+	+	+	+	+	−	+	+	+
Replicate 3										
Index-1	−	−	−	+	−	−	+	−	−	−
Index-2	−	−	−	+	+	−	+	−	−	−
Index-3	−	−	−	−	−	−	+	−	−	−
Index-4	−	−	−	−	−	−	+	−	−	−
Contact	+	+	+	+	+	+	+	+	+	−
Total	6/15	7/15	6/15	11/15	9/15	7/15	9/15	5/15	3/15	2/15

(+) — PCR positive sample; (−) — PCR negative sample; MLN — mandibular lymph node; LRLN — lateral retropharyngeal lymph node; TBLN — tracheobronchial lymph node; SLN — sternal lymph node; MILN — medial iliac lymph node; SILN — superficial inguinal lymph node; TNSL — tonsil; LNG — lung; LLVG — lung lavage; SG — salivary gland

Forty-two days postinfection, 5 more sows from the same source were delivered to the isolation facility. The 42-day interval was based on published data demonstrating PRRSV isolation from saliva and semen in the absence of a detectable viremia from experimentally infected pigs and boars 21 to 42 d postinfection (14,28). Four of the new sows (1 to 4) were designated as contact controls, and the final sow as a negative control. One contact control sow was allocated to each index room (rooms 1 to 4), while the negative control sow was housed in a separate room (room 5). Each room was divided in half using a metal gate with vertical rods, permitting nose-to-nose contact between the index and the contact sows, but not movement between pens (Figure 1).

On day 42 postinfection, index sows 1 and 2 received 5 μg/kg *Escherichia coli* LPS via the IM route, index sows 3 and 4 received a placebo (sterile cell culture media), and the 2 negative control sows in room 5 remained uninoculated. Index and contacts were housed together for 30 d and tested at regular intervals (24). At the end of this period, all 4 index sows were necropsied. Contact control sows were necropsied 14 d later to ensure that if shedding occurred late in the 30-day period, the contacts would have sufficient time to develop PRRSV-antibodies prior to necropsy. Negative control sows were not necropsied; rather their PRRSV status was based on the results of ELISA testing of sera collected throughout the replicate.

Sampling methods and diagnostic testing

Upon arrival to the isolation facility, all sows were tested to ensure a PRRSV-negative status. Sera were tested for PRRSV-nucleic acid by PCR, for infectious virus by VI and for PRRSV antibodies by ELISA (29–31). Specifically, the Taqman (Perkin-Elmer Applied Biosystems, Foster City, California, USA) PRRSV PCR assay was used throughout the entire study. Sera were collected from index animals and tested by PCR, VI, and ELISA on days 1, 3, 5, 7, 10, 14, 21, 28, 35, and 42 postinfection to document successful infection and to assess the duration of detectable PRRSV viremia. Animals were observed for signs of anorexia, lethargy, and fever (> 40°C rectal temperature); anorexia and lethargy observations were compared with those of the negative control sows. Appetite was measured as feed disappearance, and each animal was floor-fed 2.5 kg each day. Consistent personnel observed animals once daily, 5 d/wk, and a minimum time of 15 mins was spent in each room.

After introduction of the contact sows, all animals were bled on days 42, 45, 49, 52, 56, 63, and 72 postinfection. Sera were tested for PRRSV by PCR, VI, ELISA. Contact sows were sampled before index sows. Personnel did not set foot in the pens of index sow prior to testing contacts and a separate needle and syringe was used for each animal. Personnel wore hairnets, surgical face masks, and gloves while testing sows, and changed boots, coveralls, gloves, face masks, hairnets, and washed hands between rooms (32). Following removal of the index sows at the end of the 30-day period, contacts were sampled 2 more times at 7-day intervals (day 79 and 86 postinfection), and sera were tested by PCR, VI, and ELISA. Negative control sows were blood tested monthly and tested by ELISA during each replicate.

Sera, tissues, and alveolar macrophages (lung lavage) were collected at necropsy. Tissues collected were right and left sections

