**Expanding and validating the PRRSV surveillance sample options for weaning age pigs**

**Introduction: Industry Problem/Opportunity to be addressed:**

Ascertaining the porcine reproductive and respiratory syndrome virus (PRRSV) status of pigs around weaning (18 to 21 days of age) is crucial as these piglets often leave the breeding herds and are shipped to other sites. Thus, a false negative test can have grave consequences, especially in the wake of recent outbreaks with reemerging PRRSV strains.

Efficient monitoring with the reference standard sample (serum) is relatively impractical in low prevalence (requiring about 100 piglets per tested farrowing room when prevalence is below 3%).

Processing fluids are commonly submitted from breeding herds for PRRSV surveillance but do not represent the status of the pigs at the point of weaning. Family oral fluids are obtained around weaning but require that pigs interact with sampling ropes. Other alternative samples such as nasal swabs, buccal swabs, and ear vein blood swabs are also used for PRRSV monitoring and surveillance. However, there are **no guidelines for appropriate sample sizes** for those sample types.

Therefore, it is critical to establish monitoring guidelines for different sample types to increase and validate the producers’ toolbox for PRRSV monitoring and surveillance for weaning age pigs.

At the end of this project, swine practitioners will know the estimated number of any of the sample types mentioned above needed to match the reverse transcription-quantitative polymerase chain reaction (RT-qPCR) probability of detecting PRRSV in serum samples for the same set of epidemiological sampling assumptions (estimated PRRSV prevalence, confidence levels, *etcetera*).

**Preliminary Data:**

The investigators conducted a pilot study using buccal swabs (individually tested and pooled per litter) to compare the probabilities of detecting PRRSV RNA by RT-qPCR in these samples compared to FOF and serum for matched litters. Analyses were done at the litter level (the current project will be primarily at the individual pig level). A litter was considered truly positive if at least one pig within that litter had a positive serum RT-qPCR test. Some results from this study are summarized in the figures below:

Diagram

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*Figure 1: A pilot study demonstrating the probabilities of PRRSV RNA detection in sera, FOF, buccal swabs, and buccal swab pools (pooled by litter) by within-litter PRRSV prevalence. Key takeaways are: FOF and buccal swab pools had similar PRRSV detection rates at the litter level, and individual oral swab tests outperformed both.*

*Table

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*Figures 2 and 3: Two by two tables comparing PRRSV detection rates in buccal swabs and their pools to serum. Cohen’s kappa is a measure of agreement beyond chance; the closer this value gets to 1, the higher the agreement between tests. The sensitivity and specificities of both alternative sample types are also shown*.

From this study we observed that RT-qPCR PRRSV tests on buccal swabs had 62.5% sensitivity in detecting positive litters and had good agreement with serum (from Cohen’s Kappa analysis). The buccal swab pools did not perform nearly as good. There was no false positive test (100% specificity for both sample types).

The data and experiences garnered from this pilot project are valuable in guiding us towards a more refined study with minimal avenues for error, ultimately generating useful data that meet the objectives listed below.

**Objectives:**

The primary objective of this study is to evaluate the diagnostic performance (sensitivity and specificity) of matched ear vein blood swabs, buccal swabs, nasal swabs, and their pools compared to serum in the RT-qPCR detection of PRRSV-2 in weaning age pigs. The research question to be answered is: how many samples are needed for the mentioned alternative sample types to match the same probability of detecting PRRSV as serum samples?

A secondary objective of this study is to assess the agreement of family oral fluids with ear blood swabs, buccal swabs, and nasal swabs (and their pools) concerning the probability of PRRSV RNA detection by RT-qPCR. This aims to investigate the implication of the cost-saving strategy of pooling (per litter) on RT-qPCR detection of PRRSV in these alternative sample types.

**Scientific approach/procedures to achieve these objectives:**

*Study type*: This will be a cross-sectional field study conducted in three commercial breeding herds naturally exposed to wild-type PRRSV-2.

