RESEARCHREPORT
SWINE HEALTH

Title: "Family oral fluids-based PRRSV monitoring in due-to-wean piglets" NPB #18-191

Investigators: Daniel Linhares and Marcelo Almeida

Institution: Iowa State University

Date Submitted: 10-23-2019

Industry Summary

Processing fluids have been widely used in the US swine industry for PRRSV monitoring in breeding herds. Processing fluids-based testing is an efficient and reliable way to monitor newborn piglets (2-5 days of age). However, the industry still needs a practical and sensitive method to verify the PRRSV status of due-to-wean piglets.

This study evaluated the use of family oral fluids (FOF) sampling for PRRSV detection in due-to-wean piglets, compared to bleeding piglets. FOF is a sample collected by hanging a rope in the farrowing crate where both the sow and respective piglets have access to it. When hung properly, the success rate to obtain the fluids back has been >95%. In other words, you hang 20 ropes and retrieve 19 or 20 FOF. FOF can be submitted to diagnostic laboratories for testing just like a 'regular' oral fluid sample (i.e. kept on ice/refrigerated right after collecting).

This study demonstrated that FOF-based monitoring is a practical and efficient strategy to monitor for PRRSV in due-to-wean piglets (15-21 days old) in breeding herds. Using 10, 15, 20, 30 and 40 FOF samples provided an equivalent probability of PRRSV detection by PCR to testing serum samples from 90, 120, 240 and 400 piglets, respectively. When selecting which litters to hang ropes to collect FOF, results from this study suggest that litters from parity 1 sows, and/or litters with fewer number of piglets were more likely to test positive when the virus is present compared to litters from older sows or litters with a high number of piglets.

In summary, FOF is a new, practical, and efficient sample that can be used for PRRSV monitoring in due-to-wean piglets. It is a great tool to be implemented to verify PRRS status in farrowing rooms before weaning time.

Keywords: PRRSV, surveillance, due-to-wean piglets, family oral fluids, sample size.

Scientific Abstract

PRRSV status of breeding herds is commonly classified using PCR results from 30 due-to-wean piglets. According to that practice, 4 consecutive negative tests 30 days apart would qualify a herd as “PRRS stable”. However, herds have been shown to have PCR-positive piglets even after fulfilling those criteria, which demonstrates the need for better surveillance tools to detect PRRSV in low prevalence scenarios. With that, the objectives of this study were to investigate the use of family oral fluids (FOF) as an alternative for surveillance of breeding herds by a) compare the probability of PRRSV detection by PCR in litters using FOF or individual serum samples, b) assess risk factors associated with a litter being detected as positive for PRRSV, and c) estimate the number of FOF samples necessary to detect PRRSV as a function of prevalence and desired confidence level. Matching samples (FOF and serum from all piglets within a litter) were collected in six breeding herds totaling 2,177 blood samples from individual piglets and 199 FOF samples. All samples were tested by RT-qPCR for the detection of PRRSV RNA at the ISU-VDL. Information about the litter location within farrowing rooms, age, parity of the dam, and the number of piglets per litter was also recorded. Thirty-four litters had at least one viremic piglet (according to serum samples), and 28 litters had PCR-positive results on FOF samples. The overall probability of detection of a positive litter using FOF was 82.4%. Litters with one or two positive piglets had a 50% probability of being detected as positive using FOF, while litters with three or more positive piglets had a probability of being detected as positive using FOF of 97.8%. One of 163 litters was detected as positive in FOF while having all piglets testing negative on serum by PCR, accounting for a specificity of 99.4%. Parity and number of piglets were significant risk factors for a litter to have at least one viremic piglet. The odds ratio for having a litter as positive was 2.82 and 6.13 greater for parity one litters and litters with less than 12 piglets compared to litters coming from older sows, and litters with 12 or more piglets, respectively. Using 5, 7 10, 15, 20, 30 and 40 FOF samples was equivalent to testing serum samples from 30, 60, 90, 120, 240 and 400 piglets, respectively to obtain an equivalent probability of PRRSV detection by PCR. FOF is an aggregate population sample that allows an increase in the number of piglets monitored using fewer samples that are easier to collect, more practical and pig welfare-friendly compared to serum samples, providing the swine industry with an alternative sampling technique.

