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Indirect assessment of porcine reproductive and respiratory syndrome virus status in pigs prior to weaning by sampling sows and the environment

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ABSTRACT

There is a need to develop cost effective approaches to sample large populations in particular to determine the disease status of pigs prior to weaning. In this study we assessed the presence of the porcine reproductive and respiratory syndrome virus (PRRSV) in the environment (surfaces and air) of farrowing rooms, and udder skin of lactating sows as an indirect measure of piglet PRRSV status. Samples were collected at processing and weaning every three weeks for 23 weeks after a PRRSV outbreak was diagnosed in a swine breeding herd. PRRSV was detected at processing in udder skin wipes, environmental wipes and airborne deposited particle samples up to 14 weeks post outbreak and at weaning in udder skin wipes up to 17 weeks post outbreak. Similar sensitivities were observed for udder skin wipes (43% [95% CI: 23%–66%]) and surface wipes (57% [95% CI: 34%–77%]) when compared to serum at the litter level from piglets at processing. PRRSV was detected in the environment and the udder skin of lactating sows, which indicates that aggregate samples of the environment or lactating sows may be used to evaluate the PRRSV status of the herd in pigs prior to weaning. However, the use of environmental samples to detect PRRSV by RT-PCR should not be used as the single method to assess the PRRSV status at the litter level. Furthermore, our findings also highlight potential sources of PRRSV infection for piglets in breeding herds.

1. Introduction

Porcine reproductive and respiratory syndrome (PRRS) remains an important disease in the United States due to its detrimental effect on productivity and severe economic impact to the swine industry (Holtkamp et al., 2013). In breeding herds, PRRS virus (PRRSV) causes reproductive failure including abortions, stillbirths and increase in pre-weaning mortality. Piglets prior to weaning play an important role in maintaining the virus in infected breeding herds and assessing the piglet's PRRSV status at weaning is central to the American Association of Swine Veterinarians (AASV) PRRSV herd classification (Holtkamp et al., 2011).

There are management factors that help perpetuate PRRSV infections in the farrowing house. Among these, cross-fostering of piglets and use of nurse sows are two common management practices in breeding herds associated with disease spread. Cross-fostering includes the movement of piglets between litters to enhance the piglets' chances of survival and nurse sows are sows whose litters have been weaned and after that they foster another litter of younger pigs (Alvasen et al., 2017). Although both practices are directed at increasing piglet survival and herd productivity, they can also be responsible for the transmission and spread of pathogens (Influenza A and PRRSV) during lactation (Garrido-Mantilla et al., 2019b). In addition, biosecurity in the farrowing house plays an important role in preventing the spread of diseases from infected animals to susceptible newborn piglets. Current biosecurity procedures in farrowing rooms of actively infected farms include needle changing between litters, all-in/all-out, cleaning and disinfection of rooms between batches and cleaning and disinfection of shared tools (Otake et al., 2002a, b).

Monitoring swine breeding herds for PRRSV has become routine for many US producers. Herds are monitored to confirm their negative status, or to detect early infections so that mitigation and control protocols can be put in place right away (i.e. timplementation of specific biosecurity measures or disease elimination protocols). In addition, monitoring of PRRSV positive breeding herds, allows veterinarians and producers to evaluate, quantify and compare different treatments to

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Table 1
Summary of RT-PCR porcine reproductive and respiratory syndrome virus (PRRSV) test results in serum, surface wipes (SW), udder wipes (UW), and particle deposition (PD) collected every 3 weeks on a 6000 farrow-to-wean herd after an outbreak.

		Weeks post outbreak								
Age	Sample	2	5	8	11	14	17	20	23	Total
Processing (3-5 days)	**Serum**[Y]	88.9% (8/9)[*]	30% (3/10)	40% (4/10)	11.1% (1/9)	10% (1/10)	11.1% (1/9)	30% (3/10)	20% (2/10)	29.9% (23/77)
	SW	88.9% (8/9)	20% (2/10)	30% (3/10)	11.1% (1/9)	30% (3/10)	0 % (0/9)	0 % (0/10)	0 % (0/10)	22.1% (17/77)
	UW	77.7% (7/9)	10% (1/10)	0 % (0/10)	11.1% (1/9)	20% (2/10)	0 % (0/9)	0 % (0/10)	0 % (0/10)	14.3% (11/77)
	PD	100% (3/3)	25% (1/4)	33% (1/3)	0% (0/3)	25% (1/4)	0% (0/1)	0% (0/3)	0% (0/3)	25% (6/24)
Weaning (~21 days)	**SW**	70% (7/10)	70% (7/10)	50% (5/10)	0 % (0/10)	40% (4/10)	0 % (0/10)	0 % (0/10)	0 % (0/10)	28.75% (23/80)
	UW	60% (6/10)	40% (4/10)	20% (2/10)	0 % (0/10)	10% (1/10)	10% (1/10)	0 % (0/10)	0 % (0/10)	17.5% (14/80)
	PD	100% (3/3)	100% (2/2)	100% (1/1)	25% (1/4)	25% (1/4)	0% (0/3)	0% (0/3)	0% (0/4)	33.3% (8/24)

