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1 **Short Communication**

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

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19

20 **Abstract**

21           The objective of this study was to test the suitability of umbilical cord (UC)  
22 sampling and ear vein swabbing (EVS) as alternatives to jugular vein bleeding (JVB)  
23 for the assessment of vertical transmission of porcine reproductive and respiratory  
24 syndrome virus (PRRSV). Twelve farms suspected to be PRRSV-positive unstable were  
25 selected and the three types of samples were obtained from 21 batches of newborn  
26 piglets ( $n=387$ ). The proportions of positive results, viral loads and time spent to collect  
27 the samples were compared. UC yielded the highest detection rate, with 76 positives  
28 compared to 55 JVB- and 45 EV-positive results ( $P<0.05$ ). Average Ct values were  
29  $26.6\pm 8.5$  for JVB,  $30.8\pm 6.4$  for EV and  $32.1\pm 4.85$  for UC ( $P<0.01$ ). UC was the fastest  
30 collection method (mean 24 s vs. 55 s for EV and 72 s for JVB;  $P<0.05$ ). In this study,  
31 UC testing was a faster and more sensitive alternative to JVB or EV for the detection of  
32 PRRSV in newborn piglets.

33

34 *Keywords:* Diagnosis; Monitoring; PRRSV; Vertical transmission

35

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

42

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

55

56 Bleeding newborn piglets is difficult due to their small size. Since PRRSV can  
57 be found in umbilical cords (Harding et al., 2017), sampling of this tissue could be  
58 useful to determine the presence of the virus. Also, for boars and nursery piglets, ear  
59 vein puncture has been described for the detection of PRRSV (Spagnuolo-Weaver et al.,  
60 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

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

91

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

97

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

106

107 A total of 387 animals from 21 batches in 12 farms were examined. A total of  
108 105 piglets were positive in at least one of the samples, but only 27/105 piglets yielded  
109 positive results in all sample types. UC yielded the highest detection rate, with 76/387  
110 positives (19.6%; 95% confidence intervals [CI], 15.9-23.8), compared to 55/387

111 positive samples in JVB-collected samples (14.2%; 95% CI, 11.0-18.0;  $P=0.013$ ), and  
112 45/387 (11.6%; 95% CI, 0.09-0.15) positive samples when EVS was used ( $P<0.0001$ ).  
113 UC had a relative risk of detection rate of 1.9 (95% CI, 1.14-3.5;  $P=0.125$ ), which was  
114 higher than JVB, or EVS (3.4; 95% CI, 1.86-7.46;  $P<0.0001$ ). No significant  
115 differences were observed between JVB and EVS ( $P=0.133$ ). All batches were  
116 classified as positive regardless of the sample used, and there were no significant  
117 differences ( $P = 0.617 - 1$ ). Thus, 12/21 (57.1%; 95% CI, 36.5-75.5) batches were  
118 positive using UC, 11/21 (52.3%; 95% CI: 32.4-71.6) were positive using JVB, and  
119 10/21 (47.6%; 95% CI: 28.34-67.63) were positive with EVS. The classification of the  
120 farms was therefore unaffected by the type of sampling. In our study, the highest  
121 agreement for individual results was observed for JVB and EVS ( $\kappa=0.59$ ; 95% CI,  
122 0.45-0.71), followed by EVS vs. UC ( $\kappa =0.41$ ; 95% CI, 0.32-0.59), while UC and  
123 JVB showed a moderate-to-low agreement ( $\kappa =0.46$ ; 95% CI, 0.33-0.59). Similarly,  
124 the highest correlation of Ct values from positive samples was found between JVB and  
125 EVS ( $r=0.79$ ; 95% CI, 0.61-0.89;  $P<0.0001$ ), followed by JVB and UC ( $r=0.59$ ; 95%  
126 CI, 0.31-0.77;  $P=0.0003$ ), and EVS and UC ( $r=0.35$ ; 95% CI, 0.07-0.65;  $P=0.018$ ).  
127 Interestingly, UC produced the highest average Ct ( $32.1\pm 4.8$ ) compared to JVB  
128 ( $26.6\pm 8.5$ ) or EVS ( $30.8\pm 6.4$ ;  $P<0.05$ ; Fig. 1A).

129

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

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

139

140         Regarding the time needed for sampling, UC samples were the fastest to collect  
141 (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  
142 newborn piglets was difficult, and remnants of placenta and dirt made it difficult to see  
143 the ear vein clearly.

144

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

152

### 153 **Ethical statement**

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

158

### 159 **Conflict of interest statement**

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

162

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166

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208 **Table 1**  
 209 Contingency table presenting comparisons of jugular vein bleeding (JVB) and ear vein  
 210 bleeding (EVB), JVB and umbilical cord (UC) sampling, and EVB and UC, with  
 211 positive and negative agreements, during testing for porcine reproductive and  
 212 respiratory syndrome virus (PRRSV).

213

		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%		

214

215 **Figure legend**

216

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 ( $\alpha < \beta$ ;  $P < 0.05$ ).