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Impact of weaning procedures on Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) circulation in the nursery section

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Abstract

The impact of different weaning strategies on the downstream circulation of PRRSV has not been widely described. It is, however, believed that mixing pigs of different age groups is increasing the risk of PRRSV circulation in the nursery section. In this study, pigs were sampled in three herds that performed "mixed at weaning (MIX)" and three herds that performed "all in/all out at weaning (AIAO)". MIX included holding underweighted piglets back in containers for two weeks and then move them to nursery facilities with newly weaned piglets from subsequent batches. Oral fluid samples were collected from four batches of pigs in each herd, three times from weaning until 30 kg for each batch, and tested for PRRSV and PRRSV antibodies. Herds that performed MIX at weaning had an eightfold increase in risk of detecting PRRSV in oral fluids compared to herds with AIAO. In total, 41 oral fluid samples from eight batches in MIX herds and five oral fluid samples from two batches in AIAO herds tested positive for PRRSV. The PRRSV ELISA S/P ratio in oral fluid samples from weaners seem to decrease in most of the batches in the AIAO herds and to increase in most MIX herds. In addition to oral fluids, tongue tip samples were collected from dead pigs and tested for PRRSV. In 17 of 23 batches the results of the tongue tip samples correlated with the results of the oral fluid samples ($\kappa = 0.44$) indicating a good agreement between the two materials for sampling. Overall, the results of the study confirmed that the weaning strategy had a significant impact on the circulation of PRRSV post weaning.

Keywords PRRS, Virus detection, Management, Weaners, Oral fluids, Tongue samples

Background

Porcine Reproductive and Respiratory Syndrome (PRRS) is considered one of the most devastating and economically challenging diseases to the swine industry worldwide [1]. The etiological agents, the PRRS viruses (PRRSV-1 and PRRSV-2), are small enveloped RNA viruses that replicates within the monocytic lineage of the respiratory and lymphoid systems of the pigs [2]. PRRSV-1 and PRRSV-2 first appeared in Denmark in the beginning of the 1990ties and has since been one of the major health challenges in the Danish pig production [3]. In 2022, 35% of the Danish pig herds were positive for PRRSV. Of the positive herds, 37% had PRRSV-1 and 36% had PRRSV-2, whereas 26% of the declared herds were infected by both viruses.

There is a variety of strategies for the control of PRRSV within herds. The preferred strategy for most PRRSV positive herds is to establish a stable sow herd where the gilts are immunized prior to introduction into the sow herd and thereby wean PRRSV free piglets. In contrast, most herds pay less attention to virus circulating in the nursery. Sow mass vaccination is used in many herds in

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Denmark, whereas piglet vaccination is less commonly used [4]. The stabilization of the sow herd is relatively easily obtained by mass vaccination and herd closure and can be done without any or only limited change in the production cycle [5].

During recent years there has been an increasing public pressure for reducing the use of antibiotics in Danish pig herds, and many producers therefore thrive to increase the health of pigs also in the nursery period. In 2023 Danish herds with PRRS positive status had significant higher use of antibiotics than herds with PRRS negative status [6] There can also be an economic advantage in selling pigs free of PRRSV and in herds where total sanitation is not possible, the major challenge is how to implement a weaning strategy that reduce the circulation of PRRSV after weaning.

A considerable number of publications describes protocols for obtaining stability in the sow herd [7], however, there is a lack of data that describe the variations between different weaning strategies on the downstream circulation of PRRSV in the period from weaning until 30 kg. To the best of our knowledge, the impact of different weaning strategies on the downstream circulation of PRRSV has not been studied in a controlled set-up. The aim of this study was to compare the prevalence of PRRSV positive oral fluid samples (OF) and thereby the prevalence of PRRSV positive batches of weaned pigs, in herds with two different weaning strategies: All in/ all out (AIAO) and mixing (MIX) of age groups. Furthermore, the use of tongue samples from dead pigs for detection of PRRSV compared to oral fluid was evaluated.

