Risk factors associated with prolonged infection of porcine reproductive and respiratory syndrome virus determined by whole-herd sampling methods

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Abstract

Two naive sow herds became infected with porcine reproductive and respiratory syndrome virus (PRRSV) and were exposed by live virus inoculation (LVI) on week 0. Serum and tonsil scrapings were collected on week 20 post LVI and tested for PRRSV by reverse-transcriptase polymerase chain reaction (RT-PCR). For either sample type, non-negative animals were considered infected. Prolonged infection rate was 3.56% in herd 1 and 1.76% in herd 2. Age and breed were significant factors of prolonged infection. Twenty of thirty-five infected animals removed from herd 2 and resampled on week 27 were still PRRSV positive using RT-PCR on tonsil scrapings.

Keywords: swine, porcine reproductive and respiratory syndrome virus, prolonged infection, test and removal, tonsil

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Resumen - Factores de riesgo asociados a la infección prolongada por el virus del síndrome reproductivo y respiratorio porcino determinados por métodos de muestreo de toda la piara

Dos piaras con cerdas libres al virus del síndrome reproductivo y respiratorio porcino (PRRSV) se infectaron, y fueron expuestas por inoculación al virus vivo (LVI) en la semana 0. Se tomaron muestras de suero y raspados de amígdalas en la semana 20 después de la LVI y se analizaron para el PRRSV mediante la reacción en cadena de la polimerasa con transcriptasa inversa (RT-PCR). Para cualquiera de los dos tipos de muestra, los animales no negativos se consideraron infectados. La tasa de infección prolongada fue del 3.56% en la piara 1 y del 1.76% en la piara 2. La edad y la raza fueron factores significativos de la infección prolongada. Veinte de los treinta y cinco animales infectados se retiraron de la piara 2 y se volvieron a muestrear en la semana 27, estos seguían siendo positivos al PRRSV mediante RT-PCR en raspados de amígdalas.

Résumé - Facteurs de risque associés à une infection prolongée par le virus du syndrome reproducteur et respiratoire porcin déterminés par des méthodes d'échantillonnage du troupeau entier

Deux troupeaux de truies naïves sont devenus infectés par le virus du syndrome reproducteur et respiratoire porcin (VSRRP) et ont été exposés par inoculation de virus vivants (IVV) à la semaine 0. Du sérum et des grattages des amygdales ont été prélevés à la semaine 20 post-IVV et testés pour le VSRRP par réaction d'amplification en chaîne par la polymérase avec la transcriptase réverse (RT-PCR). Pour chaque type d'échantillon, les animaux non-négatifs étaient considérés comme infectés. Les taux d'infection prolongée étaient de 3.56% pour le troupeau 1 et de 1.76% pour le troupeau 2. L'âge et la race étaient des facteurs significatifs d'infection prolongée. Vingt des trente-cinq animaux infectés retirés du troupeau 2 et rééchantillonnés lors de la semaine 27 étaient toujours positifs pour le VSRRP par RT-PCR pour les grattages d'amygdales.

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Infection with porcine reproductive and respiratory syndrome virus (PRRSV) can be devastating to sow herd productivity. In most cases, maintaining productivity relies on eradicating virus circulation within a population. Until the PRRSV is eliminated, many normal production practices are altered, including the inability to introduce replacement females to the herd. Recent PRRSV strains[1] have made PRRSV control and eradication even more difficult. Reducing the time between infection and eradication can positively impact parity structure and maintaining a desired sow inventory by allowing re-introduction of naive replacement females.

The presence of prolonged PRRSV infection in animals may be a factor in herd closure length. Prolonged infection occurs when the immune system is unable to clear the infection and viral replication continues to occur.[2] When testing serum, viremia is typically detected only for several weeks post infection. However, a small portion of the herd may continue to harbor virus in the tonsil and lymph nodes.[3] The PRRSV has been isolated from tonsils of pigs up to 157 days post infection (dpi) and PRRSV RNA detected by reverse transcriptase-polymerase chain reaction (RT-PCR) in tonsils up to 251 dpi.[4,5] Age at time of infection may be a factor in duration of viral shedding. Prolonged PRRSV infection of animals is likely a key contributor to longer periods between initial infection and eradication of the virus from breeding herds.