[*] Number of positive samples/total samples.
[Y] A litter was considered positive if at least one piglet yielded an RT-PCR positive result with a cycle threshold (Ct) in serum ≤ 35. SW, UW and PD were considered positive if they had a value below 40 Ct. Ct thresholds were selected based on the receiver operating characteristic (ROC) curve analysis for sensitivity and specificity.

reach stability (i.e. time it takes after an outbreak to produce negative pigs) or to assess the disease status within the herd (i.e. prevalence or virus circulation). Sampling due-to-wean pigs is the most suitable approach to detect virus circulation in breeding herds (Cano et al., 2009). Due-to-wean piglets are easier to sample than sows and infection results indicate either vertical (i.e sow to piglet) or horizontal (i.e piglet-to-piglet) transmission of PRRSV (Cano et al., 2009). Recently, the use of processing fluids has also been indicated for PRRSV monitoring due to its representativeness of more pigs at a lower cost (Vilalta et al., 2019, 2018). Processing fluids target the piglet population at processing (~ 3–5 days of age) and are considered a good approach to monitor and evaluate PRRSV vertical transmission. However, further studies are needed to assess whether the status of piglets at processing corresponds with the status of piglets at weaning.

Despite the newer sampling types, piglet serum testing at weaning is considered the reference sample for PRRSV status classification of breeding herds (Holtkamp et al., 2011). However, bleeding pigs is time consuming, costly and only a limited subset of individuals is represented. Use of family oral fluids in pigs prior to weaning has also been suggested as a method to monitor piglets (Bjustrom-Kraft et al., 2018). This method allows sampling of more pigs at a lower cost but collection of samples is time consuming. Thus, there is still a need to develop cost effective sampling methods to determine piglet PRRSV status in the farrowing room.

An example of this is the use of udder skin wipes in lactating sows for influenza virus detection (Garrido-Mantilla et al., 2019a). This group sampling strategy is more cost-effective than individual sampling, although individual samples should still be required for virus isolation and sequencing (Garrido-Mantilla et al., 2019a) but there is no information on the detection of PRRSV on the skin of lactating sows. In the case of environmental PRRSV, Kenney and Polson (2011) were able to assess the limit of PRRSV detection after swabbing with a wipe three common surfaces of farms that had different PRRSV concentrations deposited onto them. Overall, there is limited information on the role that the environment plays as a source of PRRSV infection to newborn piglets, in particular in farms where the biosecurity procedures cannot be fully implemented (i.e complete all-in/all-out of rooms or complete drying). Sampling the environment has also been shown to be a sensitive approach to detect infectious agents such as porcine epidemic diarrhea (PED) virus (Pasma et al., 2016), influenza virus (Garrido-Mantilla et al., 2019a; Neira et al., 2016) and PRRSV in finishing and breeding herds (Kenney and Polson, 2011). Therefore, the goals of this study were to assess the presence of PRRSV in the farrowing environment and in the udder skin of sows after an active PRRSV infection was reported, and to estimate the sensitivity of sampling the environment and the sow udder to detect PRRSV at the litter level using detection of PRRSV in sera as the reference sample.

2. Material and methods

2.1. Study population and sampling strategy

Protocols and procedures followed during the study were approved by the University of Minnesota Institutional Animal Care and Use Committee (protocol number 1902-36809H).

A 6000 sow farrow-to-wean herd located in the Midwestern US that had recently reported a PRRSV outbreak was used for the study. The herd was PRRSV provisionally negative (Category 3) according to the AASV PRRSV herd classification guidelines (Holtkamp et al., 2011) before the outbreak occurrence.

The study started 2 weeks after the PRRSV outbreak was reported. A whole-herd exposure to induce whole herd immunity was performed at week 5 after the outbreak using live virus inoculation. Sampling was conducted for 23 weeks with a frequency of sampling of every three weeks following a cross-sectional design. On the same day, ten different litters were conveniently selected at both, processing (approximately 3–5 days of age) and weaning (approximately 21 days). Parity of the selected litters at processing was recorded and the following sampling was conducted at processing (bleeding, surface wipes, udder wipes and particle deposition) and at weaning (surface wipes, udder wipes and particle deposition):

- Bleeding: all piglets from the selected litters were bled. Results from piglets' sera were used as the gold standard to calculate sensitivity and specificity at the litter level.

- Surface wipes (SW): Wiping was done on surfaces of farrowing crates with attention to feeders, waterers, floor and solid partitions between crates – 1 gauze/crate with a total of 10 crates at processing and 10 crates at weaning per sampling day (n = 20 wipes/day).

- Udder skin wipes (UW): Wiping was done on the underline skin of lactating sows – 1 gauze/sow (10 sows at processing and 10 sows at weaning per sampling day (n = 20 wipes/day).

For airborne particle deposition (PD) samples, a minimum of 3 and maximum of 10 samples/room were collected at processing and at weaning on each sampling day. This method represents the deposition of airborne particles by gravitation on surfaces and is considered an indirect measure of airborne virus detection that reflects the PRRSV status of pigs in the room (Garrido-Mantilla et al., 2019a). The sample was obtained by placing a 1 m x 1 m sheet of aluminum foil on top of the crates and away from the direct contact of sows and piglets. The surface of the foil paper was swabbed using a moist gauze after 1 h of exposure.

