RESEARCH REPORT
SWINE HEALTH

Title: Evaluation, optimization, and application of "processing fluids" for PRRSV monitoring in commercial swine herds - **NPB #17-161**

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Industry Summary:

The aim of this project was to assess the use of processing fluid samples in PRRSV monitoring and surveillance. For the purpose of this project, "processing fluid" (PF) was defined as an aggregate sample composed of the fluids recovered from the tissues (testicles and tails) collected from piglets at the time of processing (Lopez et al., 2017).[1] Processing fluids are easily obtained by farm staff under field conditions and represent a unique opportunity to significantly improve current monitoring schemes.[2] The use of processing fluids 1) reduces the cost of PRRSV surveillance, 2) increases the number of piglets sampled, and 3) increases the frequency of testing.

The overall objective of this study was to evaluate the use of processing fluids for PRRSV monitoring. Our specific aims were to 1) *determine limit of PRRSV detection in processing fluids using a commercial quantitative reverse transcription polymerase chain reaction (qRT-PCR) assay*; 2) *optimize serologic assays to detect anti-PRRSV antibodies in processing fluids, and* 3) *at low prevalence (<5%), describe the probability of PRRSV detection using processing fluids tested with qRT-PCR.*

To achieve Aim 1, 6 replications of eight two-fold serial dilutions were done on a PRRSV-positive field sample with known qRT-PCR results using processing fluids from PRRS-naïve herds and the PRRSV limit of detection was assessed. For Aims 2 and 3, litter-matched processing fluids and individual pig blood samples were collected from 77 litters. Sample collection was carried out in two PRRSV-positive breeding herds at different sampling point in time following PRRS outbreaks and therefore, capture different PRRSV prevalence levels within the herds (from high to low prevalence), thus allowing for the assessment of the probability of PRRSV detection in processing fluids using the status of individual pig

sera as the reference. Samples were tested for antibody using the IDEXX PRRS X3 Ab ELISA test: first using the kit's manufacturer's directions and secondly, after making adaptations. Processing fluids obtained from PRRS-naïve herds were used for comparison.

Based on the PRRSV detection probabilities derived from the qRT-PCR results from the serially diluted processing fluid field sample, the limit of PRRSV detection by qRT-PCR in processing fluids was 1 PRRSV-positive pig out 270 total pigs in the pool, with a 95% confidence level, given that there is only one viremic piglet with a qRT-PCR CT value of 29 in the sample.

The results obtained with a modified PRRS IgG antibody ELISA showed a better and clear discrimination between positive and negative processing fluid samples compared to the original ELISA kit, designed only for IgG antibody detection in serum samples. The IgA results showed good discrimination between positive and negative processing fluid samples overall; however, not all samples detected positive by PCR were IgA-positive. Contrary, IgM results showed poor discriminatory power to detect active or recent PRRSV infection in processing fluids.

The probability of PRRSV detection in processing fluids was 72.7% at the individual litter level, as compared with the blood serum sample qRT-PCR results used as the gold standard method for comparison and to define the true status of the litters regarding PRRSV. However, when pooled by farrowing room or by the whole batch of piglets processed during a day, processing fluids had a probability of PRRSV detection of 100%.

The work hereby presented addresses all of these points and provides the industry with a reliable, fast, practical, and affordable surveillance system to detect PRRSV at low prevalence in suckling pigs. It is our expectation that this will pave the road for disease elimination efforts at regional and/or national levels. Controlling and eliminating PRRS from the U.S. swine industry will significantly improve the economical sustainability and competitive advantage of pork production in this country.

Keywords: processing fluids, porcine reproductive respiratory syndrome, diagnostic, swine, monitoring

Scientific Abstract:

Processing fluids (PF) are easily obtained by farm staff under field conditions and represent a unique opportunity to significantly improve current monitoring schemes. Our preliminary work suggests that PF provide the capacity to detect PRRSV at prevalence levels ≤ 5%. The overall purpose of this project was to evaluate the use of PF for PRRSV monitoring through specific objectives: 1) Determine limit of PRRSV detection in PF using a commercial quantitative reverse transcription polymerase chain reaction (qRT-PCR) assay, 2) Optimize serologic assays to detect anti-PRRSV antibodies in PF: compare IgM, IgA, IgG, and IgM+IgA responses and 3) Describe the probability of PRRSV detection using PF tested with qRT-PCR at low prevalence (<5%). Six replications of 8 two-fold serial dilutions were done on a PRRSV-positive PF field sample with known qRT-PCR results using PF from PRRSV-naïve herds and the PRRSV limit of detection (LoD) was assessed. Results from serially diluted PF determined the PRRSV LoD in PF being of only one (1) PRRSV-positive pig out 270 total pigs in the pool, with a 95% confidence level, having only one viremic piglet with a qRT-PCR CT value of 29 in the PF sample. For specific objectives 2 and 3, litter-matched PF and individual pig blood samples were collected from 77