Testing and removal of infected animals is a strategy that has been used successfully for viral diseases to eliminate the pathogen from the herd. Success has been demonstrated with serum testing in several cases of chronically infected PRRSV herds, but only in the later stages of infection.[6,7] Ideally, animals at risk of prolonged PRRSV infection could be identified earlier in the infection process. These infected animals are most likely characterized by the lack of virus circulation in blood, but continued presence in the tonsil and other lymphoid tissue. The location of the virus in the animal at this stage makes it difficult to develop a sampling plan to identify the infected animals. A tonsil scrape resulting in collection of tonsillar exudate has been shown to be a sensitive method for PRRSV RNA detection.[3,8] Studies have demonstrated that virus isolated from tonsils of persistently infected animals can be infectious, but repeatability of infectivity of virus isolated from the tonsil is not guaranteed in all cases.[8-10] There is no existing account that removal of tonsil-PRRSV-positive animals contributes to gaining stable herds status and reduction of virus circulation in the population. Identification of positive animals through tonsil sampling can be laborious and impractical in a production setting, which may contribute to the lack of data on test and removal of these animals.

This work contributes to identifying risk factors associated with prolonged PRRSV infection in a breeding herd undergoing herd closure. Additionally, this study demonstrates a sampling method and test-and-removal process that appears to have resulted in shortened herd closure times.

Case description

This case study evaluates the demographics of PRRSV persistence in two previously naive breeding herds (herd 1 and herd 2) and the subsequent effect of culling infected animals on herd load, close, and expose (LCE) success. Both herds experienced a natural PRRSV infection and subsequently underwent PRRSV eliminations. The elimination protocol involved introducing replacement females (load), subsequent herd closure to new animal entry (close), and live virus inoculation (LVI; expose). Date of the first LVI was defined as week 0 (day 0) for both herds. At approximately 20 weeks (day 140) following LVI, tonsil scrapings and serum were collected on the mature populations of both herds. Herd infection, LCE, and sampling occurred between February 23, 2022 and December 28, 2022.

A follow-up investigation was done to examine the status of PRRSV-infected animals removed from the original population and subjected to transport and mixing stress events. Animals in herd 2 identified by RT-PCR to be PRRSV positive at week 20 (day 142) were moved to an off-site isolation facility (herd 2 removed animal sampling) at week 25 (day 171), mixed in pens, and resampled at week 27 (day 191).

Herd 1

This herd was a 600-sow, farrow-to-finish, commercial farm with Filial 1 (F1) hybrid Landrace x Yorkshire females. Sows were individually housed, batch farrowed, and piglets were weaned to the on-site nursery and finisher every 4 weeks. Replacement gilts were raised off site. Routine weekly PRRSV testing by RT-PCR of processing fluids from all litters (up to 30 litters pooled per tube), oral fluids from nursery and finishing pigs, and weaned pig serum (if weaned pigs present) verified naive PRRSV status prior to the break. The timeline for herd 1 is shown in Figure 1.

A PRRSV infection was detected by RT-PCR, and restriction fragment length polymorphism (RFLP) 1-12-4 Lineage 1H was confirmed with open reading frame (ORF) 5 sequencing. Replacement gilts were placed into the site prior to closure. Serum was collected from suckling pigs, and 10 mL of pooled RT-PCR PRRSV-positive serum with cycle threshold (Ct) values ranging from 12.7 to 23.7 was diluted into 1000 mL phosphate buffered saline to create the inoculum. Two milliliters of the inoculum was administered intramuscularly to all sows, boars, replacement gilts, and on-site nursery and finishing pigs 16 days after the site was confirmed PRRSV positive. A second LVI was administered 28 days following the first injection using the same lot and dosage as the first LVI. Pigs were weaned off-site from week 0 until after declaration of positive stable herd status. During the closure, routine PRRSV RT-PCR testing was performed on processing fluids and family oral fluids as the batch allowed. Positive stable herd status was defined by 12 consecutive weeks (90 days) of negative weekly testing.

