Alternative samples for Porcine Reproductive and Respiratory Syndrome surveillance in an endemic PRRSV-1 infected breeding herd: a descriptive study

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Abstract

Knowing PRRSV status is essential to design herd management protocols. For this, weaning-age pigs are a key subpopulation. In the last years, several alternatives to blood sampling have been tested because of easier, welfare friendly and cost saving tools. Moreover, most of them allow to test more animals and then, seem to be more sensitive in case of low prevalence scenario. However, these studies have been implemented mainly in PRRSV-2 infected herds.

The first objective of our study was to compare the rate of detection of PRRSV-1 by RT-qPCR in individual serum samples, family oral fluid samples (FOF) and udder wipes (UW) collected the day before weaning. The second objective was to evaluate the interest of pooling.

The study was performed on a 200-sows farrow-to-finish farm, PRRSV-1 infected and unstable. 119 litters were sampled (one sample type per litter). The rate of detection of PRRSV-1 in blood samples, FOF and UW was 10.9%, 7.6% and 0.8%, respectively. The agreement between sera and FOF was almost perfect even if detection capacity of sera was numerically superior to FOF. The Ct values on sera were statistically lower than FOF ones.

Two modalities of pooling (1/3 and 1/5) were tested for sera and FOF. They showed that both modalities did not impact the qualification neither of the litter not of the batch PRRSV classification. On the other hand, even pooled by 3, most of the FOF pools gave negative results misclassifying many litters and batches.

In the conditions of our study, in a PRRSV-1 infected scenario, FOF seemed to be a good alternative to blood sampling only when analysed individually and so a more costly alternative.

Background

Porcine Reproductive and Respiratory Syndrome Virus is the most economically important disease in the swine industry (Holtkamp et al., 2013; Nathues et al., 2017; Renken et al., 2021). Before implementing any management strategy against the disease, knowing the status of the breeding herd remains an essential prerequisite. Since a long time, sampling blood of due-to-wean piglets was the recommended method to determine the stability or unstability of a sow-herd (Holtkamp et al., 2011). More recently, many studies have investigated the relative interest of other diagnostic sample types. These alternative tools wanted to answer to two main issues. The first one aimed to be more respectful of welfare as blood sampling is intrusive and therefore stressful. The second objective was to sample more animals sampled without increasing analysis total cost, especially in case of low prevalence scenario.

In particular, individual or collective oral fluids of weaners (Kittawornrat et al., 2014; Lebret et al., 2019), family oral fluids (FOF) (Almeida et al., 2021; Osemeke et al., 2022), udder wipes (UW) (Vilalta et al., 2021a), processing fluids (PF) (López et al., 2020; Vilalta et al., 2018), umbilical cord blood (UC) (Martín-Valls et al., 2018) or tongue tips (TT) (Baliellas et al., 2021; Machado et al., 2022) have been studied.
Recently, the American Association of Swine Veterinarians has reviewed its classification introducing PF and FOF alternative options to blood, alone or in combination with sera (Holtkamp et al., 2021). Different scenarios have been distinguished in the new classification taking into account the expected status and the within-herd prevalence. For example, in case of expected unstability, sampling 30 piglets at weaning in at least 4 batches over a 90-days period remains the main option.

Most of the investigations on new diagnostic tools has been done in PRRSV-2 infected conditions but more rarely in PRRSV-1 ones apart from four of them about the interest of OF (Gibert et al., 2017; Lebret et al., 2019), TT (Baliellas et al., 2021) and another one concerning UC (Martín-Valls et al., 2018).

The aim of our study was first to compare the rate of detection of PRRSV in serum, FOF and UW in an endemic herd infected by PRRSV-1 and previously known as unstable. Secondly, we also evaluated the sensitivity of pooling samples.

**Materials And Methods**

**Study design**

This descriptive study was conducted on a commercial 210-sow farrow-to-finish pig herd located in Brittany, France. The management of the farm was based on 7 batches of around 30 to 35 sows each and the age at weaning was 28 days.

This herd was PRRSV-1 positive unstable since a long time and did not use any vaccination against PRRSV nor in sows neither in piglets.

