AASV Foundation

Comparison of a novel rapid tonsil sampling method to serum, oral fluid, and tonsil scraping to detect PRRSV in sows

FINAL REPORT

Peng Li[1], Thomas Petznick[2], Emily Pratt[2], Guilherme Cezar[1], Kelly Will[1], Mafalda Mil-Homens[1], Hao Tong[1], Isadora Machado[1], Daniel Moraes[1], Rodrigo C. Paiva[1], Alexis Berte[1], Henry Osemeke[1], Christine Mainquist[3], Paul Yeske[4], Gustavo S. Silva[1], Daniel C. L. Linhares[1]

[1]College of Veterinary Medicine, ISU, IA; [2]ArkCare, NE; [3]Pillen Family Farms, NE; [4]Swine Vet Center, MN;

Date Report Submitted: 04/30/2024

Statement of the problem

There are several well-established population-based sample types, including processing fluid (PF), family oral fluid (FOF) and tongue tips (TT) to monitor porcine reproductive and respiratory syndrome virus (PRRSV) activity in suckling pigs [1-4]. These samples majorly target the suckling piglet sub-population and thus are not designed to establish PRRSV prevalence in the breeding herd. Undetected PRRSV in the breeding herd poses a great challenge to the success of virus elimination programs. Re-emergence of the same PRRSV strain is usually found in the process of PRRSV elimination after outbreak [5].

Since the sampling approaches in the farrowing may result in not identifying PRRSV-positive sows, an easy and practical tool to directly sample the sows is needed. Commonly used sample types for the sows to detect various pathogens include serum and tonsil scraping [6-8] and, occasionally, oral fluid (OF) [9, 10]. All such samples can be used to detect PRRSV at different prevalence levels and infection status. Tonsil scrapping provides superior sensitivity than serum and other sample types for detecting long-term PRRSV carrier pigs [11]. However, serum and tonsil scraping are time-consuming and labor-intensive for large scale screening purposes, especially in low prevalence scenarios. Moreover, both methods require the restraining of the sows, which causes stress impacting animal welfare. Oral fluid (OF) is often used for population-based sampling purposes [12] and very few reports documented its use in individual sows. One study showed a wide variability in the successful collection rate, ranging from 14.6% to 67.4%.[9]

We developed a novel tonsil oral scrubbing (TOSc) method which collects fluids from the oral cavity and tonsillar area. The method was developed using local supplies (figure 1) by adapting a similar sow sampling method used for the test-and-removal of sows infected with African swine fever virus (ASFV) in China[13]. It takes samples from each sow within seconds with or without snaring. Our preliminary data from an acutely infected farm in Minnesota reported a 100% positivity on TOSc samples, with lower average qPCR Ct values of PRRSV, compared with 73.3% positivity of traditional tonsil scrapings, and 10% positivity of serum for 30 matched sows. The sampling took place 4 weeks after infection. However, this study did not compare OF with TOSc, which is supposed to collect fluid from oral cavity as well. Moreover, as PRRSV detection rate varies among different

sample types and changes over time, whether TOSc shows similar PRRSV detection pattern with other sample types remains unknown.

