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

INTERIM REPORT

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Statement of the problem

There are several well-established population-based sample types such as processing fluid (PF), family oral fluid (FOF), and tongue tips (TT) to monitor PRRSV activity in suckling pigs (Almeida et al., 2020; Baliellas et al., 2021; Lopez et al., 2022; Lopez et al., 2020; Machado et al., 2022; Vilalta et al., 2018). These samples target the suckling piglet sub-population and thus are not designed to establish the true PRRSV prevalence status 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 (Linhares et al., 2014) and some reports even documented PCR-positive processing fluids (PF) and family oral fluid (FOF) after 11 consecutive PCR-negative results (Almeida, 2019).

Since the sampling approaches in farrowing may result in not identifying PRRSV-positive sows, an effective and practical tool to directly sample the sows is needed. Commonly used sample types for sows to detect various pathogens include serum and tonsil scraping (Nielsen et al., 2005; Tousignant et al., 2017; Wills et al., 2003) and occasionally, oral fluid (OF) (Brent Pepin, 2015; Pol et al., 2017). All such samples can be used to detect PRRSV at different prevalence levels and infection status. Tonsil scrapping provides superior herd sensitivity compared to serum and other sample types for detecting long-term PRRSV carrier pigs, most likely due to virus replication in tonsils at this phase of infection (Horter et al., 2002). However, serum and tonsil scraping are time consuming and labor intensive for large screenings, especially in a low prevalence scenario. Moreover, both methods require restraining of the sows, which causes stress impacting animal welfare. Oral fluid (OF) is often used for population-based sampling purposes (Henao-Diaz et al., 2020; Pol et al., 2017) and very few reports documented its use in individual sows, showing a wide variation of success rate, from 14.6% to 67.4% (Brent Pepin, 2015).

We developed a novel tonsil oral scraping (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 (Li, 2022). Samples can be taken from each sow in under one minute, with or without snaring/restraining. Our preliminary

data from an acutely infected farm in Minnesota showed that the TOSc samples had a 100% positivity 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.

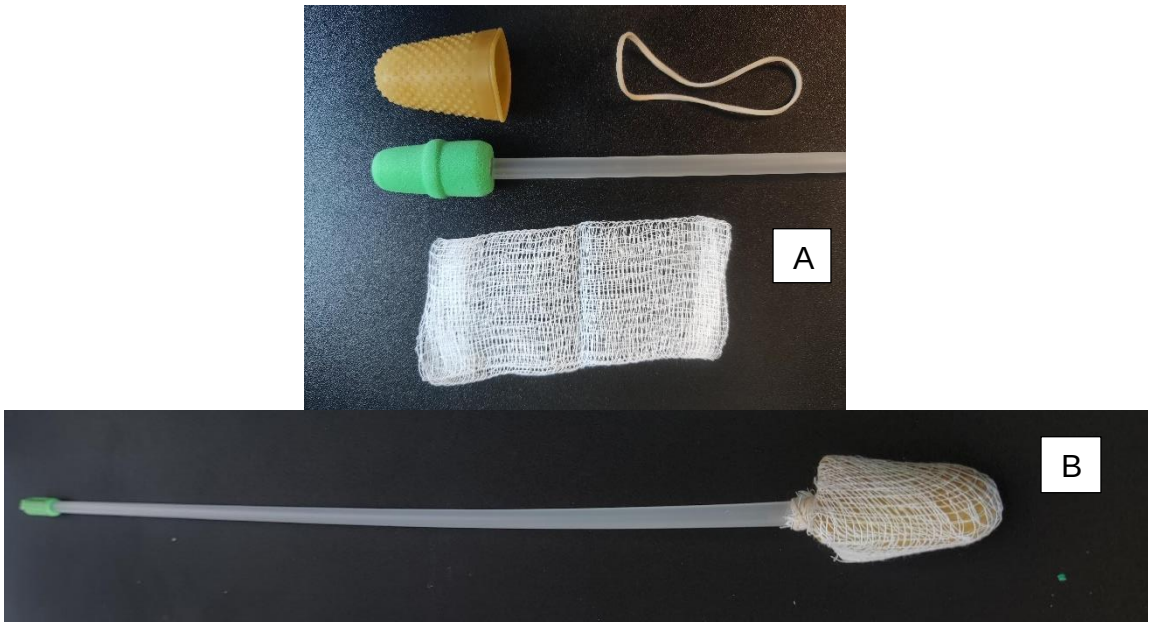

Figure 1. TOSc collector prototype adapted by our group; Figure 1A provides details of the equipment used to create the head of the collector while Figure 1B shows the entire TOSc collector.

Objective(s)

The objective of this study was to compare the new TOSc sample type with serum, oral fluid, and tonsil scrapings in terms of probability of PRRSV detection and Ct values with sows at different time points post whole-herd exposure.

