Short Communication

Testing of umbilical cords by real time PCR is suitable for assessing vertical transmission of porcine reproductive and respiratory syndrome virus under field conditions

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Abstract

The objective of this study was to test the suitability of umbilical cord (UC) sampling and ear vein swabbing (EVS) as alternatives to jugular vein bleeding (JVB) for the assessment of vertical transmission of porcine reproductive and respiratory syndrome virus (PRRSV). Twelve farms suspected to be PRRSV-positive unstable were selected and the three types of samples were obtained from 21 batches of newborn piglets (n=387). The proportions of positive results, viral loads and time spent to collect the samples were compared. UC yielded the highest detection rate, with 76 positives compared to 55 JVB- and 45 EV-positive results (P<0.05). Average Ct values were 26.6±8.5 for JVB, 30.8±6.4 for EV and 32.1±4.85 for UC (P<0.01). UC was the fastest collection method (mean 24 s vs. 55 s for EV and 72 s for JVB; P<0.05). In this study, UC testing was a faster and more sensitive alternative to JVB or EV for the detection of PRRSV in newborn piglets.

Keywords: Diagnosis; Monitoring; PRRSV; Vertical transmission

Porcine reproductive and respiratory syndrome virus (PRRSV) infection is one of the major health problems for the swine industry worldwide. The estimation of the economic impact of the disease may change over time, but is believed to be high. Holtkamp et al. (2013) estimated an economic cost of $664 million for the American industry, while Nieuwenhuis et al. (2013) estimated and average loss of €126 per sow during the outbreak period.

Control programs for PRRS are most often based on four pillars: (1) early diagnosis and monitoring; (2) immunization; (3) biosecurity; and (4) control of the pig flow (Perez et al., 2015). With regards to diagnosis and monitoring, the first step is to establish the status of the farm. Infected farms are usually categorized as unstable or stable. Unstable farms are those where the herd has had a history of positive shedding and exposure (Holtkamp et al. 2011), which, in practical terms, is usually seen as a flow of viremic piglets from maternities to nurseries because of the birth of viremic pigs. Most often the status is assessed by examining piglets at weaning. Furthermore, in farms where nurseries are in the same premises as the breeding herd, it is not uncommon to see backwards circulation of the virus from nurseries to maternities. Under these circumstances, sampling of newborn piglets is needed to determine whether or not vertical transmission has occurred.

Bleeding newborn piglets is difficult due to their small size. Since PRRSV can be found in umbilical cords (Harding et al., 2017), sampling of this tissue could be useful to determine the presence of the virus. Also, for boars and nursery piglets, ear vein puncture has been described for the detection of PRRSV (Spagnuolo-Weaver et al., 2000; Reicks et al., 2006; Patterson et al., 2007). In the present report, three different

61 sampling approaches for assessing vertical transmission of PRRSV in newborn piglets
62 were evaluated: (1) umbilical cord (UC) testing; (2) jugular vein bleeding (JVB); and
63 (3) ear vein blood swabbing (EVS).
64
65 The study was designed to compare the detection rates of PRRSV vertical
66 transmission events by real time (RT)-PCR using the abovementioned samples.
67 Assuming 95% of confidence and 80% power, it was calculated that 98 positive
68 samples would be needed to evaluate differences in the detection rates in the different
69 sample types used in this study > 10%, namely a 90% coincidence of detection rates of
70 PRRSV. We considered that was not acceptable to use a sampling method that would
71 decrease the detection rate >10% compared to the other sampling methods. According
72 to data obtained from our diagnostic laboratory and other previous samplings from 2013
73 to 2015 (data not shown), we estimated that in unstable farms, sera from at least 25% of
74 newborn piglets would test positive by RT-PCR if the weakest piglets in each litter were
75 sampled. Given that premise, it was estimated that 400 samples would be needed in
76 order to identify 100 positive animals. Selected farms were PRRSV-positive farms that
77 had stillbirths, weak-born piglets and high mortality in maternities.
78
79 Twelve farms suspected to be unstable were included in the study, with 21
80 farrowing batches examined. At least 15 samples were collected in each batch by the
81 authors, with the remaining batches sampled by trained veterinarians. A detailed
82 protocol detailing how to obtain each sample type was sent to the veterinarians. The
83 time needed for the collection of samples was recorded with the aid of a calibrated
84 chronometer. Incidents during sampling were recorded. UC were obtained from recently
85 born piglets after clamping and cutting a 3 cm portion with sterile scissors. Once the UC

was collected, it was stored in a sterile plastic bag. For each animal, ear vein blood was collected after puncture with a sterile lancet, and peripheral blood was collected by jugular venipuncture (<3mL). Ear vein blood was collected with a Dacron swab that was immersed in 1 mL of sterile phosphate buffered saline (PBS) and resuspended. Samples were refrigerated at 4° C and immediately transported to the laboratory.

UC were thoroughly sliced in sterile PBS, and the swabs were resuspended. RNA was extracted from the serum, or EVS and UC suspensions, by means of the MagMAX Pathogen RNA/DNA Kit (ThermoFisher). The extracted RNA was then examined (7 µL) using a commercial qRT-PCR system (LSI VetMAX PRRSV EU/NA Real-Time PCR Kit).

For each sample type, the proportion of PRRSV-positive samples were calculated per batch and for all samples cumulatively (the specificity of the RT-qPCR was assumed to be 100%). Assessment of relative viral loads was performed by comparing cycle threshold (Ct). Individual PRRSV detection rate and kappa values for the comparison of results were also calculated. The McNemar test and Liddell relative risks were calculated for comparing the proportion of positive results for each sample type. The non-parametric Kruskal-Wallis test was used for comparing Cts and the time needed for collection of samples.

