ORIGINAL RESEARCH PEER REVIEWED

Tonsil scrapings for porcine reproductive and respiratory syndrome virus detection in growing pigs under field conditions

Heather L. Walker, BS; Andrew S. Bowman, DVM, PhD, DACVPM; Juliana B. Ferreira, DVM, MSc, DVSc; Sarah W. Nelson, MS; Monique Pairis-Garcia, DVM, PhD; Andreia G. Arruda, DVM, PhD

Summary

Objective: The main objective of this study was to describe the use and limitations of tonsil scrapings (TS), oral fluids (OF), nasal swabs (NS), and environmental swabs (ES) to detect porcine reproductive and respiratory syndrome virus (PRRSV).

Materials and methods: Two PRRSV-positive growing pig farms using different PRRSV control strategies were enrolled in this study. Sampling began approximately 52- and 21-days post PRRSV exposure for farms 1 and 2, respectively, and occurred once a month for four months using fixed spatial sampling. Samples for OF and ES were collected at the pen level and TS and NS samples were collected at the individual level. All samples were tested using reverse transcriptase-polymerase chain reaction (RT-PCR).

Results: A total of 192 samples were collected over the study period: 48 TS, 48 OF, 48 NS, and 48 ES. Overall, 20 TS (41.6%), 0 OF (0.0%), 6 NS (12.5%), and 1 ES (2.1%) tested RT-PCR positive for PRRSV throughout this study.

Implications: Tonsil scraping samples yielded more positive PRRSV RT-PCR results for longer time periods when compared to OF, NS, and ES for PRRSV detection in growing pigs. Tonsil scraping samples tested RT-PCR positive for PRRSV up to 168 days post exposure. Oral fluids, NS, and ES sampling methods for PRRSV detection in growing pig populations, particularly months after the initial infection or vaccination, should be used with caution given low RT-PCR positive samples found in this study.

Keywords: swine, tonsil scrapings, porcine reproductive and respiratory syndrome virus detection, porcine reproductive and respiratory syndrome diagnostics

Received: June 10, 2020
Accepted: August 18, 2020

Resumen - Raspados de amígdalas para la detección del virus del síndrome reproductivo y respiratorio porcino en cerdos en crecimiento en condiciones de campo

Objetivo: El objetivo principal de este estudio fue describir el uso y las limitaciones de los raspados de amígdalas (TS), fluidos orales (OF), hisopos nasales (NS) e hisopos ambientales (ES) para detectar el virus del síndrome reproductivo y respiratorio porcino (PRRSV).

Materiales y métodos: En este estudio se registraron dos granjas de cerdos en crecimiento positivas al PRRSV que utilizan diferentes estrategias de control de PRRSV. El muestreo comenzó aproximadamente 52- y 21-días después de la exposición al PRRSV para las granjas 1 y 2, respectivamente, y se realizó una vez al mes durante cuatro meses utilizando un muestreo espacial fijo. Las muestras para OF y ES se recolectaron a nivel de corral y las muestras de TS y NS se recolectaron a nivel individual. Todas las muestras se analizaron mediante la reacción en cadena de la polimerasa con transcriptasa reversa (RT-PCR).

Resultados: Se recolectaron un total de 192 muestras durante el período de estudio: 48 TS, 48 OF, 48 NS, y 48 ES. En total, 20 TS (41.6%), 0 OF (0.0%), 6 NS (12.5%), y 1 ES (2.1%) fueron positivas a la RT-PCR para PRRSV a lo largo de este estudio.

Implicaciones: Las muestras de raspado de amígdalas produjeron resultados positivos a la RT-PCR de PRRSV durante períodos de tiempo más prolongados en comparación con OF, NS, y ES para la detección de PRRSV en cerdos en crecimiento. Las muestras de raspado de amígdalas dieron positivo en RT-PCR para PRRSV hasta 168 días después de la exposición. Los métodos de muestreo de fluidos orales, NS y EE para la detección de PRRSV en poblaciones de cerdos en crecimiento, particularmente meses después de la infección o vacunación inicial, deben usarse con precaución debido a las bajas muestras positivas para RT-PCR encontradas en este estudio.

HLW, ASB, SWN, AGA: Department of Veterinary Preventive Medicine, College of Veterinary Medicine, The Ohio State University, Columbus, Ohio.

JBF, MPG: Department of Population Health and Pathobiology, College of Veterinary Medicine, North Carolina State University, Raleigh, North Carolina.

Corresponding author: Dr Andreia G. Arruda, A194 Department of Veterinary Preventive Medicine, 1920 Coffey Rd, Columbus, OH 43210; Tel: 614-688-3277; Email: **arruda.13@osu.edu**.

This article is available online at **http://www.aasv.org/shap.html**.

Walker HL, Bowman AS, Ferreira JB, Nelson SW, Pairis-Garcia M, Arruda AG. Tonsil scrapings for porcine reproductive and respiratory syndrome virus detection in growing pigs under field conditions. *J Swine Health Prod*. 2021;29(2):72-80.

Résumé - Grattages des amygdales pour la détection du virus du syndrome reproducteur et respiratoire porcin chez des porcs en croissance dans des conditions de terrain

Objectif: Le principal objectif de la présente étude était de décrire l'utilisation et les limitations des grattages des amygdales (TS), des fluides oraux (OF), des écouvillons nasaux (NS), et des écouvillons d'environnement (ES) pour détecter le virus du syndrome reproducteur et respiratoire porcin (PRRSV).

Matériels et méthodes: Deux fermes de porcs en croissance positives pour le PRRSV et utilisant des stratégies différentes pour maitriser le PRRSV furent recrutées dans cette étude. L'échantillonnage débuta approximativement 52- et 21-jours post-exposition au PRRSV pour les fermes 1 et 2, respectivement, et fut effectué une fois par mois pendant 4 mois en utilisant un échantillonnage spatial fixe. Les échantillons d'OF et d'ES furent prélevés au niveau de l'enclos et les échantillons de TS et NS furent prélevés au niveau individuel. Tous les échantillons furent testés en utilisant une réaction d'amplification en chaîne par la polymérase avec la transcriptase réverse (RT-PCR).

Résultats: Un total de 192 échantillons furent prélevés au cours de la période d'étude: 48 TS, 48 OF, 48 NS, et 48 ES. Globalement, 20 TS (41.6%), 0 OF (0.0%), 6 NS (12.5%), et 1 ES (2.1%) se sont avérés positifs par RT-PCR pour le PRRSV au cours de l'étude.

