Assessment of abattoir based monitoring of PRRSV using oral fluids

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Abstract

Various porcine reproductive and respiratory syndrome virus (PRRSV) regional elimination projects have been implemented in the U.S., but none have yet succeeded. In part, this reflects the need for efficient methods to monitor over time the progress of PRRSV status of participating herds. This study assessed the feasibility of monitoring PRRSV using oral fluids collected at the abattoir. A total of 36 pig lots were included in the study. On-farm oral fluid (n=10) and serum (n=10) collected within two days of shipment to the abattoir were used to establish the reference PRRSV status of the population. Oral fluids (n=3 per lot) were successfully collected from 32 lots (89%) at the lairage. Three veterinary diagnostic laboratories (VDLs) tested the sera (VDL1 and VDL3: n=316, VDL2: n=315) and oral fluids (VDL1 and VDL3: n=319, VDL2: n=320) for PRRSV antibodies (ELISA) and RNA (rRT-PCR). Environmental samples (n=64, 32 before and 32 after pigs were placed in lairage) were tested for PRRSV RNA at one VDL. All oral fluids (farm and abattoir) tested positive for PRRSV antibody at all VDLs. PRRSV positivity frequency on serum ranged from 92.4% to 94.6% among VDLs, with an overall agreement of 97.6%. RNA was detected on 1.3% to 1.9%, 8.1% to 17.7%, and 8.3% to 17.7% of sera, on-farm and abattoir oral fluids, respectively. Between-VDLs rRT-PCR agreement on sera and oral fluids (farm and abattoir) ranged from 97.8% to 99.0%, and 79.0% to 81.2%, respectively. Between-locations agreement of oral fluids varied from 31.3% to 50% depending on the VDL. This study reported the application of swine oral fluids collected at the abattoir for monitoring PRRSV, and describes the between-VDL agreement for PRRS testing of serum and oral fluid field samples.

Key words: Swine, PRRS, monitoring, surveillance, abattoir, agreement.

Introduction

Porcine reproductive and respiratory (PRRS) virus infection costs over \$1 billion per year to the US swine industry (Holtkamp et al., 2013). Likewise, economic studies have reported a significant PRRS impact in Europe (Nieuwenhuis et al., 2012; Nathues et al., 2017). Thus, effectively preventing, detecting, and eliminating PRRS virus (PRRSV) infection represent a great opportunity to improve the long-term sustainability of the swine industry worldwide.

Multiple efforts to eliminate PRRS from geographic regions of the U.S. have not succeeded (Corzo et al., 2010; Perez et al., 2015; Valdes-Donoso et al., 2016), in part due to poor disease surveillance of pig populations, which delays the detection of outbreaks and favors efficient virus transmission between herds via direct and indirect routes (Wright, 2017).

One of the first steps to eliminate diseases from production systems or geographical regions (i.e., multiple farms) is to track incidence and prevalence data over time and geographical space, thereby allowing veterinarians to make informed decisions on critical aspects of disease control and elimination including pig movement, health interventions, biosecurity measures and management practices. However, to the best of our knowledge there is no on-going active monitoring system in place in the US swine population to detect PRRSV or other economically significant pathogens in growing pigs.

The scarcity of infectious disease information in the growing pig population in the US is in part attributed to the poor practicability and/or uncertainty of diagnostic accuracy of existing (conventional) surveillance protocols. The conventional sampling methods require restraining pigs to collect enough individual pig samples, such as serum, to represent the disease status of the population (e.g., 30 sera provide 95% confidence to detect pathogen at prevalence of at least 10%), which is time consuming and with limited herd sensitivity. A promising development in this regard

derives from research supporting oral fluid specimens, which are "aggregate samples" (1 sample from multiple pigs) being easy, simple, practical and animal welfare-friendly to obtain (Prickett et al., 2008a; Prickett et al., 2008b; Prickett and Zimmerman, 2010; Kittawornrat et al., 2014). Diagnostic tests have been developed and optimized for detection of PRRSV nucleic acid and antibodies in swine oral fluids since 2008 (Prickett et al., 2008a; Prickett et al., 2008b; Kittawornrat et al., 2010; Prickett et al., 2010; Prickett and Zimmerman, 2010; Ramirez et al., 2012; Kittawornrat et al., 2014; Pepin et al., 2015).

According to the United States Department of Agriculture (USDA) National Agricultural Statistics Service (NASS) census there were over 63,000 pig farms in the U.S. in 2012. Thus, it is impractical and cost-prohibitive to monitor all sites on a regular basis for infectious diseases. Therefore, there is the need to validate practical, large-scale disease monitoring systems to reliably detect PRRS activity over time and geographical space.

An abattoir is a point of concentration of swine from several sources, making it a convenient and cost-effective place for sample collection. The objective of this study was to assess the feasibility of using swine oral fluids obtained at the abattoir to monitor anti-PRRSV antibodies by serology, and PRRSV RNA by real-time reverse-transcriptase polymerase chain reaction (rRT-PCR). Feasibility was assessed by describing practicability of obtaining abattoir samples, and by comparing diagnostic agreement between locations (farm and abattoir) across 3 veterinary diagnostic laboratories (VDLs).

Material and methods

Study design

A pilot longitudinal field study was performed to assess feasibility of monitoring pigs for PRRSV at the abattoir versus the finishing sites. Serum and oral fluid samples were collected on finishing sites from 36 pig lots. Subsequently, the same pig lots were followed within 48 hours at the abattoir for swine oral fluid collection. Samples were submitted to 3 veterinary diagnostic laboratories (VDLs) for testing: Iowa State University, University of Minnesota, and South Dakota State University (Figure 1). Agreement of test results between locations (farm and abattoir), and between VDLs were assessed. Additionally, environmental (EA) samples were collected before and after study pigs entered the lairage.

