American Association of Swine Veterinarians

AASV Foundation Interim Report

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Project Title: Advancing biocontainment strategies through advanced viability qPCR for PRRSv environmental contamination assessment

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Statement of the problem

Porcine reproductive and respiratory syndrome virus (PRRSV) remains a costly disease in the United States (U.S.) (Fraile 2012, Holck & Polson, 2003, Holtkamp, et al., 2013). Annually, 20-30% of the breeding herds enrolled in the Morrison Swine Health Monitoring Project (MSHMP) report a PRRSV outbreak. Furthermore, about 30% of the breeding herds in the MSHMP persist in AASV PRRSV category 1 signifying that groups of PRRSV RT-PCR positive pigs are being weaned, filling growing pig sites which ultimately pose risk to other populations in the region. The regional transmission phenomenon is exemplified by a recent report showing that 44% of the growing pig groups weaned from stable sow herds become PRRSV positive (Angulo et al., 2023). Another study found that 24% of the sites housing near-to-market pigs were RT-PCR positive, reinforcing the hypothesis that PRRS-infected growing pig population pose a risk to neighboring farms (Montoya et al., 2021). A lingering question is why and how these groups of growing pigs are becoming infected.

Biosecurity measures and compliance play a crucial role, especially in an industry where one individual oversees large populations of growing pigs across different sites. This individual moves from site-to-site, ensuring provisions such as feed, water, heat, proper air quality, and addressing issues like dead pig removal. As most growing pig sites lack shower-in/shower-out facilities, there is a probability of fomite contamination and virus dissemination across sites of different PRRSV statuses. Understanding whether frequently touched surfaces by personnel contain viable PRRSV is crucial for raising awareness of the risk and encouraging the industry to reconsider and enforce biocontainment procedures.

Our group at the University of Minnesota has expertise in sampling and detecting swine pathogens in environmental samples to understand indirect transmission (Garrido et al., 2019). In a recent environmental sampling study in PRRSV L1C 1-4-4 positive pig farms, 14% of the samples were RT-PCR positive, but virus isolation was negative, leaving open the possibility that these positive samples contained viable virus (Melini et al., 2022). Experimental data also suggests that variants of this virus can survive ex-vivo for hours and days, emphasizing the potential for viable virus dissemination (Quinonez and Goyal et al.,

Unpublished data). Therefore, a better methodology to understand whether viruses recovered from the environment are viable is essential for assessing risk and reinforcing biocontainment and biosecurity efforts. Recently, a viability qPCR (V-qPCR) assay has been developed for ASFV and PRRSV (V-RT-qPCR) in the Schroeder Lab (Balestreri et al. in review), which provides both a rapid and sensitive (i.e., can detect at least 10 copies of virus genomic material) method to aid the industry to discriminate whether PRRSV or ASFV is still potentially infectious or not. We have already used the V-RT-qPCR to test mitigant efficacy. Similarly, such a tool used in field environmental samples will greatly advance our between and within-farm PRRSV transmission knowledge and play an important role in risk assessments. We propose conducting a project in growing pig farms across different pig production systems to assess the viability of environmentally detected PRRSV on frequently touched surfaces using this novel V-RT-qPCR assay, thus determining the risk of personnel/fomites disseminating the virus from farm to farm.

Objective(s)

- Determine whether viable PRRSV can be detected on frequently touched surfaces by farm personnel.
- Assess whether the level of PRRSV population shedding and the probability of detecting viable PRRSV are related.
- Explore the relationship between standard and viability RT-PCR for PRRSV detection under field conditions.

Brief materials and methods

Study design and eligibility criteria:

This study was designed as an observational cross-sectional study, enrolling a convenient sample of 10 growing pig farms across different pig production systems. Farm eligibility criteria was based on the following factors: 1) Growing pig barns with at least 2,000 pigs per barn, 2) Recent infection of naïve populations with a wild-type PRRSV (i.e., within 2-3 weeks of our visit), 3) Obtained or obtainable PRRSV sequence, and 4) Known PRRSV status of the sow herd.

Sample collection

At each farm, a total of 20 surface samples were collected. Such sample size will allow us to have a 95% probability of detecting at least one positive sample when the proportion of

positive samples is at least 14%. Samples will originate from specific areas of the farm based on a list of sampling areas/surfaces developed in conjunction with a group of AASV practitioners during the onset of the PRRSV L1C.5 (Table 1).

Table 1. Barn surfaces to be sampled to assess whether viable PRRSV can be detected using a viability RT-PCR.

Surfaces	Number of samples
Exterior/interior Doorknobs (main barn entry door)	2
Exterior/interior Doorknobs (pig space entry door)	2
Main entry floor (anteroom either side of bench)	2
Floor either side of pig space door	2
Mortality handling equipment handles	2
Sorting board handles	2
Exhaust fan cone	2
Doorknob loading chute / mortality removal door	2
Pig pen gating/penning	2
2 recently used bottles of injectable medicine	2

Samples were collected using a clean pair of gloves each time. Surfaces were rubbed with a PrimeStore®MTM media-moistened Swiffer pad, placed in a Ziploc bag. Through pressure, the liquid will be extracted from the pad and poured into 20 mL sterile Falcon tubes, refrigerated and transported to the Schroeder Lab for individual PRRSV standard and viability RT-qPCR testing (as described in Balestreri et. al. in review).

Simultaneously, a set of 8 ropes (16 pens) per barn will be hung to collect oral fluids to characterize the level of population shedding in the barn. Samples will be individually tested for PRRSV by RT-PCR.

