BRIEF COMMUNICATION PEER REVIEWED

An assessment of rope sampling methodologies on pen-level oral fluid samples for detection of PRRSV infection

Will Crum, DVM; Mackenzie Bartels BS; Garrett Drum, BS; Renae Kayser, BS; Lauren Skoglund, BS; Laura Munger, BS; Kitana Honkola, BS; Marisa Rotolo, DVM, PhD; Brent Pepin, DVM, MS; Karyn A. Havas, DVM, PhD

Summary

Rope sampling methodologies were assessed for porcine reproductive and respiratory syndrome virus (PRRSV) detection in 6 pens. Results showed that shared ropes detected PRRSV 50% and 66.7% of the time compared to unshared ropes. One rope provided better detection than 2 ropes per pen under the conditions of this study.

Keywords: swine, oral fluids, sensitivity, sampling

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Resumen - Una evaluación de las metodologías de muestreo con cuerdas en muestras de fluidos orales a nivel de corral para la detección de la infección por el PRRSV

Se evaluaron metodologías de muestreo con cuerdas para la detección del virus del síndrome reproductivo y respiratorio porcino (PRRSV) en 6 corrales. Los resultados mostraron que las cuerdas compartidas detectaron al PRRSV en el 50% y 66.7% de las veces en comparación con las cuerdas no compartidas. Una cuerda proporcionó mejor detección que 2 cuerdas por corral bajo las condiciones de este estudio.

Résumé - Évaluation des méthodes d'échantillonnage par la corde sur les échantillons de fluides oraux au niveau des parcs pour la détection de l'infection par le VSRRP

Les méthodes d'échantillonnage par la corde ont été évaluées pour la détection du virus du syndrome reproducteur et respiratoire porcin (VSRRP) dans 6 enclos. Les résultats ont démontré que des cordes partagées ont permis de détecter le VSRRP 50% et 66.7% du temps comparativement à des cordes non-partagées. Une corde permettait une meilleure détection que deux cordes par enclos dans les conditions de la présente étude.

Oral fluid samples are an efficient, common tool for swine diagnostics and monitoring since their introduction as a diagnostic sample in 2010.[1-3] Producers use them for surveillance of the majority of endemic swine pathogens including porcine reproductive and respiratory syndrome virus (PRRSV).[1,4,5] Oral fluid collection techniques vary within the industry, but recommendations are to hang 1 rope per pen[3] to get 80% coverage of the pen.[6] Previous research showed that an increased number of ropes increased overall chewing time, but pathogen detection was not assessed.[6] It is common practice within the industry for rope samples to be hung between pens to increase the number of animals represented within the sample. Detection of PRRSV using oral fluids increases with increasing prevalence and can be less consistent at lower prevalences.[7] Pooled oral fluids collected from pens sampled with 1 rope and oral fluids collected from a litter in a farrowing pen decreased diagnostic sensitivity when prevalence was low compared to oral fluid samples collected from unpooled rope samples and individual animal samples, respectively.[8,9]

The objective of this study was to evaluate the detection of PRRSV vaccine virus spread in pens of approximately 25 pigs using different rope sampling strategies.

Animal care and use

The Pipestone Institutional Animal Care and Use Committee approved the project (Protocol No. 2021-22).

Materials and methods

Study design and sampling

This study was conducted in an air-filtered gilt development unit in southwest Minnesota over the course of 3 weeks in November 2021. During the study, 150 isowean, PRRSV-negative gilts were housed in pens that held between 24 to 27 pigs. No other pigs were in the barn during the study. Sampling began when pigs were approximately 12 weeks of age and 12 kg.

There were 2 sets of 3 pens with an alleyway between (Figure 1) at the end of a 12-pen room. Pens were labeled 1 east (1E), 1 west (1W), 2 east (2E), 2 west (2W), 3 east (3E), and 3 west (3W). The west side pens had an additional ventilation

WC, KAH: Pipestone Research, Pipestone Holdings, Pipestone, Minnesota.

MB, GD, RK, LS, LM, KH: Professional DVM Program, South Dakota State University, Brookings, South Dakota.

MR: Pig Improvement Company, Hendersonville, Tennessee.

BP: Cytotheryx, Rochester, Minnesota.