Pig lots and participants

System A and B are two large integrated companies in the swine production industry with operations in the Midwest region of the US. A "pig lot" was defined as a population of pigs sampled at the finishing site and subsequently transported to, and sampled at, the abattoir. Thirty-six pig lots were enrolled: 16 lots from system A, and 20 lots from system B, both located in the upper Midwestern region of U.S. fulfilling the following eligibility criteria: (a) ability to confidently identify the same group of pigs on the farm, and at the abattoir, (b) less than 48 hours interval between on farm and abattoir sampling, and (c) agreement from abattoir to allow study personnel to obtain oral fluids from pigs, and environmental samples from lairage.

All pig lots were housed in finishing barns operating in an all-in/all-out system with a total capacity varying from 2,000 to 5,000 hogs. When sampled, barns had 300 to 450 hogs, which were the last group of pigs to be sent to the abattoir (i.e. last cut). Pigs were distributed in pens varying from 30

to 45 animals, according to farm design. A description of sow herd status sourcing the pigs to the wean to finish sites, pig vaccination status and timing, and premises where groups were raised until sent to the abattoir can be found in Appendix 1. Samples were collected from August 1st to September 7th of 2016. All pig lots were sent to the same abattoir. The procedures described in this study were approved by the Iowa State University Institutional Animal Care and Use Committee (IACUC – 6-16-8286-S).

Sample collection

On the farms, oral fluids (n = 10 per lot) were collected by allowing pigs to interact with 5/8-inch cotton ropes for 20-30 minutes and harvesting the fluid, as previously described (Prickett et al., 2008a; Prickett et al., 2008b). For further characterization of the infection status of the group, 10 serum samples were also collected on each farm from same pens of pigs from which oral fluid samples were collected. Blood samples were drawn from 10 conveniently selected pigs into sterile BD Vacutainer SST Venous Blood Collection Tube (Thermo Fisher scientific, Franklin Lakes, New Jersey, USA) via jugular venipuncture. To allow visual verification of study pigs at the abattoir, the same 10 pigs bled for serum collection in each lot were marked with fluorescent pig markers at the finishing farm.

At the abattoir, a subsample of each pig lot consisting on average of 150 animals had 3 oral fluid samples collected in succession using collection devices already in place: chains hanging from skywalks in individual pens allowing access to the ropes to the pigs in a specific pen only. The ropes did not touch any walls, gates or floor. In addition, environmental samples were collected from lairage before study pigs entered the pen, and after they left it. Environmental samples were taken using a Swiffer pad cloth (Dry Sweeping Refills, Bentonville, Arkansas, USA) pre-soaked with 20mM phosphate buffer (PBS) solution (Schneider et al., 2011). More specifically, at each

collection, 5 pads were used to sample 1 m^2 sections of the pen floor (lairage): one section in the middle of the pen, and one section at each corner of the pen (northeast, northwest, southeast, southwest). The 5 environmental samples were pooled into one single sample representing the lairage environment "before" or "after" being occupied by study pigs. The lairage area is completely cleaned with high volume low pressure 60 degrees Celsius water once during the weekends with a downtime period varying from 22 to 26 hours. Everyday holding pens were washed out by sprinklers 12 minutes per hour with cold water.

Sample handling

Samples were identified with key information: collection date, pen number, premises ID, pig lot ID, date, and collection person. Samples were kept at 4-8°C right after collection, and transported to the ISU campus within 24 hours. Whole blood was centrifuged during 10 min at 3,000 rpm to obtain serum. Serum, oral fluid and environmental samples were aliquoted into at least 3 cryogenic tubes and stored at -80°C until submitted for testing.

Sample size

Hypergeometric approaches for sample size calculation do not apply to pen-based oral fluids, but sample size recommendations based on a piecewise exponential survival mode are available (Rotolo et al., 2017). Therefore, oral fluids were collected from 10 pens within each finishing barn using a fixed spatial sampling approach. This number of samples provided ≥ 96% probability of ≥ 1 positive rRT-PCR result, if the prevalence in the barn was ≥ 25%. Similarly, serum samples from 10 pigs provided 90% confidence of detecting RNA (at least one positive result) when prevalence is ≥ 25%. The abattoir facilities allowed collecting 3 oral fluids from pigs at the lairage. To provide additional information on the source of nucleic acids detected in abattoir oral fluids, environmental

samples were obtained before (5 samples pooled into 1) and after (5 samples pooled into 1) study pigs occupied the pen.

Diagnostic testing

Serum samples were tested by rRT-PCR for PRRSV RNA, and for anti-PRRS antibodies by the PRRS X3 Ab ELISA test according to manufacturer's instructions (IDEXX Laboratories Inc., Westbrook, ME). Likewise, oral fluids were tested by rRT-PCR for PRRSV RNA, and for anti-PRRSV antibody using the PRRSV OF Ab ELISA according to manufacturer's instructions (IDEXX Laboratories Inc., Westbrook, ME). Environmental samples were tested for PRRSV RNA by rRT-PCR. Serum and oral fluid samples were tested in VDLs 1, 2, and 3, while environmental samples were only tested in VDL 1.