Methods

1. Study design.

This was a prospective study that followed gestating sows after a herd closure and homogenization to eliminate PRRSV. At each collection point the probability of PRRSV detection and Ct values were compared between TOSc, serum, OF, and tonsil scrapings; the four samples were taken from each sow.

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-eight sows were selected in gestation and sampled for the study period.

Sampling frequency: The sows were sampled at three different time points; the four sample types were collected from each sow at one month, two months, and three months 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 were tested at the Research and Development Laboratory for PRRSV RNA by qPCR.

Data analysis: The data were entered into 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 regression model was used to reveal the probability of detecting PRRSV RNA in TOSc, OF, and serum.

Preliminary results

As shown in Table 1, one month following LVI, there was no significant difference in detection rate between TOSc and tonsil scraping, however there was a significant difference between TOSc and oral fluid or serum sample detection rates. Samples collected two months following LVI showed a significant difference in PRRSV RNA detection between TOSc and all other sample types (tonsil scraping, oral fluid, and serum; Table 2). Similar to the results in Table 1, the 3rd month after LVI indicated a significant difference in detection between TOSc and tonsil scraping, however no significant difference between TOSc and oral fluid or serum sample detection rates.

Table 1. Differences in PRRSV detection rate and Ct values by PRRSV RNA qPCR analysis for serum, tonsil scraping, TOSc (tonsil oral scrapings), and oral fluid samples collected at 1 month post-LVI.

1 month post-LVI	Serum	Tonsil Scraping	TOSc	Oral fluid
Detection rate [95% CI]	13.2% [5.9-27.1%][a]	85.3% [71.2-93.2%][b]	66.3% [50.9-78.7%][b]	8.8% [3.3-21.9%][a]
Ct value average and range (lowest-highest Ct)	35.6 [31.4-38.8]	33.4 [26.8-39.9]	35.7 [32.3-39.8]	38.9 [37.6-39.9]

Table 2. Differences in PRRSV detection rate and Ct values by PRRSV RNA qPCR analysis for serum, tonsil scraping, TOSc (tonsil oral scrapings), and oral fluid samples collected at 2 months post-LVI.

2 months post-LVI	Serum	Tonsil Scraping	TOSc	Oral fluid
Detection rate [95% CI]	12.1% [6.2-22.4%]^{a}	74.2% [62.4-83.3%]^{c}	40.9% [29.8-53.1%]^{b}	8.0% [3.0-19.5%]^{a}
Ct value average and range (lowest-highest Ct)	36.0 [30.9-38.9]	34. 7 [28-39.6]	33.9 [30.5-39.8]	37.9 [35.5-39.5]

Table 3. Differences in PRRSV detection rate and Ct values by PRRSV RNA qPCR analysis for serum, tonsil scraping, TOSc (tonsil oral scrapings), and oral fluid samples collected at 3 months post-LVI.

3 months post-LVI	Serum	Tonsil Scraping	TOSc	Oral fluid
Detection rate [95% CI]	9.84% [3.6-24.1%]^{a}	29.5% [17.2-45.7%]^{b}	3.28% [0.5-16.9%]^{a}	17.86% [5.9-42.6%]^{ab}
Ct value average and range (lowest-highest Ct)	36.9 [32.5-39.5]	29.9 [29.7-30.2]	32.7 [25.3-37.1]	37.7 [36.8-39.1]

Discussion (how results can be applied by practitioners)

The PCR results from TOSc samples were not different from tonsil scraping. The PCR positivity of TOSc was significantly and numerically higher than oral fluid and serum at the first month following LVI, which is consistent with our previous findings. However, at the following two timepoints, there was a significant difference between TOSc and tonsil scraping. This is probably due to the fact that TOSc were collected when the sows were restrained and their mouth held open. This change in protocol aimed to meet the farm's requirement to shorten collection process and reduce the stress of the pregnant sows. However, this might reduce abrasion between TOSc and tonsillar area and decrease the amount of tonsillar material collected, thus reducing the detection rate.

As a next step, we will compare the effect of restraining and other factors that might affect the best practice of TOSc sampling.

Project timeline

The project is under the expected timeline and budget. The data analysis and the final report are expected to be completed before April 14, 2024.