Corresponding author: Dr Karyn Havas, 75 S Hwy 1300, Pipestone, MN 56164; Email: **karyn.havas@pipestone.com**.

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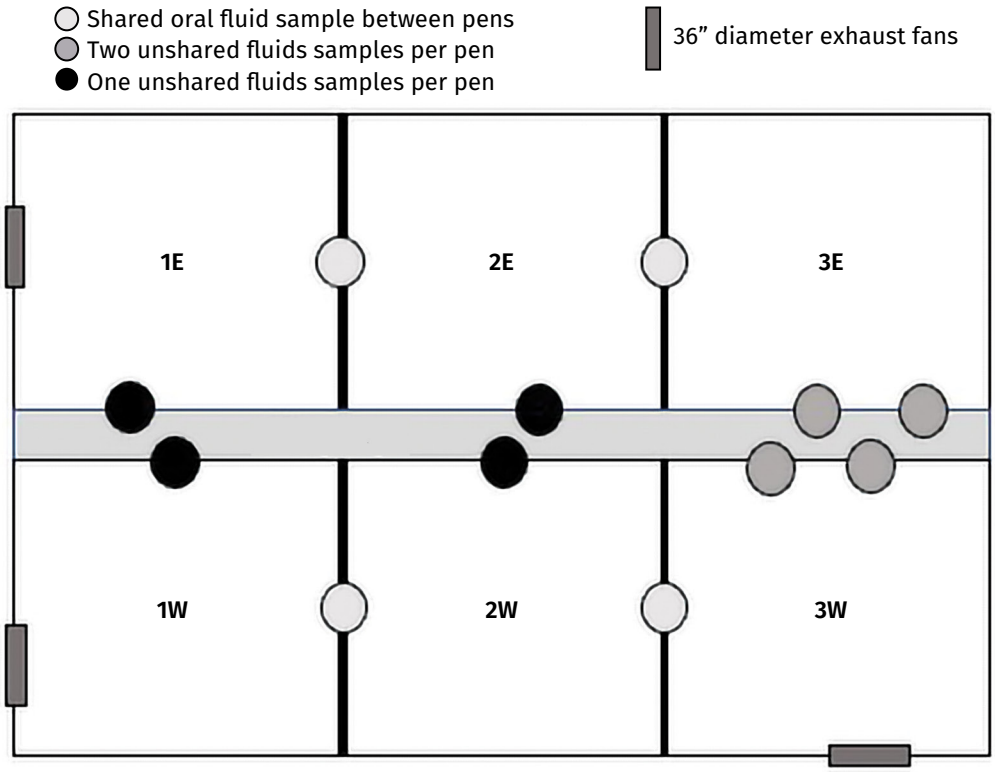
Figure 1: Schematic of the duplicate 3-pen study design used to evaluate pen-level oral fluids sampling methodologies using cotton ropes.



exhaust fan that the east side pens did not. Hard, smooth siding was placed between the pens to prevent direct contact of pigs between pens. One pig in 1E, 1W, 3E, and 3W received 2 mL of the Ingelvac PRRSV modified live vaccine (Boehringer Ingelheim) intramuscularly while restrained using a new needle for each animal. Pen 2E and 2W were control pens and had no vaccinated pigs. Husbandry activities and sample collection occurred in pens 2E and 2W with clean boots, clothes, and tools before the vaccinated pens to maintain biosecurity and reduce cross-contamination.

Individual serum and nasal swabs were collected from all pigs during the study. Serum was collected with serum separator tubes (Becton, Dickinson and Company) using jugular or vena cava venipuncture on 0, 3, 9, 15, and 21 days post vaccination (dpv). Nasal swabs were taken using polyester swabs on a plastic shaft (Fisher Scientific Company) on 0, 3, 5, 7, 9, 12, 15, 18, 21, 24, and 27 dpv. The swab was placed in a sterile polystyrene test tube (Fisher Scientific Company) with 3 mL of phosphate buffered saline (PBS; Cytiva). Pen 2E and 2W were sampled on 0 and 9 dpv (nasal swab and serum), as well as 3 and 21 dpv (nasal swabs). Pens 1W and 2W were sampled through 21 dpv, while 3W was sampled through 24 dpv due to supply shortages on 21 dpv. East pens were sampled through 27 dpv because viral transmission was limited compared to the west side.

