RESEARCH REPORT
SWINE HEALTH

Title: Assessment of sample dilution and number of ropes used for detection of swine pens infected with PRRS early in the course of infection: a pilot study – **NPB #21-121**

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Industry Summary:
This pilot study evaluated the impact of sharing ropes for sample collection between an infected pen and an uninfected pen. Further, it evaluated how the detection of a pathogen changed as the prevalence increased. Three pens of 24 to 27 pigs that were 12 weeks old and approximately 35 pounds at the beginning of the project were used. Pens #1 and #3 were infected pens, and the middle pen (Pen #2) was a control. Hard siding was placed between the pens to prevent direct contact of the pigs. To mimic infection, we used a modified live (MLV) porcine reproductive and respiratory syndrome virus (PRRSV) vaccine (Ingelvac) given intramuscularly in the neck muscle behind the ear. One pig in pen #1 and #3 were vaccinated on day 0. Individual serum and nasal swab samples were collected over a 21-day period along with pen-level oral fluid samples. Pen #1 had a single dedicated rope and a shared rope with Pen #2. Pen #3 had two dedicated ropes and one shared rope with Pen #2. Pen #2 had a dedicated rope as well. This study was done in duplicate. One set of pens successfully transmitted the vaccine strain of the virus showing a linearly increasing prevalence based on serum samples. Nasal swabs were consistently positive, but they did not show a reliably increasing prevalence. The dedicated oral fluid rope sample in Pen #1 had the most consistent and reliable detection, starting at day 6 post vaccination and remaining positive through the study. The shared rope from Pen #1 and #2 was positive on day 6, 15, and 21, but negative on day 9, 12, and 18. Pen #2 and #3 had a similar result, although Pen #3 tested positive later despite having a similar prevalence to Pen #1; it was also negative on day 18 so it had a lesser consistency. The shared rope was positive in only 66.7% of the time points that at least one of the two dedicated oral fluid rope samples from Pen #3 was positive. The other set of pens did not spread the vaccine variant as efficiently. The only difference between the sets of pens was that the side that spread the variant also had a draft form the ventilation exhaust fans, which may have added enough environmental stress to lead to shedding and direct contact transmission. Both control pens remained negative reducing consideration of air born transmission. Shared rope samples likely limit detection of pathogens in low prevalence setting and this may extend to sample pooling as well. Further studies should be done to confirm these results.

Key Findings:

- Oral fluid samples collected by sharing a rope across an infected an uninfected pen did not lead to the detection of a pathogen as readily as pen dedicated ropes.
- Using multiple dedicated ropes (2) in a pen delayed detection compared to the pen with a single dedicated rope.
- PRRSV MLV vaccine can be used to mimic infections in a pen with a mild additional stressor.

Keywords: Oral fluids, sensitivity, pooled samples

Scientific Abstract:
This pilot study compared diagnostic results for the detection of PRRSV between pens using pen-level oral fluids samples and individual animal samples in order to determine if shared samples between infected an uninfected pens limited detection of a pathogen in pens with 24-27 pigs. Two sets of three pens were used to run this study in duplicate. Pens #1 and #3 had one pig vaccinated with the PRRSV modified live vaccine (Ingelvac) intramuscularly in the neck muscle behind the ear. Pen #2 was the control pen. Individual animal samples (serum and nasal swabs) and pen-level oral fluids were collected over a 21 (set #2) to 27 (set #1) day period. Pens #1 and 3 shared ropes with Pen #2 and had one or two designated ropes for oral fluid collection, respectively. The second set of pens successfully transmitted vaccine virus in Pens #1 and #3 showing a linear increase in prevalence in the serum samples starting at 3 days post vaccination (dpv; estimated prevalence of 17.7%) and consistent rates of positive nasal swabs starting on 6 dpv (prevalence of 3.7%). The first set of pens did not successfully transmit the vaccine variant. None of the control pens had positive animals or oral fluid samples. The only difference between the sets of pigs was that second set was on the side of the room where the ventilation exhaust fans were placed, providing a mild environmental stressor to that set of pigs. Oral fluids from shared ropes detected virus at 50% of the detection of the dedicated rope in pen #1 and at 66.7% of the detection of the dedicated ropes in pen #3. Detection in the single dedicated rope sample began at 17.7% seroprevalence and 3.7% nasal swab prevalence (Pen #1) and with two dedicated ropes at 38.6% seroprevalence and 11.1% nasal swab prevalence (Pen #3). Shared rope samples are not as reliable for pathogen detection, particularly in low prevalence settings. Pooling of samples may have a similar result. Setting more than one rope in a pen of 27 animals may also dilute the sample between the two ropes. Further study can confirm and expand upon these findings.

