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1 **Vertical transmission of bluetongue virus serotype 8**
2 **virus in Dutch dairy herds in 2007**

3
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14
15 **Abstract**

16
17 In February 2008, evidence was found for transplacental infection of bluetongue virus serotype 8
18 (BTV-8) in PCR negative, seropositive heifers in Northern Ireland originating from the
19 Netherlands. The relevance of this route of transmission was studied in Dutch cow-calf
20 combinations in the Netherlands of which the calves were born in the same time period of the
21 year as the calves from the exported heifers, the first quarter of 2008. Blood samples were tested

22 from 385 cows and their calves, housed in 43 dairy farms that became naturally infected with
23 BTV-8 for the first time in 2007. All calves were at least ten days old at the moment of first
24 testing. In total 229 cows tested seropositive for BTV-8. Eight of these cows were still PCR
25 positive. Out of the 229 seropositive cows, 37 calves (16.2%; 95% CI: 11.4-21.0) were tested
26 PCR positive in the first sample taken in April 2008. In the first week of June, 34 out of the 37
27 PCR positive calves were still available for resampling. Three calves were still PCR positive; one
28 was five months old, the other two were three months old. One month later, in the first week of
29 July, all initially PCR positive calves, including the three still tested positive one month earlier,
30 were PCR negative. We showed that BTV-8 can be vertically transmitted from cow to calf and
31 can result in healthy looking viraemic calves remaining PCR positive for up to five months.
32 These PCR positive calves could play a role in the epidemiology, and in particular in
33 overwintering of BT. However, further investigations are needed to evaluate the importance of
34 this route of transmission.

35
36 *Keywords:* bluetongue, BTV-8; calves; vertical transmission

38 **1. Introduction**

39
40 In 2006, bluetongue virus serotype 8 (BTV-8) emerged in the Netherlands for the first time
41 (BTV8/net2006 in Maan et al., 2008). The infection started in August and spread until
42 November. In that year BTV-8 only spread in the southern part of the Netherlands and
43 approximately 200 cattle herds notified clinical signs to the Food and Consumer Safety Authority
44 (VWA) (van Wuijckhuise et al., 2006; EFSA, 2007; Elbers et al., 2008). Overall, BTV-8 caused

45 only limited morbidity and mortality during this first episode in 2006 (Elbers et al., 2008; van
46 Schaik et al., 2008).

47 In the spring of 2007, after the first BTV-8 episode in the Netherlands, a small scale study was
48 performed to determine the possibility of overwintering of BTV-8 by vertical transmission. In
49 this study 50 suckling cows and their newborn calves, originating from 14 BTV-8 infected herds,
50 were tested for BTV-8. From the suckling cows 36 (72%; 95% CI: 59.6-84.4) tested seropositive
51 and PCR negative in blood and colostrum. All 31 newborn calves were tested PCR negative,
52 before and after colostrum intake (van Wuijckhuise and Vellema, 2008). Based on these results, it
53 was concluded that there was no or a small risk on vertical transmission to newborn calves.

54 However, in the third week of February 2008, one heifer and three calves from a group of 21
55 pregnant heifers imported from the Netherlands were tested BTV-8 PCR positive in Northern
56 Ireland. Eight of these 21 heifers were BTV-8 seropositive, and all were PCR negative at the time
57 of departure from the Netherlands and also at arrival in Northern Ireland. More than a month after
58 arrival, one of the originally seronegative heifers tested PCR positive and three of four newborn
59 calves from seropositive but PCR negative heifers were also tested PCR positive (Menziez et al.,
60 2008). Based on these results and taken the *Culicoides*-free period into account, it was likely that
61 BTV-8 was transmitted to the fetus after infection of the pregnant cow.

62 Recently, it was shown that experimental infection with BTV-8 of a cow in the final stage of
63 pregnancy could result in the birth of a viraemic calf. In the same study, it was demonstrated that
64 infection by BTV-8 could occur after uptake of BTV-8 contaminated colostrum (Backx et al.,
65 2009). However, the relevance of transplacental and oral transmission for the epidemiology, the
66 undesired movement of a possible BTV-8 reservoir, and in particularly the overwintering of
67 BTV-8 was unknown.