litters under field conditions. Samples were collected from two PRRSV-positive breeding herds at three different sampling points in time after PRRS outbreaks to capture different PRRSV prevalence levels, thus allowing for the assessment of the probability of PRRSV detection in processing fluids using the status of individual pig sera as the reference. Samples were tested for antibody using the IDEXX PRRS X3 Ab ELISA test following the manufacturer's directions, and after making adaptations to the kits. PF samples obtained from PRRSV-naïve herds were used for comparison. IgG, IgA and IgM antibodies were detected in PF. IgG antibody detection showed a clear discrimination between positive and negative samples. The IgA antibody isotype results showed good discrimination between positive and negative samples, but not all samples detected positives by qRT-PCR were IgA positives. IgM results were not as relevant as IgA results for detection of active or recent PRRSV infection. The probability of PRRSV detection in PF was 72.7% at the individual litter level, as compared with the blood serum (BS) sample qRT-PCR results used as the gold standard method for comparison and to define the true status of the litters regarding PRRSV. However, when pooled by farrowing room or by the whole batch of piglets processed during a day, PF had a probability of PRRSV detection of 100%. At the individual litter level, the Kappa index of agreement between PF and BS sample results was of 0.79 - 95% CI (0.54 – 0.95). PF-based sampling had a lower sensitivity than bleeding all piglets in the litter, but had 100% sensitivity and 100% specificity at the whole room, or whole day level (pooled PF). Data supports that, due to the ease of implementation and the lower cost (relative to individual pig sampling), processing fluids is a robust tool for PRRSV monitoring and surveillance in 3-5 days-old piglets.

Introduction

Ongoing challenges to the detection and management of disease in the U.S. swine herd threaten the economical sustainability of the swine industry. PRRSV infection alone costs more than $1 billion per year.[3] An important step in controlling and eliminating PRRSV in production systems is interrupting the transmission cycle in breeding herds, with the goal of producing PRRSV-free piglets at weaning.[4] In this context, PRRSV monitoring is an essential tool to measure progress towards PRRS control efforts.

The current AASV guidelines for monitoring breeding herds for PRRSV call for collecting blood samples from 30 piglets at monthly intervals.[5] This monitoring scheme is not expected to detect virus at prevalence under 10%, and thus may not be sensitivity enough for early detection (because testing is only done monthly).

Studies have shown that PRRSV can sustain a prevalence of ~1% in weaned piglets for a prolonged period.[6,7] Although few in number, these infected piglets pose a risk to downstream populations and to PRRSV elimination efforts in the breeding herd. Using conventional sample size calculations, detection of PRRSV at 1% prevalence with 95% confidence would require collecting blood samples from 299 piglets. Thus, detection of infection at low prevalence is too expensive and impractical using conventional sampling methods (individual serum samples).

In contrast, aggregate samples allow sampling a greater number of animals in the population with significantly less time and resources (materials and labor). Oral fluids are one example of an aggregate sample, but collecting oral fluids from suckling pig populations has not proven to be practical. In contrast, our experience is that processing fluids are easily obtained during piglet processing and are extremely sensitive for the detection of PRRSV infections.

This research work directly addressed the following specific research priorities in the NPB 2017 Spring RFP:

1. Under *PRRS surveillance and diagnostics*, "improve the efficiency and accuracy of testing" through better "on-farm sampling methods" and higher "diagnostic sensitivity and/or diagnostic specificity".
2. Under PRRS surveillance and diagnostics, "surveillance protocols with improved efficiency and lower costs" and "sampling/testing strategies for the detection of low prevalence infection (<5%) in pigs in breeding herds".

We believe that creating a practical, affordable, simple, and accurate system to surveil PRRS in breeding herds will motivate veterinarians and producers to voluntarily increase the frequency and quality of disease surveillance, which will consequently result in improved documentation of disease activity over time, a key factor in efforts to eliminate PRRSV at regional or national levels. Thus, validation of processing fluids for farrowing room monitoring would greatly benefit the industry by facilitating surveillance of PRRSV (and potentially other pathogens).

Objectives

The overall objective of this study is to assess the use of processing fluids for PRRSV monitoring in breeding herds.

Aim 1. Determine PRRSV qRT-PCR limit of detection in processing fluids. The limit of PRRSV RNA detection (analytical sensitivity) will be assessed in processing fluids. This will determine the minimum prevalence at which PRRSV RNA can be detected by qRT-PCR in processing fluids when collected at the aggregated level (collecting all litters from a room or all litters from a whole day of processing together). This value will drive the number of pigs to be pooled for obtaining processing fluids, i.e., Can PRRSV be detected in processing fluids when 1 out of 100 pigs positive? 1/200? 1/300?

Aim 2. Optimize serologic assays to detect anti-PRRSV antibodies in processing fluids: compare IgM, IgA, IgG, and IgM+IgA responses, establish cut offs. Our preliminary work showed that PRRSV antibody could be detected in processing fluids using PRRSV ELISAs currently used for swine serum samples and oral fluids. In expected-negative weaned pig populations, IgG testing is a cost-effective way to confirm herd negativity. However, in populations originated from sow herds endemically infected and/or vaccinated with attenuated PRRSV, maternal IgG cannot be differentiated from IgG produced by the pigs in response to infection. To address this problem, we will evaluate the use of IgM- and/or IgA -specific ELISA as a means to detect active infection in the face of IgG maternal antibodies.

Aim 3. Compare the probability of PRRSV detection using processing fluids tested with qRT-PCR versus blood serum. A field study will be conducted to determine the minimum prevalence at which PRRSV RNA can be detected by qRT-PCR in processing fluids and further compare the probability of PRRSV RNA detection by qRT-PCR in processing fluids with conventional individual pig blood serum sampling schemes.

