Practical aspects of PRRSV RNA detection in processing fluids collected in commercial swine farms

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Abstract
Processing fluid samples are easily collected under field conditions and provide the means to test more piglets more frequently in a practical way, thereby improving PRRSV surveillance. However, a deeper understanding of the diagnostic characteristics of this newly described sample type is still required. Therefore, the objective of this field-based study was to determine the relationship between viremic piglets and the detection of PRRSV RNA in processing fluid samples. In two PRRSV-positive breeding herds, processing fluids (n = 77) and individual piglet serum samples (n = 834) were collected from 77 litters in three sampling events and tested for PRRSV RNA. Among the 77 litters in the study, 55 litters (71.4%) contained no viremic piglets and processing fluids tested negative for PRRSV RNA. Among the 22 (28.6%) litters with ≥1 viremic piglets, 10 litters contained a single viremic piglet and 5 of the 10 processing fluids from this group tested positive for PRRSV RNA. Based on a fitted mixed effects logistic regression model, the probability of detecting PRRSV RNA in processing fluids was highly dependent on the number of viremic piglets contributing to the sample. When the within-litter prevalence was ≥39%, the probability of detecting PRRSV RNA in processing fluids was ≥95%. By extension, the results suggest that pooling processing fluids from several litters increases the probability of PRRSV RNA detection because of the greater likelihood of including multiple litters each with ≥1 viremic piglets. In contemporary breeding herds that use processing fluid samples for PRRSV surveillance, the diagnostic costs associated with testing 100% of the processing-age piglet population can be estimated at €0.077 ($0.086 USD) per pig weaned. In contrast, to achieve an equivalent testing coverage with the use of individual piglet serum samples, the diagnostic costs associated would be €4.48 ($5.00 USD) per pig weaned. Processing fluid represents a practical, reliable and efficient method to surveil breeding herds for PRRSV because it allows for continuous surveillance at a low cost.

Keywords
Swine, PRRS virus, Processing fluids, Surveillance, Monitoring

Disciplines
Large or Food Animal and Equine Medicine | Veterinary Infectious Diseases | Veterinary Preventive Medicine, Epidemiology, and Public Health

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Abstract

Processing fluid samples are easily collected under field conditions and provide the means to test more piglets more frequently in a practical way, thereby improving PRRSV surveillance. However, a deeper understanding of the diagnostic characteristics of this newly described sample type is still required. Therefore, the objective of this field-based study was to determine the relationship between viremic piglets and the detection of PRRSV RNA in processing fluid samples. In two PRRSV-positive breeding herds, processing fluids (n = 77) and individual piglet serum samples (n = 834) were collected from 77 litters in 3 sampling events and tested for PRRSV RNA. Among the 77 litters in the study, 55 litters (71.4%) contained no viremic piglets and processing fluids tested negative for PRRSV RNA. Among the 22 (28.6%) litters with ≥ 1 viremic piglets, 10 litters contained a single viremic piglet and 5 of the 10 processing fluids from this group tested positive for PRRSV RNA. Based on a fitted mixed effects logistic regression model, the probability of detecting PRRSV RNA in processing fluids was highly dependent on the
number of viremic piglets contributing to the sample. When the within-litter prevalence was ≥ 39%, the probability of detecting PRRSV RNA in processing fluids was ≥ 95%. By extension, the results suggest that pooling processing fluids from several litters increases the probability of PRRSV RNA detection because of the greater likelihood of including multiple litters each with ≥ 1 viremic piglets. In contemporary breeding herds that use processing fluid samples for PRRSV surveillance, the diagnostic costs associated with testing 100% of the processing-age piglet population can be estimated at €0.077 ($0.086 USD) per pig weaned. In contrast, to achieve an equivalent testing coverage with the use of individual piglet serum samples, the diagnostic costs associated would be €4.48 ($5.00 USD) per pig weaned. Processing fluid represents a practical, reliable and efficient method to surveil breeding herds for PRRSV because it allows for continuous surveillance at a low cost.

