AASV Foundation

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

INTERIM REPORT

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Date Report Submitted: 10/16/2023

Statement of the problem

Cost-effective & timely detection of porcine reproductive and respiratory syndrome virus (PRRSV) in herds undergoing elimination is an ongoing challenge faced by the swine industry. Currently, processing fluids (PF) is the most frequently used sample type for PRRSV monitoring in U.S. breeding herds.[1]

PF-based PRRSV monitoring for RNA detection has been shown to have great sensitivity.[2] However, when there are unexpected results, such as a sharp drop in Ct values or persistently positive after too many weeks,[3] veterinarians are posed with questions: where did the virus come from (gestating breeding herd, or lateral infection from older piglet rooms?); is the virus widespread in most rooms, or is it concentrated in a few rooms?

Tongue tips-based sampling from dead pigs was described in 2019 in Spain and is a relatively new risk-based approach being implemented in the US.[4] Our initial results in endemically infected herds showed similar sensitivity of PRRSV RNA detection in tongue tips as compared to serum, PF, and family oral fluids.[5] However, more data is needed to understand the sensitivity of tongue tip-based sampling in herds undergoing PRRSV elimination.

Objective(s)

The overreaching objective of this proposal is to determine the dynamic of PRRSV-RNA detection in tongue tips fluids in breeding herds undergoing PRRSV elimination.

Methods

Overview of study design
The project is 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 ~3-day-old piglets are being collected daily. Additionally, serum from due-to-wean piglets are being collected weekly (Table 1). Samples are tested for PRRSV RNA by RT-PCR. The main goal is to compare the positivity of the tongue tips and PF-based samplings.

The PCR results of PF and due-to-wean serum samples will be used to establish the farm's PRRSV status as per AASV guidelines.[6] The study farms are monitored to be promoted to the stability category (AASV status 2), e.g., 13 weeks of PRRSV-negative results in PF and weaning-age serum.

Table 1. Sampling overview.

Sample type	Tongue tips	Processing fluids	Tongue tips	Serum samples
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 with the intent to reach negative status;[6] (b) producer willing to cooperate by collecting samples as prescribed.

Sample collection

Samples are being collected by farm personnel previously trained and labeled with age category and collection day. PF is collected as described by Lopez et al. (2018), and tongue tips sampling will follow the methodology described by Machado et al. (2022). Study personnel is available to train farm staff as needed. Dr. Machado visits the farms regularly and demonstrates and audits procedures to obtain, label, store, and ship samples. The samples are frozen at -20°C before shipping to Iowa State University Veterinary Diagnostic Laboratory (ISU-VDL) for testing.

Diagnostic testing

Storage and testing of PF and serum are supported by the breeding herd's production system. PF are being tested individually, and serum samples tested in pools of 5 for PRRSV RNA. Before testing, frozen tongue tips are processed to properly extract the tongue tip exudate, following standard protocols.[5] Thereafter, TTF are tested in a weekly pool per age group for PRRSV-RNA detection through RT-qPCR.

Preliminary results

Six breeding herds (breeding herds A, B, C, D, E, and F) from the same swine production system from Nebraska were initially selected for screening over five weeks, starting on October 10^{th,} 2022, with daily collection of PF, **tongue tip fluids (TTF)** from two age groups (Pre-PF and Post-PF), and weekly collection of weaning age piglet sera (n = 30). Breeding herds with negative results over five weeks were withdrawn from the study due to the PRRSV-negative results, remaining four breeding herds (A, B, C, and

D). Samples were tested for PRRSV RNA detection using RT-qPCR: PFs were individually tested, TTF was tested in a pool of seven (week pool) for each age group category, and weaning age piglet sera were tested in pools of five.

By the time the breeding herds broke with PRRSV, they were considered negative according to the AASV PRRSV classification. Following the outbreak, the breeding herds underwent an MLV and LVI exposure, followed by herd closure in order to achieve stability status for PRRSV (Table 2).

Table 2. Overall information regarding the PRRSV outbreak and breeding herds characteristics.