Materials and methods

We have designed a series of experiments to achieve the three project aims:

Aim 1. Determine PRRSV qRT-PCR limit of detection in processing fluids.

Study design. For the purpose of this study, PF is defined as an aggregate fluid sample composed of the serosanguineous drainage from the tissues collected from piglets at the time of castration and tail docking (piglet processing), typically in the first week of age. PF were collected using the protocol established in our preliminary studies (Figure 1; Lopez et al. JSHAP). PF were obtained from herds expected to be PRRSV-naïve according to the AASV criteria for breeding herd classification. PRRSV qRT-PCR were conducted in 77 PF samples and matching blood serum (BS) samples (~834 individual samples). The probability of detecting PRRSV in PF was assessed based on the true prevalence within the litter (i.e., proportion of viremic piglets as determined by PRRSV qRT-PCR on individual pig BS). Preliminary results from the aforementioned field study, revealed that litters having a single viremic piglet were the most commonly found across the dataset. The median cycle threshold (CT) value of PRRSV qRT-PCR on PF from those litters was 30.21. For the purpose of this study, litters having a single viremic piglet are defined as 'low prevalence litters'. Thus, to represent a conservative scenario, we targeted to obtain a field PF sample with a CT value of 30. PF previously tested and with CT of 28 was used to account for increase in CT value on the sample after a freeze-thaw cycle. The field PF sample was used to represent a litter of 11 pigs having a single viremic piglet in it (low prevalence litter). Using PF from PRRS-naïve herds, six replications of eight two-fold serial dilutions were performed on the PRRSV-positive sample. The serial dilutions were made to model the dilution effect of pooling PRRSV-positive PF with PRRSV-negative PF on the probability of PRRSV RNA detection by qRT-PCR. For each two-fold dilution, the number of PRRSV-negative pigs represented in the PF pool doubled relative to the previous PF sample. For instance, the first dilution would represent a PF sample from 22 pigs where only 1 piglet is viremic. After eight dilutions, the PF pool would represent 2,816 pigs with one viremic pig. The limit of PRRSV detection in PF by qRT-PCR, will be defined as the number of total pooled pigs containing one viremic pig and that produces at least 95% positive PRRSV qRT-PCR replicates, adapted from the limit of detection definition of Forootan et al. 2017[8]

All samples were tested individually for PRRSV RNA by qRT-PCR using the RealPCR IDEXX PRRS commercial kit at the Iowa State University Veterinary Diagnostic Laboratory's Research and Development laboratory.

Aim 2. Optimize serologic assays to detect anti-PRRSV antibodies in processing fluids: compare IgM, IgA, IgG, and IgM+IgA responses, establish cut offs.

In expected PRRSV-naïve weaned pig populations, IgG testing is a cost-effective way to confirm herd negativity. However, in populations from sow herds endemically infected and/or vaccinated with attenuated PRRSV, maternal IgG cannot be differentiated from IgG produced by the pigs in response to infection. To solve this problem, we are evaluating the use of IgM- and/or IgA-specific PF ELISA as a means to detect active infection in the face of IgG maternal antibodies.

Overview. To achieve Aims 2 and 3, litter-matched PF and individual pig BS samples were collected from 77 litters (Figure 2). PF were collected using the protocol described in (Figure 1), except that disposable materials (e.g. gloves, and pig processing supplies) were changed between litters to avoid cross contamination.

Eligibility of breeding herds. Litter-matched samples were collected in six farrowing rooms from two commercial breed-to-wean herds naturally infected with PRRSV. The samples were taken at two sampling points in time from farm A, and at one sampling point in time from farm B time to capture three different PRRSV prevalence levels:

a) First sampling point (Farm A): Acutely infected herd with expected high prevalence (i.e., within six weeks of the PRRS outbreak).
b) Second sampling point (Farm A): Herd with medium to low prevalence with an ongoing PRRSV elimination program (i.e., at least 10 weeks after the PRRS outbreak).
c) Third sampling point (Farm B): Herd with PRRSV prevalence under 5% in suckling pigs (At least 3 months after last PRRSV detection reported by diagnostic tests and/or no PRRS clinical signs).

The samples were categorized into three groups based on the PRRSV prevalence in suckling pigs and the sampling point in time as: 'High prevalence', 'Mid-Low prevalence' and 'Low prevalence' groups correspondingly.

Study design. Serum samples collected from the 77 litters were used to determine the true status of PF samples. Serologic assays currently available to detect PRRSV IgG antibodies in swine serum (i.e., PRRS X3 Ab Test, IDEXX Laboratories, Inc., Westbrook, ME) are being optimized for the detection of different antibody isotypes (IgM, IgA, IgG, IgM+IgA) in PF.