All females were sampled at week 20 (day 140) post LVI. Animals were restrained with a snare for sample collection. Blood was collected in serum separator tubes via standard jugular venipuncture technique. A separate needle and syringe were used for each sow. Blood was centrifuged at 3200 rpm for 10 minutes and serum was removed and stored at -80°C until testing. Tonsil scrape samples were obtained by visualizing the palatine tonsil using a speculum, scraping a long-handled spoon across the surface to collect exudate, and using a culture swab to collect and store the exudate sample. Between each sow the spoons were rinsed with water and dried. Samples were stored at -80°C from the time of collection until testing. Samples were submitted to Iowa State University Veterinary Diagnostic Laboratory (ISU VDL), and PRRSV RT-PCR was performed using their standard protocols on both the sera and the tonsil swabs. Both sample types were pooled by 5 and Ct values reported out to 40 cycles. Any non-negative pools

were split for individual polymerase chain reaction (PCR). Animals with a non-negative PRRSV RT-PCR result on either sample type were considered positive and removed from the farm on week 34 (day 236).

Herd 2

This herd was a 3000 purebred-sow, farrow-to-wean farm. Duroc, Landrace, and Meishan breeds were represented. Animals were housed in a combination of stalls and pens and were on a weekly farrowing schedule. It was a closed herd with internal multiplication flowing through detached nearby nursery and finisher farms. Routine weekly PRRSV testing by RT-PCR of all processing fluids (pooled by 30 litters), oral fluids on nursery and finishing pigs, and weaned pig serum verified naive PRRSV status prior to the break. The timeline for herd 2 is shown in Figure 2.

A PRRSV infection was detected by RT-PCR, and RFLP 1-4-4 Lineage 1C variant[1] was confirmed with ORF 5 sequencing. The LVI material was prepared and administered with the same methods as described for herd 1. All sows, boars, and replacement animals at the sow farm, nursery, and finisher were inoculated 29 days after initial infection was detected. A second LVI was administered 3 weeks (day 21) following the first injection. For 18 weeks following the LVI, litters within the weekly farrowing group were aborted. At 19 weeks post LVI, farrowings recommenced and pigs were weaned to the continuous flow nursery and finishing farms. Routine weekly PRRSV RT-PCR testing was performed on processing fluids and serum from piglets at the sow farm and nursery oral fluids after weaning began. Positive stable herd status was defined by the producer as 12 consecutive weeks (90 days) of negative testing.

All sows, gilts, and boars were selected for sampling at week 20 (day 142) post LVI. Animal restraint and testing methods were identical to those described for herd 1. Samples were stored on ice and sent to the ISU VDL immediately after collection, using the same PRRSV RT-PCR testing methods as described for herd 1. Any animal with a non-negative PRRSV RT-PCR result on either sample type were considered positive and removed from the farm by week 25 (day 171).

Herd 2 removed animal sampling

Three animals identified as PRRSV positive in herd 2 were euthanized prior to week 25. The remaining PRRSV-positive animals and PRRSV-negative cull pigs from herd 2 were shipped to an off-site isolation facility (site 3) on week 25 (day 171). Animals were transported and subsequently mixed in pens to induce stress. On week 27 (day 191), 3 weeks after arrival, all animals (n = 52) were sampled using the serum and tonsil scrape collection methods previously described. Samples were tested immediately after collection. The ISU VDL performed PRRSV RT-PCR testing on individual serum and tonsil swab samples with Ct values reported out to 37 cycles.

