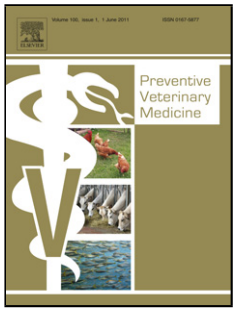

Collecting oral fluid samples from due-to-wean litters

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Collecting oral fluid samples from due-to-wean litters

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Abstract:

Oral fluids are a common diagnostic sample in group-housed nursery, grow-finish, and adult swine. Although oral fluids from due-to-wean litters could be a valuable tool in monitoring pathogens and predicting the health status of pig populations post-weaning, it is generally not done because of inconsistent success in sample collection. The objective of this study was to determine the optimum procedure for collecting oral fluid samples from due-to-wean litters. Successful collection of oral fluids from due-to-wean litters using "Litter Oral Fluid" (LOF) or "Family Oral Fluid" (FOF) sampling techniques were compared in 4 phases involving 920 attempts to collect oral fluids. Phase 1 testing showed that prior exposure to a rope improved the success rates of both LOF (33.4%) and FOF (16.4%) techniques. Phase 2 determined that longer access to the rope (4 hours vs 30 minutes) did not improve the success rate for either LOF or FOF. Phase 3 evaluated the effect of attractants and found that one (Baby Pig Restart®) improved the success rate when used with the FOF technique. Phase 4 compared the success rates of "optimized LOF" (litters previously trained) vs "optimized FOF" (litter previously trained and rope treated with Baby Pig Restart®) vs standard FOF. No difference was found between the FOF-based techniques, but both were superior to the "optimized LOF" technique. Thus, FOF-based procedures provided a significantly higher probability of collecting oral fluids from due-to-wean litters (mean success rate 84.9%, range 70% to 92%) when compared to LOF-based methods (mean success rate 24.1%, range 16.5% to 32.2%).

Keywords: Swine, oral fluids, surveillance.

Introduction

Oral fluids samples offer advantages over serum from individual pigs for surveillance of swine populations: (1) they are easily collected by a single person; (2) they can be obtained without stress or risk to pigs or people; (3) at the population level, they provide a higher probability of detection than an equal number of serum samples, and (4) they can be used to screen populations for a variety of pathogens using nucleic acid-based or antibody-based testing (Prickett et al., 2008a; Prickett et al., 2008b; Kittawornrat et al., 2010b; Detmer et al., 2011; Prickett et al., 2011; Kittawornrat et al., 2012; Romagosa et al., 2012; Giménez-Lirola et al., 2013; Goodell et al., 2013; Mur et al., 2013; Panyasing et al., 2013; Vosloo et al., 2015; Bjustrom-Kraft et al., 2016; Giménez-Lirola et al., 2016; Panyasing et al., 2016b). For these reasons, testing of oral fluids has been used extensively in group-housed growing pigs and adult animals (boars and gilts/sows). Although the collection of oral fluids from due-to-wean litters, i.e., litters within two days of weaning, would allow producers to improve surveillance in this age group and anticipate postweaning infectious disease challenges, a practical technique for collecting oral fluids from suckling piglets has not been described (Kittawornrat et al., 2014; Panyasing et al., 2016a). Thus, the objective of this study was to identify the optimum procedure for collecting oral fluids from due-to-wean litters.

Material and methods

Study design

Specific variables were evaluated for their impact on the success of 'litter oral fluid' (LOF) and 'family oral fluid' (FOF) collections in due-to-wean litters (Figure 1). LOF samples were collected by hanging a cotton rope in the farrowing crate so that the piglets, but not the dam, had access.