Breeding herd	Outbreak date	Sow head	MLV	LVI
A	1/1/2022	7500	yes	yes
B	3/22/2021	7500	yes	yes
C	11/2/2020	7500	yes	yes
D	11/8/2020	3000	yes	yes

Breeding herd A

The breeding herd A broke with PRRSV on January 1st, 2022, and is still undergoing the sampling collection until it achieves stability status. Up to now, the herd has been in the study for 49 weeks (Figure 1). Overall, the PF collection occurred over an average of 5.4 times per week (ranging from three to eight samples weekly). Additionally, from week 17 ahead, a change in the study protocol occurred, adding daily tongue tip collection from stillborn piglets separately from the Pre-PF age group collection to verify a possible PRRSV vertical transmission within the herd.

According to the PCR results obtained from the evaluated 49-week period, the breeding herd A had PRRSV-RNA positive results at 27 weeks in PF, nine weeks in the TTF stillborn age group, 27 weeks in the TTF pre-PF age group (considering the TTF stillborn week 25), 29 weeks in the TTF post-PF age group (Figures 1 and 2). Moreover, due to the high PRRSV activity in the first 40 weeks of the monitoring program, the weaning age piglet sera collection took place in the last weeks, as the positive PCR results for TTF and PF had higher CT values and were no longer frequent (Figure 2). The herd veterinarian and the production system will share their results in the next few weeks.

	Sample Type	Weeks in project																							
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
Breeding herd A (part 1)	TTF Stillborn	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	25.1	29.1	27.6	26.5	25.1	33.0	-	-	30.3
	TTF Pre-PF	24.0	26.2	24.5	22.6	24.9	24.0	20.6	23.1	21.4	20.5	21.4	21.1	23.1	24.3	25.7	25.3	23.8	23.3	23.1	26.2	29.0	30.2	24.3	-	-
	TTF Post-PF	20.8	24.1	22.4	25.7	23.0	25.1	22.5	19.4	22.1	18.2	20.7	23.0	23.3	23.8	23.8	23.9	28.2	21.6	27.6	26.0	27.3	25.6	23.7	25.6	29.5
	PF	-	24.4	28.4	22.5	-	28.2	23.1	25.5	26.9	26.9	29.7	26.1	28.1	-	26.9	28.0	29.8	28.1	28.1	30.1	30.4	24.3	27.6	31.7	34.3
	Serum	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x

	Sample Type	Weeks in project																								
		26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50
Breeding herd A (part 2)	TT Stillborn	25.0	-	-	-	-	-	-	-	35.5	-	-	-	-	-	-	-	-	-	-	-	-	x	-	-	
	TT Pre PF	33.9	-	30.3	-	25.5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	x	-	-	
	TT Post PF	28.6	33.6	24.4	-	34.6	-	-	-	-	-	-	-	-	-	-	30.5	-	-	-	-	-	x	-	-	
	PF	30.3	-	32.5	-	-	-	29.6	29.9	-	-	-	-	-	-	-	-	-	34.2	-	-	-	-	-	-	
	Serum	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x							

Figure 1. Breeding herd A PRRSV-RNA detection over time. Green cells = negative PCR results. Red cells = positive PCR results with the average CT value. White cells with an X mark = no sample was collected.