All environmental and sow samples were collected with a gauze previously impregnated with 10 mL of transport media (DMEM-Dulbecco's Modified Eagle Medium Gibco™ + antibiotics) and kept in a 4 × 6 inch Minigrip® Red Line reclosable Zip bags (Minigrip,

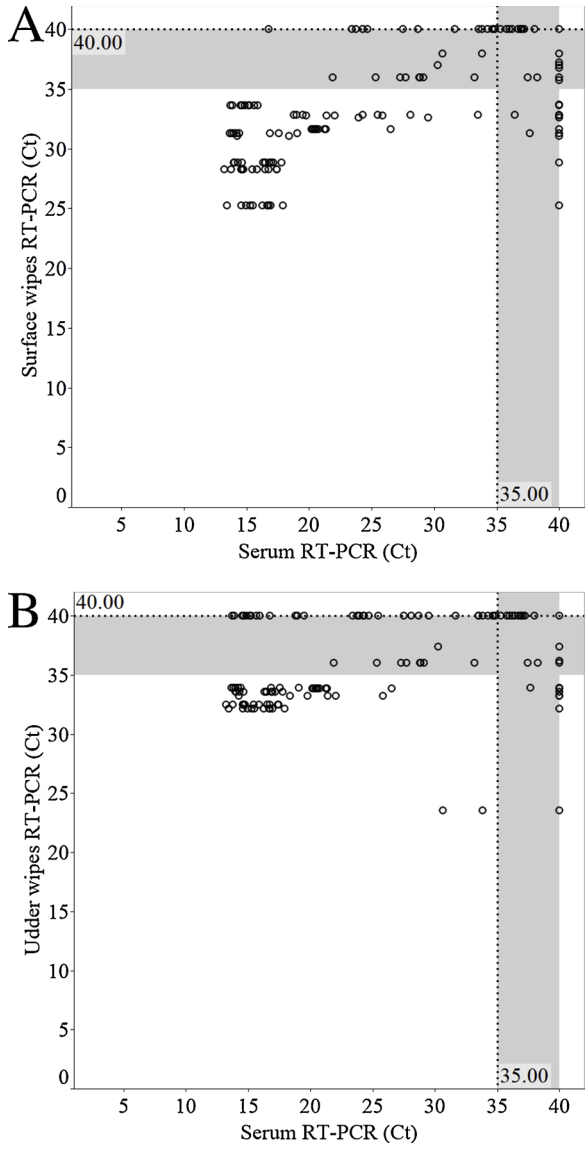

Fig. 1. Comparison of PRRSV RT-PCR cycle threshold (Ct) results of each pig serum and its associated litter surface wipe (Fig. 1A) and udder wipe (Fig. 1B) at processing on a 6000 farrow-to-wean sow farm. Grey area represents the range between 35 and 40 Ct. Negative values are depicted as 40 Ct for visualization purposes. Dotted black lines represent the Ct threshold for a sample to be considered positive in serum (< 35 Ct), udder (< 40 Ct) and surface wipes (< 40 Ct) according to the receiver operating characteristic curve (ROC) curve analysis.

Alpharetta, GA) until use. Prior to sampling, excess of liquid was removed by squeezing the gauze in the reclosable bag and then the surface of interest was sampled. After sampling, the gauze was placed back inside the bag (Garrido-Mantilla et al., 2019a). Samples were refrigerated immediately after collection and transported to the laboratory for testing. Upon arrival at the laboratory, samples were processed by squeezing and massaging the wipe and transferring the liquid to a cryovial using a sterile pipette.

All collected samples were tested using a RT-PCR for PRRSV (MagMAX™ and VetMAX™, Applied Biosystems). The standard (validated) cutoff value for this assay was a cycle threshold (Ct) of 40. A subset of 3 RT-PCR PRRSV positive samples with the lowest Ct values was further tested through open reading frame 5 (ORF5) sequencing and virus isolation.

Virus isolation was attempted by using both porcine alveolar macrophages (PAM) and MARC-145 cells. Restriction fragment length polymorphism (RFLP) comparison of the sequences was performed as previously described (Wesley et al., 1998).

3. Data analysis

The relationship between the Ct values obtained from serum samples, UW and SW at processing was assessed visually using scatter plots. A receiver operating characteristic (ROC) curve was used to assess the optimal cut-off value that maximized both sensitivity (Se) and specificity (Sp) of SW and UW RT-PCR results at processing when compared with serum RT-PCR results of the same litter. For this, two cut-offs for the individual serum sample (35 and 40) Ct-values were compared with a set of cut-off values (35, 36, 37, 38, 39 and 40) for the UW and SW Ct-values. The cut-off that provided the best possible sensitivity and specificity (closer to the top left corner of the ROC curve) was selected. A litter was considered positive when at least one pig within the litter had a RT-PCR Ct value in serum below the Ct value obtained by the ROC curve. Additionally, Se, Sp, positive predictive value (PPV) and negative predictive value (NPV) were estimated at processing for UW and SW using serum litter results as the gold standard. The agreement between udder/surface wipes and serum RT-PCR results at the selected optimal cut-offs was assessed using the Kappa statistic (Thrusfield, 2007), and the percentage of total agreement. Difference in the proportions of paired RT-PCR results were assessed using the McNemar's test. The difference in Ct values between the RT-PCR PRRSV positive SW and UW was assessed using the Mann-Whitney test.