FOF samples were collected by hanging the rope in a position that allowed access to both the sow and her piglets. For both LOF and FOF, the rope was hung between 6:00 am and 7:00 am, with three unravelled strands hanging one inch from the floor for ≥ 30 minutes, after which oral fluids were harvested by grasping the rope and pulling it from a gallon plastic bag through clenched fingers. Fluids that pooled in the bag were then poured into a 50-mL conical centrifuge tubes (Fisher Scientific, Pittsburgh, PA, USA) for transport and storage. Variables recorded for each sampling included oral fluid sample volume, litter age, sow parity, and the number of piglets within the litter.

The study was divided into 4 phases and consisted of a total of 920 attempts to collect oral fluids attempts from due-to-wean litters in a 5,500 breed-to-wean sow farm. Treatments included prior exposure (Phase 1), duration of rope exposure (Phase 2), and the use of attractants (Phase 3). Phase 4 consisted of a comparison of the optimized LOF vs. optimized FOF vs. standard FOF (SFOF). In each phase, treatments were evaluated and compared in terms of their impact on LOF and FOF success rates. A successful collection was defined as a sample containing ≥ 1 ml of fluid. The study was conducted with the approval of the Iowa State University Institutional Animal Care and Use Committee (#4-16-8240-S).

a) Phase 1 – Effect of prior experience

To evaluate the effect of prior experience, collections were attempted on litters (LOF, n = 30) and families (FOF, n = 30) on two consecutive days. The first rope exposure (Day 0) was considered the day of first experience; the second rope exposure (Day 1) was used to evaluate the effect of prior experience. On both days, litters or families were provided ≥ 30 minutes of rope exposure before oral fluids were collected, as described above. The success rates of LOF and FOF collection were compared between day 0 and 1 (no experience vs. prior experience).

b) Phase 2 – Effect of duration of rope exposure

Duration of rope exposure was evaluated for its effect on the success rate of oral fluid sampling by allocating 120 litters to either a 30-minute (FOF, n = 30 families; and LOF, n = 30 litters) or a 4-hour (FOF, n = 30 families; and LOF, n = 30 litters) exposure. The success rate by exposure time was compared within each technique.

c) Phase 3 – Effect of attractants

The effect of attractants on the success rate of oral fluid collection was evaluated using rope treated with a peanut butter solution (Jif Creamy Peanut Butter, Lexington, KY, USA) (FOF, n = 30 families; and LOF, n = 30 litters), a commercial baby pig supplement (Baby Pig Restart®, Tech Mix LLC, Stewart, MN, USA) (FOF, n = 30 families; and LOF, n = 30 litters), or untreated control (FOF, n = 30 families; and LOF, n = 30 litters). To apply the attractants to the ropes, peanut butter and commercial baby pig supplement solutions were prepared by thoroughly mixing 200 grams of product with one liter of water. Ropes were immersed in this solution until fully soaked, dried at room temperature overnight, and used on the farm the following day. All groups were exposed to cotton ropes for 30 minutes. The success rate of oral fluid collection was compared among litters and families exposed to control ropes (no attractants), peanut butter-treated ropes, and baby pig supplement-treated ropes.

d) Phase 4 – Comparison of the success rate of "optimized" LOF and FOF protocols

Based on the results from the previous phases, "optimized LOF" and "optimized FOF" protocols were established and compared to the "standard" FOF protocol, i.e., exposure of the family to a cotton rope for 30 minutes with no prior experience or added attractant to the ropes. Each protocol was tested in 100 litters.

Statistical Analysis

Descriptive analyses were performed at each phase of the study. For phases 1 through 3, the success rate of oral fluid collection (LOF or FOF) was compared using the chi-square test. The Fisher's exact test was used when one of the cells had a frequency count < 5. When the p-value of chi-square tests from phases 1-3 was ≤ 0.20, the analyzed effect was considered to have a potential impact on the success rate of FOF or LOF and that treatment was included in the protocol for Phase 4. In Phase 4, the risk ratio of successful oral fluid collection (volume greater or equal to 1 ml) was evaluated using PROC Genmod of SAS 9.4 package (SAS Institute, Inc., Cary, NC). Standard Family Oral Fluid (SFOF) sampling was used as the reference category for Phase 4.