Statistical analysis

Descriptive statistics were produced for each site to describe herd demographics and distributions by sampling result. A logistic regression model was used (PROC GLIMMIX, SAS V9.4) to evaluate individual animal factors that were associated with a PRRSV-positive result at 20 weeks post LVI. Fixed effects in the final model included animal breed (herd 2) and animal age at the time of initial LVI (herd 1 and herd 2). All interactions were tested and found to be non-significant. Results are reported in odds ratios (OR) with significance defined as $P \leq .05$. A chi-square test for independence was used (Statistix 10) to evaluate continued association of breed and age on prolonged PRRSV infection at week 27 (day 191) in herd 2.

Results

Any non-negative PRRSV RT-PCR result was considered positive for the purposes of this analysis. Any animal with a PRRSV-positive result on either serum or tonsil swab sample type was defined as infected.

Herd 1

Of the 646 RT-PCR-tested animals, 23 had PRRSV-positive tonsil scrape swabs. No serum samples were positive (Table 1). Mean Ct value on a positive sample was 35.1 (range: 28.9-38.5). Prolonged PRRSV infection rate was 3.56% for the population at week 20 (day 140; Table 2). There was a statistically significant association between age at the time of initial LVI and a PRRSV-positive result. For each week younger an animal was at the time of LVI, the odds of having a PRRSV-positive result increased (OR = 1.030; 95% CI, 1.012-1.049; $P < .001$). The number of animals with a PRRSV-non-negative result by RT-PCR at week 20 (day 140) post LVI was calculated into a proportion of infected animals by each 4-week age bracket (Figure 3). Time to positive stable herd status was 44 weeks post LVI.

Herd 2

For tonsil scrape swabs, 36 of 2049 animals tested positive for PRRSV. Only 2 of 2049 serum samples were PRRSV positive, and neither of these 2 animals had PRRSV-positive tonsil scrape swabs (Table 1). Mean Ct value of a PRRSV-positive tonsil scrape was 35.6 (range: 31.1-39.3), mean Ct value of a PRRSV-positive serum sample was 33.9 (range: 32.0-35.7). The rate of prolonged PRRSV infection was 1.85% for the population at week 20 (day 144; Table 2). There was a statistically significant association between age at the time of initial LVI and a PRRSV-positive result. For each week younger an animal was at the time of LVI, the odds of having a PRRSV-positive result increased (OR = 1.031; 95% CI, 1.009-1.053; $P = .02$). The number of animals with a PRRSV-non-negative result by RT-PCR at week 20 (day 140) post LVI was calculated into a proportion of infected animals by each 4-week age bracket (Figure 3).

Breed was also significant. Meishans had greater odds of a PRRSV-positive result than both Durocs (OR = 25.114; 95% CI, 10.094-62.484; $P < .001$) or Landrace (OR = 17.086; 95% CI, 7.547-38.684; $P < .001$). The OR between Landrace and Duroc was not different ($P = .44$). Time to positive stable herd status was 30 weeks post LVI.

Herd 2 removed animal sampling

A total of 52 animals were sampled at week 27 (day 191), 35 PRRSV-infected pigs and 17 PRRSV-negative culls from herd 2. Of the 35 PRRSV-infected animals, 20 still tested PRRSV positive by RT-PCR on tonsil scraping samples at week 27 (day191; Table 3). Mean Ct value of the PRRSV-positive samples was 33.7 (range: 26.5-38.4). A chi-square test of independence was performed on the PRRSV-infected animals at week 20 (day 142) to determine that a positive result at week 27 (day 191) was dependent on breed ($\chi^2_1 = 6.08$, $P = .01$). Table 4 shows the results of week 27 (day 191) testing by breed for the 35 PRRSV-infected animals sampled at site 3. Age at initial LVI was not a significant factor in whether a PRRSV-positive animal at week 20 (day 142) still tested positive at week 27 (day 191).

