**American Association of Swine Veterinarians (AASV) Foundation**

**Project AASV Grant Application 2023**

**Title**: Assessing the performance of tongue tips as an additional tool to monitor PRRSV in breeding herds undergoing virus elimination

## INVESTIGATORS


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### 1. Statement of the problem

Cost-effective & timely detection of porcine reproductive and respiratory syndrome virus (PRRSV) in herds undergoing virus elimination is an ongoing challenge faced by the swine industry.

Currently, processing fluid (PF) is the most frequently used sample type for PRRSV monitoring in U.S. breeding herds, as it has been shown to have great sensitivity and is easily adopted in the farms, as castration is already a common practice within herds routine.[1, 2] However, when there are unexpected results, such as a decrease in Ct values or persistently PRRSV-positive after too many weeks,[3] veterinarians are posed with questions: where did the virus come from (gestating breeding herd or horizontal infection from other litters?); is the virus widespread in most rooms, or is it concentrated

in a few rooms? Moreover, due to animal welfare and regulations, castration might not be an option in other countries.

Tongue fluids-based sampling from dead pigs was described in 2021 in Spain and is a risk-based approach being implemented in the US.[4] Our initial results in endemically infected herds showed similar PRRSV RNA detection in **tongue fluids (TF)** compared to serum, PF, and family oral fluids.[5] However, more data is needed to understand the practical use of tongue fluids-based sampling in herds undergoing PRRSV elimination.

### 1.1 Value of the research to swine veterinarians

Swine practitioners can establish strategies for PRRSV monitoring using serum, oral fluids, family oral fluids, PF, and/or TF (e.g., a combination of these sample types). Each sampling procedure answers a specific question regarding PRRSV epidemiology. For instance, PF is still the most practical, affordable, and sensitive approach for screening suckling pigs for PRRSV. However, it is directed towards the males and reflects the status of the piglet processing age (i.e., 2-7 days old). While TFs are a risk-based sampling approach since they focus on dead pigs, they can be collected from stillborns, newborns, and other age groups, including males and females. Additionally, TF can characterize the origin of infection (vertical versus horizontal transmission).[6] Therefore, understanding whether PRRSV is being transmitted vertically and/or horizontally is critical to establishing proper and efficient bio-management practices in breeding herds.

## 2. Objective

The objective of this study was to determine the dynamics of PRRSV-RNA detection in TF in breeding herds undergoing PRRSV elimination.

## 3. Material and Methods

*Overview of study design*

We conducted a longitudinal study in three PRRSV-positive breeding herds undergoing PRRSV elimination. Tongue tips from dead piglets of two different age groups (before and after piglet processing, i.e., 0 to 3 days old, and 4 to 21 days old) and PF from surgically castrated ~3-day-old piglets were collected daily. Serum samples from due-to-wean piglets were collected weekly (Table 1). Samples were tested for PRRSV RNA detection by RT-PCR.

The main goal was to compare the positivity of the TF, PF, and serum samples. The PCR results of PF and due-to-wean serum samples were used to establish the farm's PRRSV status per AASV guidelines.[7] The study farms were monitored until promoted to the stability category (AASV status 2), e.g., 13 weeks of PRRSV-negative results in PF and due-to-wean serum.

Table 1. Sampling overview. TF = tongue fluids. PF = processing fluids.

| **Sample Type** | TF-Pre-PF | PF | TF-Post-PF | Serum |
|---|---|---|---|---|
| **Piglets' age** | 0-3 days old dead piglets | 3 days old piglets – Processing age | 4-21 days old dead piglets | 21 days old piglets |
| **Collections' frequency** | Daily | Daily | Daily | Weekly |

*Eligibility criteria*

Three breeding farms were selected based on the following criteria: (a) PRRSV-positive breeding herds that implemented load-close-expose with a modified live vaccine (MLV) or live virus inoculation (LVI) exposure on similar periods with the intent to reach stability status;[7] (b) PRRSV virus exposure up to 25 weeks prior to the study enrollment; (c) producer willing to cooperate by collecting samples as prescribed.

*Sample collection*

Samples were collected by previously trained farm personnel and labeled with age category and collection day. Blood samples were collected from the jugular vein of the due-to-wean piglets, PF was collected from surgically castrated piglets as described by Lopez et al. (2018), and tongue tips (one to two inches each) were collected as described by Machado et al. (2022). The bags with tongue tip samples were frozen at -20°C on the farm before shipping to Iowa State University Veterinary Diagnostic Laboratory (ISU-VDL) for testing. At the ISU laboratory facilities, tongue tips were processed to extract the TF.

*Diagnostic testing*

All samples were tested for PRRSV-RNA detection by RT-qPCR. The breeding herd's production system supported the testing of PF and serum. PFs were tested individually, and serum samples were tested in pools of five. TFs were tested in a weekly pool per age group. The sample was considered positive when the RT-qPCR cycle threshold value (Ct) was lower than 37.