Implications: Les échantillons de grattage d'amygdales ont généré plus de résultats positifs par RT-PCR pour le PRRSV pour de plus longes périodes lorsque comparé à OF, NS, et ES pour la détection de PRRSV chez des porcs en croissance. Les échantillons de grattage d'amygdales se sont avérés positifs par RT-PCR pour le PRRSV jusqu'à 168 jours post-exposition. Les méthodes d'échantillonnage pour OF, NS et ES pour la détection de PRRSV dans les populations de porcs en croissance, particulièrement les mois après l'infection initiale ou la vaccination, devraient être utilisées avec précaution étant donné le faible nombre d'échantillons positifs par RT-PCR trouvés dans cette étude.

Porcine reproductive and respiratory syndrome (PRRS) is the costliest disease currently affecting the North American swine industry with an estimated financial damage over $600 million annually.[1] This disease is caused by an RNA virus of the same name and has two predominant strains affecting the swine industry worldwide; the Eurasian strain (Type I) and the North American strain (Type II), both of which have been found in the United States. The Type II PRRS virus (PRRSV) strain is the most prevalent in North America and thus more likely to cause outbreaks.[2] As the name implies, the virus clinically manifests in primarily two bodily systems, the reproductive and respiratory systems. At the growing and finishing phases, affected pigs show slower growth rates, lower feed conversion, and an overall weakened appearance.[3] One team estimated that grower/finisher pigs endemically infected with PRRSV could result in elevated mortality and a decreased average daily gain of 17 to 35 g/d, ultimately leading to a projected $360 million loss in revenue annually.[1]

Porcine reproductive and respiratory syndrome virus has the innate ability to reside and proliferate in the lymphatic system,[4] and after infecting a host, the virus undergoes several phases in which it travels to and infects various lymphatic organs (eg, spleen, thymus, and tonsils).[3] Bodily dissemination of the virus allows for viremia development and viral shedding through a variety of routes including saliva, nasal secretions, mammary gland secretions, urine, feces, and semen.[3] Even though these excretions can be used to detect PRRSV in infected animals, the duration of shedding for each route is usually short, transient, or both.[5]

Accurately determining herd-level PRRSV status is important for animal movement and disease prevention and control. As such, herd-level testing protocols are commonly applied to describe the disease status of a herd based on diagnostic testing from a sample population of the herd.[6] The most widely used detection methods for declaring PRRSV herd-level status in growing pig populations include serum and oral fluid (OF) testing.[7] Even though serum sampling is the gold standard for PRRSV status determination in growing pigs,[6] OF testing has become popular over the past years because it is a convenient sample type that can be conducted by farm personnel with minimal training.

Oral fluid testing has successfully shown 90% to 100% virus detection via reverse transcriptase-polymerase chain reaction (RT-PCR) at 7 to 21 days post infection (dpi); however, the sensitivity of OF testing for PRRSV is negatively proportional to the post exposure time[8] and represents a challenge for detection once PRRSV reaches low levels at the population level. In such cases, due to the potential for false negatives, a herd could be incorrectly declared PRRSV negative resulting in downstream consequences pertaining to disease spread and surveillance.

Additional sampling methods for PRRSV have also been investigated in the past including nasal swabs (NS) and environmental swabs (ES). However, studies have shown that nasal shedding may be strain-dependent, only detected via RT-PCR sporadically,[9,10] and at a maximum of 49 dpi.[11] In contrast to NS, Vilalta and colleagues[12] reported that swabbing in the farrowing environment allowed for detection of PRRSV for up to 14 and 17 weeks post exposure at processing and weaning, respectively.

It has been shown under experimental conditions that PRRSV can persist in lymphoid tissues for long periods of time[13] and can be detected over 150 dpi[14]; but for practical reasons, lymphoid tissue sampling is not commonly considered among strategies for determination of herd-level PRRSV status.[6] Tonsil scraping (TS) may be an alternative to lymphoid tissue collection and has been validated as the sampling method of choice for various foreign animal diseases.[15] In addition, tonsil sampling can be effective in isolating PRRSV in pigs infected for longer time periods. Wills et al[4] initially reported the isolation of PRRSV from experimentally inoculated pigs via TS samples up to 157 dpi. In addition, Allende et al[16] measured viral persistence from experimental PRRSV infection in a small group of pigs via tonsil biopsy samples up to 150 dpi and others[13] have detected PRRSV in tonsil samples at 251 dpi. Although these studies demonstrate efficacy of TS for PRRSV detection, no studies to date have been published using this methodology under field conditions. Therefore, the main objective of this study was to describe the use and limitations of TS, OF, NS, and ES to detect PRRSV, and to compare PRRSV detection in TS samples from a PRRSV vaccinated and nonvaccinated farm.

Materials and methods

This research project was approved under North Carolina State University IACUC protocol 18-167-T.