For phases 1 through 3 a sample size of 30 litters/families per treatment was calculated to provide an 80% confidence level for detecting a difference in proportion of 20 percentage points with 80% of statistical power. For phase 4 a sample size of 100 litters/families per treatment was calculated to provide an 95% confidence level for detecting a different in proportion of 15 percentage points with 80% of statistical power.

Univariate analyses were conducted to compare the success rates between treatment groups. Multivariate models were used to evaluate the effect of litter age, sow parity, and number of piglets in the litter on success rates. To obtain the final model, a hierarchical backward elimination approach was used. The Wald 95% confidence interval (CI) was computed around the risk ratio. The Wald Chi-Square test was used to determine the p-values of the risk ratio on a two-tailed test. The number of piglets in the litter was not recorded for phase 3 and therefore is not presented here.

Results

In Phase 1, prior experience had a positive effect on both LOF and FOF sampling with 33.4% and 16.6% improvement, respectively (Table 1). In Phase 2, longer rope exposure did not improve oral fluid collection for either LOF or FOF techniques (Table 2). In Phase 3, the use of attractant-treated rope did not result in a significant improvement of the collection success rate for LOF when compared to untreated ropes. In contrast (Table 3), the success rate for FOF collection was highest for ropes treated with the baby pig supplement (96.7%), followed by untreated ropes (86.7%), and peanut butter-treated ropes (70.0%) ($p < 0.2$). Based on the results of phases 1 - 3, the "optimized LOF" protocol consisted of exposing litters to a rope one day prior to the collection day. "Optimized FOF" consisted of giving the family prior exposure and baby pig supplement-treated ropes. Pairwise comparisons of improved LOF, improved FOF, and standard FOF (SFOF), found no significant difference in the success rates for FOF protocols, but both FOF techniques were superior to the optimized LOF protocol (Table 4). The risk ratio for a successful collection of optimized LOF and optimized FOF compared to SFOF was 0.427 (95% CI: 0.329, 0.554) and 1.056 (95% CI: 0.970, 1.150), respectively. Analyses found no association between sow parity, litter age, or litter size and successful collection of oral fluids for any protocol ($p > 0.05$). Likewise, univariate models for sow parity, litter age, or litter size were not significant ($p > 0.05$) indicating none were associated with successful collection of oral fluids from due-to-wean litters.

Discussion

Collecting and testing oral fluids from pigs for porcine reproductive and respiratory syndrom virus (PRRSV) was first described by Prickett et al. (2008a). Subsequently, oral fluid-based protocols for infectious disease diagnostics and surveillance have been described for a variety of pathogens in growing pigs and breeding stock (Prickett et al., 2008a; Prickett et al., 2008b; Kittawornrat et

al., 2010a; 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). Oral fluid sampling provides distinct advantages over individual pig sampling: 1) samples can be collected by a single person; 2) samples can be collected without stress or risk to pigs or people; 3) oral fluid samples provide for a higher probability of detection without increasing sample numbers or testing costs. Since the introduction of oral fluid testing in three US veterinary diagnostic laboratories (Iowa State University, South Dakota State University and University of Minnesota) the number of tests performed increased steadily from 20,963 tests in 2010, 70,996 in 2011, 125,202 in 2012, 190,606 in 2013, 325,481 in 2014, 352,911 in 2015, and 369,439 in 2016 (Bjustrom-Kraft et al., 2018). The temporal trend of increasing numbers of tests supports the conclusion that producers and veterinarians find utility in this approach.

Despite the advantages of oral fluid-based monitoring, there are few published reports on the collection of oral fluid samples from suckling piglets (Graham et al., 2013; Kittawornrat et al., 2014; Yeske-Livermore et al., 2014; Boulbria et al., 2016; Panyasing et al., 2016a). This can be attributed to the fact that collecting oral fluid samples from suckling piglets is not as easy and consistent as it is in older pigs. Thus, the objective of this study was to identify the optimal procedure for collecting oral fluid samples from due-to-wean litters. This process included the evaluation of prior exposure, use of attractants, and duration of rope exposure on the success rate of oral fluids collection using either of two approaches (family oral fluids versus litter oral fluids).