Two independent logistic regression models were built to assess the relationship between RT-PCR PRRSV positive litters based on UW at processing (model 1) or SW at processing (model 2) and sow parity, number of positive pigs within the litter based on serum at processing, average sera Ct value within the litter, and time from the start of the outbreak in weeks. Variables with an associated p-value of < 0.2 in the initial univariate assessment were selected for inclusion into each of the multivariable logistic models. Backward elimination of variables was performed based on the likelihood ratio test (LRT). Linearity assumption for numerical explanatory variables was checked visually using smoothed scatterplots between the logit of the outcome and the numerical variable. Numerical variables were categorized using the median, if the linearity assumption was violated.

Analyses were conducted using R software, version 3.2.5 (R Core Team, 2018).

Sequences obtained in this study were aligned and compared using the Multiple Sequence Comparison by Log-Expectation (MUSCLE) algorithm in Geneious ® 10.0.7 with default settings to compare and highlight variations across the sequences. A similarity matrix with all the collected sequences was built. Restriction fragment length polymorphism (RFLP) analysis was done to define the sequence cut pattern. Sequences obtained by the veterinarian in the initial PRRSV outbreak and ongoing monitoring were added to the analysis.

4. Results

A total of 77 litters were sampled (sera, UW and SW) at processing. UW and SW were collected from 80 litters at weaning. Overall, 10.7% (100/932) of piglets' sera tested PRRSV RT-PCR Ct value below 35. The median Ct value of those 100 samples was 20.54 with a maximum of 34.86 Ct and a minimum of 13.2 Ct The percentage of positive piglets within a litter ranged from 7.14% (1/14) to 100% (12/12). The proportion of positive litters and positive piglets by serum decreased over time but never reached zero. Additionally, the likelihood of testing positive at processing by serum was associated with males and lower parities. Further information and analysis of the serum results can be found in Vilalta et al. (2018).

The ROC curve analysis showed that the accuracy between serum RT-PCR results and SW RT-PCR results ranged from 80% to 81.81%. The best accuracy (81.81%) occurred when the serum and surface

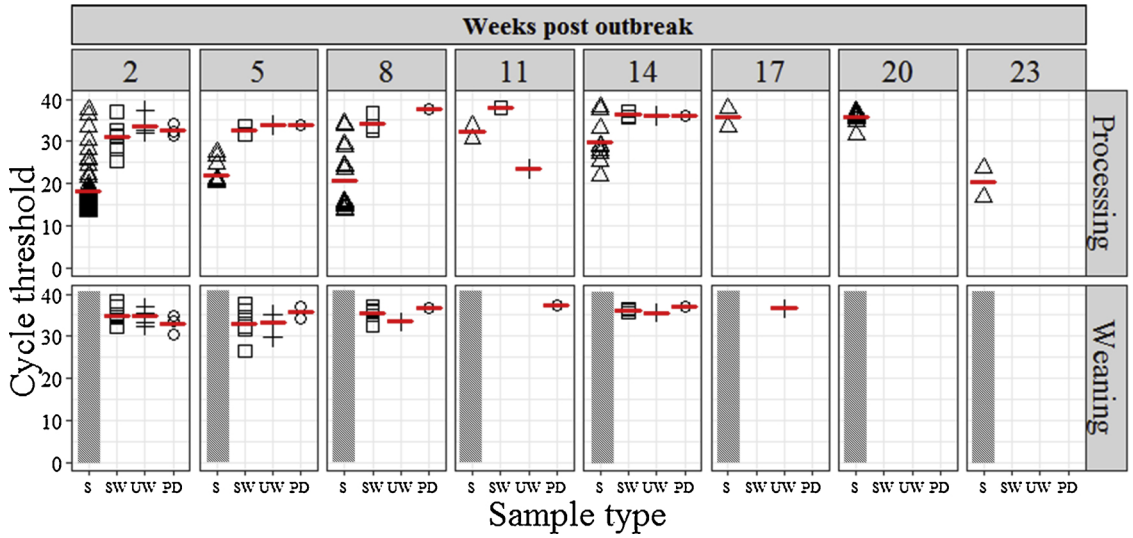

Fig. 2. Porcine reproductive and respiratory syndrome RT-PCR cycle threshold (Ct) value distribution for piglet serum (S, triangle), surface wipes (SW, square), udder skin wipes (UW, cross), and particle deposition (PD, circle) samples overtime (2, 5, 8, 11, 14, 17, 20 and 23 weeks post outbreak) on a 6000 farrow-to-wean farm. Horizontal red lines indicate the mean Ct values for each week and sample type. Negative results are not depicted. No piglets were bled at weaning (grey striped areas). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

Table 2
Summary of RT-PCR porcine reproductive and respiratory syndrome virus test results in surface wipes (SW) and udder wipes (UW) per sow parity of the litters sampled.