Results

A total of 260 oral fluid samples were collected from 23 batches of pigs from six herds. In herd B only three batches were included. In herd C, batch 4 and in herd E, batch 1, 3 and 4 were not sampled at week 11 because the pigs were moved to the finisher site or sold prior to week 11.

PRRSV in oral fluids

In MIX herds, 41 oral fluid samples from a total of 11 batches were positive for PRRSV (Table 1). All three MIX herds had at least one PRRSV positive batch. The number of positive samples at a given age group varied, but in general the number of positive batches increased with age (Fig. 1).

In the AIAO herds, five oral fluid (OF) samples from a total of 12 batches were positive for PRRSV. The PRRSV positive oral fluid samples were all collected at 11 weeks of age in herd D (Table 1 and Fig. 2). No positive samples were detected in herd E and F (data not shown).

Significant more batches of pigs were PRRSV positive in the nursery in herds with MIX weaning strategy compared to herds with AIAO weaning strategy ($p < 0.001$). The relative risk of having batches with PRRSV positive pigs in herds with MIX weaning strategy was 8.4 times the risk in AIAO herds. This indicates a significantly

Table 1 The number of oral fluid PRRSV RT-qPCR positive samples/samples tested in the three MIX herds and in one AIAO herd; Herd D. No positive samples were detected in the AIAO herds E and F

Weeks of age	Batch	Herd A	Herd B	Herd C	MIX Total	Herd D
5	1	0/4	0/4	1/4	1/12	0/4
	2	0/4	NA[a]	1/4	1/8	0/4
	3	0/4	0/4	0/4	0/12	0/4
	4	0/4	0/4	2/4	2/12	0/4
	Total	**0/16**	**0/12**	**4/16**	**4/44**	**0/16**
8	1	0/4	0/4	2/4	2/12	0/4
	2	1/4	NA[a]	2/4	3/8	0/4
	3	1/4	2/4	3/4	6/12	0/4
	4	0/4	0/4	4/4	4/12	0/4
	Total	**2/16**	**2/12**	**11/16**	**15/44**	**0/16**
11	1	0/4	4/4	2/4	6/12	0/4
	2	3/4	NA[a]	1/4	4/8	0/4
	3	4/4	4/4	4/4	12/12	2/4
	4	0/4	0/4	NA[a]	0/8	3/4
	Total	**7/16**	**8/12**	**7/12**	**22/40**	**5/16**
All samplings		**9/48**	**10/36**	**22/44**	**41/128**	**5/48**

[a] *NA* sample not available

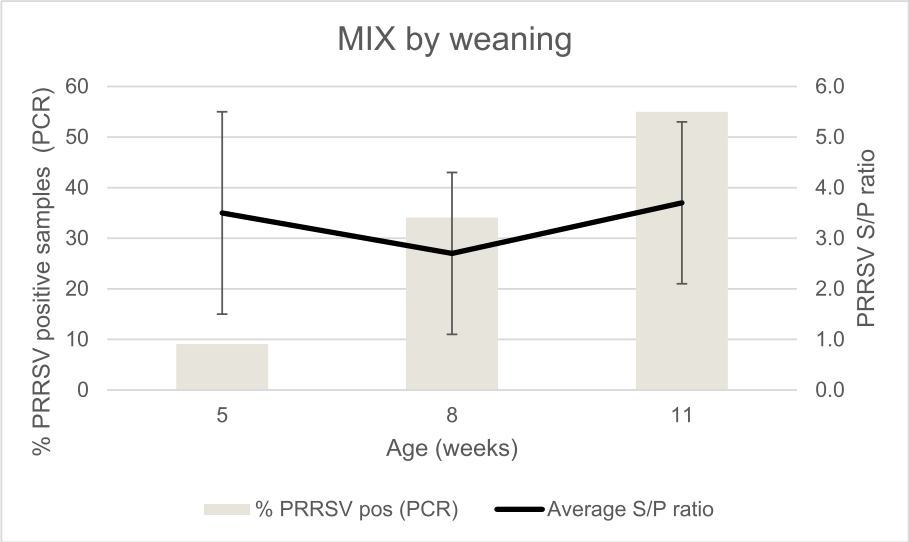

Fig. 1 Percent of PRRSV positive (RT-qPCR) oral fluid samples and average PRRSV ELISA S/P ratios collected at five, eight and 11 weeks of age when MIX by weaning