VLD 1 and 2 used the same rRT-PCR commercial assay to detect PRRSV RNA in serum and oral fluid samples as previously described by (Pepin et al., 2015). In summary RNA extraction was performed using the MagMAX™ Viral RNA Isolation Kit (Life Technologies Corporation, Carlsbad, CA, USA) and a Kingfisher 96 instrument (Thermo Scientific, Waltham, MA, USA). PRRSV rRT-PCR was performed on nucleic acid extracts using the MagMAX™ North American (NA) and European (EU) PRRSV-specific PCR assay (Life Technologies Corporation, Carlsbad, CA, USA).

VLD 3 used a real-time reverse transcription rRT-PCR assay to detect PRRSV RNA in serum and oral fluid samples as previously described (Kittawornrat et al., 2014). In summary RNA extraction was performed using the MagMAX™ Viral RNA Isolation Kit (Life Technologies Corporation, Carlsbad, CA, USA) and the EZ-PRRSV™ MPX 4.0 assay (Tetracore®, Rockville, MD).

Individual rRT-PCR results, both on serum and oral fluids, were expressed as positive, suspect or negative according to manufacturer's instruction.

For pig lots with positive rRT-PCR at the farm (serum or oral fluids) and at the abattoir (oral fluids), samples were submitted for PRRSV open reading frame (ORF)-5 sequencing to describe the genetic relationship between virus detected at each location. Environmental samples from those pig lots were also submitted for ORF-5 sequencing if tested positive by rRT-PCR. The PRRSV ORF-5 sequencing was performed at the ISU VDL using a previously described method (Zhang et al., 2017).

Statistical analysis

For the purpose of this manuscript, "general agreement" for between VDLs and between location (farm and abattoir) comparisons was defined as proportion of samples with same reported result (negative, suspect, or positive). For the agreement between locations, test results from samples collected at the farm were compared to test results from samples collected at the abattoir on a group basis. Farms were considered 'positive' when at least one sample tested positive; 'suspect' when no positive results were found for the group but one or more results were defined as 'suspect' by the VDL; or 'negative' when all samples tested negative.

The Bland Altman plot, together with a paired t-test (at significance level of 0.05) were used to describe the between VDL variation of PCR cycle threshold (Ct) values among samples with reported "positive" or "suspect" result (i.e., Ct value < 40). Statistical analyses were performed using the SAS 9.4 package (SAS Institute, Inc., Cary, NC).

Results

A total of 36 pig lots were visited on finishing barns. All on-farm samples were taken within 48 hours prior to shipment of pig lots to the abattoir. Transport time from farm to abattoir varied from 4 to 7 hours. Pig lots sampled at the abattoir consisted of a subsample of animals on each farm

with an average of 150 animals. Oral fluids were obtained from 32 (88.89%) pig lots at the abattoir. There were 316, 315 and 316 serum samples, 319, 320 and 319 on-farm oral fluids, and 96, 96 and 96 abattoir oral fluids samples submitted to VDL 1, VDL 2 and VDL 3, respectively.

Detection of anti PRRSV antibodies by ELISA

Two-hundred-ninety-five (93.35%) serum samples tested positive for anti-PRRSV antibodies on VDL 1, 291 (92.38%) on VDL 2, and 299 (94.62%) on VDL3. All oral fluids (farm and abattoir) tested positive by ELISA in all VDLs (Table 1).

Detection of PRRSV RNA by rRT-PCR

The frequency of detection of PRRSV RNA by rRT-PCR in serum was consistently below 2% in all VDLs. PRRSV RNA detection in oral fluids varied among VDLs, ranging from 8.1% to 17.2% for samples collected on-farm, and from 8.3% to 17.7% on samples collected at the abattoir (Table 1 and Figure 2). From 32 environmental samples collected before pigs entered the lairage, 3 tested positive and 4 tested suspect by rRT-PCR; 2 environmental samples tested positive (4 tested suspect) on lairage after study pigs left (Table 1).

PRRSV ORF-5 sequencing

Seven on-farm oral fluid samples, 8 abattoir oral fluid, 4 serum, and 1 environmental sample were submitted for PRRSV ORF-5 sequencing. ORF-5 sequences were obtained for four sequences from on-farm oral fluids, 2 from serum and 2 from abattoir oral fluids. The homology of the different sequences compared to commercially available vaccines range from 62.2 to 88.9% suggesting wild-type viruses. It was not possible to obtain sequences from environmental samples.

Between VDLs agreement

There was a 98.4% agreement between VDL 1 and VDL 2, 99.0% for VDL1 and VDL3 and 97.8% for VDL 2 and VDL 3 for rRT-PCR results of serum samples. Negative results accounted for 96.8% of the agreement between VDL 1 and VDL 2, 97.8% between VDL 1 and VDL 3, and 96.5% between VDL 2 and VDL 3.

For oral fluids (farm and abattoir samples combined), the agreement was 80.0%, 79.0% and 81.2% between VDLs 1 and 2, VDLs 1 and 3 and VDLs 2 and 3, respectively (Table 2).

Bland-Altman plots were used to further describe the rRT-PCR results among the VDLs on samples with reported Ct values. When comparing VDL 1 and VDL 2, there was a systematic mean difference of 1.68 (range 4.9) Cts between the two laboratories (Tukey $p<0.0001$). A systematic difference was also found when evaluating VDL 2 and VDL 3 (mean difference of 1.34, range 5.8, Tukey $p<0.0001$). When comparing VDL 1 and VDL 3 no significant difference was found (mean difference of 0.342, Tukey $p=0.1489$), and the Ct range was 6.9 (Figure 3).

Between locations agreement

The agreement between locations was 50% for VDL 1, 50.0% for VDL 2, and 31.3% for VDL 3 (Table 3).