*Eligibility criteria*: PRRSV unstable breeding herds in the Midwestern US will be selected; these herds will have high (n = 1) to low (n = 2) PRRSV prevalence as defined by the modified AASV PRRSV classification of breeding herds (Holtkamp et al., 2021). Simply put, PRRSV-unstable low prevalence (AASV status 1B) breeding herds will have at least 10 of 13 consecutive weekly PRRSV-negative RT-qPCR tests on processing fluids while PRRSV-unstable high prevalence (AASV 1A) will have less than 10 of 13 of such tests. More low prevalence herds were included in the eligibility criteria as these simulate real-world scenarios where viral loads in pigs are expected to be relatively low, and the chances of a false negative RT-qPCR test on an alternative sample type are expected to be at their highest.

*Methodology*:

At eligible farms, farrowing rooms will be selected based on an earlier PCR-positive processing fluids test and will be sampled when piglets are around weaning (18 to 21 days of age). Twenty litters (about 220 piglets) will be sampled for serum, buccal swabs, nasal swabs, and ear vein blood swabs per farm (totaling sixty litters, or about 660 pigs for all three farms). Each sample type will be pooled for each litter; at an expected average of 11 piglets per litter, 60 pools per sample type are expected. Family oral fluids will also be collected for each litter. The sample size of 20 litters provides enough sample size to detect at least 1 positive litter (by FOF) with 95% confidence when PRRSV prevalence is at least 2% (Almeida et al., 2021)

Serum will be aseptically collected via jugular venipuncture using single-use serum separation vacutainer tubes (21G, 1.5” needles) on pigs physically restrained in a supine position.

Ear vein blood swabs will be obtained from physically retrained pigs; manual pressure will be applied to the base of one ear by the restrainer to allow “bulging” of the lateral auricular vein, after a clean wipe of the skin, an 18G needle will then be used to puncture this vein and a polyester swab will thereafter be used to take up some blood seeping from the venipuncture and then transferred to an appropriately labelled tube containing transport medium.

Buccal swabs will be collected from physically restrained pigs using polyester swabs moistened in transport medium; the swab will be rotated for a few seconds as far back behind the tongue as possible. After collection, the swab will be transferred to an appropriately labelled tube containing transport medium.

Nasal swabs will be collected from physically restrained pigs; after the external snout is wiped clean, a polyester swab stick will be rotated deep into the nostrils with minimal force. After collection, the swab will be transferred to an appropriately labelled tube containing transport medium.

Family oral fluids will be collected from each litter; A single-cord cotton rope will be tied to the side rail of a farrowing crate to about the shoulder level of the piglets, the sow and piglets will be allowed to interact with the ropes for about 30 minutes, oral fluids will then be wrung from the chewed ropes into 50 ml Falcon tubes.

All matched samples will be stored on ice and transported to the Iowa State University Veterinary Diagnostic Laboratory for RT-qPCR testing for PRRSV.

*Statistical analysis*: The RT-qPCR results from the ear vein blood swabs, nasal swabs, and buccal swabs will each be compared to those of serum (the reference standard). Sensitivity and specificity for each alternative sample type will then be calculated using 2 x 2 tables on R software (R Core Team, 2019). Adequate sample sizes for effective sampling of each of the alternative sample types for PRRSV monitoring and surveillance will thereafter be proposed from these calculations. The degree of agreement between RT-qPCR tests on family oral fluids with all other sample types (and their pools) will also be assessed using Cohen’s kappa analyses (Dohoo et al., 2009).

*References:*

Almeida, M. N., Zhang, M., Zimmerman, J. J., Holtkamp, D. J., & Linhares, D. C. L. (2021). Finding PRRSV in sow herds: Family oral fluids vs. serum samples from due-to-wean pigs. *Preventive Veterinary Medicine*, *193*, 105397. https://doi.org/10.1016/j.prevetmed.2021.105397

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R Core Team. (2019). *R: A Language and Environment for Statistical Computing: R Foundation for Statistical Computing Vienna, Austria*.