Keywords: PRRSV, surveillance, due-to-wean piglets, family oral fluids, sample size.

Introduction

In 2011, the American Association of Swine Veterinarians (AASV) PRRS Task Force led by Dr. Derald Holtkamp published a paper outlining procedures for establishing the porcine reproductive and respiratory syndrome virus (PRRSV) status of infected herds and standardizing the nomenclature to describe them (Holtkamp et al., 2011). Widely adopted by producers and veterinarians, the availability of an integrated set of procedures and terminology have facilitated communication concerning PRRSV and the efforts used in its control and/or elimination. Importantly, the original document was conceived of as a "living document" subject to update and improvement, as new information became available. Herein, we provide data suggesting that such a revision is necessary.

In PRRSV elimination/eradication programs based on mass exposure of the sow herd to live virus or modified-live virus vaccine ("load-close-homogenize"), the prevalence of infectious breeding females declines over time as animals clear the infection. Eradication is achieved when all infected animals have cleared the virus or have been removed from the breeding herd. This declining prevalence in the sow herd is mirrored in a decline in PRRSV-infected piglets at weaning. For this reason, breeding herd classification was based on PRRSV RT-PCR testing of serum samples collected from due-to-wean piglets. Thus, a breeding herd was classified as PRRSV "stable" after four consecutive negative tests on due-to-wean piglet sera (n = 30) collected every 30 days (or more frequently) over a 90-day period. A minimum of 30 serum samples was required at each sampling in order to detect the virus when prevalence ≥ 10%.

Inherent in this approach is the assumption that PRRSV cannot persist in the breeding herd at a prevalence of < 10%. However, published data challenges this assumption. For example, 3 of 825 sows (0.4%) were PRRSV PCR positive on serum 15 months after the initiation of a PRRSV test-and-removal elimination project (Dee et al., 2000). In another herd, 1 of 60 sows (1.7%) was found to harbor PRRSV in lymph node by virus isolation and immunohistochemistry in a sampling done two years after the initial PRRSV outbreak, even after a 6-month period of herd closure (Bierk et al., 2001). More recently, a prospective study of 56 herds found that 5 of 56 herds (8.9%) detected a virus that matched the open reading frame-5 (ORF5) sequence of the "original" herd virus after failing to detect PRRSV in pre-weaning piglets over the course of 90 days (Linhares, 2013). Altogether, these studies suggest that PRRSV can sustain low-prevalence infection in breeding herds undergoing virus elimination indicating the need for improved methods to surveil PRRSV in breeding herds.

The collection of oral fluids from pigs was described by Prickett et al. in 2008, and has been well-studied in growing/finishing pigs and adult animals for PRRSV monitoring and surveillance (Prickett et al., 2008a; Prickett et al., 2008b; Kittawornrat et al., 2010; Kittawornrat et al., 2012; Ramirez et al., 2012; Kittawornrat et al., 2013; Olsen et al., 2013; Decorte et al., 2014; Kuiek et al., 2015; Biernacka et al., 2016; De Regge and Cay, 2016; Rotolo et al., 2017). However, there are limited reports on the use of oral fluids do detect PRRSV in suckling piglets with one study showing a very low prevalence of PRRSV (9 positives out of 600 samples tested by RT-qPCR) when collecting oral fluids from due-to-wean litters. Oral fluids have not yet been established as a sample for monitoring due-to-wean piglets, but family oral fluids have been recently described as a way to obtain oral fluids from that population with a high success rate (Almeida et al., 2019 Prev Vet Med). Thus, this study aimed to compare family oral fluids (FOF) to individual piglet serum samples for the detection of PRRSV RNA by rRT-PCR in commercial swine herds.

Objectives

The objectives of this study were to:

A. Measure the probability of detecting PRRSV by rRT-PCR in family oral fluid (FOF) samples versus serum samples in due-to-wean piglets under low PRRSV prevalence (<10%) situations.