Four consecutive batches were included and, in each of them, 30 litters were sampled.

**Sample collection**

The samples were collected between August and November 2021.

Within each batch, the day prior to weaning in the morning, 30 litters were sampled using the following methods:

- blood from one piglet, targeting the weakest piglet within the litter.
- FOF
- UW

Blood samples were collected in plain test tubes using one sterile needle per piglet from the cranial vena.

FOF were collected by presenting an untreated cotton rope to the sow and its piglets, without training the day before. One end of the 50 cm rope (0.8 cm diameter) was knotted and attached with pliers of the farrowing crate, near the sow head. The other end arrived at the shoulder level of the smallest piglet of the
litter. After 30 minutes presentation, the wet portion of the rope was inserted into a plastic bag and manually wrung to collect sow and piglets’ oral fluid. After that, the corner of the bag was cut and the saliva was transferred into a 10 mL tube, kept in cool storage until submission to the lab.

UW were collected by wiping the underline skin of sow’s udder with a 50 cm gauze previously impregnated with 5 mL of Phosphate Buffer Saline (PBS). The objective of this sample type was to indirectly collect piglets’ saliva after suckling. After collection, gauzes were inserted into a plastic bag and kept in cool storage until submission.

All samples were submitted to the laboratory within 3 hours the day of sampling.

Diagnostic testing

Diagnostic tests were performed at Labofarm (Finalab Veterinary Laboratories Group, Loudéac, France). All samples were analysed individually. After then, pools (1/3 and 1/5) were also analysed.

Blood samples were centrifuged to separate serum (4500g for 5 min). Two hundred µL of the supernatant was used for RNA extraction.

One mL of FOF was centrifuged 10 min at 95g for big particles’ sedimentation. Two hundred µL of the supernatant was used for RNA extraction. The content of the UW was suspended by kneading the wipe in 50 mL of PBS during few seconds. Two hundred µL of the suspension was then used for RNA preparation.

RNA was extracted using Indimag Pathogen kit (Indical Bioscience, Leipzig, Germany) following the manufacturer instructions.

All samples were tested for PRRSV RNA using Adiavet PRRSV real time kit (BioX Diagnostics, Rochefort, Belgium). A sample was considered positive if the cycle threshold (Ct) value was \( \leq 40 \).

Pooling

In each batch, PRRSV-1 negative samples in sera and FOF were combined and vortexed to form a homogenate negative sample for each sample type. Then, pools of 1/3 and 1/5 were created diluting one part of a positive sample with respectively 2 or 4 parts of PCR negative homogenate.

Statistical analysis

Comparison of the rate of detection between sample types and agreement between them

To assess the ability of the three sample types to detect PRRSV-1 RNA, the result of each test was compared to a reference standard which was the cumulative result of the three samples tested (at the batch or the litter level). Thus, a litter or a batch was considered positive if at least one of the three samples was positive.
Statistical analyses were conducted using R programming language 3.4.1 (R Core Team). The exact binomial test 95% was used to compute the confidence intervals (CI) of rate of detection. Rates of detection were compared between sera and FOF using a Chi-square test. The Wilcoxon paired test on average contributions was used to compare Ct between groups.

The agreement between sera and FOF was assessed using a concordance test (Kappa statistics) at the litter level using publicly available software (https://idostatistics.com/cohen-kappa-free-calculator).

Evaluation of pooling ability to detect PRRSV-1

At the batch level, the ability of pools (1/3 and 1/5) to detect the virus was assessed as the same manner as individual samples. The relation between the individual Ct value and pool Ct value was assessed using a linear model. Then, the Spearman coefficient between individual Ct value and pooled Ct value was determined. For each analyse, the different levels of pooling (1/3 or 1/5) have been taken into account.

**Results**

Tests abilities to detect PRRSV

In total, 120 litters were sampled in 4 batches. In one litter, we did not collect enough saliva with the FOF so only 119 samples of serum, FOF and UW were compared.

All the results are presented in the Tables 1 and 2.