Pen-level oral fluids were collected from all pens. Samples were collected as outlined in the nasal swab schedule using unbleached, cotton rope (Boardwalk) with seven, 0.25-inch strands bound using a generic cable tie (QC Supply) that hung for 20 minutes. The strands were placed in a clean plastic bag, fluids wrung out, and poured into a sterile polystyrene test tube (Fisher Scientific Company). One unshared rope was used in pens 1E, 1W, 2E, and 2W and 2 unshared ropes were used in pens 3E and 3W. Shared ropes between pens 1 and 2 and pens 2 and 3 on both sides of the barn were collected too. Three of the cotton strands from the shared rope were accessible to pigs in pen 1 or 3 and the other 3 strands were accessible to pigs in pen 2. The 6 strands were collected as 1 sample. One person encouraged movement in the pens during sample collection to get the greatest number of pigs chewing on all the ropes.

Samples were transported to the South Dakota State University Diagnostic Laboratory and tested using the MagMax Viral RNA extraction kit (Thermo Fisher Scientific) and a real time reverse transcriptase-polymerase chain reaction assay for nucleic acid detection (Tetracore). The cold chain was maintained from collection through testing. All samples were tested individually.

Sequencing of the 5th open reading frame (ORF5) of the genome was conducted and an alignment performed against the reference Ingelvac vaccine strain sequence. The predicted restriction fragment length polymorphisms (RFLP) were also provided.

Data analysis

Data were compiled into spreadsheets using Microsoft Excel version 16.56 (Microsoft Corporation) and analyzed using STATA version 16.1 IC (Stata Corp). The pen prevalence by sample type were compiled over time and compared to pen-level disease classification from the oral fluid results. Further statistical comparisons were not completed due to sample size limitations.

Results

Individual pig samples

Table 1 summarizes the diagnostic results. Serum was PRRSV positive in pens 3E, 1W, and 3W by 3 dpv and 1E became positive on 9 dpv. The seroprevalence numerically increased over time in pens 1W and 3W and remained consistent in 3E. Once seropositive, all pigs remained seropositive for the duration of the study, which was not the case with nasal swabs. Nasal swabs were PRRSV positive starting 6 dpv in pens 1W and 3W. The pigs in these pens remained PRRSV positive for the duration of the study. Individual animal nasal swabs did not consistently remain positive after initially becoming PRRSV positive, but the pen-level nasal swab prevalence increased over time. Nasal swabs were PRRSV negative for the east side of the barn until 24 dpv. On 24 dpv, pens 1E had a 56% (14 of 25) prevalence and pen 3E had a 25.9% (7 of 27) prevalence based on nasal swabs and then fell back to 8% (2 of 25) and 0% (0 of 27) on 27 dpv, respectively. The ORF5 sequencing of 9 samples with the lowest cycle threshold values (< 32.6) from pens 1E (4 samples), 3E (2 samples), and 3W (3 samples) had 100% homology among samples and > 99% homology to the Ingelvac vaccine strain. Serum and nasal swabs from the 2 control pens, 2E and 2W, were PRRSV negative throughout the duration of the study. Sequencing of the ORF5 segment of the genome was performed on virus found in 9 nasal swab samples taken at 24 dpv from pen 1E, 3E, and 3W. The sequences along with the predicted 2-5-2 RFLP confirmed that the PRRSV detected were vaccine strains.