Farm descriptors

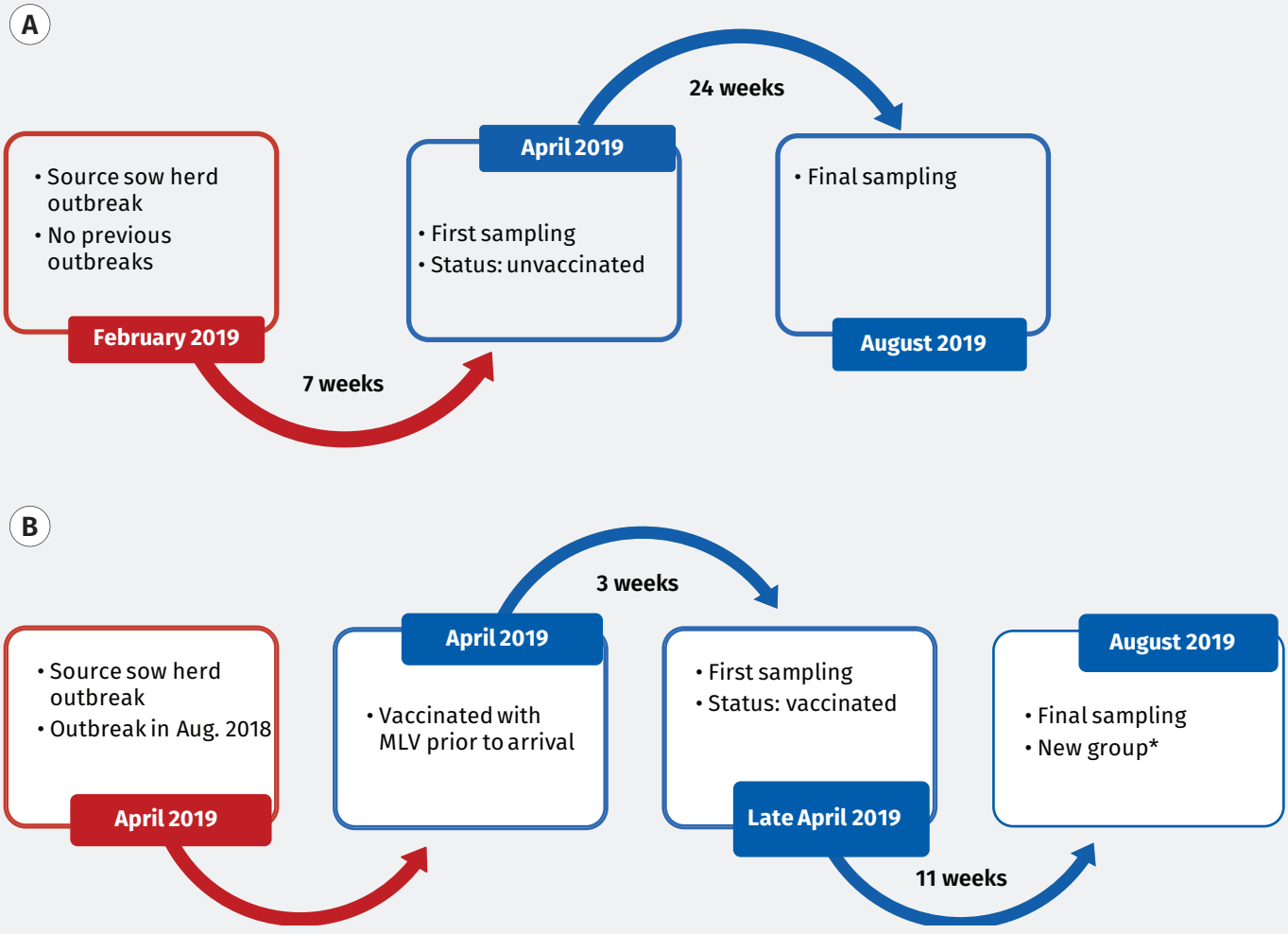
Two farms located in North Carolina were enrolled in this study. The inclusion criteria included farms located within a three-hour drive from the collaborators (for sampling purposes) that had a PRRSV outbreak within 60 days prior to the start of the study. The first farm (farm 1; unvaccinated) was a single-sourced 3500-head wean-to-finish facility composed of 4 barns with all-in/all-out pig flow. The source sow farm was presumed PRRSV negative, as no PRRS outbreaks were ever reported prior to this study. At the end of February 2019, a PRRS outbreak was confirmed on the source farm with a virus restriction fragment length polymorphism (RFLP) type 1-7-4. Transferring pigs from the source farm to farm 1 occurred throughout the month of March. No PRRSV vaccination was administered prior to or at the time of the outbreak on the source sow farm or on farm 1. The second farm (farm 2; vaccinated) was a single-source 2800-head finisher facility that was also composed of four barns utilizing an all-in/all-out pig flow. The source sow herd had a history of PRRS outbreaks with PRRSV RFLP type 1-7-4; with the last two confirmed PRRS outbreaks occurring in August 2018 and April 2019. Due to the previous PRRSV confirmation, a vaccination protocol was already in place on the source sow farm: sows were vaccinated 4 times per year and piglets were vaccinated at processing (4-6 days of age), with a 2 mL and 1 mL dose of a modified live PRRS vaccine (MLV; Ingelvac PRRS MLV; Boehringer Ingelheim), respectively.

Upon discovery of the April 2019 outbreak, the farm staff immediately began vaccinating the source sow herd with the MLV and implemented herd closure. These management strategies, along with sampling collection times, are shown in Figure 1.

Sample collection

Sampling for farms 1 and 2 was conducted between May and August of 2019, with up to 4 sampling events for each farm. Farm 1 was divided into 44 pens per barn and farm 2 was divided into 36 pens per barn, each pen consisted of 15 to 20 pigs (Figure 2). This study utilized a fixed spatial sampling technique for sample collection[17] with markers placed within each barn to indicate the sampling areas (Figure 2), representing approximately 30 to 40 pigs per sampling area (two pens of 15-20 pigs sharing a

Figure 1: Timeline for porcine reproductive and respiratory syndrome management strategies and sampling for A) farm 1 and B) farm 2. The pigs on farm 2 were vaccinated with a commercially available modified live vaccine (MLV). *Due to the timing of collections, farm 2 was in the process of shipping pigs to market during the June and July collections; thus, a new group of pigs (following the same vaccination protocol) were placed prior to the last collection in August.