**Key words:** Swine, PRRS virus, processing fluids, surveillance, monitoring.

**Introduction**

Efficient surveillance for porcine reproductive respiratory syndrome virus (PRRSV) is essential for measuring progress
towards PRRSV control and elimination efforts. Historically, serum-based surveillance has been the preferred approach for detecting PRRSV in populations and sampling schemes based on this approach are widely available (Holtkamp et al., 2011; Linhares et al., 2014). For example, the approach recommended by the American Association of Swine Veterinarians for PRRSV surveillance in breeding herds consists of collecting serum samples from 30 suckling pigs and then pooling by fives for testing by PRRSV reverse transcription quantitative polymerase chain reaction (RT-qPCR). This approach offers a 95% probability of detecting ≥ 1 viremic piglet when PRRSV prevalence is ≥ 10% (Holtkamp et al., 2011). However, PRRSV can persist in breeding herds at a prevalence approaching zero; in which situation, a sampling of 30 pigs offers a low probability of detection (Kittawornratt et al., 2014). For example, the probability of detecting PRRSV RNA in a sample of 30 piglets when prevalence is 1% would be 26%, i.e., a false negative is the most likely outcome. Compelling evidence, i.e., rebreaks with genetically homologous PRRSV, supports the idea that PRRSV control and/or elimination programs may fail because the classic serum-based surveillance approach incorrectly leads to the conclusion that the population is stable or the virus has been eliminated (Linhares, 2013; Ramírez et al., 2019).
More sensitive, yet practical, surveillance approaches are needed to assist producers and veterinarians in establishing the true PRRSV status of target populations. The use of processing fluids for PRRSV surveillance in suckling pigs was described in 2018 (Lopez et al., 2018; Vilalta et al., 2018) and has been increasingly adopted in the US swine industry for breeding herd monitoring applications (Trevisan et al., 2019a; Trevisan et al., 2019b).

Reportedly, processing fluids-based sampling provides sensitive and practical surveillance of the neonatal population at a lower cost and with less effort than required for bleeding individual piglets (Lopez et al., 2018). As a technique recently introduced to the industry, there is a need to further understand the "boundaries" of this approach and define the level of confidence that can be placed in PRRSV RNA detection using processing fluids-based surveillance. Toward that end, the objective of this field-based study was to estimate the probability of detecting PRRSV RNA in processing fluids in commercial swine herds using processing fluid samples tested by RT-qPCR.

**Materials and methods**

Individual piglet serum (n = 834) and litter processing fluid samples (n = 77) were collected from suckling piglets at the time
of processing (3 - 5 days of age) in two PRRSV-positive breed-to-wean commercial swine herds and then tested for PRRSV RNA. Test result data were analyzed using a logistic regression model with the objective of estimating the probability of detecting PRRSV RNA in processing fluids as a function of within-litter prevalence. The Iowa State University Office for Responsible Research approved the experimental design and sampling protocols used in the study.

*Study populations*

The study was conducted in two breed-to-wean herds located in the Midwest US. Both farms were managed in a continuous weekly farrowing cycle, with pigs weaned at 18 to 23 days of age. At the time of the study, neither farm used PRRSV vaccine(s) in either sows or piglets. However, Farm 1 (1,750 sows) had instituted a PRRSV control and elimination program (‘load-close-expose’) and all sows had been inoculated with viremic serum four weeks before the initiation of the study. Farm 2 (6,000 sow farm) was considered "PRRSV stable", with no clinical signs and no PRRSV-positive diagnostic tests for the previous 3 months.

To include the possibility of sampling over a range of PRRSV prevalence, 77 sets of litter samples (litter processing fluid samples
and individual piglet serum) were collected in three sampling events, as given in Table 1.

• Sampling 1: On Farm 1, a total of 21 litter samples were collected from two rooms (A, B) four weeks after live virus inoculation under the assumption that PRRSV prevalence would be high.