B. Assess litter-level risk factors associated with odds of detecting PRRSV in FOF samples by rRT-PCR.

C. Estimate the number of FOF required to detect PRRSV by rRT-PCR in due-to-wean piglet litters, as a function of 95% confidence level and different prevalence levels.

Materials & Methods

Study design: This was a field study conducted in 6 breeding herds undergoing PRRSV elimination with low PRRSv prevalence (<10%) in due-to-wean piglet populations, with the main objective of comparing PRRSv detection in serum vs. family oral fluids (FOF) collected from the same population. Blood was collected from all due-to-wean piglets housed in the farrowing room. Additionally, all due-to-wean litters were exposed to a rope to obtain FOF. Farrowing rooms varied in size from 20 to 56 crates.

Diagnostic testing: All blood serum and FOF were tested by rRT-PCR to detect PRRSv RNA at the Iowa State University Molecular Diagnostic Research and Development Laboratory.

Risk Factors: The following information was collected from each litter for risk factor analysis: parity, number of piglets per litter, mean litter age (days), and piglet gender.

Analyses: Combined results from all sampling events (6 herds) were used to calculate and compare the probability (Proc Probit; SAS Institute Inc., Cary, NC, USA) of PRRSv RNA detection by rRT-PCR using serum and/or FOF samples, given within-litter and between-litter frequency of PRRSv RNA detection in piglet serum samples. Also, the effect of risk factors was assessed for the odds of an individual piglet testing positive and/or FOF testing positive by PRRSv rRT-PCR (Proc Logistic, SAS 9.4; SAS Institute Inc., Cary, NC, USA). Moreover, we combined results from this study with findings from our previous studies to derive monitoring guidelines for the swine industry for the detection of PRRSv RNA when prevalence is < 10%. Simulation-based analyses were performed using a Bayesian latent class analysis based on field data captured in this study combined with results from our preliminary work to construct tables with sampling guidelines, i.e., the number of FOF samples required to detect PRRSv in due-to-wean piglets at a given prevalence levels (1, 3, 5, 10%) with different (e.g. 90, 95, 99%) confidence levels.

Results

Objective A

A total of 2,177 blood samples and 199 FOF samples from the same litters were collected from herds in which PRRSV prevalence was expected to be relatively low (around 10%).

Thirty-four litters were classified as positive according to serum samples (at least one positive piglet within the litter). Of those, 28 were also positive by FOF. The probability of detecting a positive litter by serum using FOF was 82.4%. When there were 3 or more viremic piglets within a litter the probability of detection of those litters with FOF was 97.8%. In one instance a litter that had all piglets testing negative on serum PCR was detected as positive in FOF (1 of 163), a false negative rate of 0.6%, or specificity of 99.4%.