Discussion

Development of practical, affordable and effective monitoring and surveillance systems (MOSS) (Salman, 2003) for tracking animal health status over time and geographical spaces is crucial for characterizing pathogen activity in livestock populations. PRRS has impacted the sustainability of the USA and global pork production since it was first described (Keffaber, 1989). The University of Minnesota's Morrison swine health monitoring project (MSHMP) reports weekly changes of

PRRS status of US breeding farms that agreed to voluntarily participate in the program (Tousignant et al., 2015; Alkhamis et al., 2016; Perez et al., 2016). However, there is very little information on PRRS incidence or prevalence over time in the US finishing pig population. The scarcity of infectious disease information in the growing pigs is in part attributed to the impracticability and/or uncertainty of the diagnostic information supporting the existing (conventional) MOSS.

Sampling pig populations in the abattoir results in a logistically superior MOSS when compared to collecting samples on-farm because several sources and many pigs can be sampled in one day by one person. In comparison, to surveil 100,000 pigs housed in 20 5,000-head finishing farms using oral fluids, one person would take 20 days if visiting one farm per day (due to downtime restrictions on movement). In an abattoir harvesting 17,000 pigs per day (1,062 animals harvested per hour in 16 hours on two shifts), receiving 113 truck loads per day (averaging 150 pigs per load), and assuming that each 5,000-head farm would send 3 loads in a particular day, 100,000 pigs could be surveilled in 8.5 hours. It follows that, in the same amount of time needed to surveil 20 farms (20 days), it would be possible to surveil 3,780,000 pigs in an abattoir. Thus, the abattoir is a practical location to sample a large number of pigs at a timely and affordable fashion.

To the best of our knowledge this was the first study demonstrating that detection of PRRSV RNA and anti-PRRSV antibodies in oral fluids was possible at the abattoir in the United States. Anti-PRRSV antibodies have been previously reported to be found in swine oral fluids collected at the abattoir in Colombia (Zimmerman et al., 2014).

Oral fluids collection at the abattoir proved to be an easy process. Chains hanging from skywalks over the lairage allowed for practical collection and limited the risk of cross-contamination of samples between pens. The few failed attempts (n=4 groups) to collect fluids were likely due to

high environmental temperatures making pigs less active (Torrey et al., 2013) and affecting pig interaction with the ropes. Thereby, a possible way to overcome this situation is to provide additional rest time for pigs after placement on lairage before starting the rope exposure. We considered that 90% of success rate to obtain oral fluids from pigs during summer time was promising.

The high frequency of antibody detection in both locations demonstrated that all pig lots were exposed to PRRSV, either by vaccination, wild-type exposure, or both. Thus, antibody tests can be used in abattoir oral fluids to monitor PRRSV exposure in pig populations, especially those non-vaccinated flows coming from PRRSV-negative breeding herds. Additionally, abattoir-based surveillance could be implemented to screen for antibodies of endemic and/or foreign diseases for which oral fluid antibody detection tests available. These include African swine fever virus (Mur et al., 2013; Giménez-Lirola et al., 2016), *Erysipelothrix rhusiopathiae* (Giménez-Lirola et al., 2013), influenza A virus (Panyasing et al., 2013; Panyasing et al., 2014; Ciacci-Zanella et al., 2015; Hughes et al., 2015; Panyasing et al., 2016), porcine circovirus type 2 (Prickett et al., 2011), and porcine epidemic diarrhea virus (Ouyang et al., 2015; Bjustrom-Kraft et al., 2016). Further studies are needed to characterize herd sensitivity and specificity of oral fluids-based testing at the abattoir for each pathogen.

The frequency of PRRSV RNA detection in farm oral fluids (range 8.1% - 17.2%) was higher than in serum samples (range 1.3 – 1.9%), which was in agreement with previous reports (Kittawornrat et al., 2010; Olsen et al., 2013), and further support the use of oral fluids as an efficient and reliable sample for PRRSV surveillance. At the abattoir, the percent of individual oral fluids testing positive for PRRSV RNA was similar to the oral fluids collected on-farm (range from 8.3% to 17.7%), even with a relatively smaller sample size. The slightly lower frequency of groups testing

positive in the abattoir compared to farm oral fluids may be due to the larger sample size collected from pig lots at the farm (n=10 ropes per pig lot) compared to abattoir (n=3 ropes per lot). It has been demonstrated that the probability of detecting PRRSV RNA by PCR-based assays in oral fluids increased as sample size increased (Rotolo et al., 2017). PCR testing on oral fluids is also available for other diseases, including African swine fever (Grau et al., 2015), classical swine fever (Grau et al., 2015; Petrini et al., 2017), foot-and-mouth disease (Grau et al., 2015; Vosloo et al., 2015), influenza A (Detmer et al., 2011; Ramirez et al., 2012; Goodell et al., 2013; Zhang and Harmon, 2014; Decorte et al., 2015; Biernacka et al., 2016; Goodell et al., 2016), porcine circovirus type 2 (Prickett et al., 2011; Ramirez et al., 2012), porcine epidemic diarrhea virus (Bjustrom-Kraft et al., 2016), porcine deltacoronavirus (Homwong et al., 2016), and others.