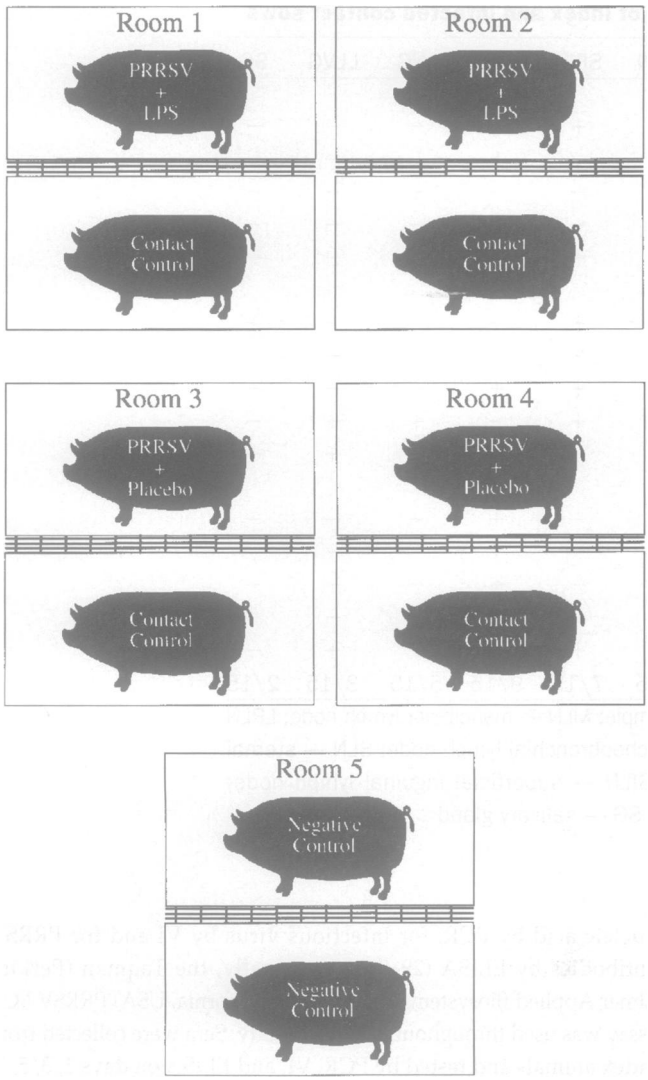

Figure 1. Experimental design: room designation and individual animal treatments

(pooled) of tonsil; apical, middle, and caudal (pooled) lung lobes; alveolar macrophages; parotid and sublingual (pooled) salivary glands; and mandibular, lateral retropharyngeal, sternal, medial iliac, tracheobronchial, and superficial inguinal lymph nodes. Tissues were tested by PCR, VI, and by immunohistochemistry (IHC) (33). Alveolar macrophages were tested for infectious virus by VI using porcine alveolar macrophages and MARC-145 cells (31). It was planned to select representative sera and tissues found to be positive by PCR or VI and nucleic acid sequence the open reading frame (ORF) 5 and a portion of ORF 6 region of the genome (34).

Results

All sows were PRRSV-negative upon arrival to the isolation facility, according to PCR, VI, and ELISA. Following infection, fever (40.2 to 41.9°C), anorexia, and lethargy were observed in all index sows for 24 h. Viremia was detected by PCR and VI in all index sows on days 3, 5, 7, 10, and 14 postinfection. Further evidence of viremia was not detected during the remainder of the 42-day testing period, and all index sows demonstrated ELISA s/p ratios > 0.4 on day 14 postinfection. Following administration of LPS, sows were febrile (41.5 to 41.9°C), anorexic and lethargic for 24 h. Index animals administered a placebo were clinically normal. During replicate 3, a prolonged period of cold weather prohibited the transport of contacts from the source farm to the isolation facility at the regularly scheduled time. Therefore, replicate 3 contacts were introduced on day 56 postinfection. Prior to arrival of the delayed contacts, the index sows were re-tested on days 42, 49, and 56 postinfection by PCR and VI, and were not viremic at the time of contact sow entry. Replicate 3 index and contact animals were subsequently blood tested on days 56, 59, 63, 66, 70, 77, and 86 postinfection. Following removal of the index sows, replicate 3 contacts were tested on days 93 and 100 postinfection.

Three episodes of PRRSV transmission were detected, and 1 episode occurred during each replicate. Transmission was detected in 2 index sows that had received LPS (replicates 2 and 3); however, shedding was not detected until 14 and 30 d following the administration of LPS. Virus was detected by PCR and infectious virus from VI was isolated from the serum of the contact controls on day 49 postinfection (replicate 1), day 56 postinfection (replicate 2), and day 86 postinfection (replicate 3). The ORF 5 and 6 regions of 1 PRRSV isolate from each infected contact was nucleic acid sequenced and found to be 99.9% homologous to the PRRSV isolate administered to the index sows. Virus nucleic acid or infectious virus was not detected in serum samples from the index sow during the shedding period during any of the 3 shedding episodes, despite extensive testing. During 2 of the 3 episodes of transmission, aggressive behavior was observed between the index sow and the contact control. This pattern of behavior consisted of growling, charging the gate, and attempting to bite each other, and head pressing against the gate in an effort to gain entry to the adjacent pen. All sera collected from the other index, contacts, and negative control sows were negative by PCR, VI, and ELISA. Rectal temperatures of negative controls ranged from 38.0 to 38.8°C throughout the replicate, and anorexia or lethargy was not observed.

Virus nucleic acid was detected by PCR in multiple tissues collected from the 12 index sows and the 3 infected contacts (Table I). Virus was isolated from alveolar macrophages collected from index sow 4 (replicate 1); alveolar macrophages, tracheobronchial lymph node, and serum from the infected contact (replicate 2); and alveolar macrophages from the infected contact in replicate 3. All tissues from index and infected contact sows were IHC negative, except for the medial iliac lymph node of the infected contact from replicate 3.