Aim 3. At low prevalence (<5%), describe the probability of PRRSV detection using processing fluids tested with qRT-PCR.
We collected 834 individual serum samples from 3-5 days old piglets, and 77 PF samples corresponding to each litter. Samples were obtained from six farrowing rooms from two breed-to-wean farms as described above. PRRSV qRT-PCR were conducted in the 77 PF and matching blood samples. The probability of detecting PRRSV in processing fluids individually by litter was assessed based on the true prevalence within the litter (i.e., proportion of viremic piglets as determined by PRRSV qRT-PCR on individual pig sera) and described for the three previously defined groups based on the PRRSV prevalence in suckling pigs ('High prevalence', 'Mid-Low prevalence' and 'Low prevalence' groups). Kappa statistic was calculated between blood serum samples and PF samples at the litter level to assess the index of agreement between sample types. Additionally, individual litter processing fluid samples were pooled by rooms within the same day of collection and by whole day of collection and tested by qRT-PCR for PRRSV RNA detection. The probability of detecting PRRSV by qRT-PCR in the pooled samples was assessed as well. All samples were tested using the RealPCR IDEXX PRRS commercial kit, with the cut-off value set at 37. All testing was performed at same laboratory facilities as described for the objective aim 1. Furthermore, using the same dataset and its results, the probability of PRRSV detection on pooled PF (pooled by rooms and/or pooled by the whole day of collection) was compared with the probability of detecting PRRSV using conventional sampling schemes (30 BS samples from individual pigs) in the three different prevalence groups.

Statistical methods. The analyses of qRT-PCR results were done using simple linear regression model for the analysis of CT values. Probability of PRRSV detection by qRT-PCR in processing fluids was calculated using Proc Probit (SAS 9.4, SAS Institute Inc. Carry, NC). Likewise, the limit of PRRSV detection by qRT-PCR was calculated using the same method. The comparison of probability of PRRSV detection in pooled processing fluids and conventional individual blood serum sampling schemes (30 blood serum samples from individual pigs) was assessed using a resampling method known as bootstrapping. The technique also known as random sampling with replacement, performed a simulation of 10,000 iterations; randomly sampling 30 serum samples from the 834 blood serum sample results to obtain the probability of detecting at least one (1) PRRSV-positive serum sample in each of the 3 prevalence groups previously described.

Results aim 1. The results of serial dilutions demonstrated an average increment of 1.37 points in CT values for each ten-fold dilution across the six replications. The mean CT values for each serially diluted group of six replications are shown in Table 1. Thus, most qRT-PCR results were negative after the 6^{th} serial dilution when the CT value were higher than the cut-off point. The raw probabilities of detecting PRRSV by qRT-PCR are presented in Table 2. The proportion of PCR-positive results decreased to 33% at the 6^{th} dilution (704 piglets), then to 17% at the 7^{th} dilution (1,408 piglets) and 0% at the 8^{th} dilution (2,816 piglets). Figure 3 shows the predicted probabilities for PRRSV detection derived from the data, by the number of pigs in the pooled sample, given that there is only one 'low prevalence litter' with a CT value of 29. Based on the probabilities described above, at 95% confidence level, the limit of PRRSV detection by qRT-PCR in PF is reached at a proportion of 1:270 pigs in the aggregate PF sample (i.e. one PRRSV-positive pig in 270 total pigs).

Results aim 2. Among the modifications performed on the IDEXX PRRS X3 ELISA kit, we evaluated the antibody response of isotypes IgG, IgA and IgM in processing fluids. The IgG results obtained with the modified kit (Figure 5. A) showed a clear discrimination between positive and negative PF samples compared to IgG results obtained with the original kit (Figure 4). The IgA results showed good discrimination between positive and negative PF samples overall; however, few samples show close proximity in the sample-to-positive (S/) ratio values and not all samples detected negatives by qRT-PCR were IgA-positives (Figure 5. B).
With the exception of few positive samples, IgM results were not as good as IgA results for detection of active or recent PRRSV infection (Figure 5. C). Tables 3-A, 3-B 3-C and 3-D show the frequency distribution of the PRRSV X3 ELISA test results in PF samples for the original kit and the modified version for different anti-PRRSV Ab detection using the PF individual litter qRT-PCR results as the reference for comparison. Cut-offs assessment and associated diagnostic performance of the isotype-specific antibody Ab ELISAs adapted for PF samples is currently ongoing.

Results aim 3. From the total 911 samples collected, 77 were litter PF and 834 were matching individual pig serum. The distribution of samples from 'Farm A' across the two sampling points was: 21 litters (212 pigs) in the 'High prevalence' group and 26 litters (289 piglets) in the 'Mid-Low prevalence' group. 30 litters (333 piglets) were in the 'Low prevalence' group from 'Farm B'. The average litter size recorded for the whole dataset was of 10.8 piglets per litter. The overall PRRSv prevalence (determined by rRT-PCR testing of individual pig sera) was 20.3%, 8.3%, and 0.0% respectively for the 'high prevalence', 'mid-low prevalence' group and the 'low prevalence' groups (Table 5). There were 22 (28.6%) litters with least 1 viremic piglet, and 16 (20.8%) litters that tested positive on PF (Table 7). The Kappa index of agreement between the results of PF tested individually per litter and the individual blood serum samples was of 0.79 (0.54 – 0.95). Using serum results as the reference method to define the true PRRSV status of the litters, the litter sensitivity and litter specificity of the PF sampling was 72.7%, and 100% respectively. The positive predictive value for PF testing was 100%, and the negative predictive value was 90%. However, when litters were pooled by farrowing room (all litters processed within a farrowing room), or by whole day of collection (all litters processed in a day) the sensitivity of the PF method was 100%. The specificity for PF testing was 100% on all analysis (litter level, whole room level, and whole day level) (Table 6). The results from the simulated sampling method performed on the 834 serum samples demonstrate a probability of detecting at least 1 PRRSV-positive pig from "conventional" sample size of 30 of 99.89% in the 'High prevalence' group, 93.53% in the 'Mid-Low prevalence' group and 0% for the 'low prevalence' group after 10,000 sampling events for each

prevalence group. In comparison, the probability of PRRSV detection of the PF method was 100% across all the prevalence groups where there was at least one PRRSV-positive pig.