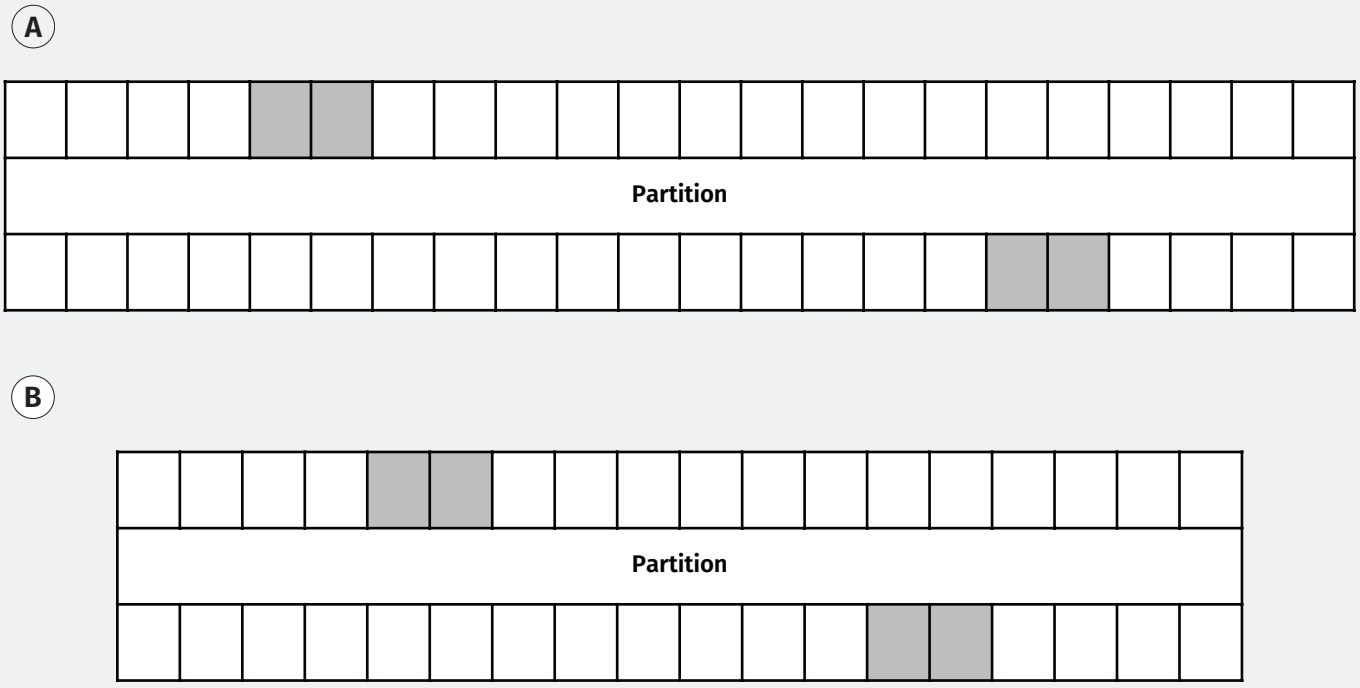
division that allowed nose-to-nose contact). Eight sampling areas (representing 2 pens each) were chosen in each farm, 2 per barn. During each monthly visit, 4 sample types were collected from each sampling area: OF, TS, NS, and ES. All sampled pens contained healthy pig populations. The OF and ES samples were collected on a group-level basis. Oral fluids were collected by placing a rope on the metal gate of two adjoining pens for approximately 15 to 20 minutes to allow pigs to chew on the rope, as stated in previous literature.[18] The end of the rope was then placed in a plastic bag to collect the fluids and poured into a glass vial for later processing. The ES were collected by wiping the feed troughs and waterers with a gauze pad as previously described.[12] The gauze pad was then placed into a vial containing 10 mL of brain-heart infusion (BHI) media for later processing. The TS and NS were collected from one individual animal in the pen using physical restraint with a snare and without any specific selection. For TS collection, a metal speculum was used to open the mouth of the pig and an elongated metal spoon was used to scrap the oropharyngeal region along the palatine tonsil of the pig[4]; the oropharyngeal fluid collected on the spoon was transferred to a vial containing 3 mL of BHI media with the aid of a sterile cotton swab as described by previous work.[4] The speculum was disinfected with Lysol or Clorox wipes prior to each use and a new spoon was used with each new TS collection. After the TS sample was collected, the same pig was used for NS sampling. A sterile NS was placed in the nose of the pig and swirled in each nostril for approximately 3 seconds per nostril; the swab was then placed in 3 mL BHI media. It should be noted that the pigs selected for the individual samplings were not specifically chosen based on any clinical signs indicative of disease; but simply according to interest in interacting with the snare and therefore being snared successfully in a timely manner. It should also be noted that pigs were not individually identified and, therefore, there is a chance that the same animal was sampled over different sampling events. After collection, all samples were placed in a cooler with ice, transported to North Carolina State University College of Veterinary Medicine within 3 hours, and kept in a refrigerator for 1 to 3 days before being shipped to The Ohio State University College of Veterinary Medicine. The OF samples were centrifuged at 1200*g* for 10 minutes (Sorvall Legend RT Centrifuge Machine; Thermo Scientific) to remove any debris prior to the RT-PCR testing.

PRRSV RT-PCR

Samples were tested for the presence of PRRSV by RT-PCR using standard protocols. Extraction of the samples were performed using the Omega Mag-Bind RNA extraction kit (Omega Bio-tek Inc) with a MagMAX Express 96 Magnetic Particle Processor (Applied Biosystems) using a laboratory-modified procedure with a company preloaded program (AM1836_DW_100_v2).[19] During the lysis step, the lysis/binding solution was combined with 10 µL of magnetic bead mix before extraction and elation in lysis enhancer (10 µL/reaction). Additionally, this procedure utilized 2 washes with 400 µL VHB Buffer (Omega Bio-tek Inc) and 500 µL SPR Wash Buffer (Omega Bio-tek Inc) for wash 1 and 2, respectively.

The samples were assayed using the VetMAX NA and EU PRRSV polymerase chain reaction kit (Applied Biosystems). Each run also contained 2 positive controls and 2 negative controls. The positive control came from a mix of 2 µL Xeno RNA Control, 2 µL NA PRRSV Control RNA, 2 µL EU PRRSV Control RNA, and 94 µL Nucleic Acid dilution solution. In the two positive-control wells, 7 µL of the positive-control mix was combined with 18 µL of the reaction mix. Each sample well included 12.5 µL Multiplex RT-PCR buffer, 2.5 µL PRRSV Primer Probe Mix V2, 2.5 µL Multiplex Enzyme Mix, 0.5 µL Nuclease-free water, and 7 µL of the sample collected for a total volume of 25 µL per well. Sample plates were loaded onto a 7500 Fast Real-Time PCR system (Applied Biosystems)

Figure 2: Barn layout for A) farm 1 and B) farm 2 sampled in this study. Each farm had four of the represented barns and each square represents one pen and the shaded area indicates the sampling area utilized within the barns.



with the following cycling conditions: stage 1 was 1 cycle of 48°C for 10 minutes, stage 2 was 1 cycle of 95°C for 10 minutes, and stage 3 was 40 cycles of 95°C for 15 seconds followed by 60°C for 45 seconds. Cycle threshold (Ct) values were calculated for each sample by setting the threshold at 5% of the positive control at cycle 40. Samples with a Ct of ≤ 37.0 were considered positive and samples with a Ct between 37.1 and 40 were considered suspect based on values described by previous work.[20]

Statistical analysis

Statistical analysis was conducted using STATA 14.2 (StataCorp LP). Descriptive statistics were used to describe the detection of PRRSV-positive samples over time for both farms and for the different sampling methods. All analyses were conducted at the sample level. First, a Fisher's Exact test was used to test the association between detection of PRRSV in TS and the predictor of interest, farm. Furthermore, to address the clustering of samples within sampling events and potential confounding effects, a multivariable exact logistic regression model was built using a forward stepwise regression approach, with estimations of median unbiased estimates (MUE).[21] This model also attempted to investigate the association between detection of PRRSV in TS and farm, but while accounting for sampling event (1-4) and total number of samples collected. Prior to addition to the final model, correlation between those variables was tested using the Spearman correlation test and a cutoff of 0.8. Confounders were defined as variables that changed the coefficient of our main variable of interest (farm) by 20% or more once removed from the model, and in such case it was retained in the final model regardless of statistical significance. Statistical significance was declared at $P < .05$, and a statistical trend was declared as $.05 \leq P < .10$.