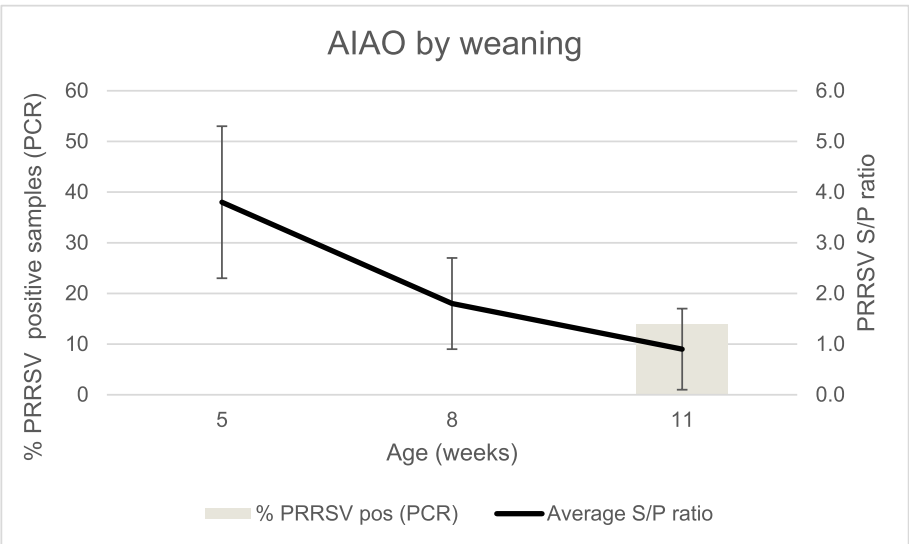

Fig. 2 Percent of PRRSV positive (RT-qPCR) oral fluid samples and average PRRSV ELISA S/P ratios collected at five, eight and 11 weeks of age when AIAO by weaning

increased risk of having PRRSV circulating in the nursery units where MIX at weaning was practiced.

PRRSV in tongue tip samples (TTS)

The number of TTS collected in herd C was mistakenly not registered. In the remaining herds, the number of collected TTS varied between batches from one to 26 (Tables 2 and 3). PRRSV was detected by RT-qPCR in five out of 11 batches in the MIX herds (Table 2). In eight of the 11 MIX batches the results of TTS correlated to the results of the test of the corresponding OF samples. In herds with AIAO at weaning, PRRSV was detected in TTS from pigs in batch 1 in herd D (Table 3). In nine of the 12 AIAO batches, the results of the TTS RT-qPCR test correlated with the outcome of the test of the corresponding oral fluid samples.

The agreement between the two test methods OF and TTS for detecting PRRSV in a batch was calculated to

Table 2 Comparison of PRRSV detection (RT-qPCR) between OF and TTS within batches in herds with MIX at weaning. The results in bold face represent batches were the results differed between the oral fluid and the tongue tests

Batch	Herd A MIX		Herd B MIX		Herd C MIX	
	Oral fluid	TTS	Oral fluid	TTS	Oral Fluid	TTS
1	Negative	Negative (0/3)	Positive (4/12)	**Negative** (0/4)	Positive (5/12)	Positive (NA)
2	Positive (4/12)	**Negative** (0/2)	NS	NS	Positive (4/12)	Positive (NA)
3	Positive (4/12)	Positive (1/1)	Positive (2/12)	**Negative** (0/1)	Positive (7/12)	Positive (NA)
4	Negative	Negative (0/2)	Negative	Negative (0/1)	Positive (6/8)	Positive (NA)