68 Here we describe a study to determine the percentage of PCR positive calves born in the first
69 quarter of 2008 from cows infected with BTV-8 in 2007. Subsequently, PCR positive newborn
70 calves were resampled to determine the length of PCR- and ELISA-positivity.

71

72 **2. Materials and Methods**

73

74 *Study population and data collection*

75 In the spring of 2008, dairy herds were selected from a region in the eastern part of the
76 Netherlands in which massive spread of BTV-8 had occurred for the first time in September and
77 October 2007 (Santman-Berends et al., 2009) (Figure 1). Based on an unknown expected
78 prevalence of 50%, an accepted error of 5% and a confidence level of 95%, 400 cows and their
79 newborn calves which were born in the first quarter of 2008, were blood sampled and BTV-8
80 PCR and serologically tested.

81 The inclusion criteria for a cow-calf combination were that both the cow and her calf remained
82 on the farm that was affected by BTV-8 in 2007. Furthermore, the calf had to be at least 10 days
83 old and had to be born in the first quarter of 2008.

84

85 (Figure 1)

86

87 Dairy herds should have at least eight calves that fulfilled the criteria to be finally selected
88 (Figure 2). Fifty-five herds met these criteria and were contacted. Eventually 43 farmers were
89 willing to cooperate in this study. From these 43 dairy herds, 388 cows and their calves were
90 sampled for serum and EDTA-blood between March 27th and April 8th (Figure 2).

91

92 (Figure 2)

93

94 Three combinations were excluded from the study because of improper samples. Eventually, 385

95 cow-calf combinations remained for analysis. Sera from cows and calves were tested for

96 antibodies to BTV-8 with a competitive VP7 ELISA (Institut Pourquier, Montpellier, France).

97 EDTA-blood samples were tested for BTV-8 with an in-house developed real time reverse

98 transcriptase polymerase chain reaction assay (PCR test) (van Rijn et al., 2009). If possible, PCR

99 positive calves in the first sampling (Figure 2) were monthly resampled for serum and EDTA-

100 blood, starting in June (Figure 2).

101

102 *Statistical analysis*

103 Proportion tests were used to determine the significance in difference between the number of

104 cows and calves positive in ELISA or PCR test.

105

106 **3. Results**

107

108 *Results of the first sampling*

109 From the 385 available combinations, 229 (8+221) cows (59.5%) and 234 (37+197) calves

110 (60.8%) were seropositive (Table 1). Of these 229 seropositive cows, eight cows were PCR

111 positive (3.5%), of which three had a relatively weak PCR-signal. Of the 234 seropositive calves,

112 37 calves were PCR positive (15.8%).

113 There was no significant difference between the number of seropositive calves and cows at first

114 testing (proportion test; P -value: 0.77). However, the number of PCR positive calves was

115 significantly higher compared to the number of PCR positive cows (proportion test; P -
116 value: <0.001).

117 All 156 seronegative cows (40.5%; 95% CI: 35.6-45.4%) also were PCR negative. Fourteen
118 calves from these seronegative cows (9.0%; 95% CI: 4.5-13.5%) were seropositive (Table 1).

119

120 (Table 1)

121

122 From 229 seropositive cows (59.5%; 95% CI: 54.6-64.4%), 37 (3+34) calves (16.2%; 95% CI:

123 11.4-21.0) were both PCR positive and seropositive. Eight of these seropositive cows were PCR

124 positive. Their respective calves were all seropositive and three out of eight were also PCR

125 positive (Table 1). The remaining 221 seropositive cows (57.4%; 95% CI: 52.5-62.3%) were

126 PCR negative. From their 221 respective calves, 178 calves (80.5%; 95% CI: 75.3-85.8%) were

127 seropositive and PCR negative, however, 34 (15.4%; 95% CI: 10.6-20.1%) calves were positive

128 in both tests and nine (4.1%; 95% CI: (1.5-6.8%) calves were negative in both tests (Table 1).