The main purpose for collecting abattoir environmental samples was to evaluate if the environment was an important source of cross-contamination for abattoir oral fluid samples. From 7 groups that had at least 1 positive sample on abattoir oral fluids, only 1 group had a positive environmental sample before the study pigs entered lairage. That same group was also detected as positive on on-farm oral fluid and serum samples. Unsuccessful attempts were made to sequence PRRSV ORF-5 from environmental samples. The failure to sequence PRRSV ORF-5 may have been due to the low amount or the poor quality of virus RNA present in the samples (Zhang et al., 2017). Thereby, it was not possible to determine the genetic relationship between the RNA detected in the environment to that detected in oral fluids. Notwithstanding, data from this study did not support the concept that cross-contamination of abattoir oral fluids with virus present in the lairage environment was frequent. Albeit, the small number of positive environmental samples may be related to the sample size (5 environmental swabs pooled to 1 sample) and may not have

represented the true status of the pens in lairage. Alternatively, the lack of PRRSV RNA detection could have meant that the virus was indeed not present at the lairage.

There was a high level of agreement among VDLs for PRRSV ELISA both for serum (97.6%, range 96.2% to 98.7%) and for oral fluids (100%) which aligns with a previous report (Kittawornrat et al., 2012). The agreement of serum rRT-PCR results was 98.4% (range 97.8% to 99.0%) showing that there was a substantial consistency among the VDLs when running PRRSV rRT-PCRs on serum. Ninety seven percent of the agreement (range 96.5% to 97.8%) was derived from negative samples.

On the other hand, the overall agreement among VDLs for oral fluids PRRSV rRT-PCR was almost 20% lower than the agreement for serum. The discordant results on oral fluids samples may be due to the relatively high Ct values (average 34, range $27.1 - 37.2$), affecting the reproducibility of results. Other factors that potentially impact test performance within and between laboratories include laboratory personnel training, as well as differences in procedures, including RNA extraction protocols, primer designs, and/or cycle optimization (Christopher-Hennings et al., 2002).

Bland-Altman plots are another method to compare laboratory results. In this chart, the difference and the average between the Ct values for two laboratories were plotted, allowing for the detection of systematic differences between laboratories. Systematic differences in performance were found between VDL 1 and VDL2, and VDL 2 and VDL 3, but not between VDL 1 and VDL 3. Nonetheless, all laboratories presented a wide range for the limits of agreement, indicating that a large variation could be expected between results from different laboratories.

The overall agreement between locations for oral fluids rRT-PCR was 43.8% (range 31.3 to 50.0%). This discrepancy between group status may be related to the number of samples collected

on farm (n=10 oral fluids) and at the abattoir (3 oral fluids). As previously observed, pig lots with a higher proportion of RNA-positive oral fluids at the farm had a higher detection rate of RNA-positive at the abattoir (data not shown). For PRRSV ELISA the agreement was 100% between locations.

PRRSV surveillance at the abattoir has already been reported using serum samples (Grunberger et al., 2015) and meat juice (Mortensen et al., 2001). Nonetheless serum and meat juice samples offer limitations that make the wide implementation of PRRSV surveillance at the abattoir difficult. Those limitations include: 1) requiring restraining pigs to collect individual pig blood (can collect after killing pigs, but requires personnel and line speed must be slow enough for personnel to get pig IDs); 2) sample size to represent the disease status of the population (e.g., 60 sera provide 95% confidence to detect pathogen by PCR at prevalence of at least 5%); which lead to 3) high cost; 4) need of intensive labor; 5) meat juice lacks uniformity of results depending on the muscle evaluated (Wallander et al., 2015), which was reported for *Toxoplasma gondii*, but has not been investigated for PRRSV; and 6) with a sensitivity of 36.4% when using 10 samples for a prevalence of 5% (Molina et al., 2008).

Oral fluid specimens on the other hand are "aggregate samples" (one sample from multiple pigs) that have been proposed as *bona fide* alternative to conventional samples (Prickett et al., 2008a; Prickett et al., 2008b; Kittawornrat et al., 2010; Prickett et al., 2010; Prickett and Zimmerman, 2010; Olsen et al., 2013; Kittawornrat et al., 2014; Pepin et al., 2015; Rotolo et al., 2017). Oral fluid samples at the abattoir can be easily collected by one person, requiring less labor than serum and meat juice, with the advantage of being readily available for sample submission to the lab, contrary to serum and meat juice, that require an extra step before sample submission (centrifugation, and freezing/thawing, respectively). As an aggregate sample, oral fluids would

also offer an economical benefit over serum and meat juice samples for population-level screening of antibodies and/or nucleic acid. A few oral fluids samples can be used to assess the disease status of pig lots at the abattoir. Conversely, multiple serum or meat juice samples would be needed for the same assessment to provide similar herd sensitivity. Additionally, multiple lots from the same farm could be used to increase sensitivity of abattoir oral fluid sampling when using rRT-PCR. PRRSV-negative pig flows that do not practice pig vaccination could use the abattoir-based monitoring system with oral fluids to monitor exposure to PRRSV after weaning. Antibody detection could also be used for foreign animal diseases as part of a national surveillance program.

Limitations to this study include the lack of evaluation of the role of pig transport as a source of contamination of pig lots to PRRSV. Also, abattoir oral fluid samples were collected using ropes hanging from a single chain, which may have led to cross-contamination of the 3 abattoir ropes within the same pig lot. Moreover, larger sampling intensity at the farm and at the abattoir would have provided additional statistical power to compare frequency and magnitude of PRRS RNA between locations.

Future research is needed to further our understanding the role of transport as a source of confounding between farm and abattoir results. Moreover, an increased sample size at the abattoir would allow better characterization of the effect of number of oral fluids per lot and the herd sensitivity to detect pathogens of choice. Also, research is needed to investigate abattoir based oral fluids sampling to screen for antibodies and/or nucleic acid of other pathogens.