• Sampling 2: On Farm 1, a total of 26 litter samples were collected from two rooms (C, D) ten weeks after live virus inoculation under the assumption that PRRSV prevalence would have declined over the previous 6 weeks.

• Sampling 3: On Farm 2, a total of 30 litter samples were collected from two rooms (E, F). No clinical signs had been observed in Farm 2 and PRRSV RT-PCR monitoring of weaned piglets had produced negative results. The assumption was that PRRSV infection, if present, would be low.

Sample collection

Individual piglet blood samples were collected using a single-use sterile system (Becton, Dickinson and Company. Franklin Lakes, NJ USA) and standard blood collection technique (Ramirez and Karriker, 2019). Processing fluids were collected from individual litters as previously described (Lopez et al., 2018), except that litter samples were placed on ice and brought to the laboratory for
processing in order to avoid cross-contamination. Serum and processing fluid samples were centrifuged at 3,000 x g for 8 min and then the supernatant was transferred into sterile polypropylene tubes for storage at -80ºC.

**PRRSV RT-qPCR**

The samples described in Table 1, i.e., individual litter processing fluids (n = 77) and individual piglet serum samples (n = 834), were tested by PRRSV RT-qPCR. In addition, pooled processing fluid samples (n = 10) were aggregated by sampling event, i.e., 1, 2, 3 (n = 3), by room within sampling events, i.e., A, B, C, D, E, F (n = 6) and an aggregate consisting of all processing fluid samples.

Nucleic acid was extracted from processing fluid and serum samples using the same commercial kit (RealPCR* DNA/RNA Magnetic Bead Kit, IDEXX Laboratories, Inc., One IDEXX Drive Westbrook, ME USA) and automated instrumentation (Kingfisher Flex System magnetic beads processor, Thermo-Fisher Scientific, Waltham, MA, USA). In brief, sample (200 µL) and lysis solution (200 µL) were incubated for 15 min followed by 5 min incubation with the bead solution (600 µL binding buffer + 20 µL magnetic beads). Magnetic beads were collected and then washed with solutions I, II, and 80% ethanol (600 µL for 3 minutes each)
followed by 10 min drying time after the final wash. Thereafter, nucleic acids were eluted from the magnetic beads (100 µL elution buffer for 5 min). All the above mentioned procedures were executed at room temperature. PRRSV RT-qPCR was performed using a commercial kit (RealPCR* PRRS Type 1 and Type 2 Multiplex RNA Mix and Master Mixes, IDEXX Laboratories, Inc.). Plates containing purified RNA, Multiplex RNA mix and master mixes were loaded onto a thermal cycler (7500 Fast Real-Time PCR System, Applied Biosystems©, Foster City, CA, USA) and the following cycling conditions with standard ramp rate were used: one cycle at 50°C for 15 min, one cycle at 95°C for 1 min, and 45 cycles of 95°C for 15 seconds and 60°C for 30 seconds. Amplification data was analyzed using the ‘auto baseline’ and ‘auto Ct’ functions. Samples with Cq values < 37 were considered PRRS-positive.

Assay controls included in each run consisted of one negative extraction control and positive and negative amplification controls. In addition, an internal sample control (ISC), i.e., endogenous, host specific genetic material, is used to monitor sample quality, extraction, and amplification. That is, ISC primers and probes target host DNA or RNA within the sample, producing an amplification curve that validates the reaction.
Data analysis

The probability of detecting PRRSV in a population using individual pig serum was estimated for each sampling (1, 2, and 3) using a conventional sampling approach, i.e., 30 serum samples from randomly selected piglets in the population, i.e., 212, 289, and 333 piglets, respectively. Using computer simulation (RStudio Team, 2018), 30 serum samples were randomly selected without replacement (hypergeometric distribution) from the testing data produced by each sampling event. Each run resulted in PRRSV RNA detection, i.e., ≥ one positive sample or not. The procedure was repeated for 10,000 iterations to estimate the probability of detection in each sampling group.