Results

There was a total of 192 samples collected over the study period: 48 TS, 48 OF, 48 NS, and 48 ES. Farm 1, the unvaccinated farm, had 12 PRRSV RT-PCR positive TS (4 positive samples occurring in each of the first and second sampling events and 2 positive samples in each of the third and fourth sampling events) and 2 PRRSV RT-PCR positive NS (1 positive sample in each of the third and fourth sampling events; Figure 3 and Table 1). The two animals that tested positive by NS were also positive by TS. Farm 2, the vaccinated farm, had 8 PRRSV RT-PCR positive TS (2 positive samples in the first sampling event and 6 positive samples in the third sampling), 4 PRRSV RT-PCR positive NS (1 positive sample in the second sampling and 3 positive samples in the third sampling), and 1 PRRSV RT-PCR positive ES (occurring in the third sampling event; Figure 3 and Table 1). From the 4 animals that tested positive by NS, 3 also tested positive by TS. While a small proportion of NS and ES tested RT-PCR positive (12.5% [6 of 48] and 2.1% [1 of 48], respectively) these sampling methods did not consistently show positive results throughout the study period. Overall, there were 20 TS, zero OF, 6 NS, and 1 ES test RT-PCR positive for PRRSV throughout this study with 48.1% (13 of 27) of the positive samples occurring on the last sampling event (Table 1). There were several samples in each sampling category that tested RT-PCR PRRSV suspect positive.

Figure 3: Total number of samples collected RT-PCR PRRSV-positive samples for the 4 different sample types (tonsil scrapings, nasal swab, oral fluid, and environmental swab) over the four sampling events. RT-PCR = reverse transcriptase-polymerase chain reaction; PRRSV = porcine reproductive and respiratory syndrome virus.

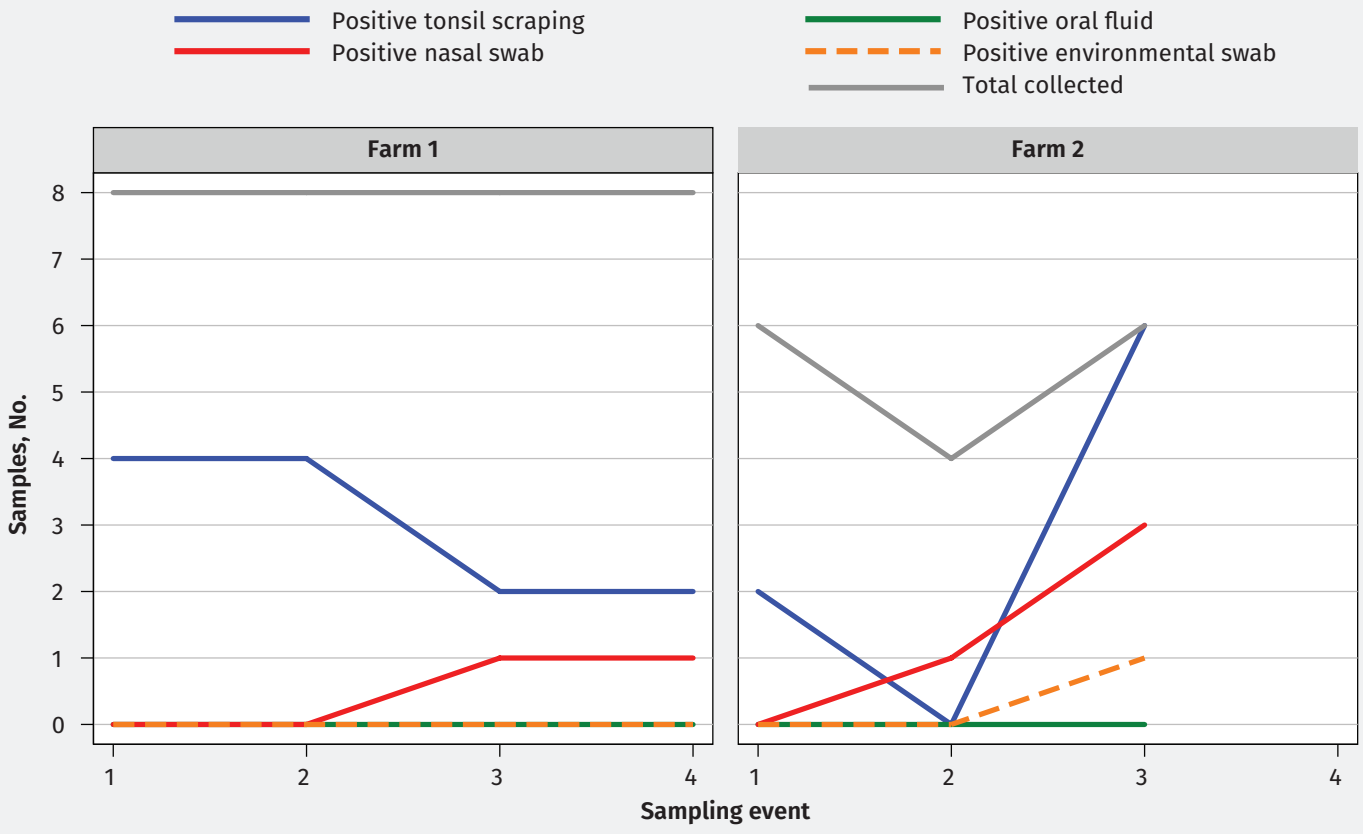


Table 1: Proportions of RT-PCR PRRSV-positive samples for the different sample types assessed in this study

