RESEARCH REPORT
SWINE HEALTH

Title: Comparison of PCR and serological assays for reliable, early and fast detection of PRRSV in boar studs – **NPB #11-058**

Investigator: Tanja Opriessnig, Dr med vet, PhD

Institution: Iowa State University

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Industry Summary

The high economic impact of porcine reproductive and respiratory syndrome virus (PRRSV) dictates the need for rapid and accurate diagnosis. The high mutation rate, rapid evolution and genetic variability of PRRSV strains complicate the development of long term reliable diagnostic assays, and consequently cases of false-negative results with commercially available assays have been reported. The objectives of this project were (1) to compare sensitivity, specificity, and time until completion of three different commercially available real-time RT PCR assays on different individual (serum, semen, blood swabs, oral fluids) or pooled sample types (serum, blood swabs) collected from boars experimentally infected with 1 of 6 diverse PRRSV isolates and (2) to compare commercial antibody detection systems for early detection of PRRSV antibodies in oral fluids and serum. Six groups of 3 boars negative for PRRSV were each inoculated with one of five PRRSV isolates (sharing 55-99% nucleotide sequence identity in ORF5). Samples were collected on days post-inoculation (dpi) -2, 1, 3, 5, 7, 14 and 21 and tested by one of three commercially available real-time RT-PCR assays: TaqMan® NA and EU PRRSV Reagents (AB, Applied Biosystems); Tetracore U.S. and Euro PRRSV Master Mix (TC, Tetracore) and the AcuPig® PRRSV real time PCR (AD, AnDiaTec GmbH). At dpi 1, all RT-PCR assays detected at least one positive sample in each group. The highest detection rates were on dpi 3 and dpi 5. Between dpi 1 and 7, serum samples had the highest detection rate (90%) with 100% agreement between tests, followed by blood swabs (Kappa = 0.97) and semen (Kappa = 0.80). Oral fluids had the lowest detection rates (AB: 55%; TC: 41%; AD: 46%) and the highest disagreement between kits (Kappa = 0.63). Pools of five samples did not reduce the detection rates if there was one high positive sample of viral RNA in the pool. Serum and blood swab samples had shorter turn-around times for RNA extraction. The AB assay had a 1.6 times shorter PCR reaction time. Serum and oral fluid samples were also tested by ELISA HerdChek PRRS X3 (E1, IDEXX laboratories), and serum samples were tested by CIVTESTsuis A/S (E2, for detection of PRRSV Type 1) and CIVTESTsuis E/S (E3 for detection of PRRSV Type 2, Laboratorios Hipra, Amer, Spain). Among serum samples, E1 were more specific and sensitive than E2 or E3. At dpi 7, 33% (5/15) pigs were positive by E1 versus none by E2 or E3. At dpi 14 and 21 all animals had detectable anti-PRRSV antibodies with E1. The E2 assay (used to detect anti-PRRSV Type II antibodies, groups 1-4) detected 1/12 false-positive samples on dpi -2. Six of 12 (50%) and 10/12 (83%) animals were positive on dpi 14 or 21. The E3 assay (used to detect anti-PRRSV Type I antibodies, group 5) had negative results throughout the experiment. From the 204 field serum samples tested, 2 (1%) tested positive using E1, 5 (2%) with E2, and none with E3. At dpi 7, 8 of 15 (53%) oral fluid samples were positive by E1, which was higher than the detection rate in serum samples.

Contact Information: tanjaopr@iastate.edu

Keywords: Porcine reproductive and respiratory syndrome virus; detection assays; comparison; sample types.