NA the number of samples was not recorded, *NS* Not sampled

Table 3 Comparison of PRRSV detection (RT-qPCR) between OF and TTS within batches in herds with AIAO at weaning. The results in bold face represent batches were the results differed between the oral fluid and the tongue tests

Batch	Herd D AIAO		Herd E AIAO		Herd F AIAO	
	Oral fluid	TTS	Oral fluid	TTS	Oral fluid	TTS
1	Negative	**Positive** (1/9)	Negative	Negative (0/7)	Negative	Negative (0/3)
2	Negative	Negative (0/11)	Negative	Negative (0/26)	Negative	Negative (0/2)
3	Positive (2/4)	**Negative** (0/8)	Negative	Negative (0/15)	Negative	Negative (0/2)
4	Positive (3/4)	**Negative** (0/23)	Negative	Negative (0/23)	Negative	Negative (0/2)

be *good* with a value of 0.44 Cohen's kappa (K). For five of the six samplings where the results differed between the oral fluid and TTS, the TTS tested negative, and the oral fluid tested positive. There was no clear relationship between the numbers of TTS collected in a given batch and the outcome of the test or the compliance with the oral fluid test results.

PRRSV antibodies in oral fluids

In herds with the MIX weaning strategy, the oral fluid tested positive for PRRSV antibodies at all time points, but the dynamics differed between herds and batches (Fig. 1; Supplementary Fig. 1). In herd A, batch 1 and 4 had increased levels of antibodies from 5–11 weeks of age, batch 3 had a steady high level and in batch 2 the antibody decreased from 5 to 8 weeks of age followed by a pronounced increase between 8 and 11 weeks of age in one sample. In herd B, batch 3 had a high antibody level at 5 and 8 weeks of age followed by a rapid increase between 8 and 11 weeks of age. The other two batches had low levels at 5 weeks of age, became negative at 8 and showed an increase between 8 to 11 weeks of age. In herd C, there was a decline in antibody levels from 5 to 8 weeks of age in all batches tested followed by a moderate increase between 8 and 11 weeks of age. As expected, there was an overall clear relationship between detection of PRRSV in oral fluid and an increase in antibody levels at subsequent samplings in all MIX herds.

In all herds with the AIAO weaning strategy, the oral fluids samples tested positive for PRRSV antibodies at five weeks of age (Fig. 1; Supplementary Fig. 1). In herd D, two out of four batches tested negative for PRRSV antibodies at week 8 and at 11 weeks of age, all batches tested negative. Batch 3 and 4 in this herd tested positive for PRRS virus in week 11. Batch 1 had one RT-qPCR positive TTS but did not seroconvert at 11 weeks of age. In herd E, there was a clear decline in levels of antibodies between 5 and 8 weeks of age. Batch 2 was sampled at 11 weeks of age and in one of the four samples a high level of antibodies was detected. No virus was detected in this herd, but the increase in antibody levels indicates that at least one batch of pigs was infected late in the nursery. In herd F there was a steady decrease in antibodies during the sampling period

which is in agreement with the negative virus tests in this herd in all batches.

Discussion

To the best of our knowledge this is the first study from Europe that investigate the impact of different weaning strategies on the risk of having PRRSV circulating in the nursery and the results clearly document that it is essential to practice AIAO to avoid virus circulating in weaners and by that support typical management advices as not moving pigs back in age, move diseased pigs away from the room, change equipment between stables and pens, optimize ventilation, wean pigs at same age directly to clean stables.

PRRSV RT-qPCR positive oral fluid samples were detected in all herds with MIX as weaning strategy and in one herd with the AIAO weaning strategy (herd D). Two out of the three herds with MIX as weaning strategy had the nursery on site and two out of three herds with AIAO as weaning strategy had the nursery off site. The effect of placement of the nurseries has not been investigated in this project, but multi site production is expected to have an impact on the circulation of diseases.