	Sampling event*	Tonsil scraping, No. (%)		Oral fluid, No. (%)		Nasal swab, No. (%)		Environmental swab, No. (%)	
		Positive	Suspect†	Positive	Suspect†	Positive	Suspect†	Positive	Suspect†
Farm 1‡	1 (n = 8)	4 (50.0)	1 (12.5)	0 (0)	1 (12.5)	0 (0)	0 (0)	0 (0)	0 (0)
	2 (n = 8)	4 (50.0)	2 (25.0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
	3 (n = 8)	2 (25.0)	2 (25.0)	0 (0)	1 (12.5)	1 (12.5)	0 (0)	0 (0)	0 (0)
	4 (n = 8)	2 (25.0)	1 (12.5)	0 (0)	0 (0)	1 (12.5)	0 (0)	0 (0)	0 (0)
	Total (n = 32)	12 (37.5)	6 (18.75)	0 (0)	2 (6.3)	2 (6.3)	0 (0)	0 (0)	0 (0)
Farm 2‡	1 (n = 6)	2 (33.3)	3 (50.0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
	2 (n = 4)	0 (0)	1 (25)	0 (0)	0 (0)	1 (25)	0 (0)	0 (0)	0 (0)
	3§ (n = 6)	6 (100.0)	0 (0)	0 (0)	2 (33.3)	3 (50.0)	1 (16.7)	1 (16.7)	1 (16.7)
	Total (n = 16)	8 (50.0)	4 (25.0)	0 (0)	2 (12.5)	4 (25.0)	1 (6.3)	1 (6.3)	1 (6.3)

* Sampling took place from May to August 2019.

† Suspect positive samples with a Ct value between 37.1 and 40.

‡ Farm 2 received the PRRSV vaccine and farm 1 did not.

§ New group of pigs.

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

The Ct values for the positive and suspect positive samples have been summarized in Table 2, highlighting the mean and range for each sampling category and event.

A reduced number of samples were collected from farm 2 due to operating procedures at the facility. During the second sampling month, the pigs at farm 2 had reached market weight and were removed from the facility. A new group of pigs from the same source sow farm were brought into the facility for the last sampling event (sampling 3). Thus, there were no samples collected on farm 2 for the fourth sampling and all samples collected during the third sampling were from a new group of pigs.

Over the 4-month study period, 13 of 16 sampled areas (considering both farms) tested PRRSV RT-PCR positive at least once with TS sampling (Table 3). All farm 2 pens that were sampled during the third event (pen 3 to pen 8) tested positive on PRRSV RT-PCR using TS (Table 3).

Analysis using a Fisher's Exact test showed no association between farm and a positive TS RT-PCR ($P = .36$). However, the multivariable exact logistic regression model accounting for sampling event and total samples taken on that sampling event showed there was a tendency ($P = .09$) for farm 2 to have higher odds of PRRSV detection on TS compared to farm 1 (odds ratio [OR] = 16.21). In this final model, the total number of samples taken in a sampling event was positively associated with the odds of PRRSV being detected in TS (OR = 3.26).

Discussion

Tonsil scraping samples yielded more positive PRRSV RT-PCR results over time for longer time periods when compared to the current commonly used sampling method, OF testing. To date, TS methods for PRRSV detection via RT-PCR have not been explored under field conditions for PRRSV diagnostic testing to determine herd-level PRRS status. This study described different sampling methods to detect PRRSV in growing pig populations under field conditions for farms utilizing different PRRS management strategies.

There was a difference in PRRSV detection between the 4 sampling methods: TS, OF, NS, and ES. Our findings corroborate similar research that showed an eventual decrease of PRRSV present in lymphoid tissues after 3 to 4 months post exposure[4,13,16]; nonetheless, we were able to detect PRRSV with TS up to 168 days post PRRSV exposure. Tonsil scraping was the only sampling method to consistently have positive samples over the four sampling events, despite being tested at the individual level in the conditions of this study. Although TS utilized only one pig per pen to determine herd status, we hypothesize that virus persistence in lymphoid tissues might explain the higher prevalence when compared to OF, which tests a larger number of pigs simultaneously. Additionally, considering the sensitivity of OF testing decreases over time,[22] TS proved to be a promising sampling method for long-term detection of PRRSV.

The determination of an accurate diagnostic method to detect PRRSV in grower pigs, especially in low PRRSV-prevalence scenarios, is vital to declare disease freedom as severe consequences can arise from inadvertently introducing PRRSV in negative populations. Our results compliment those of Horter et al[23] who reported that reverse transcriptase-nested polymerase chain reaction TS were the most effective assay-specimen combination to detect PRRSV in persistently infected animals.[23] The transition between diagnostic tools (in this case OF to TS) based on the stage of the infection and the nature of the disease is well described by Henao-Diaz et al[24] who suggests that it is vital to the relationship between the various disease transition states of PRRSV and the ability to detect infection based on those states, especially in cases of persistent infections. They

Table 2: Mean (range) of Ct values from RT-PCR PRRSV-positive and suspect positive tonsil scraping, oral fluid, nasal swab, and environmental swab samples from both farms

Sampling event	Tonsil scraping		Oral fluid		Nasal swab		Environmental swab	
	Ct, mean (range)	n	Ct, mean (range)	n	Ct, mean (range)	n	Ct, mean (range)	n
1	37.1 (35.9-38.8)	10	37.6	1	-	0	-	0
2	35.9 (31.6-38.3)	7	-	0	35.4	1	-	0
3	32.7 (27.6-38.3)	10	37.6 (37.1-37.9)	3	36.4 (34.2-38.2)	5	36.95 (36.8-37.1)	2
4	35.4 (33.2-37.7)	3	-	0	36.7	1	-	0
Total	35.2 (27.6-38.8)	30	37.6 (37.1-37.9)	4	35.97 (34.2-38.2)	7	36.95 (36.8-37.1)	2

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

Table 3: Representation of RT-PCR PRRSV-positive tonsil scraping (represented by +) samples for each pen

Sampling event	Farm 1 (unvaccinated) pen								Farm 2 (vaccinated) pen							
	1	2	3	4	5	6	7	8	1	2	3	4	5	6	7	8
1	-	-	-	+	+	+	+	-	+	-	-	-	+	-	NA	NA
2	-	+	+	-	+	+	-	-	-	-	-	-	NA	NA	NA	NA
3	-	+	-	-	-	-	+	-	NA	NA	+	+	+	+	+	+
4	-	-	-	-	+	-	+	-	NA	NA	NA	NA	NA	NA	NA	NA

PRRSV = porcine reproductive and respiratory syndrome virus; RT-PCR = reverse transcriptase-polymerase chain reaction; NA = no samples were collected for that sampling event due to pig flow from the finisher farm to slaughter.

continue to conclude that with PRRSV specifically, the probability of detecting an infection is based heavily on the diagnostic method chosen and should be recognized prior to test selection.[24] The current study also highlights the potential for additional research focusing on testing TS sampling in field conditions, as most research has only been conducted on experimentally inoculated animals or using postmortem TS procedures.