Scientific Abstract

The high economic impact of porcine reproductive and respiratory syndrome virus (PRRSV) dictates the need for rapid and accurate diagnosis. The high mutation rate, rapid evolution and genetic variability of PRRSV strains complicate the development of long term reliable diagnostic assays, and consequently cases of false-negative results with commercially available assays have been reported. The objectives of this project were (1) to compare sensitivity, specificity, and time until completion of three different commercially available real-time RT PCR assays on different individual (serum, semen, blood swabs, oral fluids) or pooled sample types (serum, blood swabs) collected from boars experimentally infected with 1 of 6 diverse PRRSV isolates and (2) to compare commercial antibody detection systems for early detection of PRRSV antibodies in oral fluids and serum. Six groups of 3 boars negative for PRRSV were each inoculated with one of five PRRSV isolates (sharing 55-99% nucleotide sequence identity in ORF5). Samples were collected on days post-inoculation (dpi) -2, 1, 3, 5, 7, 14 and 21 and tested by one of three commercially available real-time RT-PCR assays: TaqMan® NA and EU PRRSV Reagents (AB, Applied Biosystems); Tetracore U.S. and Euro PRRSV Master Mix (TC, Tetracore) and the AcuPig® PRRSV real time PCR (AD, AnDiaTec GmbH). At dpi 1, all RT-PCR assays detected at least one positive sample in each group. The highest detection rates were on dpi 3 and dpi 5. Between dpi 1 and 7, serum samples had the highest detection rate (90%) with 100% agreement between tests, followed by blood swabs (Kappa = 0.97) and semen (Kappa = 0.80). Oral fluids had the lowest detection rates (AB: 55%; TC: 41%; AD: 46%) and the highest disagreement between kits (Kappa = 0.63). Pools of five samples did not reduce the detection rates if there was one high positive sample of viral RNA in the pool. Serum and blood swab samples had shorter turn-around times for RNA extraction. The AB assay had a 1.6 times shorter PCR reaction time. Serum and oral fluid samples were also tested by ELISA HerdChek PRRS X3 (E1, IDEXX laboratories), and serum samples were tested by CIVTESTsuis A/S (E2, for detection of PRRSV Type 1) and CIVTESTsuis E/S (E3 for detection of PRRSV Type 2, Laboratorios Hipra, Amer, Spain). Among serum samples, E1 were more specific and sensitive than E2 or E3. At dpi 7, 33% (5/15) pigs were positive by E1 versus none by E2 or E3. At dpi 14 and 21 all animals had detectable anti-PRRSV antibodies with E1. The E2 assay (used to detect anti-PRRSV Type II antibodies, groups 1-4) detected 1/12 false-positive samples on dpi -2. Six of 12 (50%) and 10/12 (83%) animals were positive on dpi 14 or 21. The E3 assay (used to detect anti-PRRSV Type I antibodies, group 5) had negative results throughout the experiment. From the 204 field serum samples tested, 2 (1%) tested positive using E1, 5 (2%) with E2, and none with E3. At dpi 7, 8 of 15 (53%) oral fluid samples were positive by E1, which was higher than the detection rate in serum samples.

Introduction

Porcine reproductive and respiratory syndrome virus (PRRSV) continues to be the most important pathogen affecting pigs in North America (5). Although a variety of commercial vaccines are available on the global market, the virus remains difficult to control and the demand for PRRSV-naïve replacement boar genetics continues to grow and with it the need for highly sensitive and specific assays that can detect genetically diverse strains and information on the most appropriate samples for testing.

Presently, many boar studs in the U.S. are PRRSV negative and are routinely tested for PRRSV to assure that PRRSV-free semen is shipped to breeding herds for artificial insemination (5). If previously negative boar studs become infected with PRRSV, it is critical to detect the virus as soon as possible so that any shipments of possible PRRSV contaminated semen can be stopped.

The reverse transcription polymerase chain reaction (RT-PCR) method in the real-time format is one of the most commonly used techniques for detection of PRRSV RNA because of its sensitivity and specificity and the ability to complete testing shortly after sample processing. At the present, the earliest way to detect a positive animal is by RT-PCR. If the detection of antibodies was feasible at very early times post-infection, the cost of early diagnosis could be substantially lowered. Recently, increased rates of false negative results were

reported in several PRRSV RT-PCR assays commonly used in veterinary diagnostic laboratories in Europe and North America, and combined usage of different assays or PCR tests has been recommended to improve the diagnostic success for PRRSV (11, 24, 27).