Discussion:

The data supports that processing fluids is a robust tool for PRRSV monitoring and surveillance in suckling pigs. Regression analysis for the serially diluted PF data indicates that 1 viremic pig with a CT value of 29 could be detected in a pooled PF sample with up to 780 PRRSv-negative pigs with 61% probability. Similarly, data suggests that pooling PF from about 1 viremic piglet along with 400 non-viremic piglets (about 28 litters) gives 90% probability of detecting PRRS RNA by qRT-PCR (Figure 3). The scenario presented here models the 'worst case scenario' of having just 1 viremic pig in the PF sample which was a scenario previously described. PF sampling method is capable of detecting 1 single PRRSV-positive pig among a high number of negative pigs depending upon the magnitude of viremia of the positive pig. Regarding the optimization of serologic assays for antibody detection in PF samples, when looking at Figure 4, we can see a clear discrimination between negative and positive samples, but there is one limitation working with the IDEXX kit as it is per manufacturer's directions: it only detects IgG antibodies. The lack of concordance between qRT-PCR and IgG ELISA results was not unexpected, since it is possible to find herds that are PRRSV negative by qRT-PCR but positive for Ab if there was previous exposure to the virus. With the exception of four samples, the IgA results matched with PCR results. PF-based sampling had a lower sensitivity than bleeding all piglets at the litter level, but had 100% sensitivity and 100% specificity at the whole room, or whole day level. These results on PF also suggest that pooling litters increases the sensitivity of the method and opens the door for pooling a massive number of pigs in a single sample, depending upon the true PRRSV prevalence of the herd being sampled, making it extremely cost-efficient for qRT-PCR testing. There is an obvious risk of dilution effect due to the increased pooling of PRRSV-negative PF with the original PRRSV-positive PF field sample, which suggests that the probability of PRRSV RNA detection decreases as the number of PRRSV-free pigs in the pool increase. However, pooling PF (e.g. whole-room as opposed to PF from few litters) may also increase the coverage (number of pigs contributing to the PF sample) and this may increase the probability of PRRSV detection by qRT-PCR. Therefore, it is important to remember that only one negative qRT-PCR test result at a given point in time is not sufficient to determine the absence of PRRSV in a pig population. Continuous sampling over time is recommended to better characterize virus circulation at piglet processing time. The ability to pool large numbers of pigs in a PF sample will allow to test more pigs more frequently. Results of this study can be used as reference for designing PF-based sampling protocols.

We will continue to develop the guidelines for the best sampling scheme, to tailor it depending on the individual system's characteristics and challenges as well the expected prevalence for the disease in given herds.

References

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Tables and figures

Figure 1. Procedure for the collection of processing fluids. A clean plastic container is lined with a plastic bag. The mouth of the container is covered with cheese cloth to hold the tissues while allowing the liquid to pass through. After piglet processing is complete, processing fluids that have accumulated in the plastic bag is transferred to a storage container.

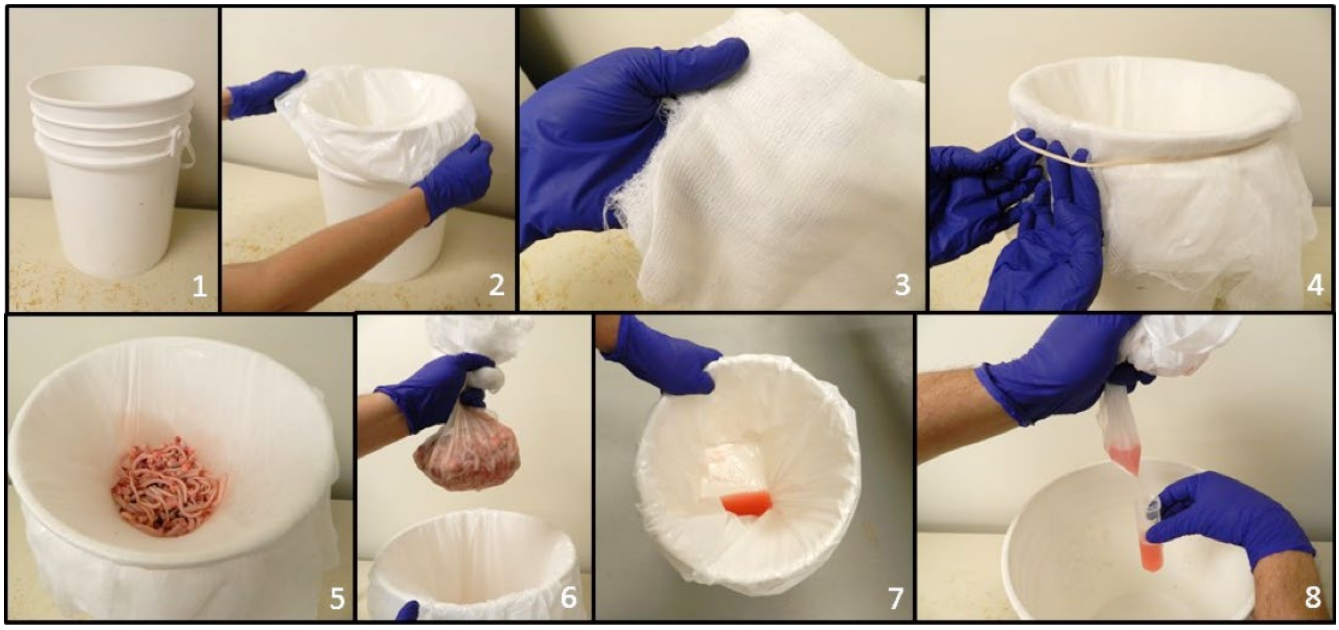


Figure 2. Processing fluid and matching individual piglet blood samples collected from 77 litters.