Figure 1: Herd 1 timeline of events during a natural porcine reproductive and respiratory syndrome virus (PRRSV) infection and subsequent elimination. Weekly site testing results and site status for herd 1 are charted by week relative to the day of live virus inoculation (LVI; day 0). For farm status, a green box indicates either a naive herd status (prior to the break) or a positive stable herd status (following the break) and a red box indicates a positive unstable herd status. For testing results, a green box designates weekly routine testing with no PRRSV-positive results and a red box designates weekly routine testing with one or more PRRSV-positive results. Weeks with NA designates no testing was performed during that week. Major study events are highlighted by week in the blue circles.

WEEKS RELATIVE TO STUDY DAY 0	-5	-4	-3	-2	-1	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
FARM STATUS	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
TESTING RESULTS	-	-	+	+	+	NA	NA	NA	NA	NA	NA	NA	+	NA	NA	NA	NA	NA	NA	+	NA	NA	NA	+	NA

WEEKS RELATIVE TO STUDY DAY 0	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44
FARM STATUS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-
TESTING RESULTS	NA	+	NA	-	NA	-	-	NA	-	+	+	+	+	-	-	NA	-	-	-	NA	-	-	-	-	-

Figure 2: Herd 2 timeline of events during a natural porcine reproductive and respiratory syndrome virus (PRRSV) infection and subsequent elimination. Weekly site testing results and site status for herd 2 are charted by week relative to the day of live virus inoculation (LVI; day 0). For farm status, a green box designates either a naive herd status (prior to the break) or a positive stable herd status (following the break) and a red box designates a positive unstable status. For testing results, a green box designates weekly routine testing with no PRRSV-positive results and a red box designates weekly routine testing with one or more PRRSV-positive results. Weeks with NA designates no testing was performed during that week. Major study events are highlighted by week in the blue circles.

WEEKS RELATIVE TO STUDY DAY 0	-6	-5	-4	-3	-2	-1	0	1	2	3	4	5	6	7	8	9	10	11	12	13
FARM STATUS	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
TESTING RESULTS	-	-	+	+	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA

WEEKS RELATIVE TO STUDY DAY 0	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34
FARM STATUS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-
TESTING RESULTS	NA	NA	NA	NA	NA	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Table 1: PRRSV RT-PCR results at week 20 (day 140) post live virus inoculation

Case	Breed	N	PRRSV-positive tonsil scraping*		PRRSV-positive serum*	
			No. (%)	Ct, mean (range)	No. (%)	Ct, mean (range)
Herd 1[†]	F1	646	23 (3.56)	35.1 (28.9-38.5)	0 (0.00)	NA[‡]
Herd 2[†]	Landrace	956	10 (1.05)	35.9 (32.0-39.3)	0 (0.00)	NA[‡]
	Duroc	973	6 (0.62)	34.6 (32.1-36.6)	1 (0.10)	32
	Meishan	119	20 (16.81)	35.7 (31.1-39.1)	1 (0.84)	35.7
	All	2049[§]	36 (1.76)	35.6 (31.1-39.3)	2 (0.10)	33.9 (32.0-35.7)

* Samples were tested using RT-PCR and Ct < 40 was considered PRRSV positive.

† Herd 1 was sampled 140 days and herd 2 was sampled 142 days post live virus inoculation.

‡ No samples were found to be PRRSV positive by RT-PCR.

§ One animal had no breed information available.

PRRSV = porcine reproductive and respiratory syndrome virus; RT-PCR = reverse transcriptase-polymerase chain reaction; Ct = cycle threshold; NA = not applicable.

Table 2: Distribution of PRRSV-infected animals at week 20 (day 140) by breed and age

Case	Breed	N	Infected animals*, No. (%)	Age at LVI, mean (range), wk	
				Negative	Infected
Herd 1[†]	F1	646	23 (3.56)	51.4 (5.6-121.6)	28.0 (6.3-77.7)
Herd 2[†]	Landrace	956	10 (1.05)	34.6 (5.7-107.0)	21.1 (9.3-53.0)
	Duroc	973	7 (0.72)	34.3 (6.9-140.1)	32.3 (7.0-103.9)
	Meishan	119	21 (17.65)	39.4 (7.1-175.1)	18.7 (7.1-72.0)
	All	2049[‡]	38 (1.85)	34.7 (5.7-175.1)	21.8 (7.0-103.9)

* Infected animals had a PRRSV-non-negative RT-PCR result on tonsil scraping or serum sample testing.