Comparison of individual to pen-level oral fluid samples

The oral fluids from unshared ropes for pen 1W were first PRRSV positive on 6 dpv and remained positive for the duration of the study. The first nasal swab positive with PRRSV collected from pen 1W occurred on 6 dpv. On 3 dpv, 2 serum samples were PRRSV positive in pen 1W. The oral fluid sample collected from shared ropes in pens 1W and 2W was PRRSV positive 50% of the time on 6, 15, and 21 dpv. The oral fluid sample collected from the unshared rope in pen 1W was positive starting 6 dpv through 21 dpv (Table 1). The oral fluid samples collected from the 2 unshared ropes in pen 3W were first positive with PRRSV on 12 dpv and were positive on all remaining days except 18 dpv. The oral fluids sample collected from the shared rope between pen 2W and 3W was not tested on 12 dpv, but samples collected on 15 and 24 dpv were PRRSV positive. The oral fluids from the shared rope were PRRSV positive 2 of 3 times (67%) that the unshared ropes were positive between 15 and 24 dpv. The nasal swabs on 12 dpv gave a pen-level PRRSV prevalence of 11.1%, when 3 days earlier the serum prevalence of the pen was 25.9%. Pen 1E and 1E-2E had a positive oral fluid result on 24 dpv, but no other oral fluids from the east side of the barn were positive.

Discussion

This study indicates that a modified live vaccine can be used as a proxy for infection and provides an effective method to evaluate viral spread in a pen as evidenced by the descriptive data collected and previous literature.[7] The major limitation to this study is the sample size. Further research using a more robust sample size is needed to confirm the results and to provide statistical relevance.

There was a difference in detectability, and potentially the transmissibility, of the vaccine strain between the east and west sides of the room. All conditions, choring procedures, and housing were identical between the east and west sides except the ventilation exhaust fans were present on the west side. The increase in airflow may have created a draft, which could act as an environmental stressor for pigs located in the west pens. This additional stress may have contributed to increased transmission (and hence detection) of the PRRSV seen in the west pens. Airborne transmission of the vaccine virus was not observed as the control pen (2W) remained negative.

The unusually high PRRSV-positivity rate in the nasal swab samples on day 24 dpv suggests contamination or natural infection. Sequencing revealed that the ORF5 sequences and the 2-5-2 RFLP pattern were homologous with the Ingelvac vaccine strain. There were no indications of contamination at the laboratory and the veterinarian and production staff could not identify any unusual stressors among the pigs. It is possible that a contamination event occurred during sampling. On 24 dpv, new supplies and PBS had been purchased and only 1 individual sampled. The PBS was reused on 27 dpv, and no further contamination was noted.

Oral fluid samples are regularly used for diagnostics in the swine industry.[1,2,7] It is common for ropes to be hung between 2 pens, but the impact of this practice on the sensitivity of detection is currently unknown. The results from this study suggest that an oral fluid sample from shared ropes may impact detection when one of the pens is negative. The shared rope between pen 1W and 2W was positive 50% of the time that the unshared rope from pen 1W was positive. The shared rope between pen 2W and 3W was positive only 67% of the time that the unshared ropes were positive. This study also suggests that having more than 1 rope per pen can reduce detection. This may be due to a decreased number of pigs chewing when multiple ropes are present despite increased chewing time,[6] or perhaps the pathogen is greatly diluted when prevalence is lower. The oral fluids from the single unshared rope in pen 1W was positive 6 days prior to the oral fluids from 2 unshared ropes from pen 3W, despite 3W having a higher nasal swab pen-level PRRSV prevalence. Pen sizes and stocking density vary across different production systems, meaning that pens of different sizes may lead to differences in detection rate. Additional research focused on increased sample size, varying pen sizes, and pathogen prevalence are needed to further elucidate the findings of this study.

Although further studies are needed, the preliminary results from this study suggest that oral fluid samples from ropes shared between a positive and negative pen can give inconsistent detection compared to oral fluid samples collected in PRRSV-positive pens from unshared ropes. The oral fluids from the PRRSV-negative pen likely dilute the analyte from the PRRSV-positive pen, decreasing the viral quantity below the limit of detection. The unshared, single rope provided the most consistent detection (Table 1). Given the likely significant health and production costs associated with undetected disease due to a false-negative result and that there is limited scientific guidance on appropriate sample collection methodologies, unshared ropes should be used until evidence shows that oral fluid samples from shared ropes returns a similar sensitivity.