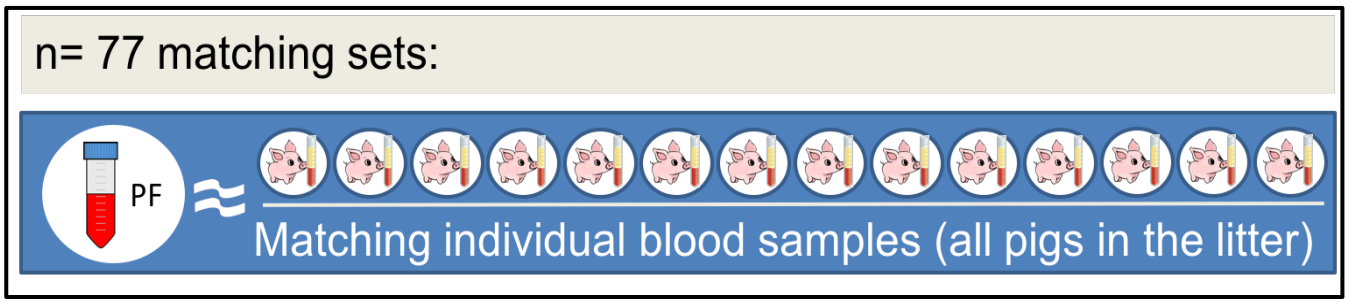


Table 1. Mean CT values for each serially diluted group of 6 replications and the equivalent number of pigs in the pool at each corresponding dilution.

CT values of serially diluted processing fluids (pooled)									
	Dilution								
	0	1	2	3	4	5	6	7	8
qRT-PCR CT Value*	29.05	30.12	31.14	32.26	33.92	35.05	37.04	38.12	39.95
№ of pigs in the pool	11	22	44	88	176	352	704	1408	2816

*Mean CT value of the 6 replications

Table 2. Raw probability for PRRSV RNA detection by qRT-PCR in different levels of pooled processing fluids.

Raw probability for PRRSV RNA detection in pooled processing fluids									
	Dilution								
	0	1	2	3	4	5	6	7	8
Probability detection (%)*	100	100	100	100	100	100	33	17	0
№ of pigs in the pool	11	22	44	88	176	352	704	1408	2816

*Proportion of PRRSV qRT-PCR positive tests from 6 replications

Figure 3. Predicted probabilities for PRRSV RNA detection in pooled processing fluids with one (1) 'low prevalence litter'

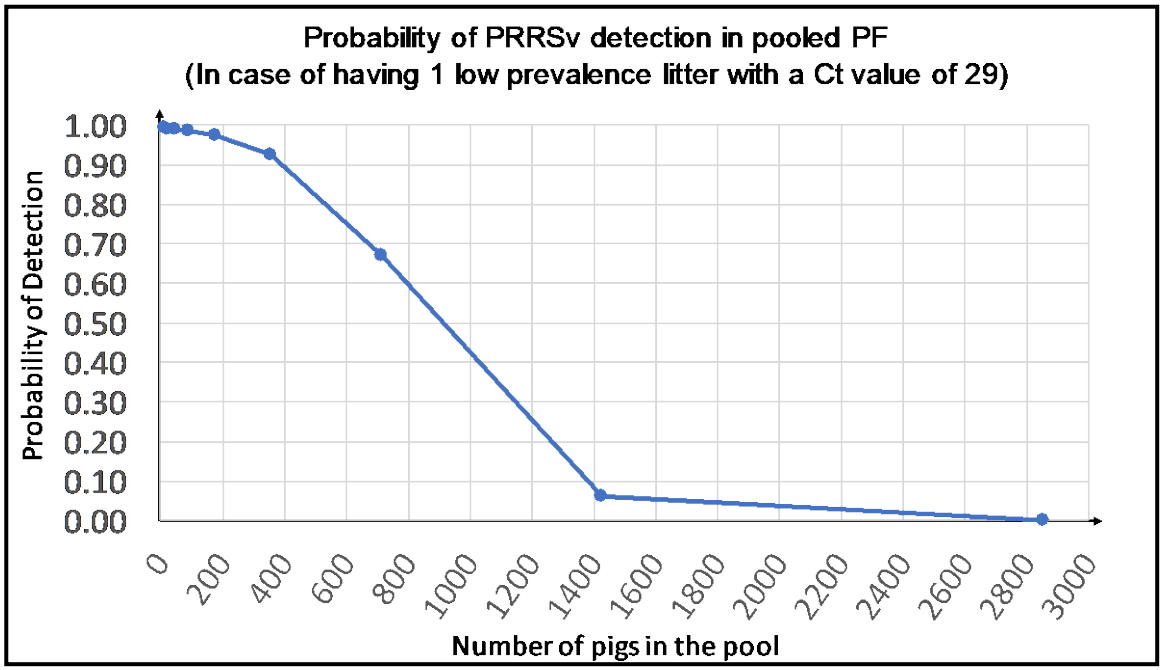


Figure 4. Distribution of know status processing fluid positive samples (affected herds confirmed by real time PCR) and negative samples (naïve herds) tested by IDEXX X3 PRRS Ab ELISA kit following manufacturer's instructions (no changes in the protocol).