The test for PRRSV antibodies in OF showed that pigs in all herds were antibody positive at weaning, which was expected due to the presence of maternal antibodies. Only two herds; herd B with MIX and herd D with AIAO had two antibody negative batches at eight weeks of age. The batches at herd B became antibody positive again at 11 weeks of age, whereas the batches at herd D remained antibody negative at week 11. A previous study from France found similar patterns in that all pigs were ELISA positive at weaning at four weeks of age, 10.5% of the pigs were ELISA-positive at 7 weeks of age whereas all the piglets were seronegative four weeks later at the age of 11 weeks [8]. Maternally derived antibodies are expected to be absent from 11 weeks of age or 7 weeks after weaning.

In herds with MIX (herd A-C) it was expected that the level of antibodies would increase during the growth period, since there was a high number of PRRSV PCR positive samples at weeks 5 and 8.

In the AIAO herd E, only one out of the four batches (batch 2) were tested at week 11 and this batch tested positive for antibodies in ELISA, after being antibody negative at weeks five and 8 and despite that PRRSV was not detected by PCR in this herd at any time point in neither oral fluid or in the 26 TTS that was tested from batch 2. This indicate that PRRSV was indeed circulating in the nursery of this farm despite the use of AIAO.

Oral fluid is in general regarded as a sensitive sampling material for PRRSV detection [9], and the unexpected negative RT-qPCR tests at some samplings in herd E can be explained by the relative low sample size or degradation of RNA by suboptimal storage of the oral fluid samples during sampling at the herds where the temperature in freezers were not calibrated, transport and/or in the laboratory. This would in other words be possible reasons to get false negative samples. Another explanation could be that the positive ELISA results of the samples taken at week 11 in batch 2 represented false positive results. Indeed, a previous study revealed that certain animal husbandry or production practices may be associated with non-specific reactions in the ELISA kit used in our study [10]. To increase the specificity of the test, they suggested to use a higher cut-off of 1.0 S/P value in contrast to 0.4 which is recommended by the vendor and used in our study. However, we decided to use the validated cut-off in our study and increasing the cut off would not have changed the outcome of the test since the S/P ratio was well above 1.0 in the samples from herd E, batch 2 at 11 weeks of age.

In herd D, all pigs tested negative for antibodies at week 11 which were in accordance with the negative RT-qPCR results at weeks 5 and 8 in all batched, but PRRSV was detected in pigs from two batches (3 and 4) at 11 weeks of age. This indicated that the pigs in the two positive batches were infected less than 7–10 days prior to the sampling in week 11. The explanation was probably that pigs in herd D were moved to a different nursery unit before trade and one section in this unit kept leftover pigs, meaning that pigs in this section was continuously mixed.

A decrease in ELISA antibodies was also seen in herd F which correlated well with the negative RT-qPCR tests, however, the samples still tested positive for antibodies at week 11 when using the S/P ratio of 0.4 as cut-off, but, interestingly, they were all below the suggested alternative cut-off of 1.0 used by Henao-Diaz et al. [10] in order to reduce the rate of unexpected positive results. Herd D performed sow mass vaccination against PRRSV with a MLV-vaccine two times a year whereas in herds E and F the sows were mass vaccinated three times a year. This difference in vaccination strategy may therefore explain the higher level of maternal antibodies at weaning and the longer persistence of antibodies in the latter two herds [8, 11].

The collection of TTS has been shown to be a reliable sample material for detection of PRRSV by RT-qPCR in piglets in the US [12], but has to our knowledge not been tested in European pig herds. TTS is regarded as a suitable sample material because it can be collected by the farmers, it represent a targeted sample strategy in that the chance of detecting PRRSV in dead pigs are regarded higher than in living pigs and because up to 40 samples

can be pooled prior to test, by that lowering the costs [12].