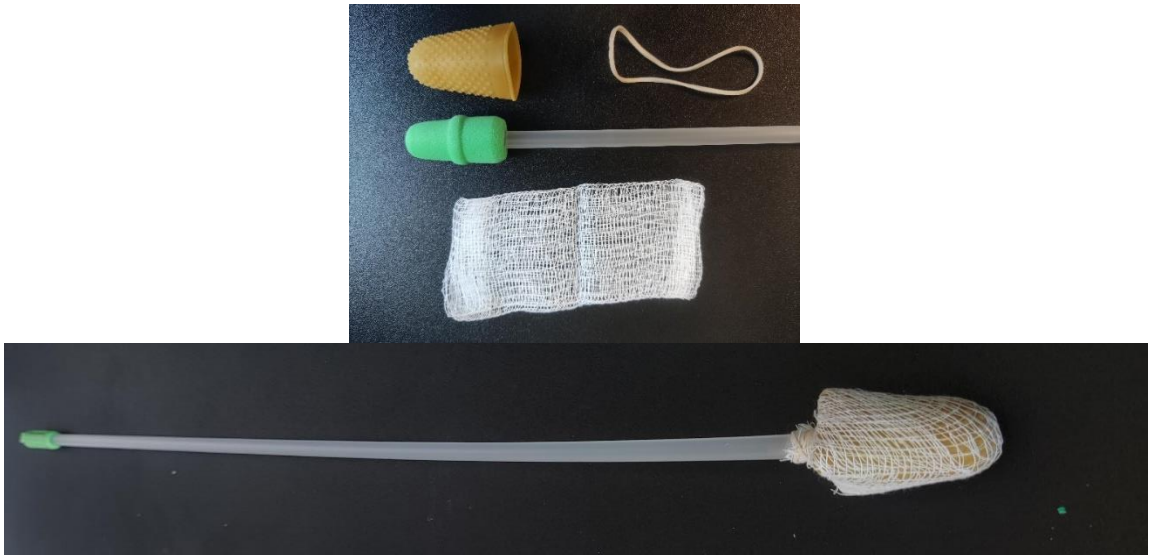

Figure 1. Tonsil-oral scrubbing collector (TOSc) prototype. Overall picture of the TOSc collector (bottom), and specific parts of the collector (up).

Objective

Thus, the objective of this study is to compare the new TOSc sample type to serum, oral fluid and tonsil scraping in terms of probability of PRRSV detection and Ct values in sows at different time points post whole-herd exposure in one farm.

Methods

1. Study design.

This was a prospective field study that followed gestating sows after a herd closure and live virus exposure to eliminate PRRSV. At each collection point the probability of PRRSV detection and Ct values was compared between TOSc, serum, OF, and tonsil scraping. The four samples were taken from each sow. The institutional Animal Care and Use Committee IACUC of Iowa State University, IA approved this study (IACUC-22-101).

2. Materials and methods

Farm selection: A naive herd seeking PRRSV elimination after the outbreak was selected. Whole-herd exposure with live virus inoculation (LVI) was carried out one month before the first sampling point. Sixty-one conveniently selected sows in gestation were identified and sampled for the study period.

Sampling frequency: The sows were sampled at three different time points. Different sample types were collected from each sow in the order of OF, TOSc, tonsil scraping, and serum at 30 days, 60 days and 90 days after whole herd exposure to live virus.

Diagnostic testing: All samples were collected by study collaborators, chilled, and shipped on ice to the Iowa State University Veterinary Diagnostic Laboratory (ISU-VDL) and tested for PRRSV RNA by qPCR. Test results having cycle threshold value (Ct value) <40 were considered PRRSV RNA-positive.

Data analysis: The data were imputed to the R program (R Core Team, 2019). Descriptive statistics was used to report the frequency of PRRSV RNA detection and Ct values by qPCR in each sample type. Tonsil scraping was used as the reference standard for comparison. A logistic mixed regression model was used to assess the difference in the PRRSV RNA detection rate as a function of different sample types, different collection times, and interaction between sample types and collection times using sow ID as a random effect, and the Tukey-Kramer test was used to compare the post hoc pairwise differences in the detection rate between sample types. The Dunn's Test was performed to assess if there was a difference in the Ct values from positive samples among different sample types. All analyses were performed using the package lme4 from R program 4.2.2 (R Core Team, 2019).

Results

1. Comparison of PRRSV RNA detection rate among sample types at 30, 60, and 90 days after LVI.

There was a similar pattern of PRRSV detection rate for TOSc and tonsil scraping, which decreased over time, while that of OF and serum remained relatively flat (table 1).

At 30 days after LVI, tonsil scraping (85.3%) had significantly higher PRRSV RNA positivity than TOSc (66.3%, p=0.045), and both tonsil scraping and TOSc had significantly higher detection rate than OF (8.8%) and serum (13.2%) ($p<0.001$ for all pairwise comparisons). At 60 days after LVI, similarly, tonsil scraping (74.2%) had significantly higher detection rate than TOSc (40.9%) ($p<0.001$), and TOSc had significantly higher detection rate than OF (8.0%) and serum (12.1%) ($p<0.01$ for all pairwise comparisons). At 90 days after LVI, there was a significant difference between TOSc (3.28%) and tonsil scraping (29.5%) (p=0.046), and no significant difference between TOSc and OF (17.86%) or serum (9.84%) ($p>0.05$ for both pairwise comparisons) (table 1).

Table 1. PRRSV RT-rtPCR detection rate among sample types at 30, 60, 90 days after live virus inoculation.