† Herd 1 was sampled at 140 days and herd 2 was sampled 142 days post LVI.

‡ One animal had no breed information available.

PRRSV = porcine reproductive and respiratory syndrome virus; LVI = live virus inoculation; RT-PCR = reverse transcriptase-polymerase chain reaction

Table 3: PRRSV RT-PCR results at week 27 (day 191) post live virus inoculation

Week 25 results, No.	No. sampled	Week 27 results, No.	
		Positive	Negative
Positive	35	20	15
Negative	17	1	16

PRRSV = porcine reproductive and respiratory syndrome virus; RT-PCR = reverse transcriptase-polymerase chain reaction.

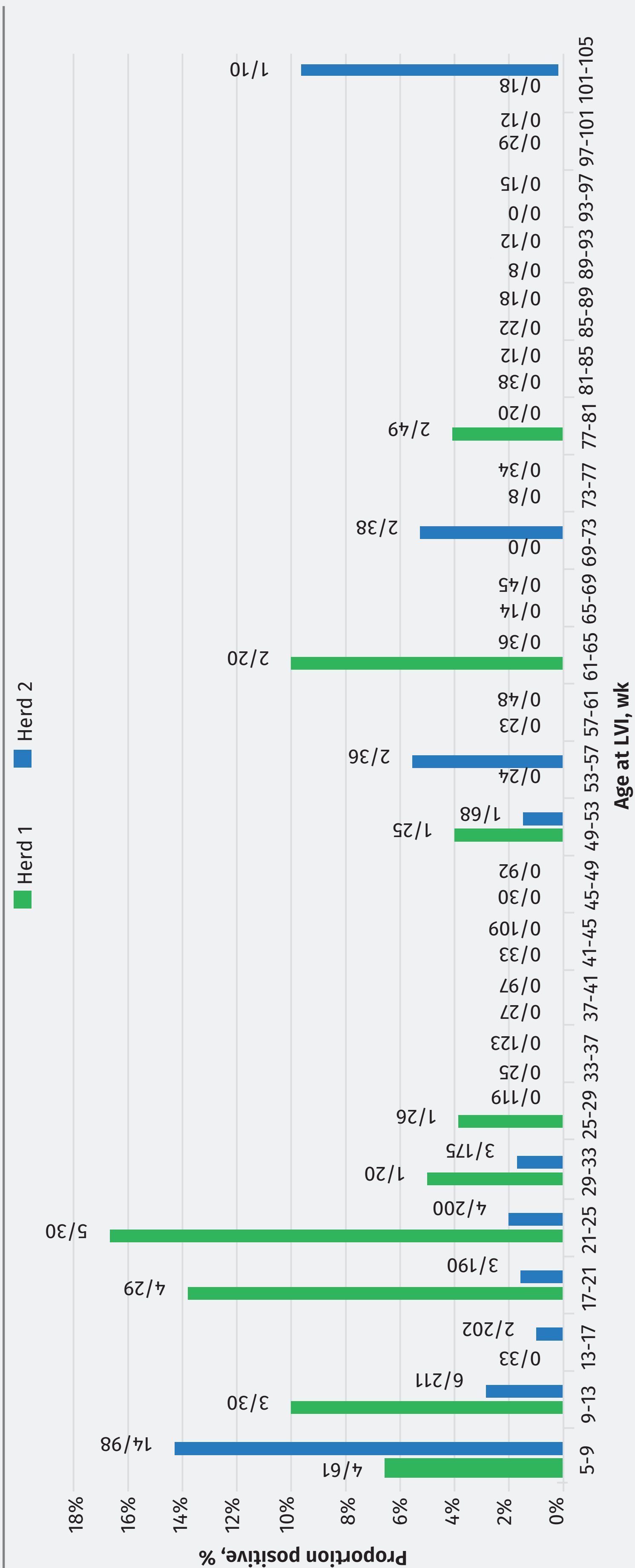

Figure 3: Proportion of PRRSV-infected animals at week 20 (day 140) post LVI in herds 1 and 2 by age at the time of first LVI. The 4-week age bracket was the pig age in weeks at the time of LVI (week 0). The number of PRRSV-positive animals per number of animals tested within each herd and age group is shown. No PRRSV was detected after 105 weeks of age at the time of LVI. PRRSV = porcine reproductive and respiratory syndrome virus; LVI = live virus inoculation; RT-PCR = reverse transcriptase-polymerase chain reaction.

Of the 17 PRRSV-negative cull animals, 1 tested PRRSV positive by RT-PCR on tonsil scraping samples at week 27 (day 191). None of the 52 animals had PRRSV-positive serum at week 27 (day 191).

Discussion

Age at the time of infection is a critical factor in whether an animal demonstrates prolonged infection. Results from both herds showed similar odds of a PRRSV-positive result as age at time of LVI decreased. Animals under 33 weeks of age were over-represented as infected at week 20 (day 140) when compared to the rest of the population (Figure 3) as the mean age of PRRSV-positive pigs in herds 1 and 2 were 28.0 and 21.8 weeks, respectively. Other studies have evaluated age as a factor for prolonged PRRSV infection and also found that age at time of infection was a significant contributor. This could be due to differences in mechanisms of the innate immune response, which appear to be more effective in animals exposed at 15 weeks of age or older.[11] This information may help inform herd closure strategies. In many cases, a site may choose to load their farm with as many young gilts as possible to have replacement females during the extended weeks of herd closure. One should carefully consider gilt loading strategies to reduce the odds of prolonged infection. Insuring gilts are as old as possible prior to LVI will reduce the odds of prolonged PRRSV infection of animals in the population.