**Expected Outcome or Impact:**

With the results of this study, pork producers will better understand how they can surveil/monitor **effectively** with any of the sample choices and be confident about the PRRSV status of their herds given the results of RT-qPCR tests. This information will also help forestall the significant consequences of the unintended movement of misdiagnosed PRRSV positive pigs to nursery/finishing sites. Results from this study will also highlight the effect of pooling (at the litter level) on the probability of detection, demonstrating possible cost-saving avenues for effective PRRSV surveillance to producers.

The Fieldepi team ([www.fieldepi.org](http://www.fieldepi.org)) at the ISU CVM has worked on and built an open-source easy-to-use application to practitioners calculate sample size for detecting PRRSV. The results from the proposed research will be added to the application which is already live.

A well-revised SOP for collecting each of the alternative sample types during the study will be shared. This SOP will provide the most practical and efficient ways/methods to successfully obtain optimum diagnostic quality alternative sample types from swine herds.

**SAMPLING PLAN FOR JULY/AUGUST**

**Overview**

* We plan to arrive and begin our sampling before 8 AM to improve our chances of obtaining FOF
* The goal is to sample **20 litters**.

**Materials**

* Markers for pigs and sharpies for materials
* Swab sticks (800 per farm) for collecting swabs
* 5 ml Falcon tubes (800 per farm) with PBS for swab sticks post-collection
* 25 50 ml Falcon tubes and Ziploc bags
* Vacutainer tubes and needles (300 per farm)
* Needles for pricking the ear vein of piglets (300 per farm)
* Individual boxes for storing samples collected from each litter.
* Scissors for cutting the swab sticks
* Disinfecting alcohol
* Trash bags

**Work plan**

* Firstly, ropes will be tied across 30 randomly selected farrowing crates.
* We should get at least 20 of those to chew the ropes.
* 20 litters from which FOF is successfully collected will be documented. The 50 ml Falcon tubes containing the collected FOF will be labelled and stored on the same rack.
* The crates that gave successful FOF will then have all the piglets sampled for oral swabs, nasal swabs, ear vein blood swabs, and then serum (preferably in this order). It is advisable for oral swabs to be collected first because if the piglet screams long enough, the mouth will be dry.
  + Oral swabs:
    - With the pig manually restrained, insert the swab stick as far back behind the mouth along the cheek and rotate. If the mouth is dry, please dampen the swab stick in PBS from the 5ml falcon tube you intend to use for the swab stick.
    - Then, store the portion of the swab stick not touched with the hand (by cutting it with scissors or breaking along the weak spot) into the 5ml falcon tube.
    - Tube is labelled as: crate number-piglet number-sample type, e.g. **A5-1-OS**
  + Nasal swabs
    - The restrainer may wish to wrap one hand around the mouth of the pig just behind the snout.
    - insert the swab stick as far back into the nostril as possible and rotate (it is not uncommon for the pig to bleed slightly, no worries!)
    - Then, store the portion of the swab stick not touched with the hand (by cutting it with scissors or breaking along the weak spot) into the 5ml falcon tube.
    - Tube is labelled as: crate number-piglet number-sample type, e.g. **A5-1-N**
  + Ear vein blood swabs
    - The restrainer may apply a slight pressure to the base of the ear
    - A new needle is then used to prick the top of the ear vein.
    - A swab stick is then used to pick some of the blood that seeps out from the tiny wound
    - Then, store the portion of the swab stick not touched with the hand (by cutting it with scissors or breaking along the weak spot) into the 5ml falcon tube
    - Tube is labelled as: crate number-piglet number-sample type, e.g. **A5-1-E**
  + Serum
    - As usual
    - Tube is labelled as: crate number-piglet number-sample type, e.g. **A5-1-S**
* After sampling a piglet for all samples, the pig is then marked clearly on the back to avoid picking it again
* All the above steps are repeated for every piglet within each litter, changing the labeling for the next piglet e.g., **A5-2-E**
* The sampler and restrainer changes gloves for the next litter.
* This continues until all litters are done.