Active PRRSV surveillance in boar studs relies mainly on collection and testing of serum, semen, blood swabs and more recently, oral fluids (19, 12). Sample type choice should consider the availability and ease of collection in addition to the sensitivity and specificity of the RT-PCR assay to detect PRRSV RNA. Studies have shown that PRRSV RNA can be detected in boar serum, oral fluids, and blood swabs as early as 24 to 48 h post-infection, and as early as 48 to 120 h post-infection in semen samples (3, 19, 20, 12). Isolate-specific differences in the levels of PRRSV replication have been reported (8), and as alternative sampling methods are being described and used, it is important to conduct an unbiased comparison for the ability of different commercial real-time RT-PCR tests to detect genetically diverse isolates of PRRSV.

The sample collection process, transport and testing are time and labor consuming. In order to test a large number of animals and reduce cost, pooled sample analysis has been used successfully in recent years for detection and surveillance of infectious diseases (20, 6, 13). Pooling of serum and blood swabs is used on a regular basis by many boar stud owners to monitor PRRSV status by RT-PCR. While a single study demonstrated a decrease in sensitivity, especially during the first days of PRRSV infection, when pools of 3 and 5 were used in serum and blood samples (20), the effects of processing and analyzing samples individually or pooled have never been comprehensively compared.

A complete understanding of the sensitivity of the test used to detect PRRSV RNA would better inform decisions on boar stud PRRSV monitoring protocols. The aims of this study were (1) to compare the sensitivity and specificity of three commercially available PRRSV diagnostic assays to detect genetically diverse isolates of PRRSV in different sample types (serum, semen, blood swabs, oral fluids), (2) to evaluate the effects of pooling serum and blood swab samples on diagnostic accuracy, and (3) to compare the turn-around time of the three assays.

Objectives

The objectives of this project were (1) to compare sensitivity, specificity, and time until completion of three different commercially available real-time RT PCR assays on different individual (serum, semen, blood swabs, oral fluids) or pooled sample types (serum, blood swabs) collected from boars experimentally infected with 1 of 5 diverse PRRSV isolates and (2) to compare commercial antibody detection systems for early detection of PRRSV antibodies in oral fluids and serum.

Materials and Methods

A. Experimental samples

Animals and housing. Eighteen, 6-month-old, boars were acquired from a commercial PRRSV naïve breeding herd. Upon arrival at the Iowa State University Livestock Infectious Disease Isolation Facility, the boars were randomly divided into six groups of three boars and housed in separate rooms each containing three individual pens for housing of the boars and a collection pen within the same room.

Experimental design, sample collection, and storage. After a short acclimation period, the boars were infected with one of five different PRRSV isolates as summarized in Table 1 by slowly dripping 4 ml of the inoculum containing a median tissue culture infective dose ($TCID_{50}$) of $10^{4.5}$ into the nostrils. Prior to inoculation at -2 day post-inoculation (dpi) and 1, 3, 5, 7, 14 and 21 dpi, serum, semen, blood swabs, and oral fluids were collected. Semen was collected using the gloved-hand technique. Concurrently, blood was collected by venapuncture of the saphena vein. Immediately after blood collection, blood swabs were collected as described (20). Oral fluids were individually collected by using a cotton robe as described (22). All samples were stored on ice after collection until arrival at the laboratory. Samples were stored at -80°C until tested.

Table 1. Experimental design.

Group designation	N. boars	PRRSV isolate	PRRSV type	Year isolated	Reference
1	3	VR2385	2	1991	Halbur et al. (1995)
2	3	SDSU73	2	1996	Megeling et al. (1998)
3	3	JA142	2	1996	Megeling et al. (1998)
4	3	FL12	2	2004	Truong et al. (2004)
5	3	2010011381	1	2010	This manuscript

B. Field samples

A total of 204 serum and 200 oral fluid samples from individual boars from 29 different herds were selected from routine submissions from known PRRSV negative herds to the Veterinary Diagnostic Laboratory at Iowa State University to perform the serological tests. Samples were stored at -80°C until tested.

C. Molecular diagnostic assays

*RNA extraction***.** Total nucleic acids of serum and blood swabs samples were extracted by using the King Fisher® Flex 96 Ambion® from Thermoscientific using the MagMAX^{TM}-96 viral RNA isolation kit according to manufacturer's directions. Prior to automated extraction, semen samples were centrifuged at 1000 x g for 10 min and the cell pellets were re-suspended in 300 µL of physiological saline. Oral fluid and semen samples were extracted as previously described (13).