Results from this study should be considered given the limitations of the study. Firstly, it should be noted that the BHI mixture utilized in this study was not precisely measured prior to adding it to the TS, NS, and ES samples. While every effort was made to ensure that the proper volume was used for each sample, some samples could have been more diluted than others, which could play a role in PRRSV RT-PCR detection from the samples. This is specifically true for the ES samples, which were diluted in a larger volume of media and could explain the lower detection rate for that sample type. Additionally, even though we utilized a relatively large number of samples (over 100), it is important to note that there were only two farms enrolled in this study. This complicated further analysis of potential farm-level confounders such as farm size, facility-specific characteristics, and detailed management. We attempted to address this by building a robust model that partially accounted for clustering effects, but the effect of vaccination versus other farm-level characteristics cannot be disentangled. Interestingly, farm 2 (vaccinated) had increased odds for PRRSV detection in TS when compared to farm 1 (unvaccinated). This was unexpected since viral shedding of vaccine virus strains has been shown to be shorter compared to wild types.[25,26] However, we hypothesize that this increase could be due to the presence of the vaccine strain in the lymphoid tissue as farm 2 was vaccinated with an MLV three weeks prior to arrival at the grower facility. These pigs were at least 84 days post inoculation with the MLV, which can show varying shedding results as time increases. For example, Linhares et al[26] showed that the viral shedding from TS and OF can vary in both a control and vaccinated group. This team demonstrated that for both the vaccinated and control groups OF PCR was only detected up to 36 days post inoculation, while TS PCR was detected until the end of the study (118 days).[26] Furthermore, it could also be the case that the farm still had field viruses in the facility; which were being detected by the assay.

Another limitation of our study was that the study design did not allow for calculations of sensitivity or specificity for TS sampling, since samples were not collected from the same animal for head to head comparison. However, the aim of

this paper was to describe the use and limitations of TS, OF, NS, and ES to detect PRRSV; and not to validate TS as a gold standard compared to other methods. Furthermore, this study was conducted from May to August, therefore, we do not know whether these results would differ during cooler months. Nevertheless, we would not anticipate major deviations in our conclusions considering that PRRSV has been shown to survive and infect animals throughout the year[27] and that modern swine farms are commonly able to provide a well-controlled climate inside the barns year round.

Lastly, under the conditions of this study, we were not able to obtain an open reading frame (ORF) 5 sequences from the samples we had collected to differentiate whether the PRRSV being detected via PCR corresponded to vaccine-like or wild-type viruses. This information would have been important to differentiate between potential lateral PRRSV introduction and vaccine or previous outbreak strains.

To continue to understand the potential benefits of TS sampling for PRRSV detection, we recommend that future research focus on comparing OF and TS sampling from individual, known positive swine herds. This will allow for additional discussion surrounding the effectiveness of TS vs OF testing. Furthermore, performing ORF5 or whole genome sequencing and virus isolation would likewise be of value as they would provide further information on which viruses are being detected and whether they could cause infection in other pigs. These were not successfully conducted in this study, likely due to high overall Ct values.

Implications

Under the conditions of this study:

- Tonsil scrapings yielded more positive PRRSV results overall.
- Tonsil scrapings tested positive for PRRSV up to 168 days post exposure.
- In this study, OF, NS, and ES showed lower PRRSV detection than TS.

Acknowledgments

Funding was provided in part by the National Pork Board. Student research was supported through the National Institute of Health T35 training grant and the National Veterinary Scholars Program.

Conflict of interest

None reported.

Disclaimer

Scientific manuscripts published in the *Journal of Swine Health and Production* are peer reviewed. However, information on medications, feed, and management techniques may be specific to the research or commercial situation presented in the manuscript. It is the responsibility of the reader to use information responsibly and in accordance with the rules and regulations governing research or the practice of veterinary medicine in their country or region.

References

1. Holtkamp DJ, Kliebenstein JB, Neumann EJ, Zimmerman JJ, Rotto HF, Yoder TK, Wang C, Yeske PE, Mowrer CL, Haley CA. Assessment of the economic impact of porcine reproductive and respiratory syndrome virus on United States pork producers. *J Swine Health Prod*. 2013;21(2):72-84.

2. Perez AM, Davies PR, Goodell CK, Holtkamp DJ, Mondaca-Fernández E, Poljak Z, Tousignant SJ, Valdes-Donoso P, Zimmerman JJ, Morrison RB. Lessons learned and knowledge gaps about the epidemiology and control of porcine reproductive and respiratory syndrome virus in North America. *J Am Vet Med Assoc.* 2015;246(12):1304-1317. **doi:10.2460/javma.246.12.1304**

3. Zimmerman JJ, Dee SA, Holtkamp DJ, Murtaugh MP, Stadejek T, Stevenson GW, Torremorell M, Yang H, Zhang J. Porcine reproductive and respiratory syndrome virus (Porcine Arteriviruses). In: Zimmerman JJ, Karriker LA, Ramirez A, Schwartz KJ, Stevenson GW, Zhang J, eds. *Diseases of Swine*. 11th ed. Ames, IA: Wiley-Blackwell; 2019:665-708.

4. Wills RW, Zimmerman JJ, Yoon KJ, Swenson SL, Hoffman LJ, McGinley MJ, Hill HT, Platt KB. Porcine reproductive and respiratory syndrome virus: routes of excretion. *Vet Microbiol.* 1997;57(1):69-81.

5. Pileri E, Mateu E. Review on the transmission porcine reproductive and respiratory syndrome virus between pigs and farms and impact on vaccination. *Vet Res*. 2016;47(1):108. **doi:10.1186/s13567-016-0391-4**

6. Holtkamp DJ, Polson DD, Torremorell M, Morrison B, Classen DM, Becton L, Henry S, Rodibaugh MT, Rowland RR, Snelson H, Straw B, Yeske P, Zimmerman JJ. Terminology for classifying swine herds by porcine reproductive and respiratory syndrome virus status. *J Swine Health Prod.* 2011;19(1):44-56.

*7. Swine Health Information Center. Swine Disease Reporting System Report 25. **https://www.swinehealth.org/wp-content/uploads/2020/03/SHIC-109-DSDMR-3-4-2020.pdf.** Published March 2020. Accessed May 3, 2020.