Introduction:
Oral fluid samples have become very popular for diagnostics and monitoring within the swine industry (Kittawornrat et al, 2010; Bjustrom-Kraft et al, 2018). There is not an established protocol for optimal sampling methods based on the expected prevalence in a pen. Many individuals hang ropes between two pens to cover as many animals as possible with a single sample, but without considering the analytic sensitivity impacts. In addition, some practitioners pool oral fluid collected within an airspace into one sample. There is a significant change in diagnostic sensitivity of pooled samples, thus pooling may be inappropriate in low prevalence situations (Lebret et al, 2019; Trevisan et al, 2020).

It has been shown that the sensitivity of oral fluid samples vary by pen size (Prickett et al, 2008; Olsen et al, 2013). It is possible to safely conduct this research as vaccination with the modified live vaccine for PRRSV results in viral shedding that is detectable with PRRSV PCR assays (Olsen et al, 2013). The shed vaccine virus will also infect other in-contact pigs as well (Martínez-Lobo, 2013).

Objectives:

1. Determine if oral fluid samples collected aggregately from a rope hung between a positive and negative pen reduces the diagnostic sensitivity of the sample in detecting the positive pen at different levels of pen prevalence.

2. Determine the prevalence limit of detection for within pen prevalence using a Type 2 (North American) PRRSV modified live vaccine when using one rope per pen, two ropes per pen, or sharing a rope between a positive and negative pen.
3. Determine the agreement between detection of virus in oral fluids, serum, and nasal swabs when a single rope per pen is used, two ropes per pen are used, or a rope is shared between a negative and positive pen?

Materials & Methods:

All pigs used in this study were approximately 12 weeks old, 35 kilograms, and were housed a gilt development unit undergoing repopulation. These were the first 150 pigs reintroduced to the site and they were introduced at the same time, quarantined, held for a five week growth period and then moved to the pens used for this study at a density of 24-27 pigs per pen. The Pipestone Institutional Animal Care and Use Committee approved the protocol (Protocol # 2021-22).

Two sets of three pens were used and the sets of pens were separated by an aisle. Pens in each set were separated using hard siding to prevent direct contact. A pig in pen #1 and #3 was inoculated with a modified live Ingelvac PRRS vaccine per manufacturer's instructions: two milliliters were inoculated into the neck muscle behind the ear. Pen #2 was the control pen for both sets. Barn staff and the study team all worked to prevent exposure of PRRSV by sampling and conducting chores in pen #2 first were sampled first and had chores done first.

Individual pig serum samples were collected on days 0, 3, 9, 15, and 21. Individual nasal swabs were collected on days 0, 3, 5, 7, 9, 12, 15, 18, 21, 24, and 27. Pen #2 was sampled on day 0 (nasal swab and serum), day 9 (nasal swab and serum), day 21 (nasal swabs), and pen #2 in set one had nasal swabs collected on day 27 as well. Pens #1 and #2 in set two were sampled through day 21, Pen #3 in set two also had nasal swabs taken on day 24, and all pens in set one had nasal swabs taken on day 24 and 27 as well. Set one had limited transmission of the vaccine MLVvariant compared to set two pens, and nasal swabs were collected for more days. Pen #3 in set two was sampled on day 24 due to supply shortages on day 21 that limited samples.

Pen-level oral fluids were sampled from all pens on days 0, 3, 5, 9, 12, 15, 18, 21, 24, and 27. Pen #1 in both sets had one unshared oral fluid sample taken and another sample with three of the strands in Pen 1 and three of the strands in Pen 2. The shared rope sample was pooled and submitted as one sample. Pen #3 in both sets had two unshared rope samples taken and a shared sample with Pen 2. Pen #2 in both sets had the shared samples taken at each sampling day and an unshared rope sample taken as well.

All samples were transported on ice to the South Dakota State University Laboratory immediately after sampling and were tested using the laboratory protocols form PRRSV. Sequencing of the ORF5 portion of the genome was also done on nine samples in pens # 1 and #3 in set one and pen #3 in set two. The predicted restriction fragment length polymorphism (RFLP) was returned to ensure all strains detected were vaccine strains when positive results spiked on day 24. The samples were not pooled.

Data was compiled into spreadsheets using Microsoft Excel version 16.56 (Microsoft Corporation, Redmond, Washington) and analyzed using STATA version 16.1 IC (Stata Corp, College Station, Texas) with graphs created in Microsoft Excel version 16.56 (Microsoft Corporation, Redmond, Washington). Prevalence was calculated and estimated from the lines of best fit (serum only) and compared to pen-level disease classification from the oral fluid results. Statistical analysis was not completed due to sample size limitations. Serum prevalence was plotted by day post vaccination and the line of best fit calculated estimate prevalence.