The most significant finding of this study was the observation that family oral fluids were the key to oral fluid sampling in due-to-wean litters (Table 4). Pig behavior is paramount to successful oral fluid sampling, regardless of pig age. That is, pigs pay close attention to the behaviors of conspecifics in their social group and actions by one member of the group often induce the same

or similar behavior in other animals (Stolba and Woodgush, 1989). In family oral fluid collection, the sow in each family chewed on the rope first and piglets followed suit. Whether or not they had previously been involved in oral fluid sampling, sows are experienced explorers and willing to evaluate new objects in their environment. In contrast, litter oral fluid sampling requires one or more piglets in the litter to initiate an interaction with the rope; hopefully prompting littermates to do likewise. The fact that observing or participating in a behavior is an important mechanism of social vertical learning for piglets (Oostindjer et al., 2011) may explain why the success rate of the litter oral fluid protocol was consistently lower than the family oral fluid protocol. The absence of a statistical difference, despite a numeric difference, between the optimized family oral fluid and standard oral fluid protocols (Table 4) may be explained by sample size (n = 100 for each), plus the fact that all 'families' were present in the same farrowing rooms, i.e., sows and piglets in the "standard" family oral fluid group may have learned by watching the behavior of the optimized FOF group because both groups were in the same environment (farrowing rooms).

Longer exposure time did not improve the success rate of oral fluid collection for either LOF or FOF protocols, whereas prior exposure was shown to increase the success rate for both. This is in agreement with other studies in which prior exposure of individually housed boars (Kittawornrat et al., 2010b), sows (Pepin et al., 2015), and feeder pigs (White et al., 2014) improved oral fluid collection, thereafter. White et al. (2014) found that groups of pigs without prior exposure took more time (approximately 60 min) to achieve a similar level of rope interaction (around 70% of the group) compared to pigs with prior exposure (approximately 30 minutes). In the same study, about 30% of the animals never interacted with the rope during the observation period. In the present study, the FOF the success rate was ~70% for both the 30 minute and 4 hour exposures,

which was similar to (White et al., 2014). In contrast, LOF achieved 23.3% and 33.6% success rates with 30 minute and 4 hours of rope exposure, respectively.

A commercial baby pig supplement (Baby Pig Restart®) was effective in increasing the success rate of oral fluid for FOF, but not LOF. In a review of the literature (Kittawornrat and Zimmerman, 2011) found that sweet tastes, e.g., sucrose, glucose, and lactose, were generally preferred by pigs. The commercial baby pig supplement contained both sucrose and lactose, which may explain the higher collection success rate in families (sow and piglets) exposed to that treatment versus peanut butter or control ropes. The piglets exposed to LOF should have had the same taste preferences, but young piglets learn from conspecifics (Figueroa et al., 2013) and these piglets lacked experienced conspecifics, e.g., sows, to demonstrate rope chewing. The attractants in this study were limited to peanut butter and baby pig supplement, but there is a wide range of possibilities and it may be productive to evaluate other attractants in future research. Pigs prefer baits containing plant-derived compounds, especially corn meal, over animal-derived compounds, with no aroma preference among apple, corn, almond, hazelnut, truffle or potatoes (Rossi et al., 2015).

Altogether, these results suggested that the family protocol is the best option for collecting oral fluids from due-to-wean litters. Collection can be successful using a standard technique (success rate 89%), or enhanced by adding flavor to the ropes (baby pig supplement: success rate 96.7%), with prior exposure (success rate 83.3%), or combining both (optimized FOF using baby pig supplement and prior exposure: success rate of 94%).