The probability of detecting PRRSV using serum vs processing fluids was compared within sampling group (i.e. litter prevalence). Based on classical probability theory, the probability of drawing ≥ 1 positive piglets from a positive litter is described in Equation 1 as,

\[
1 - \frac{\left( \frac{\text{no. neg}}{\text{sample size}} \right)}{\frac{\text{no. total}}{\text{sample size}}} = 1 - \frac{\left( \frac{\text{no. total (1 - p)}}{\text{sample size}} \right)}{\text{no. total}} \quad \text{Equation 1)}
\]
where \textit{no. total} is the total number of piglets in the litter, \textit{sample size} is the number of randomly selected piglets, \textit{no. neg} is the number of non-viremic piglets, and \textit{p} is the within-litter prevalence. Based on the data from this study, the \textit{no. total}, i.e., litter size, is 11 piglets.

Statistical software (RStudio Team, 2018) was used to calculate the probability of detecting PRRSV RNA in processing fluids as a function of within-litter prevalence, i.e., the proportion of viremic piglets in a litter, using a mixed effects logistic regression model. This was done using data from all sampling groups.

\[
\text{logit}(p_i) = \log \left( \frac{p_i}{1-p_i} \right) = \beta_0 + \beta_1 \times \text{Within-litter prevalence}_i + \delta_i \quad (\text{Equation 2})
\]

In Equation 2, \(p_i\) is the probability of a positive processing fluid result for litter \(i\) \((i = 1, 2, \ldots, 77)\), \(\beta_0\) and \(\beta_1\) are unknown model parameters to be estimated from the data, where \(\beta_0\) would represent the log odds of obtaining a processing fluid positive result for PRRSV if the within litter prevalence is 0 and \(\beta_1\) represents the increase in log odds of obtaining a processing fluid positive result for PRRSV per unit increase in within litter prevalence. That is, each one percent increase in within litter
prevalence is associated with $e^{\beta_1/100}$ times the odds of a positive processing fluid response. Finally, $\delta$ represents the random effect within sampling group (1, 2, 3), which was assumed to follow a normal distribution centered at mean 0.

**Results**

A summary of piglet serum (n = 834) PRRSV RT-qPCR testing results is given in Table 2. Among serum samples collected from piglets at processing (3-4 days of age), 67 piglets from 22 litters were viremic (8.0%). The rate of positivity varied among samplings, with the highest proportion of PRRSV-positive piglets and litters in Sampling 1 and with no positive piglets or litters detected in Sampling 3. In addition, Table 2 reports the probability of detecting PRRSV in a population using 30 individual pig serum samples based on the R software simulations previously described.

A summary of results at the litter level are given in Table 3. Among the 77 litters in the study, 55 litters (71.4%) contained no viremic piglets and processing fluids tested negative for PRRSV RNA. Among the 22 (28.6%) litters with $\geq$ 1 viremic piglets, 10 litters contained a single viremic piglet and 5 of the 10 processing fluids from this group tested positive for PRRSV RNA. Among the 12 litters with $>$ 1 viremic piglet, 11 of 12 processing fluids
tested RT-qPCR positive. Figure 1 shows the distribution of Cq values for individual viremic piglets based on the litter processing fluids PRRSV RT-qPCR results. The overall mean Cqs for individual piglets from PRRSV-negative and PRRSV-positive litters by processing fluids status was 30.0 and 24.8, respectively.

The probability of detecting PRRSV RNA in one processing fluid sample versus serum samples from randomly selected piglets (1, 2, 3 or 4 per litter) is given in Figure 2 as a function of within-litter PRRSV prevalence. Based on a fitted mixed effects logistic regression model, the probability of detecting PRRSV RNA in processing fluids was $\geq 95\%$ ($\geq 99\%$) when the within-litter prevalence was $\geq 39\%$ ($\geq 50\%$).

Table 4 reports the detailed composition and PRRSV RT-qPCR results of pooling processing fluid samples by sampling event (1, 2, 3), room (A through F), and overall (77 litters). In each case, testing of samples pooled by room produced the same result as the corresponding sampling event pool.