Sample types	PRRSV mean detection rate		
	30 days after LVI	60 days after LVI	90 days after LVI
Tonsil scraping	85.3%	74.2%	29.5%
TOSc	66.3%	40.9%	3.28%
Serum	13.2%	12.1%	9.84%
OF	8.8%	8.0%	17.86%

TOSc, tonsil-oral scrubbing, OF, oral fluid. LVI, live virus inoculation.

2. Comparison of PRRSV RT-rtPCR Ct values from positive samples among sample types at 30, 60, and 90 days after LVI.

Numerically, tonsil scraping and TOSc had lower mean Ct values than OF and serum in RT-rtPCR-positive samples at all sampling points (table 2).

At 30 days after LVI, tonsil scraping showed significantly lower mean Ct values (33.3) than TOSc (35.5, $p<0.01$) and OF (38.9, $p<0.001$). TOSc also showed significantly lower mean Ct values than oral fluid ($p=0.014$). At 60 days after LVI, there was no significant difference between any sample types in terms of Ct values ($p> 0.05$). At 90 days after LVI, the mean Ct values of tonsil scraping (32.7) and TOSc (29.9) were significantly lower ($p<0.01$ for all pairwise comparisons) than oral fluid (36.9) and serum (36.9), while there is no significant difference between tonsil scraping and TOSc, or between OF and serum ($p>0.05$).

Table 2. Ct value mean and range of PRRSV RT-rtPCR-positive samples among sample types at 30, 60, 90 days after live virus inoculation.

	PRRSV RT-rtPCR Ct value average		
Sample type	30 days after LVI	60 days after LVI	90 days after LVI
Tonsil scraping	33.3	34. 7	32.7
TOSc	35.5	33.9	29.9
Serum	35.7	36.0	36.9
OF	38.9	37.9	37.7

TOSc, tonsil-oral scrubbing, OF, oral fluid. LVI, live virus inoculation.

Discussion (how results can be applied by practitioners)

In general, TOSc exhibited a similar PRRSV RNA detection pattern with tonsil scraping, but a distinct detection mode compared with individual OF and serum over time after live virus exposure, when both TOSc and tonsil scraping showed a decreased PRRSV RNA detection rate over time while that for OF and serum remained relatively constant. Moreover, the median Ct values of TOSc and tonsil scraping were numerically lower than OF and serum at each sampling points.

Specifically, tonsil scraping had significantly higher PRRSV RNA detection rate than other sample types, including TOSc at all sampling points, except at 90 days after LVI when there is no significant difference of detection rate between tonsil scraping and OF. This was inconsistent with our previous study in which TOSc showed numerically higher but not statistically different PRRSV RNA detection rate than tonsil scraping in 30 acutely infected sows[13] This was likely because in the current study TOSc was collected when the sows were restrained, and mouth held open to decrease the amount of time required for all samples to reduce stress of each pregnant sow as per the farm management request. However, in the previous study, TOSc were collected when sows were not snared, and they were more likely to chew the TOSc collector and increase the contact

between the collector and tonsillar area than when the sows were restrained, and mouth held open. In another study (data not published), around 90 days after LVI, we collected TOSc from the same 61 sows as in this study, but without snaring them. Results showed that 13 sow TOSc were tested PRRSV RNA positive (21.3%, (10.4%-34.2%)) without being snared as contrast to 2 sows TOSc were tested positive when they are snared (3.28% (0.5-16.9%)) in the current study.

TOSc showed significantly higher detection rate than OF and serum at 30 and 60 days after LVI. This was within expectation because TOSc can collect fluid and cells including immune cells from tonsillar area, and tonsil samples showed extended PRRSV RNA detection until 251 days after live virus inoculation [8]. In contrast, PRRSV RNA was reported to be positive in OF and serum from 8 weeks up to 12 weeks post live virus exposure in sows [14-16]. No significant difference of PRRSV RNA detection rate between OF and tonsil scraping or TOSc at 90 days after LVI might be due to the fact that only 26 OF samples were successfully collected from 61 sows. Similarly, 45 OF were successfully collected from 61 sows at 60 day after LVI. This decreases the sample size of OF for valid comparison. Moreover, OF was normally used as a population-based sample type, which was collected from a group of animals rather than from individual animals [1, 17]. This was consistent with previous report documenting various success rate of OF collection in individual adult pigs [1]. *The ease of collection and similar PRRSV RNA detection pattern with tonsil scraping suggested the potential usefulness of TOSc as a proxy of tonsil scraping for PRRSV surveillance in sow populations.*

Taken together, in this study TOSc had a similar pattern of PRRSV RNA detection than tonsil scraping and higher positivity compared to OF and serum, suggesting the potential use of TOSc as a proxy of tonsil scraping to detect PRRSV in sows. Further studies are needed to characterize the “best practice” of TOSc collection process to improve PRRSV detection rate at different PRRSV infection stages for a better PRRSV surveillance plan in sow population.