Real time RT-PCR. Real-time RT-PCR was performed on RNA extracts using VetMax NA and EU PRRSV reagents (abbreviated here with AB; Applied Biosystems, Foster City, CA, USA); VetAlert NA and EU PRRSV PCR reagents (abbreviated here with TC; Tetracore, Rockville, MD, USA), and AcuPig PRRSV real time PCR reagents (abbreviated here with AD; AnDiaTec GmbH, Kornwestheim, Germany). Reactions were performed according to the manufacturers' instructions using the 7500 Fast Real-Time PCR system (ABI, Foster City, CA, USA). Based on the cutoff that is currently used by the Veterinary Diagnostic Laboratory at Iowa State University, a cycle threshold (Ct) < 37 cycles was used for considering samples as positive for all three assays. A negative and a positive control provided in each kit were added to each PCR plate. Internal control amplification was evaluated to validate the result as recommended by the manufacturers' protocol.

Sequencing. The open reading frame (ORF) 5 gene fragment amplified from a PRRSV positive pig in each group on dpi 21 and the inocula used for the experimental infections were sequenced (11).

Pooling strategy. Serum and blood swab samples individually tested were classified as high positive when the Ct was ≤ 29.9; moderate positive when the Ct was between 30.0 and 34.9; and low positive when the Ct was between 35.0 and 37.0. One sample of each positive range per virus isolate was selected and diluted in appropriate amount of negative control samples of serum or blood swabs to simulate pools of 2, 3, 5 and 10 with one positive sample with the respective number of negative samples.

Turn around-time. The turn-around time of each assay was calculated manually by recording specific time points: sample preparation, if needed; extraction protocol used according each sample type and the real-time PCR cycle conditions of each set of reagents.

D. Serology assays

ELISA 1. Serum samples were tested for anti-PRRVS antibodies using the commercial ELISA HerdChek PRRS X3 (E1; IDEXX laboratories, Inc., Westbrook, ME, USA) according to the manufacturer's label instructions. Oral fluids were tested by ELISA 1 using an optimized protocol as previously described (Kittawornrat et al., 2012). Samples were considered positive if the calculated sample-to-positive (S/P) ratio was equal to 0.4 or greater.

ELISA 2 and 3 .Serum samples were tested for anti-PRRVS antibodies using the commercial ELISA CIVTESTsuis A/S (E2) for detection of PRRSV Type II (strains VR2385, SDSU73, JA142, FL12 and field samples) and CIVTESTsuis E/S (E3) for detection of PRRSV Type 1 (strain 2010011381 and field samples)

(Laboratorios Hipra, Amer, Spain) according to the manufacturer's label instructions. Samples were considered positive if the IRPC (relative index x 100) were higher than 20.

E. Statistical analysis

Cochran's Q test for matched data, followed by McNemar's test for pairwise comparisons was used to determine whether the proportions of RT-PCR positive samples were significantly different by assays or sample type. Differences between groups were considered significant if $p < 0.05$. Kappa index was performed to determine the agreement of positive/negative results between assays and samples types. The strength of agreement was considered as previously described (14), ≤ 0 = poor, 0.01-0.2 slight, 0.21-0.4 = fair, 0.41-0.60 = moderate, 0.61-0.80 = substantial, and 0.81-1 = almost perfect. Statistical analyses were performed using SAS Version 9.2 (SAS Institute, Inc., Cary, NC, USA).

Results

A. Confirmation of PRRSV infection

To determine whether the experimentally-inoculated boars had prior exposure to PRRSV and to assess the status of experimental PRRSV infection, all serum samples were tested for presence of anti-PRRSV negative antibodies and all sera, blood swabs, semen samples, and oral fluid samples on dpi -2 were tested by all three PCR assays prior to experimental infection. All serum samples collected on dpi -2 tested negative for anti-PRRSV antibodies and all samples were PRRSV RNA negative by the three assays. All 15 animals in groups 1 to 5 seroconverted between dpi 7 and 14. Sequencing of the ORF5 gene of PRRSV and comparison with the original inoculum confirmed that the correct PRRSV isolates.