Samples collected using “family” based techniques contain oral fluids from both the sow and piglets. It was not the goal of the current study to investigate to what proportion sow and piglets contribute to the final sample. It is also important to note that not all piglets within a litter may add to the oral fluid samples either on FOF or LOF techniques. Yeske-Livermore et al., (2014) reported

that 54% of piglets interacted with ropes using FOF without prior exposure (Yeske-Livermore et al., 2014). The sow contribution to the oral fluid and the uncertainty about the number of piglets contributing to the sample may have significant implications on the probability of infectious disease detection for FOF and LOF based monitoring and surveillance protocols. However the detection of anti-PRRSV antibodies in FOF (Yeske-Livermore et al., 2014) demonstrated the potential of this sample type for surveillance in breeding herds.

This study was executed in one farm only and does not account for other farm-specific factors that could influence (for better or worse) the success rate of oral fluid collection.

Conclusion and implications

The success rate for family oral fluid (FOF) sampling was significantly and consistently higher (84.9%, range 70% to 92%) than for litter oral fluid (LOF) sampling (24.1%, range 16.5% to 32.2%). The use of prior exposure or Baby Pig Restart enhanced the success rate of oral fluid collections and could be used in addition to FOF in herds with a low success rate (<90%). Family oral fluid-based techniques can be a new tool applied to monitoring and surveillance systems of pathogens in swine breeding herds.

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References

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.

Bjustrom-Kraft, J., Christopher-Hennings, J., Daly, R., Main, R., Torrison, J., Thurn, M., Zimmerman, J., 2018. The use of oral fluid diagnostics in swine medicine. Journal of Swine Health and Production 26, 262-269.

Bjustrom-Kraft, J., Woodard, K., Giménez-Lirola, L., Rotolo, M., Wang, C., Sun, Y., Lasley, P., Zhang, J., Baum, D., Gauger, P., Main, R., Zimmerman, J., 2016. Porcine epidemic diarrhea virus (PEDV) detection and antibody response in commercial growing pigs. BMC Vet Res 12, 99.

Boulbria, G., Lebret, A., Leblanc-Maridor, M., Gin, T., Berton, P., Le Guennec, J., Belloc, C., Normand, V., 2016. Is it feasible to collect oral fluids from litters of piglets before weaning? In, 24th International Pig Veterinary Society Congress, Dublin, Ireland, 366.

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.

Detmer, S.E., Patnayak, D.P., Jiang, Y., Gramer, M.R., Goyal, S.M., 2011. Detection of Influenza A virus in porcine oral fluid samples. J Vet Diagn Invest 23, 241-247.

Figueroa, J., Sola-Oriol, D., Manteca, X., Perez, J., 2013. Social learning of feeding behaviour in pigs: Effects of neophobia and familiarity with the demonstrator conspecific. Applied Animal Behaviour Science 148, 120-127.

Giménez-Lirola, L.G., Mur, L., Rivera, B., Mogler, M., Sun, Y., Lizano, S., Goodell, C., Harris, D.L., Rowland, R.R., Gallardo, C., Sánchez-Vizcaíno, J.M., Zimmerman, J., 2016. Detection of African Swine Fever Virus Antibodies in Serum and Oral Fluid Specimens Using a Recombinant Protein 30 (p30) Dual Matrix Indirect ELISA. PLoS One 11, e0161230.

Giménez-Lirola, L.G., Xiao, C.T., Zavala, M., Halbur, P.G., Opriessnig, T., 2013. Improving ante mortem diagnosis of Erysipelothrix rhusiopathiae infection by use of oral fluids for bacterial, nucleic acid, and antibody detection. J Microbiol Methods 92, 113-121.

Goodell, C.K., Prickett, J., Kittawornrat, A., Zhou, F., Rauh, R., Nelson, W., O'Connell, C., Burrell, A., Wang, C., Yoon, K.J., Zimmerman, J.J., 2013. Probability of detecting influenza A virus subtypes H1N1 and H3N2 in individual pig nasal swabs and pen-based oral fluid specimens over time. Vet Microbiol 166, 450-460.