Parity	SW	UW
1	30.4% (7/23)*	21.7% (5/23)
2	30.8 % (4/13)	23.1 % (3/13)
3	14.3 % (2/14)	14.3 % (2/14)
4	20 % (2/10)	0 % (0/10)
5	16.7 % (1/6)	16.7 % (1/6)
5+	10 % (1/10)	0 % (0/10)

* Percentage (Number of positive samples/total samples).

Table 3
Final multivariable logistic regression model results between surface wipes RT-PCR results (dependent variable) and significant predictors retained in the model. Odds ratio (OR) and 95% confidence interval (95% CI) are reported.

Variable	Levels	n litters	OR (95% CI)	p
Intercept			0.26 (0.07 - 1.02)	0.053
Number positive pigs	> =1	23	11.9 (3.02 - 46.84)	< 0.001
	0	54	Reference	
Week	> =8	58	0.2 (0.05 - 0.81)	0.024
	< 8	19	Reference	

Table 4
Final multivariable logistic regression model results between udder wipes RT-PCR results (dependent variable) and significant predictors retained in the model. Odds ratio (OR) and 95% confidence interval (95% CI) are reported.

Variable	Levels	n litters	OR (95% CI)	p
Intercept			0.07 (0.01 - 0.64)	0.018
Number positive pigs	> =1	23	27.91 (3.06 - 254.27)	< 0.001
	0	54	Reference	
Week	> =8	58	0.13 (0.02 - 0.71)	0.018
	< 8	19	Reference	

thresholds to classify a sample as positive were < 35 Ct and < 40 Ct, respectively. Accuracy between serum RT-PCR results and UW RT-PCR ranged from 77.9% to 81.81%. Likewise, the best accuracy (81.81%) was observed when the same thresholds of < 35 Ct and < 40 Ct were used to define positive samples in serum and UW results, respectively. Thus, these values were used to define positive and negative samples. A threshold value of < 40 Ct was also used to classify a PD sample as positive.

Out of the 77 litters sampled at processing, 23 (29.87% [95% CI 20.0%–41.4%]) had at least one positive piglet in serum, 17 (22.1% [95% CI 13.4%–33.0%]) SW samples and 11 (14.28% [95% CI 7.4%–24.1%]) UW samples tested RT-PCR positive at processing (Table 1). Udder skin wipes average Ct values (33.23 [95% CI 30.8–35.65]) were similar (p = 0.68) than the SW average Ct values (33.08 [95% CI 31.28–34.90]) at processing. The relationship between serum and SW Ct values, and serum and UW Ct values showed a slightly different distribution when assessed using scatterplots for the litters at processing. More specifically, SW appeared to have lower Ct values when piglet serum Ct values were lower. On the other hand, all UW except one sample yielded a Ct value above 30 (Fig. 1).

At weaning, 28% (23/80) (95% CI 19.2%–40.0%) and 17.5% (14/80) (95% CI 9.9%–27.6%) of the SW and UW samples tested RT-PCR PRRSV positive, respectively (Table 1). Average Ct values from UW (34.36 [95% CI 33.28–35.44]) were not significantly different (p = 0.55) than average Ct values from SW (34.6 [95% CI 33.43–35.76]) at weaning.

PRRSV was detected at processing in SW and UW of lactating sows up to 14 weeks after the outbreak. At weaning, PRRSV was detected up to 17 weeks post outbreak using UW. The number of positive samples decreased over time and the Ct values of the positive samples increased over time (Fig. 2).

The unconditional association between RT-PCR results and parity showed that the RT-PCR positive proportion of P1 and P2 was marginally not significantly higher than the RT-PCR positive proportion observed in P3+ parity litters using either UW (p = 0.08) or SW (p = 0.1) (Table 2). However, after accounting for the effects of the number of positive piglets within the litter and the number of weeks from the outbreak, parity was largely not associated (p > 0.8) with both the proportion of positive SW and UW.

The odds of detecting a positive wipe (SW and UW) at processing significantly increased when at least one positive piglet was present in the litter (Tables 3 and 4). Wipes from litters with a larger number of positive piglets within a litter tended to have lower Ct values (Fig. 3).