In eight out of the 11 batches with MIX as weaning procedure and in nine out of 12 batches with AIAO as weaning procedure, the result of the test of TTS showed a *good* correlation to the results of the OF tests on the batch level in terms of the 0.44 Cohen's kappa (K) but unfortunately the sample size was to low for a robust statistical analysis. In five of the six samplings where the results of the oral fluid and the TTS tests differed, the TTS tested negative and the oral fluid tested positive indicating that TTS is less sensitive than oral fluid. In a previous study in weaners it was found that TTS was more sensitive than family oral fluid in two out of three herds, but less sensitive in one herd [12]. Despite the lack of relationship between the number of TTS collected at each sampling and the compliance with the OF test results in the present study, it may be that the number of dead pigs is too low to give a reasonable sample size or that other factors than PRRSV is the cause of pig mortality in the herds. Another explanation for the lower sensitivity of the TTS results could be degradation of RNA since the stability of the PRRSV RNA in TTS under different storage conditions using the assay conditions of the present study, have not yet been validated. Some pigs might have been dead for 24 h before the tongues were sampled. The sample that tested positive in TTS and negative in oral fluid were collected in Herd D, batch 1. In this batch, no other samples were RT-qPCR positive and all the samples tested negative for PRRSV antibodies at week 11. This could indicate that this test result represented a false positive result and by that compromise the specificity of the TTS, however, the detection of PRRSV in oral fluid in two subsequent batches (3 and 4) in this herd indicated that PRRSV were indeed circulating in the nursery section of at least some of the batches in this herd. The negative TTS tests in herd F and results from the earlier study do on the other hand support that the specificity of TTS tests is high.

Conclusions

The results of this study confirmed that the weaning strategy had a clear impact on the circulation of PRRSV post weaning. Statistically significant more batches from herds where pigs were mixed at weaning were PRRSV positive compared to herds performing AIAO at weaning in the nursery section.

The preliminary results of the use of TTS for the detection of PRRSV in weaners supported previous findings that TTS is a relatively sensitive sampling material, but due to the variability in number of dead pigs it cannot be used alone. Thus, overall the most sensitive approach would be to combine TTS with OF sampling for optimized surveillance of pig herds for PRRSV.

Methods

Herds

Six PRRSV positive sow herds with a production of 30 kg pigs and weekly weaning with an average weaning age of four weeks were included. Number of sows per herd ranged from 570 to 1800 and all herds were positive for *Mycoplasma hyopneumoniae* and either PRRSV1 or PRRSV2 (Table 4). Some herds had a long history of circulation of PRRSV, whereas others had only been infected for a few years (Table 4). All herds performed mass vaccination against PRRSV with an MLV-vaccine two or three times a year. The nursery section was either on or off site (Table 4).

Three of the herds performed strict all-in/all-out in the nursery (AIAO) and three herds performed mixed by weaning in the nursery (MIX). During the project period, the MIX herds included placing underweighted pigs

Table 4 Description of the herds including number of sows, health status, time of first PRRS infection, vaccination protocol and placement of the nursery

Herd	Size	Health status	Infected	Mass Vaccination	Nursery
A MIX	570	SPF +M.Hyo+PRRS2	Q1 2018	3×yearly since 2018	On site
B MIX	1025	SPF +M.Hyo+PRRS2	Q1 2020	3×yearly in 2020	On site
C MIX	1510	+M.Hyo+App2+PRRS1	Before 2010	2×yearly since 2016	Off site
D AIAO	1050	+M.Hyo+PRRS2	Before 2010	2×yearly since 2017	On site
E AIAO	1800	SPF +M.Hyo+App6+PRRS1	Q1 2010	3×yearly since 2016	Off site
F AIAO	1000	SPF +M.Hyo+App12+PRRS1	Q3 2019	3×yearly since 2019	Off site

at weaning in containers for two weeks, until they were mixed with newly weaned pigs two weeks later. One to two weeks age difference between newly weaned pigs and pigs from containers was a fact. In the MIX herds underweighted pigs were kept close to the farrowing unit all though one of the herds had the nursery off site. It was personnel from the farrowing unit that took care of the underweighted pigs and internal biosecurity could be limited.

The sampled pigs from the six herds were privately owned by producers having a herd health advisory agreement with the swine practice HyoVet I/S.