**Discussion**

On-going surveillance is required to track progress toward PRRSV control and elimination efforts, and to detect virus introduction to
swine populations. In breeding herds, conventional surveillance approaches are challenged by PRRSV prevalence levels approaching zero in suckling and weaned pigs (Graham et al., 2013; Kittawornrat et al., 2014; Redalen et al., 2009). Under these circumstances, effective routine surveillance based on individual pig sampling is impractical because of the time and cost of bleeding and testing the number of pigs required for detection in low prevalence situations. It follows that PRRSV surveillance protocols based on bleeding a subsample of piglets may fail to detect PRRSV infection and lead to faulty decision-making (Linhares, 2013; Ramírez et al., 2019).

Despite the fact that operational decisions require accurate information, surveillance is often neglected in commercial herds. Processing fluids provide the means to perform routine surveillance at low cost. That is, samples are easily collected and prepared for testing by farm staff; sample aggregation results in wholesale coverage of the piglet population with a high probability of virus detection. First reported in 2017 (Lopez et al., 2017), the use of processing fluid samples has been rapidly adopted by the swine producers and veterinarians (Trevisan et al., 2019b). In 2017, the Swine Disease Reporting System (SDRS) reported 211 processing fluid submissions to 4 US veterinary diagnostic
laboratories, then 7,100 processing fluid submissions in 2018, and 11,608 in 2019 (Trevisan et al., 2019b). Processing fluids may be a viable alternative to serum sampling in breeding herds, but there is a need to better understand the diagnostic characteristics of this approach.

In this study, RT-qPCR testing of serum samples from all processing-age piglets provided complete data on the true PRRSV infection status of each member of the sampled population and provided for accurate analysis of the effect of within-litter PRRSV prevalence on the probability of PRRSV RNA detection in processing fluid samples. Corroborating previous reports (Almeida et al., 2018; Cano et al., 2007; Vilalta et al., 2018), most PRRSV-positive litters (10 of 22 positive litters, Table 3) contained a single viremic piglet. Unexpectedly, pooling processing fluids from several litters was more likely to result in detection than testing individual litter samples, i.e., all processing fluid samples from positive herds aggregated at the sampling level or room level were positive for PRRSV RNA (Table 4). Thus, consistent with the observation that inclusion of ≥ 2 viremic piglets in a sample increased the probability of PRRSV detection, the data support the practice of aggregating processing fluids from several
litters for the purpose of increasing the probability of detecting PRRSV-viremic piglets.

The cost of surveillance includes both sample collection (labor) and testing costs. The labor costs of processing fluid-based surveillance consist of the time (a few minutes) required to prepare the sample(s) for submission - a negligible cost. The diagnostic costs associated with testing 100% of the processing-age piglet population in a 5000-sow PRRSV-stable commercial breeding herd farrowing ~235 sows weekly can be estimated at €0.077 ($0.086 USD) per pig weaned ($13.000 USD annually), assuming: 1) 3,170 piglets are processed each week; 2) 10 processing fluids produced per week by aggregating 23 - 25 litters; 3) PRRSV RT-qPCR tests cost of €22.3 ($25.00 USD); and 4) pre-weaning mortality of 9.0%. In contrast, to achieve the same sampling coverage of the piglet population (100%) using an individual serum sampling approach, the estimated costs associated with testing would be €4.48 ($5.00 USD) per pig weaned ($750.000 USD annually). The cost of acquiring the data needed to move toward PRRSV control and elimination (€0.077 or $0.086 USD per pig) may be also compared with the estimated cost of living in perpetuity with PRRSV, i.e., €5.62 - 13.71 ($6.25 - 15.25 USD) (Holck and Polson 2003); €4.13 ($4.60 USD) (Holtkamp et al.,
Conclusions