A total of 387 animals from 21 batches in 12 farms were examined. A total of 105 piglets were positive in at least one of the samples, but only 27/105 piglets yielded positive results in all sample types. UC yielded the highest detection rate, with 76/387 positives (19.6%; 95% confidence intervals [CI], 15.9-23.8), compared to 55/387

positive samples in JVB-collected samples (14.2%; 95% CI, 11.0-18.0; P=0.013), and 45/387 (11.6%; 95% CI, 0.09-0.15) positive samples when EVS was used (P<0.0001). UC had a relative risk of detection rate of 1.9 (95% CI, 1.14-3.5; P=0.125), which was higher than JVB, or EVS (3.4; 95% CI, 1.86-7.46; P<0.0001). No significant differences were observed between JVB and EVS (P=0.133). All batches were classified as positive regardless of the sample used, and there were no significant differences (P = 0.617 - 1). Thus, 12/21 (57.1%; 95% CI, 36.5-75.5) batches were positive using UC, 11/21 (52.3%; 95% CI: 32.4-71.6) were positive using JVB, and 10/21 (47.6%; 95% CI: 28.34-67.63) were positive with EVS. The classification of the farms was therefore unaffected by the type of sampling. In our study, the highest agreement for individual results was observed for JVB and EVS (kappa=0.59; 95% CI, 0.45-0.71), followed by EVS vs. UC (kappa =0.41; 95% CI, 0.32-0.59), while UC and JVB showed a moderate-to-low agreement (kappa =0.46; 95% CI, 0.33-0.59). Similarly, the highest correlation of Ct values from positive samples was found between JVB and EVS (r=0.79; 95% CI, 0.61-0.89; P<0.0001), followed by JVB and UC (r=0.59; 95% CI, 0.31-0.77; P=0.0003), and EVS and UC (r=0.35; 95% CI, 0.07-0.65; P=0.018). Interestingly, UC produced the highest average Ct (32.1±4.8) compared to JVB (26.6±8.5) or EVS (30.8±6.4; P<0.05; Fig. 1A).

Major discrepancies between the UC and JVB sample types were identified for the low positive UC samples that were negative on JVB. A detailed examination of this result showed that Ct-values from positive UC samples obtained from JVB-positive piglets (29.4±1.8) were significantly lower (P<0.05) than those from JVB-negative piglets (34.3±1.0, respectively; Figs. 1B and 1C). These results suggested some surface contamination of the UC samples. Since samples were obtained shortly after birth (<12

h) and before cross-fostering, it is likely that the source of PRRSV contamination was the sow, birth materials or other littermates; this strongly suggests vertical transmission of PRRSV in that litter.

Regarding the time needed for sampling, UC samples were the fastest to collect (24.1±1.2 s), followed by EVS (55.5±5.3 s), and JVB (73.1±5.7 s; $P<0.05$). Bleeding of newborn piglets was difficult, and remnants of placenta and dirt made it difficult to see the ear vein clearly.

The results of the present report indicate that UC sampling was a simple, fast, sensitive method to assess vertical transmission of PRRSV compared to JVB or EVS. UC collection respects animal welfare and this sampling method could be especially useful when collecting specimens from weak-newborn piglets, or in research to determine sow populations where vertical transmission of PRRSV has occurred. UC could also be used for comparison with other sampling methods, such as oral fluids or bleeding at weaning, for PRRSV farm categorization.

Ethical statement

The present paper has been done on the basis of clinical testing. Then, according to Directive 63/2010 -Article 1, point 5(b) “non-experimental clinical veterinary practices”- the procedure carried out to obtain data is not within the scope of named directive.

Conflict of interest statement

None of the authors has any financial or personal relationship that could inappropriately influence or bias the contents of the paper.

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Table 1

Contingency table presenting comparisons of jugular vein bleeding (JVB) and ear vein bleeding (EVB), JVB and umbilical cord (UC) sampling, and EVB and UC, with positive and negative agreements, during testing for porcine reproductive and respiratory syndrome virus (PRRSV).

		EVS		
		Positive (%)	Negative (%)	Total
JVB	Positive (%)	32	23	55
	Negative (%)	13	319	332
	Total	45	342	387
	Positive agreement	47.1%		
	Negative agreement	89.8%		
		UC		
		Positive (%)	Negative (%)	Total
JVB	Positive (%)	33	22	55
	Negative (%)	43	289	332
	Total	76	311	387
	Positive agreement		33.6%	
	Negative agreement		81.6%	
		EVS		
		Positive (%)	Negative (%)	Total
UC	Positive (%)	33	12	45
	Negative (%)	43	299	342
	Total	76	311	387
	Positive agreement	37.5%		
	Negative agreement	87.8%		

215 **Figure legend**

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217 Fig. 1. Box and whisker plots of Ct-values observed for each type of sample taken for
218 the detection of porcine reproductive and respiratory syndrome virus (PRRSV). Mean
219 (x), median, 25 and 75% quartiles, 95% confidence intervals and outliers are shown.
220 Plot A show that Ct values for jugular vein bleeding (JVB) were significantly lower
221 than ear vein swabbing (EVS) and umbilical cord (UC) sampling a<b<c; $P < 0.05$).
222 Plots B and C show Ct values for UC and EVS that were also positive and negative for
223 JVB, respectively. Positive UC and EVS from viremic animals had significantly lower
224 Ct values than UC and EVS that were negative for JVB ($\alpha < \beta$; $P < 0.05$).