Results:

Table 1 presents data from pens 1W, 2W and 3W, the second set of pens of the two-set experiment. The first set of pens (1E, 2E, and 3E) did not efficiently spread the PRRSV MLV variant. Oral fluids were positive for 1W on day 6. The nasal swabs had a 3.7% prevalence that day and there was an 17.7% estimated seroprevalence. The seroprevalence was estimated based on the line of best fit equation calculated by plotting seroprevalence against days post vaccination. The correlation coefficient was 0.99. The dedicated oral fluid sample from that pen remained positive for the duration of the study (6 sampling days), while the oral fluid sample from the rope shared between pens 1 and 2 (control pen) was positive for only half of the sampling days that the dedicated rope was positive. Pen 3 had two dedicated rope samples, and they became positive on 12 dpv, when the pen had an estimated seroprevalence of 38.6% and nasal swab prevalence of 11.1%. The oral fluid sampled shared between pen 3 and 2 was positive for 66.7% of the days that the dedicated oral fluid sample positives were positive. One shared oral fluid sample was not tested as well.

	Day 0		Day 3		Day 6		Day 9		Day 12		Day 15		Day 18		Day 21		Day 24	
NASAL SWABS																		
Pen	**n**	**# pos (%)**	**n**	**# pos (%)**	**n**	**# pos (%)**	**n**	**# pos (%)**	**n**	**# pos (%)**	**n**	**# pos (%)**	**n**	**# pos (%)**	**n**	**# pos (%)**	**n**	**# pos (%)**
1W	27	Negative	27	Negative	27	Positive	27	Positive	26	Positive	27	Positive	27	Positive	26	Positive		
1W-2W	54	Negative	54	Negative	54	Positive	54	Positive	53	Positive	54	Positive	54	Positive	53	Positive		
2W	27	Negative					26	Negative							27	Negative		
2W-3W	54	Negative	54	Negative	52	Positive	53	Positive	54	Positive	53	Positive	54	Positive			53	13 (24%)
3W	27	Negative	27	Negative	25	Positive	27	Positive	27	Positive	26	Positive	27	Positive			26	13 (50%)
SERUM																		
Pen	**n**	**# pos (%)**	**n**	**# pos (%)**	**n**	**# pos (%)**	**n**	**# pos (%)**	**n**	**# pos (%)**	**n**	**# pos (%)**	**n**	**# pos (%)**	**n**	**# pos (%)**	**n**	**# pos (%)**
1W	27	Negative	27	Positive			27	Positive			27	Positive			26	Positive		
1W-2W	54	Negative	54	Positive			53	Positive			54	Positive			53	Positive		
2W	27	Negative					26	Negative							27	Negative		
2W-3W	54	Negative	54	Positive			53	Positive			53	Positive			54	Positive		
3W	27	Negative	27	Positive			27	Positive			25	Positive			27	Positive		
Pen	**ORAL FLUIDS**																	
1W	--		--		Positive		Positive		Positive		Positive		Positive		Positive		N/A	
1W-2W	--		--		Positive		--		--		Positive		--		Positive		N/A	
2W	--		--		--		--		--		--		--		--		N/A	
2W-3W	--		--		--		--		Not Tested		Positive		--		--		Positive	
3Wa	--		--		--		--		Positive		Positive		--		Positive		Positive	
3Wb	--		--		--		--		Positive		Positive		--		Positive		Positive	

Discussion:

PRRSV MLV was successful as a proxy for infection and transmission on the half of the room where there was a mild environmental stressor, in this case ventilation exhaust. Seroprevalence measures using real-time PCR detection of PRRSV showed a positive linear relationship between prevalence and time. Serum was the most reliable sample type, but it is the most challenging to collect. Use of oral fluids had replaced individual animal samples and has grown in popularity in the swine industry (Bjustrom-Kraft et al, 2018). The methodology for collecting pen-level oral fluid samples has not been examined. Many producers place ropes between pens so that one sample covers more animals. This practice appears to impact detection and may reduce the reliability of the sample based on these preliminary results. Shared ropes to collect oral fluids samples detected PRRSV in pens at half to two-thirds the rate of dedicated pen ropes. A single dedicated rope also resulted in a detection six days earlier than a pen with two dedicated ropes; these pens had similar levels of seroprevalence as well. Sharing of ropes between pens to collect oral fluids samples reduces pathogen detection in low-to-moderate prevalence situations. Pooling of samples may have a similar impact. Using one dedicated rope per pen had the best detection in this pilot study. Further work should be done to verify and expand on these results.

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