Porcine reproductive and respiratory syndrome continues to impose major costs on the global swine industry (Holtkamp et al., 2013). Previous studies have shown that processing fluids are a valuable, practical and cost-efficient sample for monitoring PRRSV in breeding herds (Lopez et al., 2018; Vilalta et al., 2018; Trevisan et al., 2019a). This study is the first to report the probability of PRRSV RNA detection in processing fluids as a function of within-litter prevalence. The study was performed under standard field conditions, i.e., routine management programs were maintained as usual, but the design enabled exact comparisons between piglet serum (n = 834) and processing fluid (n = 77) testing results at the litter level. Due to the ease of implementation of processing fluid sampling method (Lopez et al., 2018) its herd-level sensitivity and its strong potential for pooling massive quantities of piglets, processing fluid represents a practical, reliable and efficient method to surveil breeding herds for PRRSV.

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**Conflict of interest**

The authors declare no conflicts of interest with respect to the conduct, authorship, and/or publication of this study. Co-author JZ has served as a consultant to IDEXX Laboratories, Inc. on areas of diagnostic medicine independent of this research. The terms of the consulting arrangement have been reviewed and approved by Iowa State University in accordance with its conflict of interest policies.

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**References**


Figure 1. Box plots showing median, 10th, 25th, 75th and 90th percentiles of individual piglet PRRSV RT-qPCR Cq values in the context of the number of viremic piglets in the litter and processing fluid PRRSV RT-qPCR status.

Figure 2. Probability of PRRSV RNA detection in processing fluid and serum sample(s) from randomly selected piglet(s) as a function of within-litter PRRSV prevalence.
Table 1. Number of piglets and litters sampled by room, sampling event, and farm.

<table>
<thead>
<tr>
<th>Farm</th>
<th>Sampling Event</th>
<th>Location within the farm</th>
<th>Number of litters sampled</th>
<th>Number of piglets in the sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Farm 1</td>
<td>1</td>
<td>Room A</td>
<td>4</td>
<td>39</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Room B</td>
<td>17</td>
<td>173</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>Room C</td>
<td>7</td>
<td>67</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Room D</td>
<td>19</td>
<td>222</td>
</tr>
<tr>
<td>Farm 2</td>
<td>3</td>
<td>Room E</td>
<td>23</td>
<td>256</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Room F</td>
<td>7</td>
<td>77</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Total</td>
<td>77</td>
<td>834</td>
</tr>
</tbody>
</table>
Table 2. Piglet data: Summary of PRRSV RT-qPCR testing of piglet sera collected at processing

<table>
<thead>
<tr>
<th>Sampling event</th>
<th>Piglets positive/total</th>
<th>Litters positive/total</th>
<th>Mean positive pigs/positive litter</th>
<th>Probability of detection - 30 randomly selected piglet serum samples*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>43/212 (20.3%)</td>
<td>15/21 (71.4%)</td>
<td>2.9</td>
<td>99.9%</td>
</tr>
<tr>
<td>2</td>
<td>24/289 (8.3%)</td>
<td>7/26 (26.9%)</td>
<td>3.4</td>
<td>93.5%</td>
</tr>
<tr>
<td>3</td>
<td>0/333 (NA)</td>
<td>0/30 (NA)</td>
<td>0</td>
<td>NA</td>
</tr>
<tr>
<td>Total</td>
<td>67/834 (8.0%)</td>
<td>22/77 (28.6%)</td>
<td>3.1</td>
<td>92.1%</td>
</tr>
</tbody>
</table>

* Probability of detecting ≥ 1 viremic piglet estimated by computer simulation (RStudio Team, 2018) using the data derived from each sampling event. Simulations were based on random selection of 30 serum samples without replacement (hypergeometric distribution). NA stands for "not applicable".
Table 3. Litter data: Summary of PRRSV RT-qPCR testing of piglet sera and processing fluids