B. Molecular diagnostic assays

PRRSV detection by the three commercial real-time assays in experimental samples. To obtain the prevalence of PRRSV RNA detection in each sample type over time, data from the 15 infected boars (groups 1 to 5) were used. At dpi 1, all assays were able to detect at least one positive sample in each group, with the highest detection rates on dpi 3 and dpi 5 for all three assays. On dpi 14 and dpi 21, PRRSV RNA detection rates decreased in all sample types except for oral fluids, which coincided with the detection of anti-PRRSV antibodies. (A) *Serum samples.* Among the sample types, the ability to detect PRRSV RNA was highest in serum samples throughout the study reaching a positive detection rate of 90% (54/60) during the acute phase of infection (dpi 1 through 7) with 100% agreement among the three assays tested. (B) *Blood swabs.* PRRSV RNA detection rates in blood swab samples were similar to those in serum samples throughout the study (Kappa = 0.73, $p = 0.11$). Considering the acute phase of infection (dpi 1 through 7), PRRSV RNA was detected in 86.6% (AB), 86.6% (TC), or 81.6% (AD) of the samples, with near complete agreement between the tested assays (Kappa = 0.97). (C) *Oral fluids.* Oral fluid samples had the lowest detection rate and the highest disagreement between assays throughout the study (Kappa = 0.40 to 0.73), with 55% (AB), 41% (TC), and 46% (AD) of positive detection from dpi 1 through 7. In contrast to the other sample types that showed a decreasing trend in PRRSV RNA detection after dpi 7, there was an increase in detection rates of PRRSV RNA in oral fluids on dpi 14 and dpi 21, showing the highest detection rates among sample types on dpi 21. Among all tested samples, the TC assay had the lowest detection rate (41.1% 37/90, $p = 0.02$). There was no difference between the AB and the AD assays (56.7% 51/90 vs. 50.0% 45/90, $p = 0.06$). (D) *Semen samples.* During the acute phase of infection (dpi 1 through dpi 7), the detection rates in semen samples were 81.6% (AB), 73.3% (TC), and 78.3% (AD). The AB test had a higher detection rate than TC (67.7% 61/90 vs. 60.0% 54/90, Kappa 0.83, $p = 0.02$). There was no difference between AB and AD assays (67.7% 61/90 vs. 65.50% 59/90, $p = 0.61$).

Detection of PRRSV RNA and sensitivity of the three commercial real-time assays for heterologous PRRSV isolates. Considering all five PRRSV strains tested, AB detected 67.2% of positive samples (242/360), followed by AD (62.5%; 225/360), and TC (61.7%; 221/360). The AB assay had the highest PRRSV RNA detection rate among the three assays ($p < 0.01$). There was no difference between detection rates of the AD and the TC assays ($p = 0.72$). The AB test had a higher detection rate than TC for group 3 ($p < 0.01$) and a higher detection rate than AD for group 4 ($p = 0.03$).

Effects of pooling. To evaluate the effect of the size of sample pools on sensitivity of the RT-PCR, pools of 2, 3, 5 or 10 serum or blood swab samples which contained only one low, moderate or high positive sample per pool were tested. There was no difference in detection rate among strains or assays. For high positive samples (Ct ≤ 29.9), pooling of 2, 3, 5 or 10 serum or blood swab samples did not reduce the probability of identifying a single positive sample among them. For moderate positive samples (Ct between 30.0 and 34.9), pools of 5 serum or blood swabs samples still resulted in a positive signal (Ct < 37). When samples were low positive (Ct between 35.0 and 37.0), pools of 2 were positive in 4/5 strains of serum and blood swab samples. Larger pool sizes gave negative results with low positive samples, although 4/5 and 3/5 serum samples produced Ct > 37 in pools of 3 and 5 respectively. Among blood swab samples, 2/5 and 1/5 produced Ct > 37 in pools of 3 and 5, respectively.

Negative oral fluids and serum samples from the field. To evaluate the prevalence of false positive results between assays, oral fluid and serum samples obtained from farms considered PRRSV-negative on the basis of regular PRRSV-negative serological test results. All of the 200 serum samples and the 200 oral fluid samples tested negative with the AB and the TC assays. Three of 200 (1.5%) oral fluid samples and 2 of 200 (1.0%) serum samples were positive using the AD assay. Sequencing of the amplified products was attempted to confirm the presence of PRRSV; however, none of the three "positive samples" yielded a sequence consistent with PRRSV.