Graham, J., Rademacher, C., Swalla, R., 2013. Use of oral uid sampling in suckling pigs for PRRSV monitoring. In, 44th AASV Annual Meeting, San Diego, CA, 83.

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., Panyasing, Y., Goodell, C., Wang, C., Gauger, P., Harmon, K., Rauh, R., Desfresne, L., Levis, I., Zimmerman, J., 2014. Porcine reproductive and respiratory syndrome virus (PRRSV) surveillance using pre-weaning oral fluid samples detects circulation of wild-type PRRSV. Vet Microbiol 168, 331-339.

Kittawornrat, A., Prickett, J., Chittick, W., Wang, C., Engle, M., Johnson, J., Patnayak, D., Schwartz, T., Whitney, D., Olsen, C., Schwartz, K., Zimmerman, J., 2010a. 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 Res 154, 170-176.

Kittawornrat, A., Prickett, J., Chittick, W., Wang, C., Engle, M., Johnson, J., Patnayak, D., Schwartz, T., Whitney, D., Olsen, C., Schwartz, K., Zimmerman, J., 2010b. 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.

Kittawornrat, A., Zimmerman, J.J., 2011. Toward a better understanding of pig behavior and pig welfare. Anim Health Res Rev 12, 25-32.

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.

Mur, L., Gallardo, C., Soler, A., Zimmermman, J., Pelayo, V., Nieto, R., Sánchez-Vizcaíno, J.M., Arias, M., 2013. Potential use of oral fluid samples for serological diagnosis of African swine fever. Vet Microbiol 165, 135-139.

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.

Panyasing, Y., Goodell, C., Kittawornrat, A., Wang, C., Levis, I., Desfresne, L., Rauh, R., Gauger, P.C., Zhang, J., Lin, X., Azeem, S., Ghorbani-Nezami, S., Yoon, K.J., Zimmerman, J., 2016a. Influenza A Virus Surveillance Based on Pre-Weaning Piglet Oral Fluid Samples. Transbound Emerg Dis 63, e328-338.

Panyasing, Y., Goodell, C.K., Giménez-Lirola, L., Kittawornrat, A., Wang, C., Schwartz, K.J., Zimmerman, J.J., 2013. Kinetics of influenza A virus nucleoprotein antibody (IgM, IgA, and IgG) in serum and oral fluid specimens from pigs infected under experimental conditions. Vaccine 31, 6210-6215.

Panyasing, Y., Rungprasert, K., Anakkul, N., Kedkovid, R., Thanawongnuwech, R., Kittawornrat, A., Zimmerman, J., 2016b. Detection of classical swine fever virus (CSFV) in oral fluid samples by RT-PCR. In, 47th Annual Meeting of the American Association of Swine Veterinarians, New Orleans, Louisiana, 320.

Pepin, B., Liu, F., Main, R., Ramirez, A., Zimmerman, J., 2015. Collection of oral fluid from individually housed sows. Journal of Swine Health and Production 23, 3.

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., Johnson, J., Murtaugh, M.P., Puvanendiran, S., Wang, C., Zimmerman, J.J., Opriessnig, T., 2011. Prolonged detection of PCV2 and anti-PCV2 antibody in oral fluids following experimental inoculation. Transbound Emerg Dis 58, 121-127.

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.

Romagosa, A., Gramer, M., Joo, H.S., Torremorell, M., 2012. Sensitivity of oral fluids for detecting influenza A virus in populations of vaccinated and non-vaccinated pigs. Influenza Other Respir Viruses 6, 110-118.

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.

