



This document is a postprint version of an article published in The Veterinary Journal © Elsevier after peer review. To access the final edited and published work see <https://doi.org/10.1016/j.tvjl.2018.01.008>

1 **Short Communication**

2

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**

6

7 Gerard E. Martín-Valls ^{a,b*}, Mireia Hidalgo^b, Esmeralda Cano^b, Enric Mateu^{a,b}.

8

9

10 *^a Department Sanitat i Anatomia Animals, Facultat de Veterinària, Universitat*
11 *Autònoma de Barcelona, 08193 Cerdanyola del Vallès, Spain*

12 *^b Centre de Recerca en Sanitat Animal (CRESA-IRTA-UAB), edifici CRESA, campus*
13 *UAB, 08193 Cerdanyola del Vallès, Spain.*

14

15

16 * Corresponding author. Tel.: +34 93 5812807.

17 *E-mail address:* gerard.martin@uab.cat (G.Martin-Valls).

18

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 ± 8.5 for JVB, 30.8 ± 6.4 for EV and 32.1 ± 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 ± 4.8) compared to JVB
128 (26.6 ± 8.5) or EVS (30.8 ± 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 ± 1.8) were significantly lower ($P<0.05$) than those from JVB-negative
134 piglets (34.3 ± 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

163 **Acknowledgements**

164 This study was funded by Boehringer-Ingelheim as the 2015 European PRRS
165 Research Award.

166

167 **References**

- 168 Harding, J., Ladinig, A., Novakovic, P., Detmer, S., Wilkinson, J., Yang, T., Lunney,
169 J., Plastow, G., 2017. Novel insights into host responses and reproductive
170 pathophysiology of porcine reproductive and respiratory syndrome caused by
171 PRRSV-2. *Veterinary Microbiology* 209, 114-123.
- 172
- 173 Holtkamp, D., Polson, D., Torremorell, M., Morrison, B., Classen, D., Becton,
174 L., Henry, S., Rodibaugh, M., Rowland, R., Snelson, H., Straw, B., Yeske,
175 P., Zimmerman, J., 2011. Terminology for classifying swine herds by porcine
176 reproductive and respiratory syndrome virus status. *Journal of Swine Health and
177 Production* 19, 44-56.
- 178
- 179 Holtkamp, D., Kliebenstein, J., Neumann, E., Zimmerman, J., Rotto, H., Yoder, T.,
180 Wang, C., Yeske, P., Mowrer, C., Haley, C., 2013. Assessment of the economic
181 impact of porcine reproductive and respiratory syndrome virus on United States
182 pork producers. *Journal of Swine Health and Production* 21, 72-84.
- 183
- 184 Nieuwenhuis, N., Duinhof, T., van Nes, A., 2012. Economic analysis of outbreaks of
185 porcine reproductive and respiratory syndrome virus in nine sow herds.
186 *Veterinary Record* 170, 225.
- 187
- 188 Patterson, A., Karriker, L., Evans, R., Yoon, K., 2007. Evaluation of alternative
189 antemortem diagnostic samples for porcine reproductive and respiratory
190 syndrome virus. *Journal of Swine Health and Production* 15, 339–345.
- 191
- 192 Perez, A., Davies, P., Goodell, C., Holtkamp, D., Mondaca-Fernández, E., Poljak,
193 Z., Tousignant, S., Valdes-Donoso, P., Zimmerman, J., Morrison, R., 2015.
194 Lessons learned and knowledge gaps about the epidemiology and control
195 of porcine reproductive and respiratory syndrome virus in North America.
196 *Journal of the American Veterinary Medical Association* 246, 1304-1317.
- 197
- 198 Reicks, D., Muñoz-Zanzi, C., Rossow, K., 2006. Sampling of adult boars during early
199 infection with porcine reproductive and respiratory syndrome virus for testing
200 by polymerase chain reaction using a new blood collection technique (blood-
201 swab method). *Journal of Swine Health and Production* 14, 258-264.
- 202

203 Spagnuolo-Weaver M., Walkerb, I., Campbella, S., McNeillyb F., Adairb B., Allan G.,
204 2000. Rapid detection of porcine reproductive and respiratory syndrome viral
205 nucleic acid in blood using a fuorimeter based PCR method. Veterinary
206 Microbiology 76,15-23.
207

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%		

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