Turn-around time. Semen samples required an additional centrifugation and re-suspension step prior to nucleic acid extraction. Extraction protocol for semen samples and oral fluids required an additional lysis step before the automated extraction on the KingFisher platform compared to serum samples and blood swabs. Considering the amount of time from start of sample processing until the end of nucleic acid extraction, serum and blood swab samples required 1 h. For processing, the time was increased by 25 min for oral fluids and by 55 min for semen samples. Regarding the time to complete the PCR reaction, the AB assay required 1 h 30 min, and the TC and the AD assays required an additional 51 min in the run length time.

C. Serology assays

Anti-PRRSV antibody detection by the three ELISAs in experimental samples. (A) *Serum.* The sensitivity of the assays depended on the PRRSV strain inoculated. At dpi 7, 33% (5/15) pigs were detected as positive by E1 versus none by E2 or E3. Regarding E1 results, at dpi 7 anti-PRRSV antibodies were detected in strains SDSU73 (3/3), VR2585 (1/3) and JA142 (1/3); at dpi 14 and 21 all animals presented anti-PRRSV antibodies. E2, used to detect anti-PRRSV Type II antibodies (groups 1 to 4), detected one positive animal on dpi -2, which was negative on dpi 5 and 7. At dpi 14, 40% (6/15) were detected as positive by E2. On dpi 21, E2 detected all animals infected with SDSU73 or JA142 strains as positive; and 2 of 3 infected animals with the strains VR2385 or FL12. E3, used to detect anti-PRRSV Type I antibodies failed to detect any animals infected with 2010011381 throughout the experiment. (B) *Oral fluid.* Table 2 summarizes results obtained from oral fluid experimental samples tested by E1. The sensitivity depended on which PRRSV strain the boars were inoculated with. At dpi -2, one of 15 samples was positive. At dpi 7 at least one animal from each group was detected as positive, with 100% detection rate for strains VR2385 and SDSU73. The strain with the lowest detection rate was FL12.

Table 2. Number of positive anti- PRRSV antibody samples and sample-to-positive (S/P) values (mean ± standard deviation) from -2 to 21 days post infection using ELISA 1 (IDEXX PRRS X3 Ab Test) on boars experimentally infected with PRRSV.

Strain	-2	5	7	14	21
	(1/3)	(0/3)	(1/3)	(3/3)	(1/3)
VR2385	0.2 ± 0.22	0.00 ± 0.03	0.85 ±1.57	2.38 ± 1.61	1.26 ± 2.06
	(0/3)	(0/3)	(3/3)	(3/3)	(3/3)
SDSU73	0.08 ± 0.06	0.00 ± 0.04	6.25 ±1.61	2.32 ± 1.64	1.34 ± 0.61
	(0/3)	(0/3)	(1/3)	(3/3)	(2/3)
JA142	0.09 ± 0.14	0.03 ± 0.00	0.93 ±1.47	1.96 ± 0.37	0.62 ± 0.25

	(0/3)	(0/3)	(1/3)	(0/3)	(1/3)
FL12	0.02 ± 0.06	0.00 ± 0.04	0.43 ± 0.70	0.23 ± 0.07	0.79 ± 0.92
	(0/3)	(0/3)	(2/3)	(3/3)	(2/3)
2010011381	0.10 ± 0.24	0.03 ± 0.03	0.55 ± 0.58	2.79 ± 2.25	0.72 ± 0.85
Total	0/15	0/15	8/15	12/15	9/15

Anti-PRRSV antibody detection by the three ELISAs in field samples. To evaluate the prevalence of false positive results between assays, oral fluid and serum samples were obtained from farms considered PRRSV-negative. From the 204 serum samples tested, 2 (0.98%) tested positive using E1, 5 (2.45%) with E2, and none with E3. From the 200 oral fluids tested with E1, 4 (2.00%) tested positive.