*Statistical Analysis*

Descriptive statistics were performed to report each breeding herd's PRRSV positivity across different sample types using Microsoft Excel®.

For statistical analysis purposes, the herds were simultaneously analyzed as herd-weeks. A mixed-effect logistic regression model (P-value <0.05) was employed to assess PCR positivity, with "Sample type" as the fixed effect and "breeding herd" as the random effect. A non-parametric analysis using the Kruskal-Wallis and Dunn test (P-value <0.05) was employed to assess differences in Ct values per sample type (Pre- and Post-PF and PF). The statistical analysis was performed in R Studio software.[8]

## 4. Results

Six breeding herds (A, B, C, D, E, and F) from the same swine production system located in Nebraska were initially selected for PRRSV screening over five weeks, starting on October 10th, 2022, with daily collection of PF, **TF** from two age groups (Pre- and Post-PF), and weekly collection of due-to-wean piglets (n = 30). Breeding herds with PRRSV-negative results over five weeks were withdrawn from the study, remaining three breeding herds (A, B, and C) with 3,000 to 7,500 sow heads each.

The three selected herds broke with PRRSV 1-8-4 Lineage 1H on different time points: The Breeding herd A on January 1st, 2022; Breeding herd B on March 22nd, 2021; and Breeding herd C on November 15th, 2021. Before the outbreak, the herds were considered PRRSV-stable category II-vx according to the AASV PRRSV classification, i.e., PRRSV-stable with MLV vaccination protocol. Following the outbreak, the system discontinued the MLV protocol, and the breeding herds underwent an LVI exposure and herd closure on April 15th, 2022, to achieve PRRSV-stable status (Table 2). All herds were enrolled in this study on week 24 after the LVI and herd closure.

Table 2. Overall information regarding the PRRSV outbreak and breeding herds characteristics. LVI = live virus inoculation. MLV = modified live virus.

| Breeding herd | Sow heads | Outbreak date | PRRSV strain | LVI post-outbreak | MLV post-LVI | LVI and Herd-closure date |
|---|---|---|---|---|---|---|
| **A** | 7,500 | 1/1/2022 | 1-8-4 L1H | yes | no | 04/15/2022 |
| **B** | 7,500 | 3/22/2021 | 1-8-4 L1H | yes | no | 04/15/2022 |
| **C** | 3,000 | 11/15/2021 | 1-8-4 L1H | yes | no | 04/15/2022 |

### 4.1 Herd-weeks: PF and TF PRRSV-RNA detection

Breeding herd C was withdrawn from the statistical analysis as it had a single positive PF over the project. For breeding herds A and B, 76 herd-weeks were analyzed (Table 3). There was no statistical difference ($P < 0.05$) between TF-Pre-PF, TF-Post PF, and PF for PRRSV-RNA detection, of which TF-Pre-PF was positive over 35 weeks, TF-Post-PF 43 weeks, and PF 33 weeks.

Regarding the Ct value distribution, TF-pre-PF ($P < 0.05$) and TF-post-PF ($P < 0.001$) had significantly lower Ct values than PF. The median Ct value for TF-pre-PF was 25.35, TF-post-PF 24.80, and PF 28.32.

Table 3. Herd-weeks: TF and PF PRRSV-RNA detection results.

| **Sample Type** | TF-Pre-PF | Processing fluids | TF-Post-PF |
|---|---|---|---|
| **RT-qPCR detection** | 35 positive weeks | 33 positive weeks | 43 positive weeks |
| **PCR-positive over a total of weeks** | 46.0% (35/76) | 43.4% (33/76) | 56.5% (43/76) |
| **Median Ct value** | 25.35 | 28.32 | 24.80 |

### 4.2 Descriptive results by breeding herd

*Breeding herd A*

The breeding herd A was followed in the study over 55 weeks. The PF collection occurred over an average of 5.4 times per week (ranging from three to eight samples weekly). The number of tongue tips per daily bag ranged from 9 to 104.

Considering the weeks with sampled samples, the breeding herd A had PRRSV-positive results over 28 weeks in the TF-pre-PF (51.8%), 26 in PF (49.0%), and 31 in TF-post-PF (57.4%). The last PRRSV-positive result for TF-pre-PF occurred on week 58 post-LVI (Ct value = 35.5), followed by TF-post-PF on week 65 (Ct value = 30.5), and PF on week 66 (Ct value = 34.2). The due-to-wean blood collection occurred in the last 13 weeks, as the positive PCR results for TF and PF had higher Ct values and were no longer frequent, with no PRRSV-positive results. The herd reached PRRSV-stable status 79 weeks after the LVI exposure and herd closure, totaling 93 weeks from the initial outbreak until stability.