After taking into consideration the effects of the number of weeks after the outbreak, the proportion of positive SW and UW were

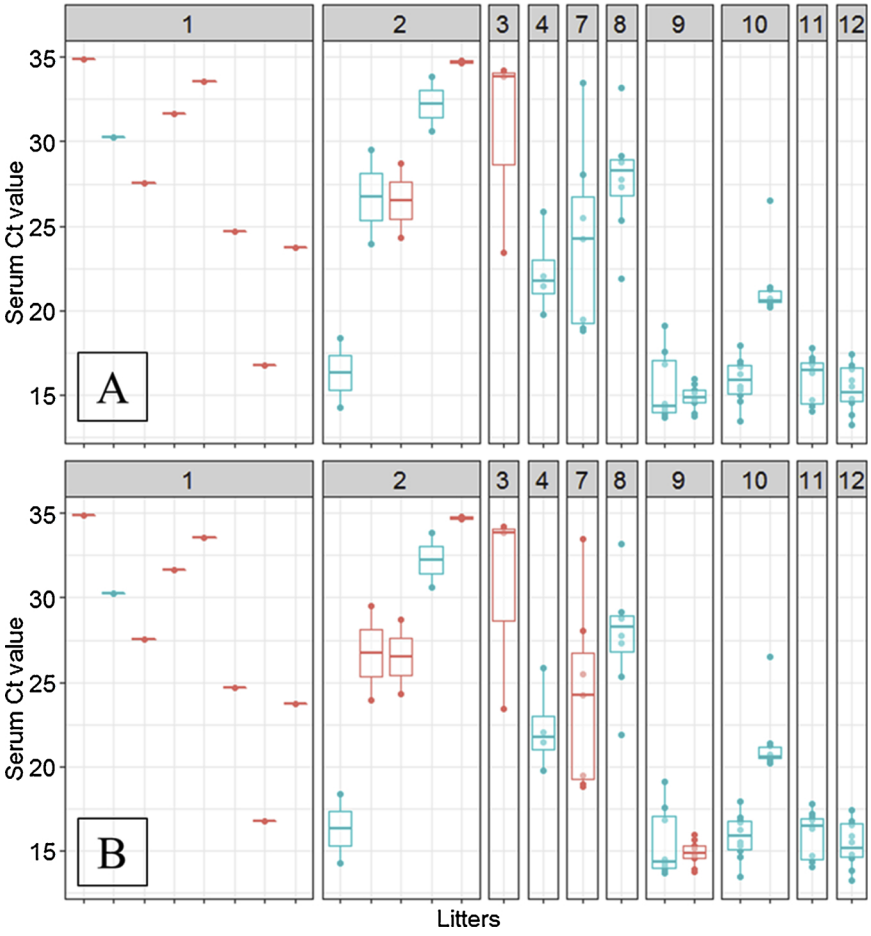

Fig. 3. Boxplot cycle times (Ct) values of porcine reproductive and respiratory virus RT-PCR positive piglets per litter. Each mark in the X axis corresponds to one litter. Litters grouped per number of positive piglets within a litter (top grey strip). Points and boxplots colored according to the result (blue: positive; red: negative) of the surface wipes (A) and the udder skin wipes (B). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

Table 5
Agreement between porcine reproductive and respiratory syndrome virus RT-PCR results in serum (gold standard) with surface wipes (SW) (A) and udder wipes (UW) (B). Se: sensitivity, Sp: specificity, PPV: positive predictive value and NPV: negative predictive value. 95% confidence intervals of values are within brackets.

A		Serum		
		Litter +	Litter -	
Test Result	**SW +**	13	4	PPV 76% (50–93)
	SW -	10	50	NPV 83% (71–92)
		Se 57% (34-77)	Sp 93% (82-98)	Kappa 0.53 (0.31–0.75)

B		Serum		
		Litter +	Litter -	
Test Result	**UW +**	10	1	PPV 91% (50–93)
	UW -	13	53	NPV 80% (69–89)
		Se 43% (23-66)	Sp 98% (90-100)	Kappa 0.49 (0.29–0.69)

positively associated with the number of positive piglets within a litter (Table 3 and 4). Additionally, the odds of positivity of both SW and UW were significantly lower after the eighth week after the outbreak than during the first eight weeks after the outbreak.

Surface wipes (SW) had a sensitivity and specificity of 57% (95% CI: 34%–77%) and 93% (95% CI: 82%–100%), respectively when compared with serum results of the same litter. Similar values were observed for sensitivity (43% [95% CI: 23%–66%]) and specificity (98% [95%CI: 90%–100%]) of udder skin wipes. When compared to serum (gold standard), the kappa statistic for SW and UW at processing was 0.53 and 0.49 respectively (Table 5). The percentage agreement between SW and UW with serum was 81.8% in both cases. The difference in the proportion of positive samples between serum and SW was not statistically significant (McNemar test, $p = 0.1814$). On the other hand, the same difference was statistically significant in the case of UW (McNemar test, $p = 0.0033$).

The estimated sensitivity was above 85% in weeks 2, 11 and 14 after the outbreak in both types of samples. Sensitivity dropped to 0% after week 14 in both SW and UW. UW specificity point estimate was higher than SW specificity in all the weeks (Table 6).

Virus isolation and ORF5 sequencing was attempted in three samples with the lowest Ct values of each sample type, UW (23.54, 29.74 and 32.12 Ct), SW (25.23, 28.28 and 26.45 Ct) and PD (31.46, 32.32 and 30.54 Ct); however, these attempts were not successful. Direct sequencing of the sample was possible in one PD and one SW sample collected at 2 and 5 weeks post-outbreak, respectively. All of the SW samples (weeks 2 and 5 post-outbreak) yielded a sequence. Sequences from SW, UW and PD had a similarity of nucleotides ranging between 99.58 and 99.91% when compared to the original outbreak sequence with all having the same 1-3-4 RFLP cut pattern.