Significant results

All 10 growing pig farms were visited, and samples were collected during the summer of 2024. All samples have been tested through the screening process (UMN-VDL) and half are currently being tested using the viability RT-PCR (Table 1).

Farms were visited within 3-5 weeks of the outbreak, with at least 50% of the oral fluids collected testing positive, suggesting that these populations were still shedding virus. Interestingly, most of the farms (i.e., 80%) tested positive for the L1C.5 strain, highlighting the importance of this variant in our industry.

The detection of viral particles depended on the test used. Our screening test detected virus particles in 10% of the 200 samples collected. Positive samples originated from 7 of the visited farms. The maximum number of positive samples detected on a given farm was 7 which occurred on 2 farms. The remaining farms had 1 or 2 positive samples. Positive samples originated from pig pen penning housing sick pigs, general pig pen penning, mortality handling equipment, exhaust pit fan cone dust, sorting board handle, and main entry floor close to bench or line of separation.

At the time of writing, only 100 out of the 200 samples had been tested at the Schroeder Lab. Of these, 42% were found to contain viral particles and 10% had detectable viable virus.

Table 1. Summary of PRRSV RT-PCR and viability RT-PCR test results from environmental samples collected in 10 recently infected growing pig farms.

Farm ID	State	First PRRS Positive result	UMN sampling date	Days between samplings	VDL OF RT-PCR Pos/Total (%)	Variant	VDL RT-PCR Pos/Total (%)	Schroeder RT-PCR Pos/Total (%)	Schroeder viability RT-PCR Pos/Total (%)
A1	MN	6/7/2024	6/25/2024	18	8/8 (100%)	1C.5	7/20 (35%)	6/14 (43%)	0/14 (0%)
A2	IA	5/24/2024	6/25/2024	32	6/8 (75%)	1C.5	1/20 (5%)	3/11 (27%)	0/11 (0%)
A3	IA	5/30/2024	6/25/2024	26	7/7 (100%)	1C.5	7/20 (35%)	5/14 (36%)	0/14 (0%)
B1	MN	5/28/2024	6/27/2024	30	5/8 (63%)	1C.5	0/20 (0%)	1/6 (17%)	0/6 (0%)
B2	MN	5/23/2024	6/27/2024	35	7/8 (88%)	1C.5	0/20 (0%)	2/6 (33%)	0/6 (0%)
B3	MN	6/3/2024	6/27/2024	24	7/7 (100%)	1C.5	2/20 (10%)	2/11 (18%)	0/11 (0%)
C1	IA	6/4/2024	7/8/2024	34	7/8 (88%)	1C.5	1/20 (5%)	1/10 (10%)	0/10 (0%)
C2	IA	6/5/2024	7/8/2024	33	4/8 (50%)	1C.5	1/20 (5%)	5/7 (71%)	2/7 (29%)
D1	IA	6/7/2024	7/10/2024	33	8/8 (100%)	1A	1/20 (5%)	6/7 (86%)	3/7 (43%)
D2	IA	6/7/2024	7/10/2024	33	5/8 (63%)	1C	0/20 (0%)	11/14 (79%)	5/14 (36%)
							20/200 (10%)	42/100 (42%)	10/100 (10%)

Discussion of how results can be applied by practitioners

While the probability of detecting PRRS viral particles on the surfaces of PRRS-positive growing pig farms is low, the preliminary results of this study confirm that in some cases, the virus was viable at the time of sampling. These results clearly highlight the importance of reviewing biosecurity procedures for exiting barns, as individuals could become carriers of viable viral particles. Furthermore, personnel barn entry procedures should also be reassessed to ensure staff understand the risks, as viable virus can be present on surfaces.

Practitioners can use the results of this study to raise awareness among farm personnel. Reminding individuals who frequently move between farms of these risks is crucial to reduce the probability of pathogen dissemination.

REFERENCES

1. Angulo, J., Yang, M., Rovira, A., Davies, Torremorell, M. (2023). Infection dynamics and incidence of wild-type porcine reproductive and respiratory syndrome virus in growing pig herds in the U.S.. Prev Vet Med. 217. 105976.
2. Fraile, L. (2012). Control or eradication? Costs and benefits in the case of PRRSV. *Vet Rec*, *170*(9), 223–224.
3. Garrido-Mantilla, J., Alvarez, J., Culhane, M., Nirmala, J., Cano, J.C., Torremorell, M. (2019). Comparison of individual, group and environmental sampling strategies to conduct influenza surveillance in pigs. BMC Veterinary Research. 15(1), 61.
4. Holck, J.T., Polson, D, 2003. The financial impact of PRRS virus.
5. Holtkamp, D. J., Kliebenstein, J. B., & Neumann, E. J. (2013). Assessment of the economic impact of porcine reproductive and respiratory syndrome virus on United States pork producers. *Swine Health and Prod*, *21*(2).
6. Montoya, J., Kikuti, M., Vilalta, C., Sanhueza, J., Corzo, C.A. (2021). PRRSV farm prevalence in near-to-market-pigs in the United States. Poster, AASV conference 2021.
7. Melini, C. M., Kikuti, M., Torremorell, M., Bruner, L., Stahl, C., Roggow, B., Yeske, P., Leuwerke, B., Alerson, M., O'Brien, K., Schwartz, M., Corzo, C.A. (2022). Addressing biocontainment through environmental contamination assessment in farms housing PRRS Lineage 1C 1-4-4 positive pigs. Poster, NAPRRS conference 2022.