<table>
<thead>
<tr>
<th>Viremic piglets w/n litter</th>
<th>Litter count</th>
<th>PRRSV RT-qPCR Cq mean</th>
<th>Probability of a positive processing fluid sample ($p_i$)*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Viremic piglets w/n litters</td>
<td>Processing fluids (pos of total tested)</td>
</tr>
<tr>
<td>0</td>
<td>55</td>
<td>&gt; 37.0</td>
<td>&gt; 37.0</td>
</tr>
<tr>
<td>1</td>
<td>10</td>
<td>29.1</td>
<td>30.9 (5 of 10)</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>27.1</td>
<td>28.5 (1 of 1)</td>
</tr>
<tr>
<td>3</td>
<td>4</td>
<td>27.0</td>
<td>27.2 (3 of 4)</td>
</tr>
<tr>
<td>4</td>
<td>2</td>
<td>22.2</td>
<td>26.8 (2 of 2)</td>
</tr>
<tr>
<td>5</td>
<td>2</td>
<td>28.5</td>
<td>27.7 (2 of 2)</td>
</tr>
<tr>
<td>6</td>
<td>1</td>
<td>17.1</td>
<td>25.2 (1 of 1)</td>
</tr>
<tr>
<td>9</td>
<td>1</td>
<td>25.9</td>
<td>17.1 (1 of 1)</td>
</tr>
<tr>
<td>10</td>
<td>1</td>
<td>23.6</td>
<td>23.0 (1 of 1)</td>
</tr>
</tbody>
</table>

*As described in materials and methods, $p_i = (1 + e^{b_0 + b_1 \times \text{Within-litter prevalence}})^{-1}$
Table 4. Effect of pooling processing fluids on PRRSV detection in processing fluid: individual litter samples vs pooled samples

<table>
<thead>
<tr>
<th>Processing fluid Pools</th>
<th>Pooled Processing fluid composition</th>
<th>Pooled RT-qPCR Cq values</th>
<th>PRRSV RT-qPCR qualitative test result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sampling 1</td>
<td>9/21 Litters in pool (positive/total)</td>
<td>29.3</td>
<td>Positive</td>
</tr>
<tr>
<td></td>
<td>43/212 Piglets in pool (positive/total)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Room A</td>
<td>1/4 Litters in pool (positive/total)</td>
<td>28.9</td>
<td>Positive</td>
</tr>
<tr>
<td></td>
<td>7/39 Piglets in pool (positive/total)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Room B</td>
<td>8/17 Litters in pool (positive/total)</td>
<td>29.0</td>
<td>Positive</td>
</tr>
<tr>
<td></td>
<td>36/173 Piglets in pool (positive/total)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sampling 2</td>
<td>7/26 Litters in pool (positive/total)</td>
<td>21.6</td>
<td>Positive</td>
</tr>
<tr>
<td></td>
<td>24/289 Piglets in pool (positive/total)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Room C</td>
<td>4/7 Litters in pool (positive/total)</td>
<td>20.7</td>
<td>Positive</td>
</tr>
<tr>
<td></td>
<td>14/67 Piglets in pool (positive/total)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Room D</td>
<td>3/19 Litters in pool (positive/total)</td>
<td>24.5</td>
<td>Positive</td>
</tr>
<tr>
<td></td>
<td>10/222 Piglets in pool (positive/total)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sampling 3</td>
<td>0/30 Litters in pool (positive/total)</td>
<td>&gt; 37.0</td>
<td>Negative</td>
</tr>
<tr>
<td></td>
<td>0/333 Piglets in pool (positive/total)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Room E</td>
<td>0/23 Litters in pool (positive/total)</td>
<td>&gt; 37.0</td>
<td>Negative</td>
</tr>
<tr>
<td></td>
<td>0/256 Piglets in pool (positive/total)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Room F</td>
<td>0/7 Litters in pool (positive/total)</td>
<td>&gt; 37.0</td>
<td>Negative</td>
</tr>
<tr>
<td></td>
<td>0/77 Piglets in pool (positive/total)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pool of all litters</td>
<td>16/77 Litters in pool (positive/total)</td>
<td>22.0</td>
<td>Positive</td>
</tr>
<tr>
<td></td>
<td>67/834 Piglets in pool (positive/total)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>