Conclusions

This study reports, for the first time, the use of oral fluids obtained at an U.S. abattoir for monitoring and surveilling PRRSV in growing swine populations in a practical and affordable

way. We demonstrated detection of anti-PRRS antibodies and PRRS nucleic acid from swine oral fluids obtained at the abattoir. The general agreement of ELISA testing between locations was 100%. Likewise, it was demonstrated the ability to detect PRRSV RNA in swine oral fluids collected at the abattoir, even with limited sampling (n=3 oral fluids per lot) compared to on-farm sampling (n=10 oral fluids per lot). Further studies are needed to establish sample size requirements to detect PRRSV RNA at low prevalence in abattoir lairage (i.e. large pens). Moreover, it was not possible to rule out the role of environment as contaminant of oral fluids at the abattoir for molecular testing. Nonetheless, data showed that PRRS RNA detection in environmental samples was not frequent event under study conditions.

Acknowledgments

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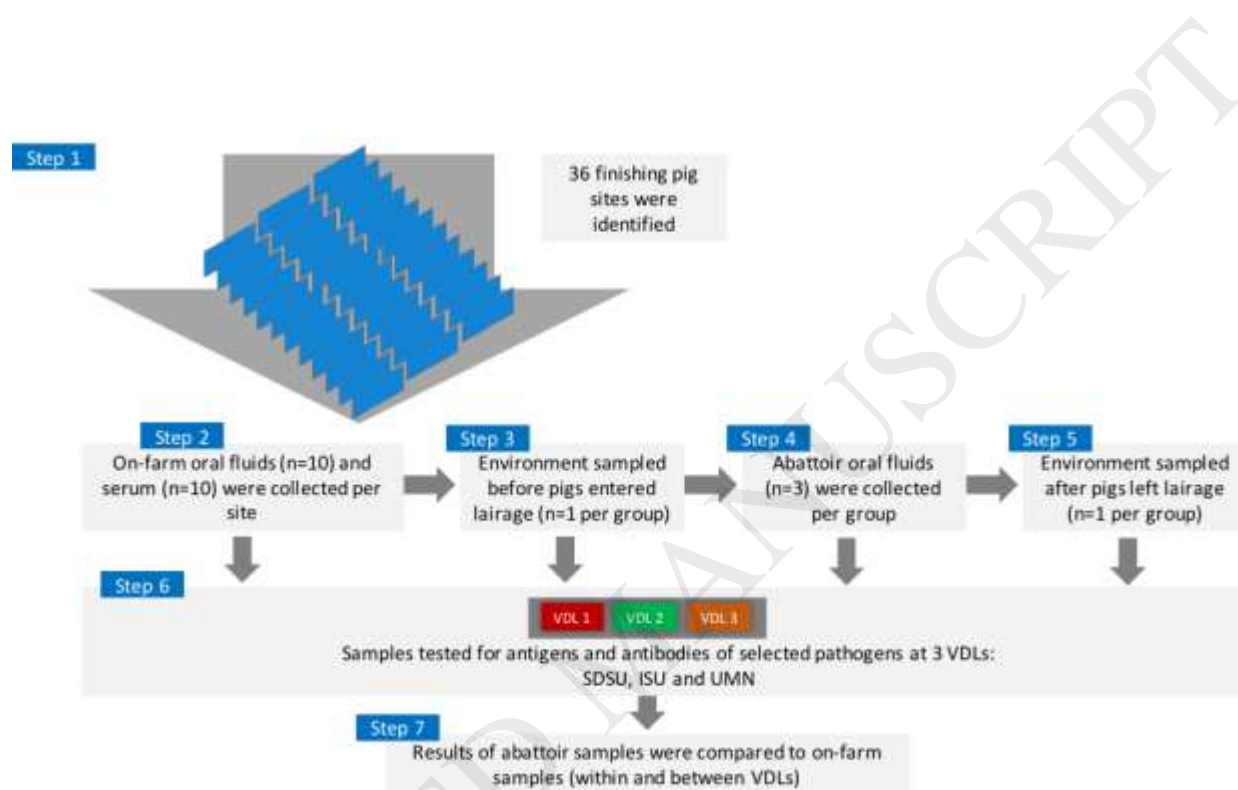
Fig 1. Experimental design: Assessment of oral fluid-based abattoir surveillance.
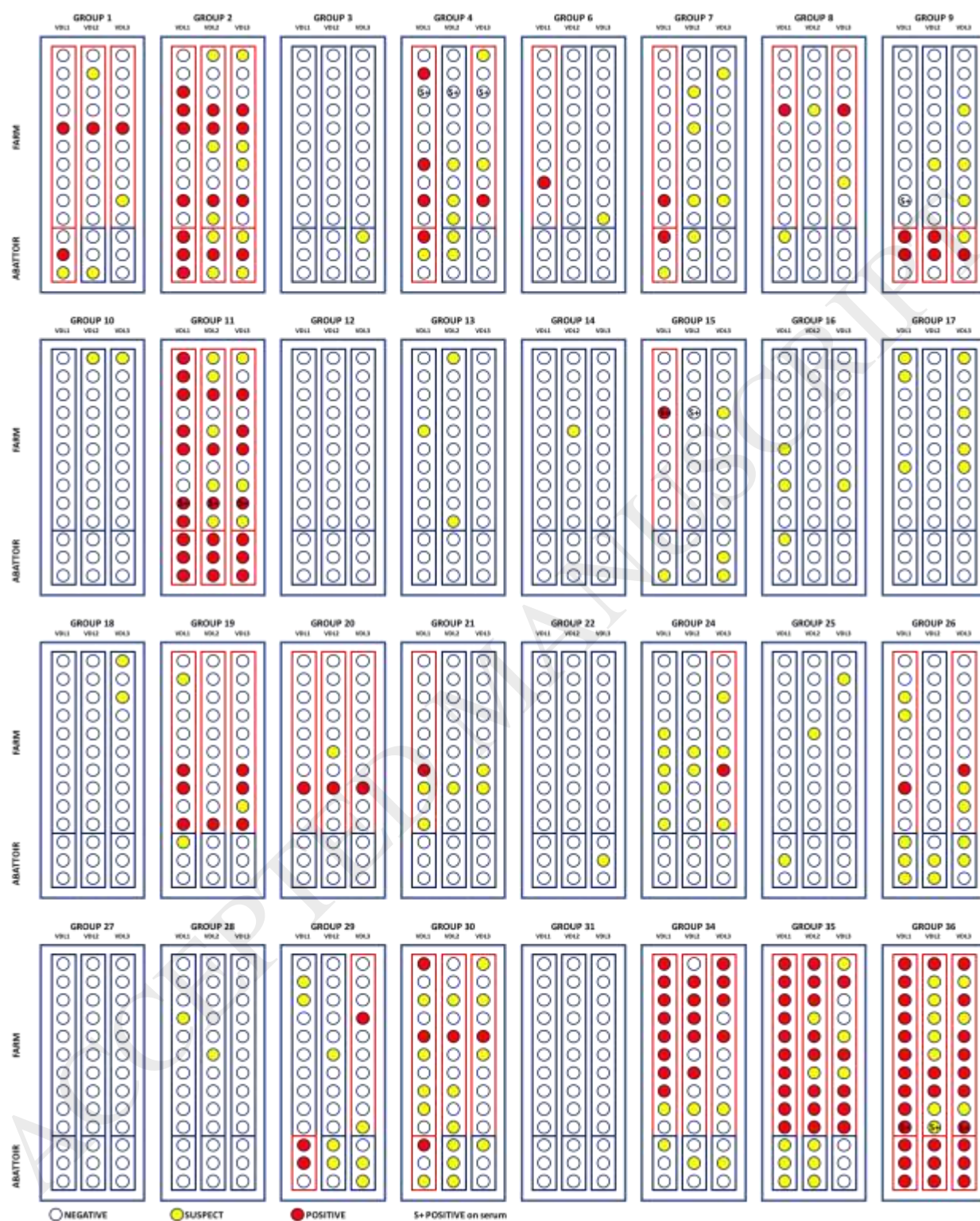
Fig 2. Detection of PRRSV RNA by rRT-PCR in on-farm and abattoir oral fluids from 32 pig lots in three different Veterinary Diagnostic Laboratories (VDLs). Circles represent