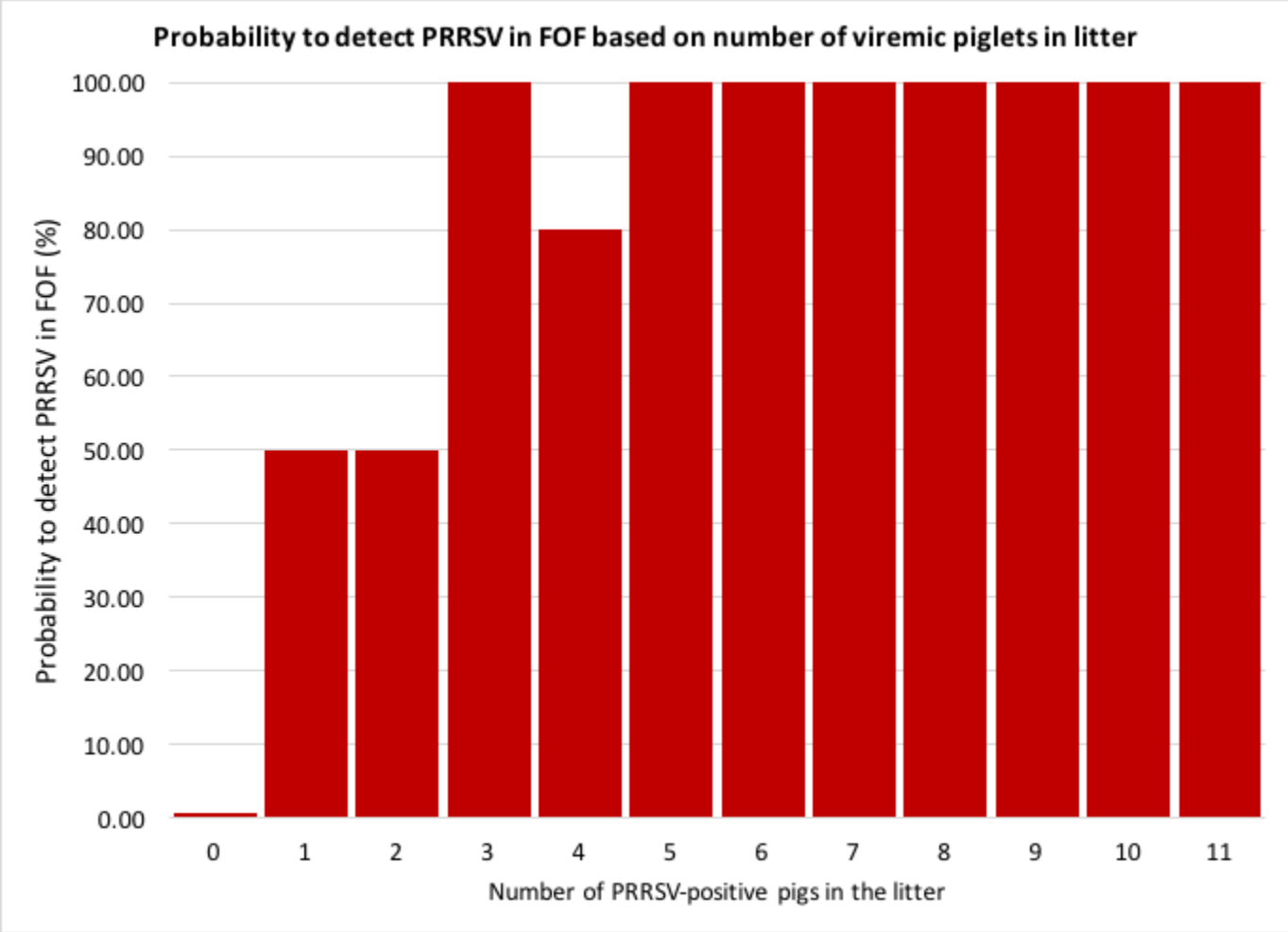

Figure 1. The probability to detect PRRSV in FOF based on the number of viremic piglets in a litter.

Objective B

Thirty-four litters of 199 (17.1%) had at least one viremic piglet. Within those "positive" litters, there were 399 piglets, from which 184 were viremic (46.1%). Overall, 184 piglets were positive from 2177 (8.5%).

Parity information was recorded for 185 litters. From those, 34 were parity one litters, from which 9 (26.5%) had at least one positive piglet within the litter. There were 151 parity ≥ 2 litters, from which 17 (11.3%) had at least one positive piglet within the litter. The odds ratio for having a litter as positive was 2.82 (95% CI: 1.14 – 6.95, P=0.0246) times greater for litters coming from parity one sows compared to litters coming from older sows.

Piglet gender information was recorded for 1,692 piglets in 127 litters. However, only 12 of those litters were positive for PRRSV by PCR on serum which did not allow to perform statistical analysis.

The number of piglets per litter ranged from 3 to 15 in the 199 litters, averaging 11 piglets per litter. For the 34 PCR-positive litters, the number of piglets ranged from 4 to 12, averaging 9.97 piglets per litter, while litters that had no positive piglets ranged from 3 to 15, averaging 11.21 piglets (T-test P=0.0013).

Litters that had < 12 piglets (average 9.2) had an odds ratio 6.13 greater (95% CI 2.27 – 16.52, P=0.003) than litters with ≥ 12 piglets (average 12.9) to have at least one viremic piglet.