Project timeline

The project was performed under the proposed timeline and budget. A draft manuscript was prepared, and the AASV Foundation will be acknowledged for funding. Results of this study will be shared with the swine industry in the form of oral presentations at swine conferences, giving preference to the AASV annual meeting.

References

1. Almeida, M.N., et al., Collecting oral fluid samples from due-to-wean litters. Prev Vet Med, 2020. 174: p. 104810.
2. López, W.A., et al., Practical aspects of PRRSV RNA detection in processing fluids collected in commercial swine farms. Prev Vet Med, 2020. 180: p. 105021.
3. Baliellas, J., et al., Porcine Reproductive and Respiratory Syndrome Surveillance in breeding Herds and Nurseries Using Tongue Tips from Dead Animals. Vet Sci, 2021. 8(11).
4. Machado, I.F., et al., Porcine reproductive and respiratory syndrome virus RNA detection in tongue tips from dead animals. Front Vet Sci, 2022. 9: p. 993442.

5. Daniel C. L. Linhares, Giovani Trevisan, Rodger Main, D.H., Leticia Linhares, Cesar Corzo, Swine Disease Management Information Program # 20-109. 2022.
6. Nielsen, E.O., et al., Use of a novel serum ELISA method and the tonsil-carrier state for evaluation of Mycoplasma hyosynoviae distributions in pig herds with or without clinical arthritis. Vet Microbiol, 2005. 111(1-2): p. 41-50.
7. Tousignant, S.J.P., et al., Longitudinal study of Senecavirus a shedding in sows and piglets on a single United States farm during an outbreak of vesicular disease. BMC Vet Res, 2017. 13(1): p. 277.
8. Wills, R.W., et al., Duration of infection and proportion of pigs persistently infected with porcine reproductive and respiratory syndrome virus. J Clin Microbiol, 2003. 41(1): p. 58-62.
9. Kikuti, M., et al., Emergence of a New Lineage 1C Variant of Porcine Reproductive and Respiratory Syndrome Virus 2 in the United States. Front Vet Sci, 2021. 8: p. 752938.
10. Prickett, J.R., W. Kim, and R. Simer, Oral-fluid samples for surveillance of commercial growing pigs for porcine reproductive and respiratory syndrome virus and porcine circovirus type 2 infections. Journal of Swine Health and Production, 2008. 16(2): p. 86-91.
11. Horter, D.C., et al., Characterization of the carrier state in porcine reproductive and respiratory syndrome virus infection. Vet Microbiol, 2002. 86(3): p. 213-28.
12. Henao-Diaz, A., et al., Understanding and interpreting PRRSV diagnostics in the context of "disease transition stages". Res Vet Sci, 2020. 131: p. 173-176.
13. Li, P., et al., Comparison of a novel rapid sampling method to serum and tonsil scraping to detect PRRSV in acutely infected sows. Prev Vet Med, 2024. 223: p. 106082.
14. Trang, N.T., et al., Detection of porcine reproductive and respiratory syndrome virus in oral fluid from naturally infected pigs in a breeding herd. J Vet Sci, 2014. 15(3): p. 361-7.
15. Woonwong, Y., et al., Oral fluid samples used for PRRSV acclimatization program and sow performance monitoring in endemic PRRS-positive farms. Trop Anim Health Prod, 2018. 50(2): p. 291-298.
16. Batista, L., et al., Assessing the duration of persistence and shedding of porcine reproductive and respiratory syndrome virus in a large population of breeding-age gilts. Can J Vet Res, 2002. 66(3): p. 196-200.
17. Hernandez-Garcia, J., et al., The use of oral fluids to monitor key pathogens in porcine respiratory disease complex. Porcine Health Manag, 2017. 3: p. 7.