individual oral fluid samples collected from specific groups on-farm and at abattoir, aliquoted and tested by rRT-PCR in each VDL. The outside rectangle represents a group (10 farm and 3 abattoir samples tested in each VDL), the inner rectangles represent each VDL. When at least one sample tested positive by rRT-PCR, the rectangle has a red outline. Conversely, when there were only negative or suspect results the outline is blue.

Fig 3. Bland-Altman plots of PRRSV rRT-PCR Ct values by VDL1 versus VDL2, VDL1 versus VDL3 and VDL2 versus VDL3. Upper 95% LOA = Upper Limit of Agreement; Lower 95% LOA = Lower Limit of Agreement; No bias line = zero line, or line of equality, where all dots would lie if there was a perfect agreement.

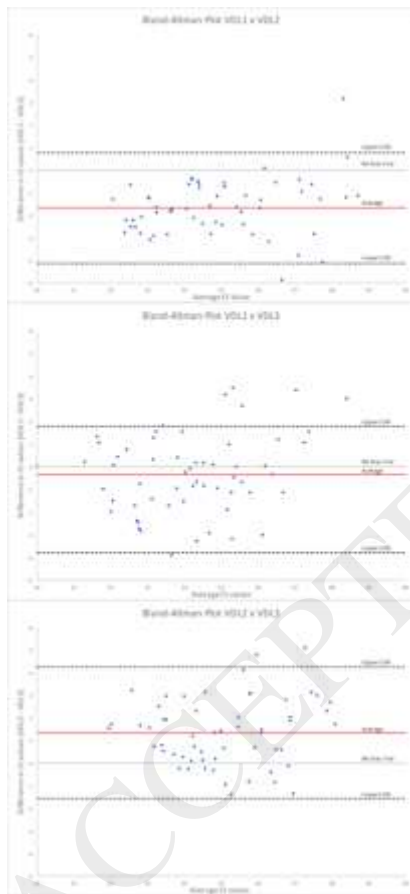**Table 1**. Frequency of PRRSV antibodies (ELISA) and RNA (rRT-PCR) detection in serum and oral fluids collected on-farm, and

oral fluids and environmental samples collected at abattoir.

	VDL1		VDL2		VDL3	
	RNA (+)	ELISA (+)	RNA (+)	ELISA (+)	RNA (+)	ELISA (+)
On-farm sampling						
Serum	6 of 316 (1.9%)	295 of 316 (93.4%)	5 of 315 (1.6%)	291 of 315 (92.4%)	4 of 316 (1.3%)	299 of 316 (94.6%)
Oral fluids by sample	55 of 319 (17.2%)	319 of 319 (100%)	26 of 320 (8.1%)	320 of 320 (100%)	35 of 319 (11.0%)	319 of 319 (100%)
Oral fluids by pig lot group results	16 of 32 (50%)	32 of 32 (100%)	9 of 32 (28.1%)	32 of 32 (100%)	14 of 32 (43.8%)	32 of 32 (100%)
Abattoir sampling						
Environmental – before placement in lairage	3 of 32 (9.4%)	-	-	-	-	-
Oral fluids by sample	17 of 96 (17.7%)	96 of 96 (100%)	9 of 96 (9.4%)	96 of 96 (100%)	8 of 96 (8.3%)	96 of 96 (100%)
Environmental – after removal from lairage	2 of 32 (6.3%)	-	-	-	-	-
Oral fluids – by pig lot group results	9 of 32 (28.1%)	32 of 32 (100%)	4 of 32 (12.5%)	32 of 32 (100%)	4 of 32 (12.5%)	32 of 32 (100%)

Table 2. Between VDLs agreement for PRRSV rRT-PCR on oral fluid samples (farm and abattoir).