5. Discussion

Monitoring pigs in breeding herds is important to assess herd stability (Holtkamp et al., 2011), implementation of biosecurity measures and success of PRRSV control and elimination efforts. PRRSV status of due to wean pigs is key to determine herd stability and blood samples are still the sample of choice. Thus, there is a need to continue identifying more cost effective methods to sample the herd for PRRSV detection. In this study we compared RT-PCR results of two sample types,

Table 6
Summary of sensitivity (Se) and specificity (Sp) of surface wipes (SW) and udder skin wipes (UW) compared to serum results from the litter, and percentage of serum porcine reproductive and respiratory virus positive litters and pigs at processing during the weeks after the outbreak.

		Weeks post outbreak								
		2	5	8	11	14	17	20	23	Total
Se	**SW**	100% (8/8)*	33% (1/3)	50% (2/4)	100% (1/1)	100% (1/1)	0% (0/1)	0% (0/3)	0% (0/2)	57% (13/23)
	UW	87.5% (7/8)	33% (1/3)	0% (0/4)	100% (1/1)	100% (1/1)	0% (0/1)	0% (0/3)	0% (0/2)	43% (10/23)
Sp	**SW**	100% (1/1)	85.7% (6/7)	80% (4/5)	100% (9/9)	77.8% (7/9)	100% (8/8)	100% (7/7)	100% (8/8)	93% (50/54)
	UW	100% (1/1)	100% (7/7)	100% (5/5)	100% (9/9)	88.9% (8/9)	100% (8/8)	100% (7/7)	100% (8/8)	98% (53/54)
Positive litters		88.9% (8/9)	30% (3/10)	40% (4/10)	11.1% (1/9)	10% (1/10)	11.1% (1/9)	30% (3/10)	20% (2/10)	29.9% (23/77)
Positive pigs		51.4% (55/107)	9.4% (12/128)	13.6% (16/118)	2% (2/102)	6.5% (8/124)	0.9% (1/112)	3.3% (4/121)	1.6% (2/127)	10.6% (100/939)

*Se: Percentage (Number of positive litters by either SW or UW/total positive litters by serum).
Sp: Percentage (Number of negative litters by either SW or UW/ total negative litters by serum).
Positive litters: Percentage (Number of positive litters by serum/ total number of litters sampled).
Positive piglets: Percentage (Number of positive pigs by serum with a Ct value below 35/ total number of piglets bled).

namely UW and SW to the RT-PCR results of serum samples at the litter level.

Overall, we found that UW and SW samples are good indicators to assess PRRSV status of the herd prior to weaning in particular after a recent PRRSV outbreak, and offer an alternative to bleeding. PRRSV was detected in the environment up to 14 and 17 weeks after the outbreak at processing and weaning, respectively. To our knowledge this is the first study that describes the use of environmental samples and udder wipes to monitor and detect PRRSV in a breeding herd after an acute infection.

In this study, sampling surfaces that are in contact with the pigs (SW and UW) or clean surfaces exposed to the same room environment (PD) were used to explore the PRRSV status in pigs at processing (˜3–5 days of age) and at weaning (˜21 days of age). Sp of UW and SW was above 90% when compared with that of serum results at the litter level using samples collected at processing. However, UW and SW showed a poor Se at processing compared to serum samples from the same litters. Se of detection of PRRSV at the litter level using SW (57% [95% CI: 34%–77%]) was higher than Se using UW (43% [95% CI: 23%–66%]). However, an important point to make is that all pigs within a litter were bled when establishing the sera as the gold standard, which is not commonly done in routine samplings under field conditions. Usually only a subset of pigs is bled within a litter or farm to determine PRRSV status at weaning. It is important to highlight that all the calculations in this study were done using sera from pigs at processing and may not be extrapolated to those at weaning. How the Se and Sp of UW and SW compare to sera under those conditions still needs to be assessed but we speculate that in those cases the Se of UW and SW will be closer to that of sera as percentage of positive piglets may increase from processing to weaning

A Ct-value of 35 was used as cut-off for serum RT-PCR classification. We choose this value because it provided the highest possible sensitivity and specificity. However, we acknowledge that the probability of having false negative results may have increased by this selection of serum cut-off. Nonetheless, if a cut-off of 40 would have been chosen for serum Ct-values, sensitivity and specificity at the litter level would have been 40% (95% CI: 21%–61%), and 98% (95% CI: 87%–100%) for UW, respectively and 52% (95% CI: 31%–72%), and 92% (95% CI: 81%–98%) for SW, respectively. These values are not different (overlapping 95% CI) to the ones reported using a cut-off of 35. Therefore the selection of serum cut-off did not affect the overall inference for UW and SF sensitivity and specificity.