References

Almeida. (2019). Update on PRRSV monitoring in suckling pigs. ISU Swine Disease Conference for Swine Practitioners,

Almeida, M. N., Rotto, H., Schneider, P., Robb, C., Zimmerman, J. J., Holtkamp, D. J., Rademacher, C. J., & Linhares, D. C. L. (2020). Collecting oral fluid samples from due-to-wean litters. Prev Vet Med, 174, 104810. https://doi.org/10.1016/j.prevetmed.2019.104810

Baliellas, J., Novell, E., Enric-Tarancon, V., Vilalta, C., & Fraile, L. (2021). Porcine Reproductive and Respiratory Syndrome Surveillance in breeding Herds and Nurseries Using Tongue Tips from Dead Animals. Vet Sci, 8(11). https://doi.org/10.3390/vetsci8110259

Brent Pepin, F. L., Roger Main, Alejandro Ramirez, Jeffrey Zimmerman;. (2015). collection of oral fluid from individually housed sows. Journal of Swine Health and Production,, 23.

Henao-Diaz, A., Gimenez-Lirola, L., Baum, D. H., & Zimmerman, J. (2020). Guidelines for oral fluid-based surveillance of viral pathogens in swine. Porcine Health Manag, 6, 28. https://doi.org/10.1186/s40813-020-00168-w

Horter, D. C., Pogranichniy, R. M., Chang, C. C., Evans, R. B., Yoon, K. J., & Zimmerman, J. J. (2002). Characterization of the carrier state in porcine reproductive and respiratory syndrome virus infection. Vet Microbiol, 86(3), 213-228. https://doi.org/10.1016/s0378-1135(02)00013-5

LI, Z. J. Y. X. L. W. W. P. (2022). Eliminating African Swine Fever Virues in Four Large Sow Herds by New Generation Test-Removal Technology in China from 2018 to 2019. International Pig Veterinary Conference,

Linhares, D. C., Cano, J. P., Torremorell, M., & Morrison, R. B. (2014). Comparison of time to PRRSv-stability and production losses between two exposure programs to control PRRSv in sow herds. Prev Vet Med, 116(1-2), 111-119. https://doi.org/10.1016/j.prevetmed.2014.05.010

Lopez, W., Zimmerman, J., Gauger, P., Harmon, K., Magtoto, R., Bradner, L., Holtkamp, D., Zhang, M., Zhang, J., Ramirez, A., Linhares, D., & Gimenez-Lirola, L. (2022). Considerations in the use of processing fluids for the detection of PRRSV RNA and antibody. J Vet Diagn Invest, 34(5), 859-863. https://doi.org/10.1177/10406387221114855

Lopez, W. A., Zimmerman, J. J., Gauger, P. C., Harmon, K. M., Bradner, L., Zhang, M., Gimenez-Lirola, L., Ramirez, A., Cano, J. P., & Linhares, D. C. L. (2020). Practical aspects of PRRSV RNA detection in processing fluids collected in commercial swine farms. Prev Vet Med, 180, 105021. https://doi.org/10.1016/j.prevetmed.2020.105021

Machado, I. F., Magalhaes, E. S., Poeta Silva, A. P. S., Moraes, D. C. A., Cezar, G., Mil-Homens, M. P., Osemeke, O. H., Paiva, R., Moura, C. A. A., Gauger, P., Trevisan, G., Silva, G. S., & Linhares, D. C. L. (2022). Porcine reproductive and respiratory syndrome virus RNA detection in tongue tips from dead animals. Front Vet Sci, 9, 993442. https://doi.org/10.3389/fvets.2022.993442

Nielsen, E. O., Lauritsen, K. T., Friis, N. F., Enoe, C., Hagedorn-Olsen, T., & Jungersen, G. (2005). 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, 111(1-2), 41-50. https://doi.org/10.1016/j.vetmic.2005.08.009

Pol, F., Dorenlor, V., Eono, F., Eudier, S., Eveno, E., Liegard-Vanhecke, D., Rose, N., & Fablet, C. (2017). Individual and pen-based oral fluid sampling: A welfare-friendly sampling method for group-housed gestating sows. Prev Vet Med, 147, 58-65. https://doi.org/10.1016/j.prevetmed.2017.08.011

Tousignant, S. J. P., Bruner, L., Schwartz, J., Vannucci, F., Rossow, S., & Marthaler, D. G. (2017). 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, 13(1), 277. https://doi.org/10.1186/s12917-017-1172-7

Vilalta, C., Sanhueza, J., Alvarez, J., Murray, D., Torremorell, M., Corzo, C., & Morrison, R. (2018). Use of processing fluids and serum samples to characterize porcine reproductive and respiratory syndrome virus dynamics in 3 day-old pigs. Vet Microbiol, 225, 149-156. https://doi.org/10.1016/j.vetmic.2018.09.006

Wills, R. W., Doster, A. R., Galeota, J. A., Sur, J. H., & Osorio, F. A. (2003). Duration of infection and proportion of pigs persistently infected with porcine reproductive and respiratory syndrome virus. J Clin Microbiol, 41(1), 58-62. https://doi.org/10.1128/JCM.41.1.58-62.2003