One other implication was the observation that when the prevalence was low, PCR-positive piglets were not randomly distributed in the farrowing room, but rather clustered in spatial locations within a room and within in litters (Figure 2).

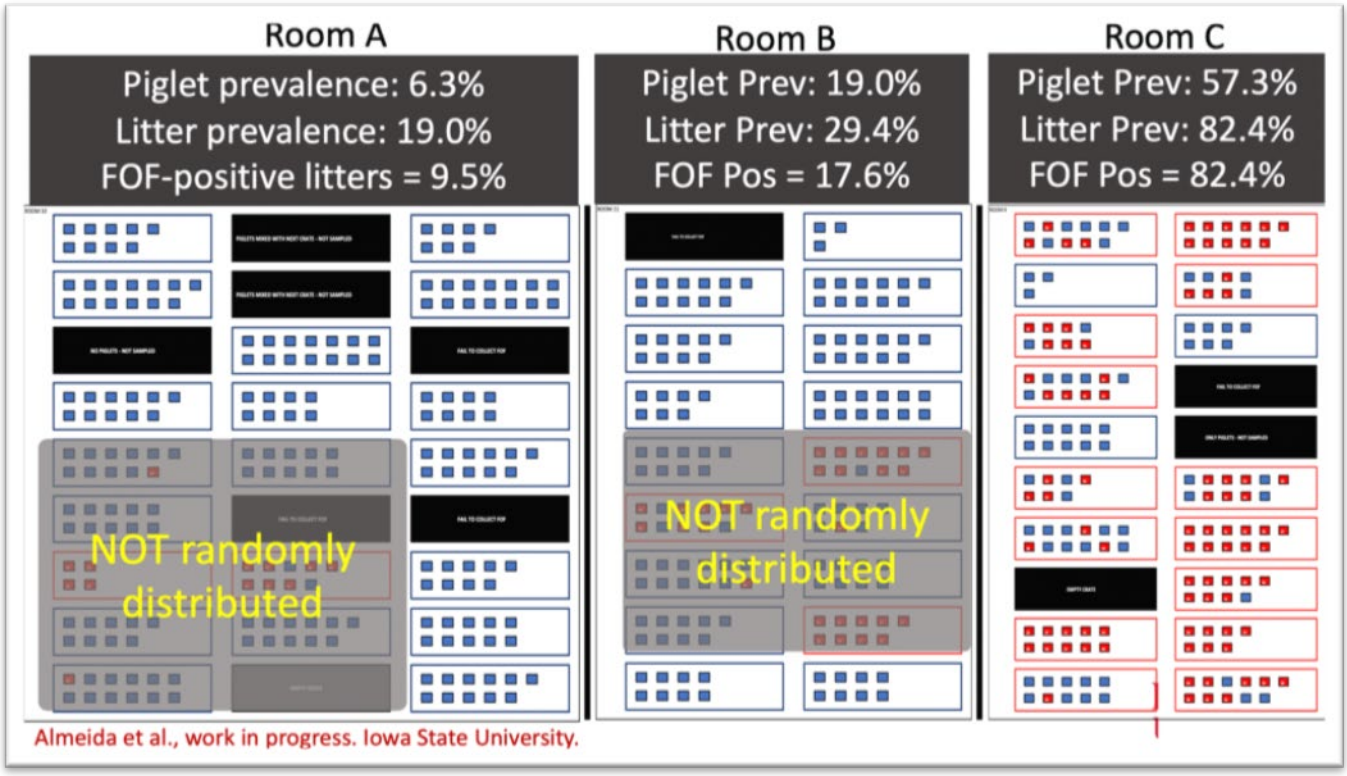

Figure 2. Spatial distribution of positive piglets within farrowing rooms.

Objective C

Using a Bayesian latent class analysis (simulation-based statistics), we were able to estimate the PRRSV prevalence and 95% credible interval for different sample sizes for both FOF and serum samples. Using that method FOF-based monitoring was able to match the probability of PRRSV detection to individual pig serum-based monitoring; however, using a much lower number of samples (Table 1). For example, 10 FOF samples were equivalent to using 90 serum samples.