<table>
<thead>
<tr>
<th>Batch</th>
<th>No. Litters</th>
<th>PCR +</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Serum</td>
<td>FOF</td>
<td>UW</td>
<td>Serum et FOF</td>
<td>Reference standard</td>
</tr>
<tr>
<td>1</td>
<td>30</td>
<td>3</td>
<td>1</td>
<td>0</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>2</td>
<td>29</td>
<td>7</td>
<td>5</td>
<td>0</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>3</td>
<td>30</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>4</td>
<td>30</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>119</td>
<td>13</td>
<td>9</td>
<td>1</td>
<td>16</td>
<td>16</td>
</tr>
</tbody>
</table>
Table 2
Rate of detection of PRRSV RNA in the three index tests at the litter level

<table>
<thead>
<tr>
<th>Test</th>
<th>Reference standard</th>
<th>Rate of detection (%)</th>
<th>CI 95%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Lower limit</td>
</tr>
<tr>
<td>Serum</td>
<td>13</td>
<td>81.25</td>
<td>54</td>
</tr>
<tr>
<td>FOF</td>
<td>9</td>
<td>56.25</td>
<td>30</td>
</tr>
<tr>
<td>UW</td>
<td>1</td>
<td>6.25</td>
<td>0</td>
</tr>
<tr>
<td>Serum + FOF</td>
<td>16</td>
<td>100</td>
<td>80</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Upper limit</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>96</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>80</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>30</td>
</tr>
</tbody>
</table>

The reference standard is the cumulative result of the three sample types tested.

In each batch, at least one sample type was positive, confirming the unstable status of the farm. In each batch, it was possible to detect at least one positive sample with sera and FOF but not with UW with only one positive sample out of 119. It means that UW was unable to detect PRRSV in three out of four batches. In total, we found respectively 13 and 9 positive samples out of 119 in sera and FOF.

Sixteen different litters were tested positive with at least one sample type. Rates of detection in blood, FOF and UW were 81% (95% Confidence Interval (CI): 54–96%), 56% (95% CI: 30–80%) and 6% (95% CI: 0.2–30%) respectively. Taking together the results of blood and FOF, we detected 100% of positive litters (95% CI: 80–100%). Detection capacity of serum was superior to FOF but it was not statistically significant.

The Cohen's Kappa between sera and FOF was calculated at 0.84 indicating an almost perfect concordance.

UW results are not presented in the rest of this paper due to their poor capacity to detect PRRSV in our conditions.

Comparative Ct in samples analyzed individually

Ct values for blood samples and FOF are presented in Fig. 1.

The minimum Ct value in blood and FOF were 24 and 31 respectively. Ct were significantly lower in sera than in FOF (p = 0.0006).

Evaluation of pooling

Pooling was evaluated only for sera and FOF due to the lack of positivity in UW. It was possible in all but one positive serum and for all positive FOF.

For sera, 2 out of 12 serum samples returned negative after pooling by 3 or 5. This result did not impact the qualification of the batch. After pooling by 3, regarding FOF, 7 out of 9 samples returned negative...
misclassifying 2 batches out of 4. After pooling by 5, 8 out of 9 samples returned negative, misclassifying 3 batches (Table 3).

<table>
<thead>
<tr>
<th>Table 3</th>
<th>PRRSV status classification of each batch with individual and pooled samples (+ means at least one positive sample, - means all samples negative)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Batch 1</td>
</tr>
<tr>
<td>Individual serum</td>
<td>+</td>
</tr>
<tr>
<td>Sera pooled by 3</td>
<td>+</td>
</tr>
<tr>
<td>Sera pooled by 5</td>
<td>+</td>
</tr>
<tr>
<td>Individual FOF</td>
<td>+</td>
</tr>
<tr>
<td>FOF pooled by 3</td>
<td>+</td>
</tr>
<tr>
<td>FOF pooled by 5</td>
<td>+</td>
</tr>
</tbody>
</table>

Statistical analyses were only performed for sera due to the small number of positive FOF. There was a strong correlation between individual Ct and pool Ct values ($r = 0.96$, $p < 0.001$). Indeed, individual Ct values impacted significantly pools results ($p = 0.007$).