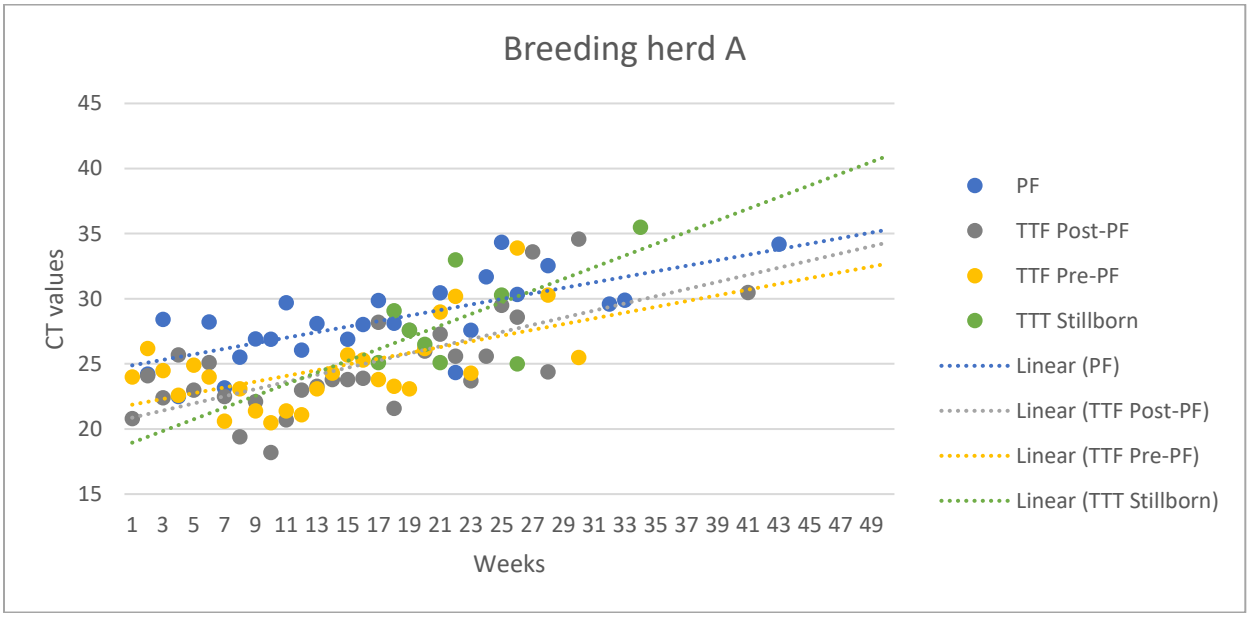

Figure 2. Breeding herd A PRRSV-RNA detection over time.

Breeding herd B

The breeding herd B broke with PRRSV on March 22nd, 2021, and was followed in the study over 21 weeks. Overall, the PF collection occurred over an average of 7.3 times per week (ranging from four to 20 samples weekly). From week 10 to week 15, the system opted to collect PF from sows separately from gilts, resulting in a bigger PF sample size.

Analyzing the results obtained from the evaluated 21-week period, the breeding herd B had PRRSV-RNA positive results at eight weeks in PF, eight weeks in the TTF pre-PF age group, 13 weeks in the TTF post-PF age group, and ten weeks in weaning age piglet sera (Figures 3 and 4). Over the eight positive weeks for PF, 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). Additionally, similarly to breeding herd

A, in week 17, a change in the protocol occurred by adding daily tongue tip collection from stillborn piglets separately from the Pre-PF age group, resulting in four of five weeks of positive results.

The breeding herd B was withdrawn from the study on week 22, as opted by the herd veterinarian and production system, to faster achieve stability.

Breeding herd B	Sample Type	Weeks in project																					
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
	TTF Stillborn	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	-	22.2	-	26.0	22.1	DEPOPULATION
	TTF Pre-PF	-	-	-	35.4	-	-	-	-	-	-	-	29.1	-	31.4	-	35.6	-	32.8	36.5	22.1	19.3	
	TTF Post-PF	-	23.4	23.2	-	-	33.9	-	-	27.1	31.3	-	-	-	26.0	27.9	24.8	26.2	24.2	35.8	22.0	24.8	
	PF	-	37.7	-	-	-	-	27.5	-	-	-	-	-	33.8	29.1	27.8	-	31.1	-	26.2	35.0	x	
	Serum	30.6	-	24.2	-	-	-	31.2	-	21.7	-	25.5	-	-	22.2	26.6	20.5	-	26.4	-	25.5	x	

Figure 3. Breeding herd B PRRSV-RNA detection over time. Green cells = negative PCR results. Red cells = positive PCR results with the average CT value. White cells with an X mark = no sample was collected.