Discussion

In this study, a head-to-head comparison of three commercially available RT-PCR assays and two commercially available ELISAs were performed to compare early detection of PRRSV RNA in boars infected experimentally with 1 of 5 genetically different strains of PRRSV. All of the PRRSV strains used in the study were detected by the three assays. Whereas the AB test had the overall highest detection rate among the assays, the TC and AD tests had overall similar detection rates across all strains.

When considering the specificity among assays using field samples from historically PRRSV ELISA negative herds, the AD assay resulted in 1.0% (serum samples) to 1.5% (oral fluid) false positive results. E1 resulted in 0.9% (serum) to 2.0% (oral fluid) and E2 in 2.5% (serum) false positive results. False positive results can have a great economic impact for the producers and industry due to the immediate hold of all semen samples from the stud, re-testing of all the boars and likely culling of suspect positive boars.

Under the conditions of the present study, serum and blood swabs had the best overall performance based on the detection rates during the acute phase of infection, the near complete agreement between the three diagnostic assays tested, and the assay turn-around time. This result is in agreement with previous studies which compared PRRSV RNA detection in serum, blood swabs and semen (20, 21).

Recently, oral fluid sample submissions for surveillance and diagnosis have increased due to the ease of this collection method and cost effectiveness of virus surveillance in pig herds (18). A previous study has shown similar PRRSV RNA detection rates between oral fluids and serum samples of experimentally infected boars (13). However, in this study, oral fluids had the lowest overall RT-PCR detection rates, the highest disagreement between assays and an increased turn-around time when compared with blood swabs and serum samples. In contrast to the previous study, in which PRRSV detection increased from 10% (7/69) on dpi 1 up to 100% (67/67) on dpi 4; the detection rate on dpi 1 in the present study was 60% (9/15) with the lowest detection on dpi 7 (5/15). As both studies used the same extraction method and the AB reagents, the difference in results may be due to differences between inoculation methods and strains used and oral fluid collection procedures.

Oral fluid matrix is known to contain inhibitors that can cause reaction failure or can reduce the analytical sensitivity (1). Although an optimized extraction protocol to overcome this was used in addition to the use of double the recommended PCR enzymes for the AB test (1), due to the limited amount of this reagent in the TC and the AD reagents, this strategy could not be used for these two assays. This could explain the clear difference in detection rates between the assays used in this study, in which the AB assay presented the highest detection rates on oral fluids using the optimized PCR protocol while the detection rates were lowest for the TC test. This difference was not noticed in the other sample types tested indicating that PRRSV PCR optimization for oral fluid may also be required for the other two assays.

Sample pooling can result in major savings, particularly of consumables and labor, thereby reducing cost. A pool size of 10 for both serum and blood swab samples substantially increased the number of false negative results for moderate and low positive samples and should be avoided. There was also a significant decrease in sensitivity when low positive samples were tested in pools of 3 and 5. This is consistent with another study that determined up to 14% of positive samples would be missed if pools of 5 serum or blood swabs were used during the first 5 days of PRRSV infection (21). Due to the dilution effect, it has been suggested that increasing the cut-off value for preliminary evaluation of pools should be used and re-testing

pooled samples with Ct values close to cut-off value as single samples should be conducted to avoid false negative results (6). Under the conditions of the present study, the detection rate in low positive samples would have increased substantially, and the detection in serum pools of 5 would have increased from no positive samples to 3/5 positive results if a Ct > 37 were considered. As the use of a higher cut-off would likely decrease the specificity of the test thus potentially increasing the number of false positive results.

Among the serology assays, E1 had an earlier detection of experimental serum samples than E2 and E3. Due to the lack of an optimized protocol for oral fluid samples testing with E2 and E3 assays, this sample were only tested by E1 test. At dpi 7, 8 of 15 (53%) oral fluid samples were positive by E1, which was higher than the detection rate in serum samples (5/15, 33%). However, oral fluid samples also presented a false-positive sample on dpi -2 and two false-positive samples among the field samples (2/204 versus 4/200).

In summary, the detection rate for PRRSV RNA varied depending on the sample type and virus isolate used under the conditions of this study. Serum and blood swabs had the best overall performance with the highest detection rates and agreement between kits. The AB reagents had the highest detection rate across the PRRSV isolates used in this study. Testing pooled samples can compromise the detection rates when low positive samples are included. The E1 test had highest specificity and sensitivity.

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