Breed also appears to be a factor in prolonged PRRSV infection. Meishans had statistically greater odds than Landrace or Duroc of demonstrating prolonged infection at week 20 (day 140). This is important because of the role Meishans play in the commercial breeding herd. Boars from the Meishan breed are popular for use in estrus detection and are often moved throughout the farm each day. If Meishans are more likely to stay infected, they could be a source of constant virus shedding and exposure to many other animals on the site. Although both male and female Meishans were sampled in herd 2, limited observations and age as a confounding variable prevented analysis of sex as a factor in prolonged infection. With the follow up sampling of removed animals from herd 2, the association of continued persistence at week 27 (day 191) with the Meishan breed further supports the impact of breed on PRRSV infection dynamics. Differences of early response to PRRSV

Table 4: Breed distribution of PRRSV RT-PCR results at week 27 (day 191) post live virus inoculation

	Breed		
Week 27 result	**Meishan**	**Landrace/Duroc**	**Total**
Positive, No.	15	5	20
Negative, No.	5	10	15
Total, No.	20	15	35

PRRSV = porcine reproductive and respiratory syndrome virus; RT-PCR = reverse transcriptase-polymerase chain reaction.

between breeds has been observed in the literature, but evaluation of prolonged infection rates were not followed in this analysis.[12] Meishans were present in herd 1 but were not included in the sampling at week 20 (day 140).

In addition to characteristics associated with prolonged infection, this study reported the effects of the test-and-removal process using tonsil scrapings on herd closure success and time to negative. Tonsil scrapings are well documented as a sensitive method for detection of prolonged PRRSV infection in the tonsils.[3,8] Collection of tonsil samples in large sow herds requires skilled labor and physical endurance from a significantly sized team. To help alleviate the challenges of a tonsil sample, there is now evidence that oropharyngeal samples may be an appropriate sample for PRRSV detection.[13] Further exploration of these techniques is necessary for whole-herd testing and removal to be a practical approach to PRRSV eradication.