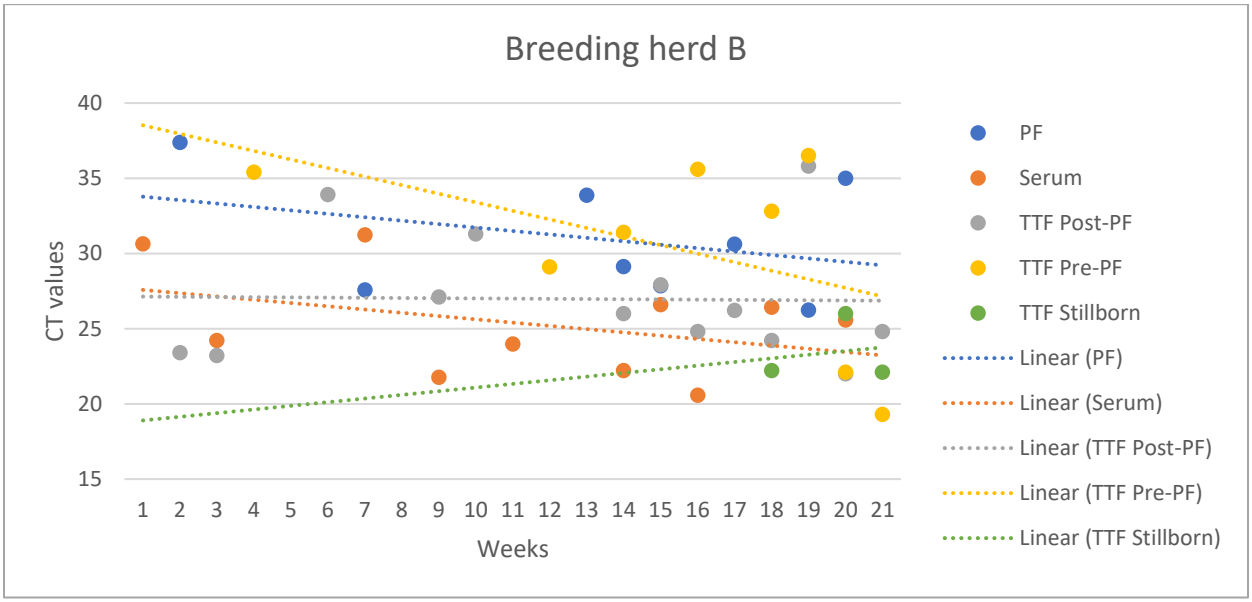

Figure 4. Distribution of PRRSV-RNA detection over time in breeding herd B.

Breeding herd C

The breeding herd C broke with PRRSV on November 2nd, 2020, and was followed over 40 weeks in the study. Overall, the PF collection occurred over an average of 5.6 times per week (ranging from four to eight samples weekly).

Regarding the timeline collection, the herd was monitored for over five weeks, and after all PRRSV-PCR negative results, the herd was withdrawn from the study. However, PRRSV-RNA was detected on PF in week 16, with a single positive day out of seven days of collection (CT = 33.4), followed by another positive result in week 18, with a single positive day out of six days of collection (CT = 29.2). Subsequently, the breeding herd returned to the study. After nine weeks, on week 28, another PRRSV-positive result was detected in a PF on a single day out of five days of collection (CT = 31.9). No

TTF nor weaning age piglet sera detected PRRSV RNA across the study (Figures 5 and 6).

After 40 weeks in the study, breeding herd C was considered stable according to the AASV PRRSV classification, and PRRSV-negative gilts were introduced into the system.

Breeding herd C (part 1)	Sample Type	Weeks in project																			
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
	TTF Pre-PF	-	-	-	-	out	x	x	x	x	x	x	x	x	x	x	x	x	x	x	-	x
	TTF Post-PF	-	-	-	-	out	x	x	x	x	x	x	x	x	x	x	x	x	x	x	-	x
	PF	-	-	-	-	out	-	-	-	-	-	-	-	-	-	-	33.4	-	29.2	-	-	-
	Serum	-	-	-	-	out	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Breeding herd C (part 2)	Sample Type	Weeks in project																			
		22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41
	TTF Pre-PF	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	Gilts in
	TTF Post-PF	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	PF	x	-	-	-	-	x	32.0	-	-	-	-	-	-	-	-	-	-	-	-	
	Serum	-	-	-	-	-	x	-	-	-	-	-	-	-	-	-	-	-	-	-	

Figure 5. Breeding herd C PRRSV-RNA detection over time. Green cells = negative PCR results. Red cells = positive PCR results with the average CT value. White cells with an X mark = no sample was collected.