8. Kittawornrat A, Prickett J, Chittick W, Wang C, Engle M, Johnson J, Patnayak D, Schwartz T, Whitney D, Olsen C, Schwartz K, Zimmerman JJ. Porcine reproductive and respiratory syndrome virus (PRRSV) in serum and oral fluid samples from individual boars: will oral fluid replace serum for PRRSV surveillance? *Virus Res.* 2010;154(1-2):170-176. **doi:10.1016/j.virusres.2010.07.025**

9. Duan X, Nauwynck HJ, Pensaert MB. Virus quantification and identification of cellular targets in the lungs and lymphoid tissues of pigs at different time intervals after inoculation with porcine reproductive and respiratory syndrome virus (PRRSV). *Vet Microbiol.* 1997;56:9-19.

10. Rossow KD, Bautista EM, Goyal SM, Molitor TW, Murtaugh MP, Morrison RB, Benfield DA, Collins JE. Experimental porcine reproductive and respiratory syndrome virus infection in one-, four-, and 10-week-old pigs. *J Vet Diagn Invest.*1994;6:312.

11. Charpin C, Mahé S, Keranflec'h A, Belloc C, Cariolet R, Le Potier M-F, Rose N. Infectiousness of pigs infected by the porcine reproductive and respiratory syndrome virus (PRRSV) is time-dependent. *Vet Res.* 2012;43(1):69. **doi:10.1186/1297-9716-43-69**

12. Vilalta C, Sanhueza J, Garrido J, Murray D, Morrison R, Corzo CA, Torremorell M. Indirect assessment of porcine reproductive and respiratory syndrome virus status in pigs prior to weaning by sampling sows and the environment. *Vet Microbiol*. 2019;237:108406. **doi:10.1016/j.vetmic.2019.108406**

13. Wills RW, Doster AR, Galeota JA, Sur J-H, Osorio FA. Duration of infection and proportion of pigs persistently infected with porcine reproductive and respiratory syndrome virus. *J Clin Microbiol.* 2003;41(1):58-62. **doi:10.1128/JCM.41.1.58-62.2003**

14. Hess AS, Lunney JK, Abrams S, Choi I, Trible BR, Hess MK, Rowland RR, Plastow GS, Dekkers JC. Identification of factors associated with virus level in tonsils of pigs experimentally infected with porcine reproductive and respiratory syndrome virus. *J Anim Sci.* 2019;97(2):536-547. **doi:10.1093/jas/sky446**

*15. United States Department of Agriculture Animal and Plant Health Inspection Service. Procedure Manual for Classical Swine Fever (CSF) Surveillance. **https://www.aphis.usda.gov/vs/nahss/swine/csf/CSF_procedure_manual_2007.pdf**. Published April 1, 2007. Accessed May 3, 2020.

16. Allende R, Laegreid WW, Kutish GF, Galeota JA, Wills RW, Osorio FA. Porcine reproductive and respiratory syndrome virus: description of persistence in individual pigs upon experimental infection. *J Virol*. 2000;74(22):10834-10837. **doi:10.1128/JVI.74.22.10834-10837.2000**

17. Rotolo ML, Sun Y, Wang C, Giménez-Lirola L, Baum DH, Gauger PC, Harmon KM, Hoogland M, Main R, Zimmerman JJ. Sampling guidelines for oral fluid-based surveys of group-housed animals. *Vet Microbiol*. 2017;209:20-29. **doi:10.1016/j.vetmic.2017.02.004**

18. White D, Rotolo M, Olsen C, Wang C, Prickett J, Kittawornrat A, Panyasing Y, Main RG, Rademacher C, Hoogland M, Zimmerman JJ. Recommendations for pen-based oral-fluid collection in growing pigs. *J Swine Health Prod*. 2014;22(3):138-141.

19. Bliss N, Nelson SW, Nolting JM, Bowman AS. Prevalence of influenza A virus in exhibition swine during arrival at agricultural fairs. *Zoonoses Public Health*. 2016;63(6):477-485. **doi:10.1111/zph.12252**

20. Gerber PF, O'Neill K, Owolodun O, Wang C, Harmon K, Zhang J, Halbur PG, Zhou L, Meng X-J, Opriessnig T. Comparison of commercial real-time reverse transcription-PCR assays for reliable, early, and rapid detection of heterologous strains of porcine reproductive and respiratory syndrome virus in experimentally infected or noninfected boars by use of different sample types. *J Clin Microbiol.* 2013;51(2):547-556. **doi:10.1128/JCM.02685-12**

21. Hirji KF, Tsiatis AA, Mehta CR. Median unbiased estimation for binary data. *Am Stat.* 1989;43(1):7-11.

22. Prickett J, Simer R, Christopher-Hennings J, Yoon K-J, Evans RB, Zimmerman JJ. Detection of porcine reproductive and respiratory syndrome virus infection in porcine oral fluid samples: a longitudinal study under experimental conditions. *J Vet Diagn Invest*. 2008;20(2):156-163. **doi:10.1177/104063870802000203**

23. Horter DC, Pogranichniy RM, Chang C-C, Evans RB, Yoon K-J, Zimmerman JJ. Characterization of the carrier state in porcine reproductive and respiratory syndrome virus infection. *Vet Microbiol.* 2002;86(3):213-228. **doi:10.1016/S0378-1135(02)00013-5**

24. Henao-Diaz A, Ji J, Giménez-Lirola L, Baum DH, Zimmerman J. Understanding and interpreting PRRSV diagnostics in the context of "disease transition stages". *Res Vet Sci*. 2020;131:173-176. **doi:10.1016/j.rvsc.2020.04.023**

25. Madapong A, Temeeyasen G, Saengchuto K, Tripipat T, Navasakuljinda W, Boonsoongnern A, Tantituvanont A, Nilubol D. Humoral immune responses and viral shedding following vaccination with modified live porcine reproductive and respiratory syndrome virus vaccines. *Arch Virol*. 2017;162(1):139-146. **doi:10.1007/s00705-016-3084-4**

26. Linhares DC, Cano JP, Wetzell T, Nerem J, Torremorell M, Dee SA. Effect of modified-live porcine reproductive and respiratory syndrome virus (PRRSv) vaccine on the shedding of wild-type virus from an infected population of growing pigs. *Vaccine*. 2012;30(2):407-413. **doi:10.1016/j.vaccine.2011.10.075**

27. Zimmerman JJ, Jacobs AC, Hermann JR, Muñoz-Zanzi C, Prickett JR, Roof MB, Yoon K-J. Stability of porcine reproductive and respiratory syndrome virus at ambient temperatures. *J Vet Diagn Invest*. 2010;22(2):257-260. **doi:10.1177/104063871002200216**

* Non-refereed references.