Table 1: Individual and pen-level results from pens with 1 pig inoculated with a modified live PRRSV vaccine, day 0 to 27*

	PRRSV-positive samples/Total samples collected, No. (%)									
Pen	**0 DPV**	**3 DPV**	**6 DPV**	**9 DPV**	**12 DPV**	**15 DPV**	**18 DPV**	**21 DPV**	**24 DPV**	**27 DPV**
Nasal swabs										
1E	0/25 (0.0)	0/26 (0.0)	0 /25 (0)	0/25 (0.0)	0/25 (0.0)	0/25 (0.0)	0 /25 (0.0)	0/25 (0.0)	14/25 (56.0)	2/24 (8.3)
2E	0/27 (0.0)	NA	NA	0/27 (0.0)	NA	NA	NA	NA	NA	0/27 (0.0)
3E	0/27 (0.0)	0/27 (0.0)	0/27 (0)	0/27 (0.0)	0/27 (0.0)	0/27 (0.0)	0/27 (0.0)	0/26 (0.0)	7/27 (25.9)	0/27 (0.0)
1W	0/27 (0.0)	0/27 (0.0)	1/27 (3.7)	2/27 (7.4)	4/27 (14.8)	4/27 (14.8)	2/27 (7.4)	6/26 (23.1)	NA	NA
2W	0/27 (0.0)	NA	NA	0/27 (0.0)	NA	NA	NA	NA	NA	NA
3W	0/27 (0.0)	0/27 (0.0)	3/27 (11.1)	2/27 (7.4)	3/27 (11.1)	3/27 (11.1)	7/27 (25.9)	NA	14/27 (51.9)	NA
Serum										
1E	0/26 (0.0)	0/26 (0.0)	NA	1/25 (4.0)	NA	2/25 (8.0)	NA	1/25 (4.0)	NA	NA
2E	0 /27 (0.0)	NA	NA	0 /27 (0.0)	NA	NA	NA	0 /27 (0.0)	NA	NA
3E	0/27 (0.0)	1/27 (3.7)	NA	1/27 (3.7)	NA	1/27 (3.7)	NA	1/26 (3.8)	NA	NA
1W	0/27 (0.0)	2/27 (7.4)	NA	8/27 (29.6)	NA	11/27 (40.7)	NA	17/26 (65.4)	NA	NA
2W	0/27 (0.0)	NA	NA	0 /27 (0.0)	NA	NA	NA	0/27 (0.0)	NA	NA
3W	0/27 (0.0)	2/27 (7.4)	NA	7/27 (25.9)	NA	11/27 (40.7)	NA	20/27 (74.1)	NA	NA
	Oral fluids qRT-PCR results for PRRSV									
Pen	**0 DPV**	**3 DPV**	**6 DPV**	**9 DPV**	**12 DPV**	**15 DPV**	**18 DPV**	**21 DPV**	**24 DPV**	**27 DPV**
1E	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive	Negative
1E-2E	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive	Negative
2E	NA	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
2E-3E	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
3Ea	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
3Eb	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
1W	Negative	Negative	Positive	Positive	Positive	Positive	Positive	Positive	NA	NA
1W-2W	Negative	Negative	Positive	Negative	Negative	Positive	Negative	Positive	NA	NA
2W	NA	Negative	Negative	Negative	Negative	Negative	Negative	Negative	NA	NA
2W-3W	Negative	Negative	Negative	Negative	Not Tested†	Positive	Negative	Negative	Positive	NA
3Wa	Negative	Negative	Negative	Negative	Positive	Positive	Negative	Positive	Positive	NA
3Wb	Negative	Negative	Negative	Negative	Positive	Positive	Negative	Positive	Positive	NA

* Pen 1E began with 26 pigs, but one died after day 3 from non-PRRSV related causes. Pens with less than 26 pigs tested did not have tubes with labels that could be matched to an animal ID tag or an animal was missed during sampling.

† This sample was collected but compromised, and so was not tested.

PRRSV = porcine reproductive and respiratory syndrome virus; DPV = days post vaccination; NA = samples were not collected or tested.

Implications

Under the conditions of this study:

- Shared rope samples between positive and negative pens may decrease detection.
- Two unshared rope samples per pen may reduce viral detection compared to 1.
- Studies using a more robust sample size are needed to further elucidate results.

Acknowledgments

The National Pork Board provided funding for this study (NPB 21-121). Spronk Brothers provided access to a gilt development unit, and South Dakota State University helped coordinate students and provided feedback on laboratory results when needed.

Conflict of interest

The study site and the pigs involved are a customer of Pipestone Management.

Disclaimer

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