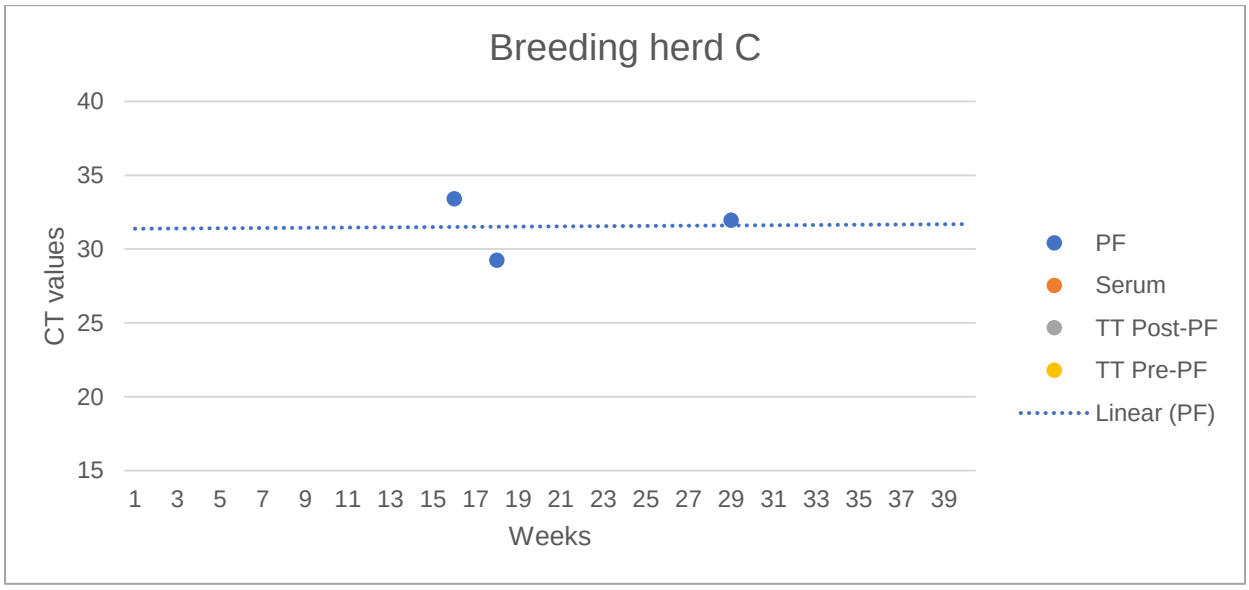

Figure 6. Breeding herd C PRRSV-RNA detection over time.

Breeding herd D

The breeding herd D broke with PRRSV on November 8th, 2020, and was followed in the study over 15 weeks. Although the scope of the study was to measure daily collection for PF, together with TTF, for routine purposes, the breeding herd B performed castration protocol over two or five days in a week due to a small size population (sow heads = 3000), with an average of over 2.6 times a week (ranging from one to five samples weekly).

During the study period, the first week had PCR-positive in one of the two PF samples collected (CT = 34.0). No TTF nor weaning age piglet sera detected PRRSV RNA across the study (Figures 7 and 8).

After 15 weeks in the study, breeding herd D was considered stable according to the AASV PRRSV classification, and PRRSV-negative gilts were introduced into the system.

	Sample Type	Weeks in project															
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Breeding herd D	TTF Pre-PF	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	Gilts in
	TTF Post-PF	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	PF	34.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	Serum	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	

Figure 7. Breeding herd D PRRSV-RNA detection over time. Green cells = negative PCR results. Red cells = positive PCR results with the average CT value.