Microscopically, germinal centers with blast-lymphocytes were observed in the sternal lymph node of index sows 1 and 2; the superficial inguinal lymph node of index sow 3; and the medial iliac, tracheobronchial, and sternal lymph nodes from the infected contact (replicate 1). Similar lesions were observed in the tracheobronchial, sternal, medial iliac, and lateral retropharyngeal lymph node from index sow 2 and the infected contact (replicate 2). Finally, the sternal lymph node of index sow 1 (replicate 3) was characterized by germinal centers with blast-lymphocytes. Cystic degeneration and a single polykaryocyte, and germinal centers with blast-lymphocytes were detected in the lateral retropharyngeal

lymph node of the infected contact from replicate 3. There were no gross lesions observed in any tissues from all index or infected contacts. All sera from all negative control sows tested throughout the study were negative by ELISA.

Discussion

The objective of this study was to establish a model to determine if PRRSV could persist in non-pregnant sows and if persistently infected sows could transmit virus to contact controls. We defined PRRSV persistence as the maintenance and replication of the virus in the host beyond the acute symptomatic phase of infection (1,2). This definition was based on published information on viral persistence and documented clinical and diagnostic parameters of PRRSV infection in the non-pregnant sow (1,2,6,7). Following experimental infection of PRRSV-naive sows, we used these parameters to ensure that index animals had progressed beyond the acute phase of infection and to classify them as persistently infected. Finally, we used diagnostic testing of selected tissues at necropsy to determine if this classification was accurate.

The virus was detected in non-pregnant index sows for up to 86 days postinfection, and a subset of the sows shed virus were capable of infecting naive contacts. PRRSV was detected by multiple methods in infected contacts. This virus proved to be homologous to the strain of PRRSV used to infect the index animals. These results are important to the swine industry because they verify that PRRSV can persist in sows and persistently infected sows can transmit PRRSV over extended periods. It was also interesting to note that during all 3 shedding periods, PRRSV was not detected in sera from the index sows. Potential explanations for this observation may have been the schedule of sampling, or the possibility that virus was transmitted through other routes such as saliva, in the absence of detectable viremia (14).

Regarding factors that initiate shedding, this study demonstrated that aggressive behavior may play a role in certain cases. Therefore, the weaned sow area in the breeding facility may be a potential site to house PRRSV-naive sentinel animals when assessing if intervention strategies have influenced viral shedding. In commercial swine facilities, weaned sows are frequently group-housed in pens, resulting in maximum animal interaction, in contrast to individual gestation stalls. If seronegative sentinel boars were allowed nose-to-nose contact with pens of weaned sows on a daily basis, the detection of PRRSV shedding may be improved. The role of lipopolysaccharide in the initiation of shedding is not clear. Although 2 of the 3 index sows that shed virus did receive LPS, shedding was detected 14 and 30 d following administration, long after the observable clinical signs subsided.

The primary limitation of this study is that certain parameters used to define PRRSV persistence are subjective and can be difficult to measure. The clinical signs seen in non-pregnant sows infected with PRRSV may be very mild, of limited duration, and are not unique to PRRS. It was difficult to objectively measure lethargy, and we did not quantify feed disappearance in infected versus non-infected sows. Rectal temperatures were only recorded once daily. While results may have been due to the normal fluctuations in body temperature throughout the day, this measurement occurred at approximately the same time each day. Regarding the detection of viremia, while we attempted to sample frequently, it was impossible to sample sows every day. Therefore, we can only conclude that the sows were not viremic on the days we collected samples.

Our persistence model is based on published clinical and diagnostic parameters of PRRSV infection in non-pregnant sows (6,7). In support of the diagnostic parameters, the duration of viremia in the index sows was consistent across all replicates and homologous PRRSV nucleic acid and infectious virus was recovered from the sera of infected contacts at various times during contact with index sows. Furthermore, homologous PRRSV was again detected in multiple tissues from all 12 of the index sows and the 3 infected contacts, and lesions of PRRSV infection were observed microscopically in multiple tissues collected from index and contact sows (35).

In support of the clinical parameters, index and contact sows were evaluated using consistent personnel, at a specific time of day, and the use of negative controls provided comparative assessments of rectal temperatures, anorexia, and lethargy. The fact that animals were housed individually and floor-fed a limited amount of feed each day enhanced our ability to detect any change in appetite or attitude.

In conclusion, to further the knowledge relating to PRRSV persistence in the breeding herd, future studies should focus on evaluating the duration of PRRSV persistence in larger groups of breeding age females over longer periods of time to understand what occurs under conditions representative of today's commercial swine industry. Studies of this nature would be especially helpful to determine the length of time breeding herds need to be closed to the introduction of replacement stock to reduce the risk of carrier animals existing in farms that are attempting PRRS eradication.

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