		VDL1			VDL3		
		Positive no. (%)	**Suspect no. (%)**	**Negative no. (%)**	**Positive no. (%)**	**Suspect no. (%)**	**Negative no. (%)**
VDL2	Positive	35 (8.4)	0 (0.0)	0 (0.0)	29 (7.0)	3 (0.7)	2 (0.5)
	Suspect	24 (5.8)	14 (3.4)	20 (4.8)	8 (1.9)	23 (5.5)	27 (6.5)
	Negative	13 (3.1)	26 (6.3)	283 (68.2)	6 (1.4)	32 (7.7)	285 (68.7)
VDL3	Positive	40 (9.7)	1 (0.2)	2 (0.5)	-	-	-
	Suspect	19 (4.6)	12 (2.9)	26 (6.3)	-	-	-
	Negative	12 (2.9)	27 (6.5)	275 (66.4)	-	-	-

Table 3. Agreement of PRRSV rRT-PCR results between farm and abattoir oral fluids at different

VDLs

	FARM	Abattoir			
		Positive no. (%)	**Suspect no. (%)**	**Negative no. (%)**	
Farm	**Positive**	7 (21.9)	6 (18.8)	3 (9.4)	**VDL1**
	Suspect	1 (3.1)	1 (3.1)	4 (12.5)	
	Negative	1 (3.1)	1 (3.1)	8 (25.0)	
Farm	**Positive**	3 (9.4)	4 (12.5)	2 (6.3)	**VDL2**
	Suspect	1 (3.1)	3 (9.4)	8 (25.0)	
	Negative	0 (0.0)	1 (3.1)	10 (31.3)	
Farm	**Positive**	3 (9.4)	4 (12.5)	7 (21.9)	**VDL3**
	Suspect	1 (3.1)	1 (3.1)	8 (25.0)	
	Negative	0 (0.0)	2 (6.3)	6 (18.8)	

Appendix 1. Description of sow herd status, piglet vaccination status and timing, and site ID of pig lots sampled at finishing site and abattoir.

Group	System	Finishing site	Sow farm PRRSV status	Piglets Vaccinated	Vaccination timing
1	A	A	NEGATIVE	NO	
2	A	B	NEGATIVE	NO	
3	A	C	NEGATIVE	NO	
4	A	D	POSITIVE	YES	AT PLACEMENT
5	A	E	POSITIVE	YES	AT PLACEMENT
6	A	F	POSITIVE	YES	AT PLACEMENT
7	A	G	POSITIVE	YES	AT PLACEMENT
8	A	H	NEGATIVE	NO	
9	A	I	POSITIVE	YES	AT PLACEMENT
10	A	J	NEGATIVE	NO	
11	A	K	POSITIVE	YES	AT PLACEMENT
12	A	L	POSITIVE	YES	AT PLACEMENT
13	A	M	POSITIVE	YES	AT PLACEMENT
14	A	N	NEGATIVE	NO	
15	A	O	POSITIVE	YES	AT PLACEMENT
16	A	P	POSITIVE	YES	AT PLACEMENT
17	B	Q	NEGATIVE	YES	2 WEEKS AFTER PLACEMENT
18	B	Q	NEGATIVE	YES	2 WEEKS AFTER PLACEMENT
19	B	R	NEGATIVE	YES	2 WEEKS AFTER PLACEMENT
20	B	R	NEGATIVE	YES	2 WEEKS AFTER PLACEMENT
21	B	S	NEGATIVE	YES	2 WEEKS AFTER PLACEMENT
22	B	S	NEGATIVE	YES	2 WEEKS AFTER PLACEMENT
23	B	S	NEGATIVE	YES	2 WEEKS AFTER PLACEMENT
24	B	S	NEGATIVE	YES	2 WEEKS AFTER PLACEMENT
25	B	R	NEGATIVE	YES	2 WEEKS AFTER PLACEMENT
26	B	R	NEGATIVE	YES	2 WEEKS AFTER PLACEMENT
27	B	T	NEGATIVE	NO	2 WEEKS AFTER PLACEMENT
28	B	T	NEGATIVE	NO	2 WEEKS AFTER PLACEMENT

29	B	U	POSITIVE	NO	2 WEEKS AFTER PLACEMENT
30	B	U	POSITIVE	YES	2 WEEKS AFTER PLACEMENT
31	B	U	POSITIVE	YES	2 WEEKS AFTER PLACEMENT
32	B	V	NEGATIVE	YES	2 WEEKS AFTER PLACEMENT
33	B	V	NEGATIVE	YES	2 WEEKS AFTER PLACEMENT
34	B	V	NEGATIVE	YES	2 WEEKS AFTER PLACEMENT
35	B	V	NEGATIVE	YES	2 WEEKS AFTER PLACEMENT
36	B	V	NEGATIVE	YES	2 WEEKS AFTER PLACEMENT