Vosloo, W., Morris, J., Davis, A., Giles, M., Wang, J., Nguyen, H.T., Kim, P.V., Quach, N.V., Le, P.T., Nguyen, P.H., Dang, H., Tran, H.X., Vu, P.P., Hung, V.V., Le, Q.T., Tran, T.M., Mai, T.M., Singanallur, N.B., 2015. Collection of Oral Fluids Using Cotton Ropes as a Sampling Method to Detect Foot-and-Mouth Disease Virus Infection in Pigs. Transbound Emerg Dis 62, e71-75.

White, D., Rotolo, M., Olsen, C., Wang, C., Prickett, J., Kittawornrat, A., Panyasing, Y., Main, R., Rademacher, C., Hoogland, M., Zimmerman, J.J., 2014. Recommendations for pen-based oral-fluid collection in growing pigs. Journal of Swine Health and Production 22, 138-141.

Yeske-Livermore, L., O'Neil, K., Main, R., Zimmerman, J., 2014. Improved pre-weaning surveillance using oral fluids: a pilot study. In, 44th AASV meeting, Dalla, TX, 317-318.

Figure Caption

Fig 1

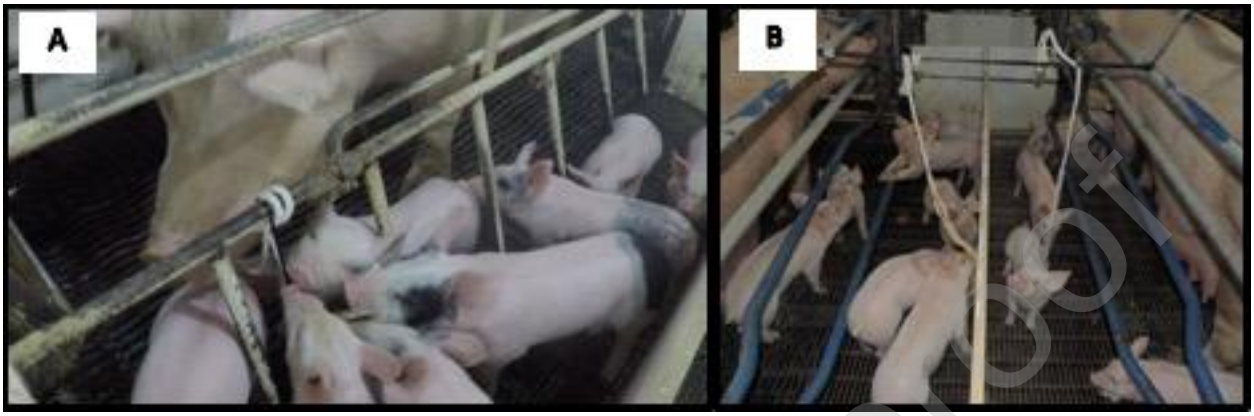

Table 1. Effect of prior exposure on oral fluid collection success rates in due-to-wean litters and description of study population.

	Litter Oral Fluid Protocol		**Family Oral Fluid Protocol**	
	Before exposure	After exposure	Before exposure	After exposure
Success rate	1/30 (3%)[a]	11/30 (37%)[b]	20/30 (67%)[a]	25/30 (83%)[b]
Median sow parity (P_{25}, P_{75})[1]	3 (1, 6)	3 (1, 6)	4 (1, 6)	4 (1, 6)
Median sample (ml) (P_{25}, P_{75})	0 (0, 0)	0 (0, 2)	14 (0, 21)	13 (6, 23)
Median litter age (days) (P_{25}, P_{75})	17 (16, 18)	18 (17, 19)	17 (16, 18)	18 (17, 19)
Median litter size (P_{25}, P_{75})	11 (10, 12)	11 (10, 12)	10 (9, 11)	10 (9, 11)

[1] P_{25}, P_{75} denote the 25th and 75th percentiles.

a,b Within sampling protocol, superscripted letters denote $p < 0.20$.

Table 2. Effect of sampling time on oral fluid collection success rates in due-to-wean litters and description of study population.