**Discussion**

Monitoring PRRSV on farms allows to determine the herd status regarding shedding and exposure. For this, sera still remain the reference sample (Holtkamp et al., 2021). RT-qPCR is the most commonly used test for the diagnosis of PRRSV because of its high sensitivity and specificity. Our study confirmed both the minimum number of piglets sampled and the detection capacity of PRRSV in sera, according to AASV recommendations (Holtkamp et al., 2021, 2011).

Sampling FOF is a way to increase the number of pigs sampled and its collection is easy, quick and not stressful. Using FOF for detection of PRRSV has been well documented in the United States (US) (Almeida et al., 2021; Osemeke et al., 2022). It has been currently added as a part of the AASV diagnostic guidelines for classification of breeding herds regarding PRRSV status (Holtkamp et al., 2021). Our results confirmed the interest of FOF compared to serum at the litter level as at the batch level. The Cohen's Kappa calculated was almost the same that one calculated in previous study conducted in PRRSV-2 infected farms (Almeida et al., 2021). However, the conditions of both studies were different especially regarding sample collection. Indeed, Almeida et al. (2021) sampled all piglets in each litter where FOF were collected and compared the results between both and we sampled serum from only one piglet per litter.

In a previous study, our team has already demonstrated that piglets collective oral fluid (COF) was an interesting alternative to detect PRRSV in unstable herds at the litter as at the batch level (Lebret et al.,
However, the success rate of collection depended on several conditions, especially age at weaning and training (Boulbria et al., 2020). In a previous study, Almeida et al., (2020) have also shown that collecting FOF was easier than COF. They assumed that piglets had a tendency to mimic the behavior of their mother which interacted first with the rope, showing their piglets how to do. In our study, we had also a high rate of success (119/120) of collection for FOF but we did not compare them with COF.

Regarding UW, we found a very poor capacity of these sample type to detect PRRSV. This is in agreement with previous studies with a poor agreement between sera, PF and UW to detect PRRV-2 (Vilalta et al., 2021b, 2019b). It is also the reason why AASV did not retained this sample type in the new classification (Holtkamp et al., 2021).

Finally, the effects of pooling serum and FOF samples on PRRSV detection was evaluated. In order to test a large number of pigs, pooled samples are routinely used for the monitoring of PRRS, especially in order to lower the cost of analysis (Lebret et al., 2019; Lopez et al., 2019; Osemeke et al., 2022; Rovira et al., 2007; Vilalta et al., 2019a). The interest of pooling serum samples has been widely investigated and there is no longer any debate at this time. Regarding FOF, our results are in contraction with the study of Osemeke et al., (2022). Indeed, they showed that for instance in low prevalence scenario like in our study, pooling FOF up to 1/10 was valuable. Even if they demonstrated an increase of Ct values after pooling, it didn’t change the final classification of the farm (Osemeke et al., 2022). In our study, pooling FOF conducted to a misclassification of 3 batches out of 4 which is not acceptable.

Conclusion

In the conditions of our study, FOF seemed to be a good alternative to blood samples but only when there are analysed individually and not after pooling. All UW but one returned negative showing that this kind of sample was not suitable for PRRSV-1 surveillance. Finally, we confirmed that blood samples gave the higher rate of detection even after pooling by five, confirming that, at this time, they seem to be the gold standard.

Declarations

Ethics approval and consent to participate

This study was performed in accordance with the French national current legislation on ethical and welfare recommendations. All samples analysed in this study come from a veterinary practice for general diseases diagnosis. No specific authorization or declaration was needed in this case.

Consent for publication

All authors gave their consent for publication.

Competing interests
The authors declare no conflict of interest.

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Availability of data

All datasets used in this study are available from the corresponding author on reasonable request.

Authors’ contribution

Study conception and design: AL, GB. Data acquisition: AL, GB, TN. Data analysis and interpretation: AL, GB, CTC. Drafting the manuscript: AL, GB. All authors read, critically revised and approved the final manuscript.

References


Figures
Figure 1

Distribution of Cycle threshold (Ct) values for detection of PRRSV on blood samples and Family Oral Fluids using RT-qrtPCR. Boxplots show median, quartiles, minimum and maximum values.