Table 1. Number of serum and FOF samples and maximum prevalence when all samples are negative.

Prevalence (%)*	# serum samples	# FOF samples
~9	30	5
~5	60	7
~3	90	10
~2	120	15
~1	240	30
~0.5	400	40

* Prevalence cut off to provide 95% confidence to detect at least one PCR-positive sample. For example, when prevalence is above 9%, 30 serum or 5 FOF would provide 95% confidence to detect at least one PCR-positive sample.

Discussion

The overall sensitivity of FOF compared to the status of the litter defined by individual piglet sera testing was 82.4%. This means that sampling a random litter within a farrowing room would give a chance of 82.4% of detecting that litter as positive if it had viremic piglets. More specifically, there was a 50% chance of detection when a litter had 1 or 2 viremic piglets, and 97.8% probability of detection when there were ≥ 3 viremic piglets within a litter. Depending on the size of a farrowing room current individual sampling practices would collect 1-2 piglets per litter. With that in mind, the probability to sample the correct viremic piglet in a litter of 12 piglets with one or two viremic individuals sampling 1 or 2 pigs varies from 8.3% to 33.3%. If all piglets within a litter are sampled individually, and the sensitivity and specificity of the PCR are assumed to be 100%, the probability of detection of such litter would be 100%. However, that is not how surveillance and monitoring of due-to-wean piglets in breeding herds are being done today. FOF is, therefore, an alternative to increase the sample size of piglets for PRRSV monitoring in a practical and welfare friendly way.

It was not possible to perform an extensive risk factor analysis with the dataset accumulated here due to the fact that over 80% of litters were negative for PRRSV, and over 90% of piglets were not detected as positives. However, litters from parity 1 sows and litters with < 12 piglets had greater odds to have at least one viremic piglet compared to litters from older sows and litters with ≥ 12 piglets. Breeding herds that experience an outbreak usually also experience increased pre-weaning mortality rate. Additionally, Jean Paul Cano demonstrated that piglets in parity 1 litters had a higher probability to have PCR-positive results.

Finally, using the field data collected in this study we were able to run simulation analysis using a Bayesian approach to estimate the sample size requirement for FOF compared to individual pig sera o detect PRRSV in due-to wean piglet in a low prevalence scenario. In that regard, testing 5, 7, 10, 15, 30 and 40 FOF samples was equivalent to testing 30, 60, 90, 120, 240 and 400 individual piglet sera. These results provide guidelines for the use of FOF samples for surveillance of due-to-wean litters as an alternative to the traditional individual piglet sampling in a more practical, welfare-friendly way allowing for an increased sample size across the population and higher sensitivity compared to the current industry common practice of sampling 30 piglets at weaning.

References

Bierk, M., Dee, S., Rossow, K., Collins, J., Guedes, M., Pijoan, C., Molitor, T., 2001. Diagnostic investigation of chronic porcine reproductive and respiratory syndrome virus in a breeding herd of pigs (vol 148, pg 687, 2001). Veterinary Record 149, 90-90.

Biernacka, K., Karbowiak, P., Wróbel, P., Charęza, T., Czopowicz, M., Balka, G., Goodell, C., Rauh, R., Stadejek, T., 2016. Detection of porcine reproductive and respiratory syndrome virus (PRRSV) and influenza A virus (IAV) in oral fluid of pigs. Res Vet Sci 109, 74-80.

De Regge, N., Cay, B., 2016. Comparison of PRRSV Nucleic Acid and Antibody Detection in Pen-Based Oral Fluid and Individual Serum Samples in Three Different Age Categories of Post-Weaning Pigs from Endemically Infected Farms. PLoS One 11, e0166300.

Decorte, I., Van Breedam, W., Van der Stede, Y., Nauwynck, H.J., De Regge, N., Cay, A.B., 2014. Detection of total and PRRSV-specific antibodies in oral fluids collected with different rope types from PRRSV-vaccinated and experimentally infected pigs. BMC Vet Res 10, 134.