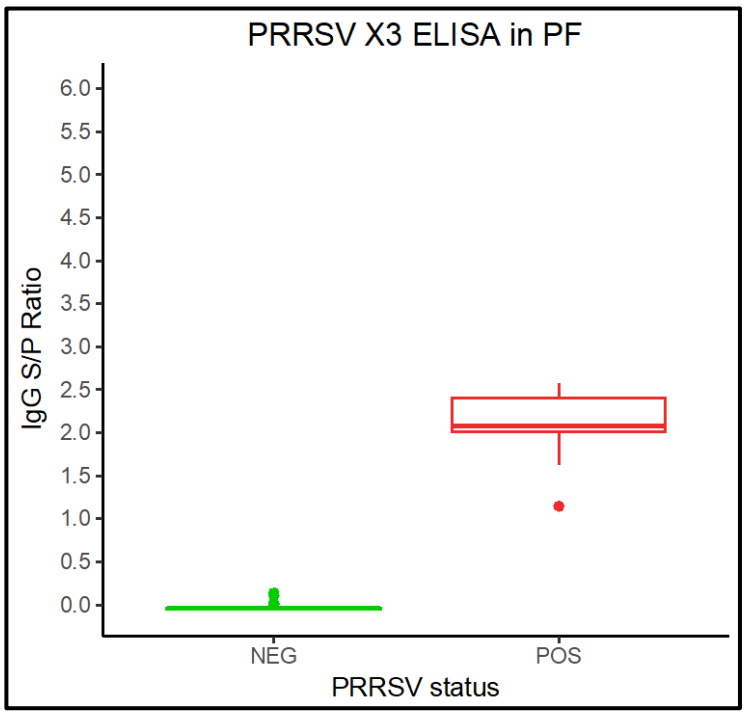
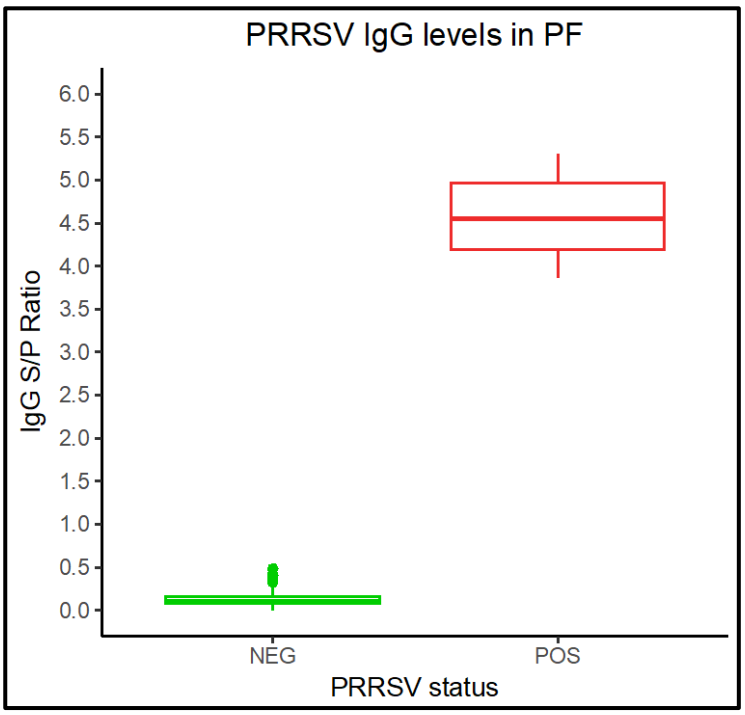
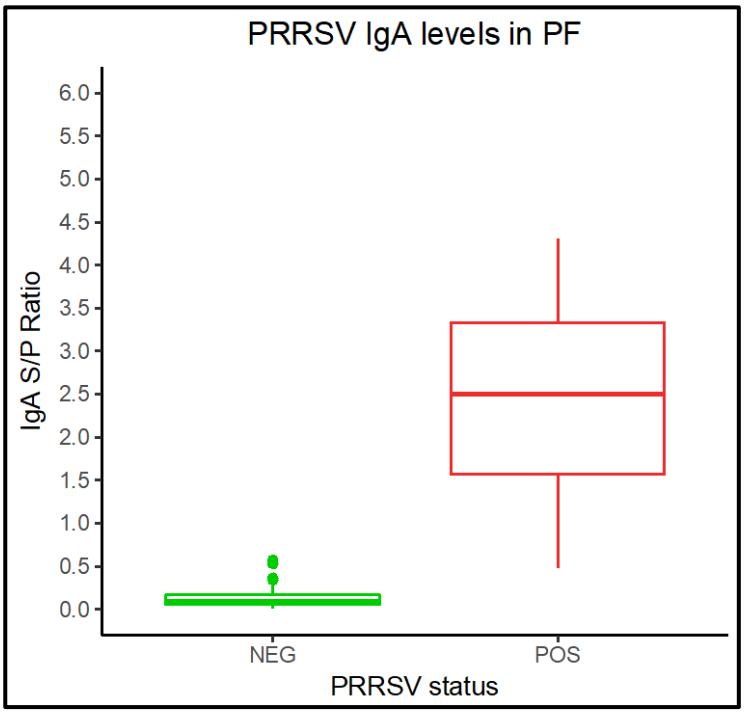


Figure 5. Distribution of know status processing fluid positive samples (affected herds confirmed by real time PCR) and negative samples (naïve herds) tested by a modified version of the IDEXX X3 PRRS Ab ELISA for the three different antibody isotypes in processing fluids: A: IgG, B: IgA and C: IgM.