We were interested in assessing the detection of PRRSV in UW given the recent reports that show the viability of IAV in the skin of lactating sows (Garrido-Mantilla et al., 2019b). We speculate that the udder skin is contaminated by saliva and nasal secretions of suckling piglets that ultimately represent a risk to littermates as PRRSV can be found in saliva (Wills et al., 1997) and the amount of virus and shedding duration in saliva follows a similar pattern to the presence of the virus in blood (Charpin et al., 2012; Kittawornrat et al., 2010). Therefore, it seems reasonable to sample the skin of lactating sows to assess the PRRSV status of the litter. At sample collection, we did not consider whether pigs had suckled recently. However, given the frequency when piglets suckle (˜ once/hour), we do not think that time of sampling in relation to suckling should have a major influence in the RT-PCR results. Although our efforts did not yield a viable PRRSV isolate from the UW, it is plausible to still consider the udder skin as a source of virus and a risk of contamination for negative pigs in the litter (Garrido-Mantilla et al., 2019b). Also, contaminated udder skin could play a role in PRRSV transmission to other litters if the sow is used as a nurse sow. Further research is needed to elucidate this point.

PRRSV detection on SW and UW was associated with the number of RT-PCR positive piglets within the litter and the low Ct values as seen in Figs. 2 and 3. Thus, the odds of detecting a positive litter by using a SW or a UW when at least one positive piglet was present within the litter had 12 and 28 times the odds in litters with no positive piglets, respectively. All SW were positive when the number of positive piglets were equal or above 4. However, detection by UW was less sensitive than SW in those litters having 4 or more positive piglets.

In our study we were able to detect PRRSV by RT-PCR in SW for 17 weeks after the outbreak and in UW until 14 weeks after the outbreak. These results suggest that these methods are mostly applicable to monitor recently infected populations likely within the first four months after the outbreak.

It was possible to detect PRRSV in surfaces of crates occasionally using the SW where all the pigs yielded a negative result from serum. Even though we do not know the source of this contamination, we speculate that the positives could be the result of the deposition of airborne particles in those surfaces, or the mechanical movement of contaminated particles into the crate in some other way while conducting chores or by cross-contamination with the adjacent crates. Nevertheless, the significance of the role that the environment plays as a potential source of infection in pigs is still unclear. Hosting negative piglets in a positive environment can potentially lead to a PRRSV infection as reported by Dee et al. (2005). In our study, we were not able to isolate PRRSV from the environmental samples even though their Ct values were low (25.23, 28.28 and 26.45). These results are similar to those described by Dee et al. where PRRSV could be detected but not isolated when evaluating viability outside the host on different surfaces at different temperatures and different times (Dee et al., 2003, 2002). In those studies pigs that were later inoculated with samples that were RT-PCR positive and isolation negative became infected and generated PRRSV-antibodies. Bioassay with collected samples was not done in our study and further research should be done to determine if RT-PCR positive samples from the environment could infect naïve pigs.

The particle deposition technique may offer a valuable alternative for sampling enclosed air spaces where animals are housed. PRRSV could be detected in PD up to week14 after the break. Also, PRRSV was

detected in 4 and 5 out of the 8 sampling events at processing and weaning, respectively. Thus, when small numbers of positive animals (< 6.5%) were PRRSV positive in serum in the room, the PD yielded a negative result. A factor that may have influenced this result was the number of tinfoil pieces per room since only 3–4 sheets were conveniently placed in each room. A larger number of tin foils should increase the probability of detection at the room level. In addition, collection time may have also influenced the results. PRRSV is associated to coarse aerosol particles larger than 1 μm (Alonso et al., 2015). Large aerosol particles settle faster than smaller ones, for instance, a 4 μm diameter particle takes 33 min to settle 1 m with no air currents (Lindsley et al., 2010). Therefore, longer sampling times may increase the amount of particles carrying viruses above the detection limit and the sensitivity of this method. Either way, this sample type also appeared to work better during the acute phase of the outbreak when generation of aerosols is considered significant. Furthermore, if this sample type is further validated, it could allow the detection of airborne PRRSV particles without having to use air collectors. Air collectors are expensive and may be difficult to operate but other factors such as ventilation rates and type of building need to be taken into consideration before recommending the use of PD to monitor PRRSV in farrowing rooms.

Obtaining an ORF 5 sequence of SW, UW and PD samples was possible. However, sequencing was related with the Ct value of the sample. All the attempts to sequence samples with Ct values over 30 gave negative results (five out of five). Similar results were reported by other researchers where only one out of thirteen environmental samples with a Ct value over 30 could be sequenced in contrast to samples that had Ct values below 30 (three out of three) (Kenney and Polson, 2011).

6. Conclusions

SW, UW and PD offer a valuable and practical sampling methodology to monitor piglets in breeding herds, especially in the first stages of an outbreak. This work demonstrated that PRRSV could be detected in the environment and the udder skin of sows and they could have a potential role in the transmission and maintenance of PRRSV in piglets prior to weaning. Sampling the environment or the udder skin of the lactating sow could be a less invasive alternative sampling method to determine the PRRSV status of the herd or the presence of the virus at weaning. Further research is needed to compare environmental sampling methods with other existing procedures such as serum or oral fluids to determine PRRSV detection during the same days/weeks. However, these environmental techniques should not be used as a sole indicator of PRRSV status at the litter level.

Declaration of Competing Interest

None.

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