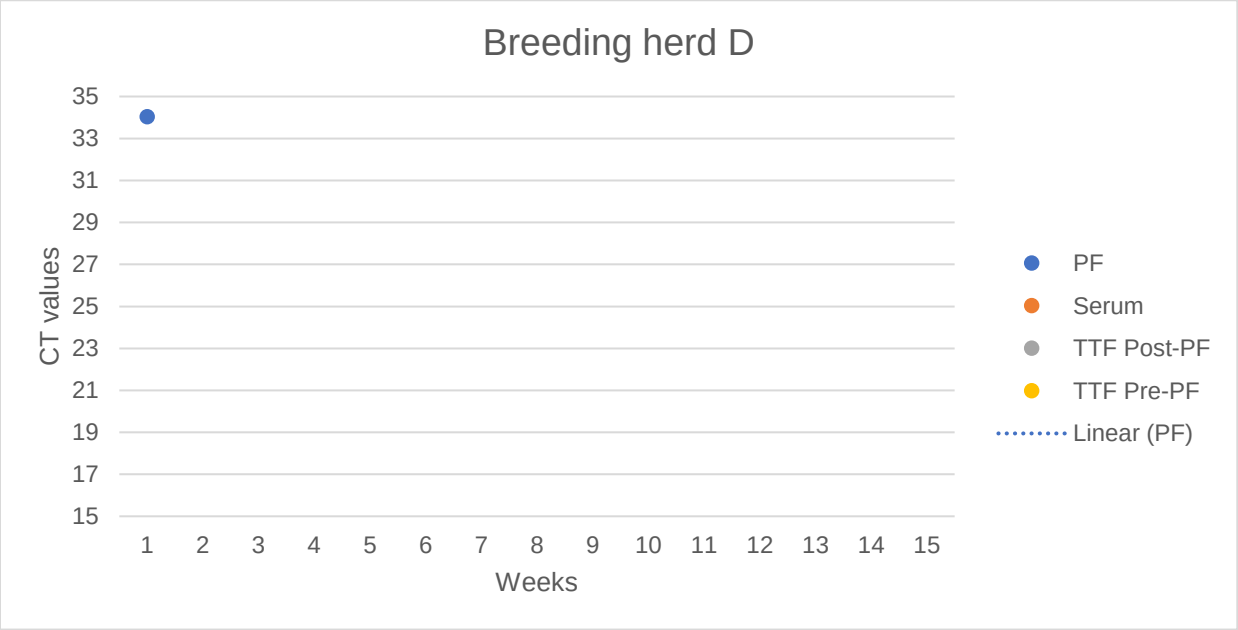

Figure 8. Breeding herd D PRRSV-RNA detection over time.

Discussion (how results can be applied by practitioners)

Preliminary results showed that TTF could be used as an extra sample type to monitor breeding herds for PRRSV-RNA detection over different piglet age categories in breeding herds.

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 the tongue tips collection. Following the training, tongue tip SOPs were shared by the herd veterinarian with all breeding herds enrolled in the study. Throughout the study, the daily availability of tongues from dead animals

was not reported as an issue. Additionally, the collection method was reported as practical and fast by the farm personnel, and it was not interrupted at any time of the study for such reasons as routine issues, demonstrating the full compliance of the system to collect the new sample type on a routine basis.

In breeding herds A and B, the detection rate of the TTF pre-PF group was similar to the processing fluids (55% and 38% positive weeks, respectively). In breeding herd B, the TTF post-PF group had higher positive results over the weeks (61%) compared to weaning age piglet sera (47%). In breeding herds C and D, no weaning-age piglet sera detected PRRSV RNA. It demonstrates that by relying only on the weaning age category to monitor positive unstable IB or stable herds, production systems may not be able to detect PRRSV circulation, decreasing the herd-level sensitivity.

Initially, the goal was to achieve stability following MLV and LVI exposure, followed by herd closure. However, the herd veterinarian and the pig producer aimed to understand if the PRRSV was circulating exclusively among the farrowing room population or if the virus was also coming from the gestation herd. Therefore, a change in the study protocol was performed in breeding herds A and B by collecting daily tongue tips from stillborn piglets separately from the Pre-PF group collection to verify a possible PRRSV vertical transmission. In breeding herd B, after four positive results out of five, together with a higher PRRSV activity by PCR results and continuous CT values in the range of 25 compared to the initial thirteen weeks of the study, the production system opted for a depopulation protocol in order to achieve a negative status more rapidly. Therefore, the breeding herd B was withdrawn from the project on week 22.

Conversely, in breeding herds C and D, PRRSV RNA was not detected in TTF, unlike PF. In breeding herd C, there was a gap in TTF collection from week seven to week 19, which might have negatively influenced the results regarding PRRSV-RNA detection, as two of the three positive PF occurred in the same gap period. The gap period on TTF collection occurred due to the breeding herd being withdrawn from the study, as there was a lack of PRRSV clinical signs and detection according to several previous laboratory tests, as the farm joined the study 101 weeks after the outbreak week. This circumstance demonstrated that the collection of TTF should be followed on a continuous basis collection, with a higher frequency when the breeding herd is reaching stability. In breeding herd D, there was a unique PRRSV-RNA detection in the first week of the study in a PF sample from a single day (CT = 34.03), demonstrating that the farm was about to reach the stable category by the time it joined the study (100 weeks post outbreak).

The results highlighted that veterinarians and pig producers could adopt the TTF collection in breeding herd systems for PRRSV monitoring. Additionally, by collecting TTF from the stillborn piglet category, it could be possible to understand the PRRSV dynamics within the herd, as stillborn piglets reflect the gestation population, in which PF samples may not represent this population, requiring further research focused on this age group category. The dynamic of sampling different age group piglets allows the herd veterinarians to intervene early and plan PRRSV control and elimination.

Project timeline

The project is under the expected timeline. The data analysis and the final report are expected to be completed before March 31, 2024.

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