*Breeding herd B*

The breeding herd B was followed in the study over 21 weeks. The PF collection occurred 7.3 times per week (ranging from four to 20 samples weekly), and the number of tongue tips per daily bag ranged from 2 to 48.

The breeding herd B had PRRSV-positive results at eight weeks in the TF-pre-PF (38.1%), eight in PF (38.1%), 13 in TF-post-PF (61.9%), and ten in due-to-wean serum (50%). Over the seven PF-positive weeks, four had a single positive PF within the week, and the other four weeks had more than two positive results (ranging from two to four positive results).

On week 43 post-LVI, all sample types were PRRSV-positive, and on week 45, breeding herd B was withdrawn from the study, as the herd veterinarian and production system opted for faster stability through depopulation management.

*Breeding herd C*

The breeding herd C was followed in the study over 15 weeks. The herd performed castration protocol one to five days a week, with an average of 2.6 PF samples per week. Moreover, the number of tongue tips per daily bag ranged from 2 to 50.

The herd had a single PCR-positive result in the study, specifically in one of the two PF samples collected in the first week (6.7%), with a Ct of 34.0. No PRRSV RNA was detected in TF or due-to-wean serum. Thus, the herd reached PRRSV-stable status 38 weeks after LVI exposure and herd closure, with a total of 59 weeks from the initial outbreak until stability.

## 5. Discuss the most significant findings and your recommendations

The results from this study demonstrated that **tongue fluids (TF)** can be used as an alternative sample type to monitor breeding herds for PRRSV-RNA detection over

different piglet age categories in breeding herds undergoing PRRSV monitoring programs.

Regarding the collection process, a graduate researcher from Iowa State University went to one of the breeding herds in the swine production system to instruct the farm personnel and herd veterinarian about tongue tip collection with proper Standard Operational Procedure (SOP), which training was replicated to the other breeding herds enrolled in the project. Throughout the study, the daily availability of tongues from dead animals was not reported as an issue. Moreover, the farm personnel reported the collection method as quick and practical. It was not interrupted at any time of the study for such reasons as routine issues, except one week in Breeding herd A due to refrigerator failures, demonstrating the full compliance of the system to collect the new sample type on a routine basis.

Considering the 76 herd-weeks for breeding herds A and B, there was no statistical difference (P-value <0.05) regarding positivities across TF-pre-PF, TF-post PF, and PF samples. In breeding herd B, the only herd with positive due-to-wean serum, the TF-post-PF had higher positive results over the weeks (62%) than due-to-wean serum (50%), PF (35%), and TF-pre-PF (38%). Combining those results, we can conclude that TF is a great tool to assess PRRSV circulation within the farrowing room population.

On the other hand, regarding Ct value distribution, TF-Pre-PF and TF-Post-PF had significantly lower Ct values compared to PF (P-value <0.05), with a difference of up to 3.5 Ct value. TF are aggregate risk-based samples, as they come from multiple dead pigs, and we expected to have a higher viral load within this sample, as dead animals are more likely to harbor PRRSV than live animals.

Unfortunately, in breeding herd B, due to a high PRRSV activity within the farrowing rooms (PF, serum, and TF PCR results) and continuous Ct values below 30, the production system opted for a depopulation protocol on week 45 post-LVI to achieve a negative status.

Conversely, PRRSV RNA was not detected in TF and serum samples in breeding herd C. In this herd, there was a unique PRRSV-RNA detection in the first week of the study in a PF sample from a single day, with a high Ct of 34.0, supporting that the farm was about to reach the stable category by the time it was enrolled in the study.

## 6. Describe how your findings will assist the practicing veterinarians

The results highlighted that veterinarians and pig producers can adopt the TF collection in breeding herd systems for PRRSV monitoring. Also, the dynamic of sampling different age piglets allows the herd veterinarians to intervene early and plan PRRSV control and elimination, as TF can cover all piglet ages.

## 7. State what we can learn from this case, or the methods used to work up this case

This study showed that tongue fluids is a promising sample type for PRRSV detection. The veterinarian should adapt the sampling protocol to benefit the PRRSV monitoring program, i.e., the sample can be collected from different pig ages, rooms, or batches, depending on the research question and budget.

## 8. Itemize the take-home message(s) for the audience

From this study, the take-home messages for the audience were:

A. Tongue fluids (TF) can be used in the PRRSV monitoring program, as they were shown to be an effective population-based sample for PRRSV-RNA detection within the studied population, with its positivity comparable to PF but with higher viral load.
B. TF can be collected at any piglet age (from stillborn to due-to-wean age piglets), broadening the PRRSV monitoring program, as presented in this study with TF-pre-PF and TF-post-PF.
C. TF offers an innovative approach for PRRSV monitoring in breeding herds as an alternative to traditional PF without the need for castration and enhancing animal welfare practices.
D. The dead piglet availability within a breeding herd is not a limitation.

## 9. Project timeline

The project was completed under the proposed timeline.

## References


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