Dee, S.A., Molitor, T.W., Rossow, K.D., 2000. Epidemiological and diagnostic observations following the elimination of porcine reproductive and respiratory syndrome virus from a breeding herd of pigs by the test and removal protocol. The Veterinary record 146, 211-213.

Holtkamp, D., Polson, D., Torremorell, M., Morrison, R., Classen, D., Becton, L., Henry, S., Rodibaugh, M.T., Rowland, R.R., Snelson, H., Straw, B., Yeske, P., Zimmerman, J., 2011. Terminology for classifying swine herds by porcine reproductive and respiratory syndrome virus status. J Swine Health Prod 19, 44-56.

Kittawornrat, A., Engle, M., Panyasing, Y., Olsen, C., Schwartz, K., Rice, A., Lizano, S., Wang, C., Zimmerman, J., 2013. Kinetics of the porcine reproductive and respiratory syndrome virus (PRRSV) humoral immune response in swine serum and oral fluids collected from individual boars. BMC Vet Res 9, 61.

Kittawornrat, A., Prickett, J., Chittick, W., Wang, C., Engle, M., Johnson, J., Patnayak, D., Schwartz, T., Whitney, D., Olsen, C., Schwartz, K., Zimmerman, J., 2010. Porcine reproductive and respiratory syndrome virus (PRRSV) in serum and oral fluid samples from individual boars: Will oral fluid replace serum for PRRSV surveillance? Virus research 154, 170-176.

Kittawornrat, A., Prickett, J., Wang, C., Olsen, C., Irwin, C., Panyasing, Y., Ballagi, A., Rice, A., Main, R., Johnson, J., Rademacher, C., Hoogland, M., Rowland, R., Zimmerman, J., 2012. Detection of Porcine reproductive and respiratory syndrome virus (PRRSV) antibodies in oral fluid specimens using a commercial PRRSV serum antibody enzyme-linked immunosorbent assay. J Vet Diagn Invest 24, 262-269.

Kuiek, A.M., Ooi, P.T., Yong, C.K., Ng, C.F., 2015. Comparison of serum and oral fluid antibody responses after vaccination with a modified live (MLV) porcine reproductive and respiratory syndrome virus (PPRSV) vaccine in PRRS endemic farms. Trop Anim Health Prod 47, 1337-1342.

Linhares, D., 2013. Evaluation of Immune Management Strategies to Control and Eliminate Porcine Reproductive and Respiratory Syndrome Virus (PRRSv). University of Minnesota, Saint Paul, MN, 138.

Olsen, C., Wang, C., Christopher-Hennings, J., Doolittle, K., Harmon, K.M., Abate, S., Kittawornrat, A., Lizano, S., Main, R., Nelson, E.A., Otterson, T., Panyasing, Y., Rademacher, C., Rauh, R., Shah, R., Zimmerman, J., 2013. Probability of detecting Porcine reproductive and respiratory syndrome virus infection using pen-based swine oral fluid specimens as a function of within-pen prevalence. J Vet Diagn Invest 25, 328-335.

Prickett, J., Simer, R., Christopher-Hennings, J., Yoon, K.J., Evans, R.B., Zimmerman, J.J., 2008a. Detection of Porcine reproductive and respiratory syndrome virus infection in porcine oral fluid samples: a longitudinal study under experimental conditions. J Vet Diagn Invest 20, 156-163.

Prickett, J.R., Kim, W., Simer, R., Yoon, K.-J., Zimmerman, J., 2008b. 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 16, 86-91.

Ramirez, A., Wang, C., Prickett, J.R., Pogranichniy, R., Yoon, K.J., Main, R., Johnson, J.K., Rademacher, C., Hoogland, M., Hoffmann, P., Kurtz, A., Kurtz, E., Zimmerman, J., 2012. Efficient surveillance of pig populations using oral fluids. Prev Vet Med 104, 292-300.

Rotolo, M.L., Sun, Y., Wang, C., Giménez-Lirola, L., Baum, D.H., Gauger, P.C., Harmon, K.M., Hoogland, M., Main, R., Zimmerman, J.J., 2017. Sampling guidelines for oral fluid-based surveys of group-housed animals. Vet Microbiol.