In this study, neither herd had a PRRSV-positive result by RT-PCR on routine weekly testing after infected animals were culled. The timelines for herd 1 and herd 2 are displayed in Figures 1 and Figure 2, respectively, with positive stable status declared at 30- and 44-weeks post LVI, respectively. This producer follows the AASV PRRSV herd classification guidelines for defining positive stable status.[14,15] As reported by the producer, both closures were shorter than the production system's 46-week average. The timing of testing and culling may have impacted closure length. If animals had been identified and culled immediately, it is possible closure lengths could have been reduced further. There is evidence in the literature to suggest an association with clearance rates in the serum by day 35 to 42 and the ability of the pig to clear virus from tissues.[16] This indicates an opportunity to attempt a test-and-removal strategy sooner than the 20 weeks (140 days) implemented in these two cases. The literature has historically shown that persistence occurs between 3 to 4 months following infection.[5] Both herds in this study have re-opened, brought in negative replacement animals, and remained negative to their respective PRRSV strains on routine weekly testing.

The observed persistence of PRRSV-positive animals at 27 weeks (day 191) as measured by RT-PCR demonstrates that presence of PRRSV RNA can remain present in adult swine for at least 27 weeks (191 days) following exposure. Stressful conditions associated with the shipping process and mixing in pens may indicate that stress could play a role in delayed clearance of PRRSV from the tonsil, but there were not enough animals in this data set to draw strong conclusions. Several attempts to evaluate stress on PRRSV infection dynamics have been reported, which demonstrate little significant impact of stress on shedding or transmission.[17,18] The mean Ct values from the 20 animals that tested positive at both timepoints, week 25 (day 142) and week 27 (day 191), numerically decreased from 35.5 to 33.7, which could fall within normal variation in the sampling methods. Additionally, the occurrence of one negative animal at week 20 (day 142) testing positive at week 27 (day 191) may suggest re-infection or further support stress as a factor in viral load in tonsils. Further analysis with a larger sample size and identical sample handling protocols is warranted for better understanding of these observations.

The main strength of this study was the ability to capture cross-sectional data on two sow populations on a large scale. At the time of writing, this appears to be the largest sample size collected to date for evaluation of prolonged PRRSV infection. Additionally, several breeds were able to be compared, which is unusual in a normal production setting.

Several limitations exist with this case study. Since both herds were a result of natural infection, a true time zero to establish a dpi timeline on each individual animal is an assumption. The date of the first LVI was assigned as day zero, but some animals may have been infected several days or even weeks prior to LVI. This may lead to an underestimation of the true prevalence of PRRSV persistence at week 20 (day 140). Additionally, neither of these two sites were allowed to farrow continuously during the closure. One site only farrowed every 4 weeks and the second site aborted 18 weeks of farrowings. This may have impacted viral load and spread within the population thus potentially impacting closure times. Empty farrowing rooms and lack of continued presence of viremic piglets on site between these two herds should not be underestimated when interpreting herd closure timelines.

Subsequent research includes the investigation of breed differences in their response to PRRSV infection. Breed effects suggest some level of natural genetic variation exists for the response to this virus that could be exploited through genetic selection programs. In addition, work that defines sampling techniques that can be applied early post infection to identify animals at risk for prolonged PRRSV infection would allow for effective test-and-removal strategies resulting in shorter herd closures that more reliably produce the desired outcome of eradication.

Implications

Under the conditions of this study:

- Pigs infected at a younger age had greater odds of prolonged PRRSV infection at week 20.
- Meishans had greater odds of prolonged PRRSV infection than Landrace or Duroc.
- Tonsil scraping was a good method to identify prolonged PRRSV infection.

Acknowledgments

Conflict of interest

None reported.

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