A:



B:



C:

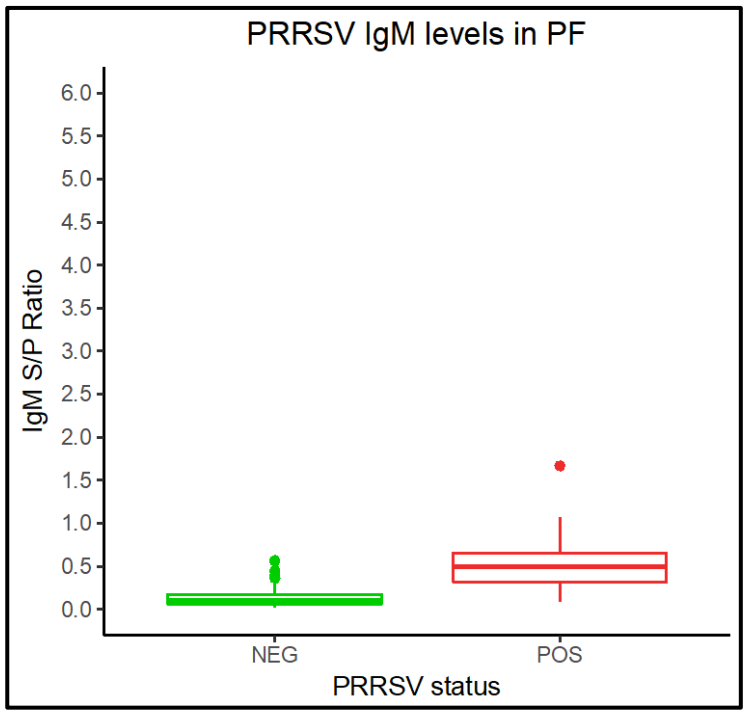


Table 3. Frequency distribution of ELISA test results of different anti-PRRSV Ab isotypes vs corresponding qRT-PCR test results as the gold standard in processing fluid field samples. A: IgG (original), B: IgG (Modified), C: IgA and D: IgM.

A:

PRRSV X3 ELISA (Original kit)		**Litter true status**		
		qRT-PCR +	qRT-PCR -	
ELISA status PF	IgG +	20	9	29
	IgG -	0	0	0
		20	9	29

B:

PRRSV X3 ELISA (Improved for IgG)		**Litter true status**		
		qRT-PCR +	qRT-PCR -	
ELISA status PF	IgG +	20	9	29
	IgG -	0	0	0
		20	9	29

C:

PRRSV X3 ELISA (Modified for IgA)		Litter true status		
		qRT-PCR +	qRT-PCR -	
ELISA status PF	IgA +	20	9	29
	IgA -	0	0	0
		20	9	29

D:

PRRSV X3 ELISA (Modified for IgM)		Litter true status		
		qRT-PCR +	qRT-PCR -	
ELISA status PF	IgM +	15	4	19
	IgM -	5	5	10
		20	9	29

Table 4. Sensitivity and specificity of ELISA test for the original PRRSV X3 ELISA kit and for the modified tests to detect different anti-PRRSV Ab isotypes in processing fluid field samples.

Test	Ab Isotype	Sensitivity	Specificity
PRRSV X3 ELISA (Original kit)	IgG	100 %	0 %
PRRSV X3 ELISA (Improved for IgG detection)	IgG	100 %	0 %
PRRSV X3 ELISA (Modified for IgA detection)	IgA	100 %	0 %
PRRSV X3 ELISA (Modified for IgM detection)	IgM	75 %	56 %

Table 5. Prevalence of PRRSV in piglets of each study group, as determined by PRRSV RNA detection by qRT-PCR in piglet sera.

Sampling Group	Total pigs	Total Litters	# of PRRSV Positive pigs by serum	Observed PRRSV Prevalence
High prevalence	211	21	43	20.4%
Mid-Low prevalence	286	26	24	8.4%
Low prevalence	332	30	0	0%
Total	829	77	67	8.1%

Table 6. Sensitivity and specificity of processing fluids sampling for PRRSV qRT-PCR, as compared to testing of matching serum samples.

Sampling scheme	Description	Sensitivity	Specificity
Litter level sampling	Litters tested individually	72.7%	100%
Room level sampling	Pool of all litters processed in a room	100%	100%
Whole day sampling	Pool of all litters processed in a day	100%	100%

Table 7. Frequency of PRRSV detection by qRT-PCR on processing fluid and blood serum samples.

Overall 77 litters		**Blood serum status**		
		BS+	BS-	
Processing fluids status	PF+	16	0	16
	PF-	6	55	61
		22	55	77