Sampling

Four consecutive batches of weaned piglets were included in each of the six herds. The pigs were four weeks old at arrival and was in the room until they were sold at a maximum of 12 weeks of age. In each of the 24 batches, OF was collected at five, eight and 11 weeks of age. The OF was collected from four double pens representing around 60 pigs/pen. By hanging a cotton rope between the two pens and allowing the pigs to chew on the rope for 30 min. It was not necessarily the same pens that were sampled at each sampling.

TTS were collected from pigs within the included batches, that died or were euthanized during the period from weaning at 4 weeks old until the pigs were sold at maximum 12 weeks of age.

The employees at the farms decided if pigs should be euthanized for animal welfare reasons, following the procedures at the herds. Clinical signs resulting in low animal welfare were mainly wasting, lameness and large hernias. Pigs found dead without former clinical signs observed were also sampled. All euthanizing's were done by using a penetrating captive bolt at the middle of the forehead of the pig followed by a deep cut across the throat to cut at least one of the two arteria carotis communis. The samples were taken from as many dead animals as possible. The employees at the herds were instructed in cutting of a large piece of the tongue as possible by opening the mouth of the pig and pulling the tongue out with one hand while cutting the tongue with a scalpel in the other hand.

The OF was rescued into plastic containers by twisting the cotton ropes and the TTS were placed in plastic bags. All the material was stored at -20°C in the herds before shipped to the laboratory on ice. When arriving at the laboratory at the University of Copenhagen the samples were stored at -80°C until test.

Laboratory analyses

All the oral fluid samples were tested for PRRSV antibodies using ELISA (IDEXX PRRS OF Ab Test) as described by the manufacturer. The ELISA results were expressed as the sample-to-positive ration (S/P) and the ratios were considered positive if >0.40 as recommended. Total viral RNA was extracted from oral fluid and fluids from TTS and tested for PRRSV-1 and PRRSV-2 by RT-qPCR as described previously [13]. The sensitivity of the RT-PCR has been estimated to be 1–10 TCID50/ML [14]. The diagnostic sensitivity and specificity of the Oral Fluid antibody assay has been assessed to be 94.7% (95% confidence interval [CI]: 92.4, 96.5) and 100% (95% CI: 99.0, 100.0), respectively, at a sample to positive ratio cutoff of ≥ 0.40 [15].

Statistics

To evaluate whether there was a significant association between the number of batches positive and negative for PRRSV in OF and the management at weaning (AIAO and MIX), a Chi-square test was used, without taking repeated measuring within the herds into account. The relative risk calculated as Risk in MIX/Risk in AIAO).

The degree of accuracy between batches, that were PRRSV positive or negative in both OF and TTS, was evaluated based on calculation of the statistical coefficient "Cohens' kappa" (Cohen, 1969) [16].

$$\kappa = \frac{observed\ agreement - expected\ agreement}{maximum\ agreement - expected\ agreement}$$

For the statistical analysis the website for statistical Computation (http://vassarstats.net/) was used together with Excel from Microsoft.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12917-025-04623-0.

Supplementary Material 1.

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Authors' contributions

L.L. wrote the background text. C.K. calculated sample sizes and did statistics. L.K. handled samples at lab and wrote the text about laboratory analyses. P.H. wrote the main manuscript. P.H. collected samples at farms and advised personel at farms how to collect samples. P.H. prepared figures. All authors reviewed, edited and and approved the manuscript.

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Data availability

Raw data apart from the presented data can be shared upon reasonable request by contacting the corresponding author and requires prior acceptance from the involved herd owners.

Declarations

Ethics approval and consent to participate

Participating owners of the herds where guaranteed anonymity of their participation and data. Informed consent was obtained from the owners of the pigs used in this study. The owners had a herd health advisory agreement with swine practice HyoVet I/S.
According to the regulation law Council Directive 76/768/EEC of 27 July 1976 on the approximation of the laws of the Member States relating to cosmetic products the Ethics approval was not required.
https://www.retsinformation.dk/eli/lta/2022/1107
https://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2010:276:0033:0079:en:PDF

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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