	Litter Oral Fluid Protocol		**Family Oral Fluid Protocol**	
	30 minutes	4 hours	30 minutes	4 hours
Success rate	7/30 (23%) [a]	10/30 (34%) [a]	22/30 (73%) [a]	20/30 (67%) [a]
Median sow parity (P_{25}, P_{75})[1]	2 (1, 4)	2 (1, 5)	2 (1, 5)	2 (1, 4)
Median sample (ml) (P_{25}, P_{75})	0 (0, 0)	0 (0, 1)	7 (0, 15)	5.5 (0, 14)
Median litter age (days) (P_{25}, P_{75})	17 (17, 19)	17 (16, 18)	17 (16, 19)	18 (17, 19)
Median litter size (P_{25}, P_{75})	10.5 (9, 11)	11 (10, 12)	10.5 (9, 11)	10.5 (9, 12)

[1] P_{25}, P_{75} denote the 25th and 75th percentiles.

Table 3. Effect of attractants[1] on oral fluid collection success rates in due-to-wean litters sampled with the "Family Oral Fluid" protocol and description of study population.

	Litter Oral Fluid Protocol			**Family Oral Fluid Protocol**		
	Control	A	B	Control	A	B
Success rate	9/29 (31%)[a]	9/29 (31%) [a]	10/29 (35%) [a]	26/30 (87%)[a]	21/30 (70%)[b]	29/30 (97%)[c]
Median sow parity (P_{25}, P_{75})[2]	1 (1, 6)	3 (1, 5)	2 (1, 4)	3 (1, 5)	3 (1, 6)	4 (2, 6)
Median sample (ml) (P_{25}, P_{75})	0 (0, 1)	0 (0, 1)	0 (0, 2)	13 (4, 20)	5 (0, 17)	12.5 (6, 27)
Median litter age (days) (P_{25}, P_{75})	18 (18, 19)	18 (18, 19)	18 (17, 19)	18 (17, 19)	18 (17, 19)	18.5 (17, 19)
Median litter size (P_{25}, P_{75})	ND[3]	ND	ND	ND	ND	ND

[1] Cotton ropes were immersed in a (A) peanut butter solution (200 grams in one liter of water) or (B) a commercial baby pig supplement (Baby Pig Restart®, Tech Mix LLC, Stewart, MN), dried at room temperature overnight, and used the following day.

[2] P_{25}, P_{75} denote the 25th and 75th percentiles.

[3] ND = Not Done

[a,b,c] Within sampling protocol, superscripted letters denote $p < 0.20$.

Table 4. Effect of sampling protocol on oral fluid collection success rates in due-to-wean litters and description of study population.

	"Optimized" Litter Oral Fluid Protocol[1]	**"Optimized" Family Oral Fluid Protocol[2]**	**"Standard" Family Oral Fluid Protocol[3]**
Success rate	38/100 (38%)[a]	94/100 (94%)[b]	89/100 (89%)[b]
Median sow parity (P_{25}, P_{75})[4]	3 (2, 5)	3 (1, 6)	3 (2, 6)
Median sample (ml) (P_{25}, P_{75})	0 (0, 3)	22 (9.5, 30)	19 (8, 29)
Median litter age (days) (P_{25}, P_{75})	18 (17, 19)	18 (17, 19)	18 (17, 18)
Median litter size (P_{25}, P_{75})	11 (10, 12)	11 (9, 12)	11.5 (10, 13)

[a,b] Different superscripted letters denote $p < 0.05$.

[1] Litter previously exposed to ropes and then sampled for 30 minutes using an untreated cotton rope.

[2] Sow and piglets previously exposed to ropes and then sampled for 30 minutes using a cotton rope treated with a commercial baby pig supplement (Baby Pig Restart®, Tech Mix LLC, Stewart, MN).

[3] Sow and piglets sampled for 30 minutes without prior exposure and using an untreated cotton rope.

[4] P_{25}, P_{75} denote the 25th and 75th percentiles.