129

130 *Results of the follow-up period*

131 At first sampling, 37 out of 385 calves (9.6%; 95% CI: 6.6-12.6) were PCR positive (Table 1).

132 These PCR positive calves were resampled monthly from June 2008 until the end of the study

133 period in September 2008.

134 At second sampling in the first week of June, 34 calves were available for resampling; two had

135 died for unknown reasons and one was no longer present on the farm. Three out of 34 initially

136 PCR positive calves were still PCR positive (8.8%; 95% CI: 1.9-23.7) (Figure 3). From these

137 three, one calf was born in January 2008 and the other two calves were born in March 2008.

138

139 (Figure 3)

140

141 In the first week of July, all 34 initially PCR positive calves were PCR negative and remained
142 PCR negative in following resamples (Figure 3).

143 All 34 initially PCR positive calves available for resampling were also seropositive in April. Of
144 these 34 calves, 32 calves (94.1% 95% CI: 80.3-99.3) remained seropositive during the whole
145 study period which was until September. Two initially PCR positive calves originating from
146 different herds had no detectable levels of antibodies against BTV as determined by ELISA in
147 September 2008 (Figure 3).

148

149 **4. Discussion**

150

151 In February 2008, BTV-8 seropositive but PCR negative heifers imported into Northern Ireland
152 gave birth to PCR positive healthy looking calves during the *Culicoides*-free period, providing
153 evidence of transplacental transmission of the virus (Menzies et al., 2008). Before this event, it
154 was generally assumed that infection *in utero* by field strains of BTV could not result in healthy
155 looking calves. *In utero* infection resulting in healthy looking calves seemed only possible for
156 laboratory-adapted or vaccine strains (Osburn et al., 1971; Jochim et al., 1974; Waldvogel et al,
157 1992), whereas infection with field strains of BTV would result in abortion, malformed or weak
158 calves, or dummy calves (De Clercq et al., 2008; MacLachlan and Osburn, 2008; Wouda et al.,
159 2008; MacLachlan and Osburn, 1983).

160 A small scale study performed in the Netherlands in the spring of 2007 (van Wuijckhuise and
161 Vellema, 2008), demonstrated that there was no or a small risk on transplacental transmission.

162 Hence, it was unclear whether the findings of Menzies et al. (2008) were a common or rare

163 observation and therefore a larger study was performed. From an area in the Netherlands which
164 was heavily affected for the first time in 2007, 385 cow-calf combinations were selected in order
165 to find cows which were likely to have been infected during gestation. In parallel to the
166 observation in Northern Ireland, calves in this study were born in the first quarter 2008. From the
167 385 combinations selected for this study, 229 cows were seropositive at the end of March. This
168 indicated that they were infected before calving, because the *Culicoides*-free period, which was
169 based on outdoor temperatures and *Culicoides* activity, started the 13th of December 2007 and
170 lasted until the 21st of April 2008. Furthermore, these cows probably were infected during
171 gestation, as the area was first affected in 2007. Eight cows were still PCR positive at the end of
172 March which is in agreement with previous observations that cattle can be PCR positive for more
173 than 200 days post infection. The calves from these eight PCR positive cows were all
174 seropositive and three out of eight were also PCR positive. This latter finding indicated that
175 BTV-8 was vertically transmitted from cow to calf, since birth and sampling occurred in the
176 period without *Culicoides* activity. In other words, it is most unlikely that these newborn calves
177 were infected by midges. From the remaining 221 calves, originating from BTV-8 infected cows,
178 178 were seropositive, but PCR negative, and 34 calves were PCR positive and seropositive. The
179 first group of 178 seropositive calves can be explained by the uptake of colostral antibodies, or
180 by seroconversion in combination with clearance of BTV-8.

181 In April 2008, the number of PCR positive calves (37) was significantly larger than the number
182 of PCR positive dams (8). Apparently, the PCR-signal remained longer in the newborn calves
183 than in the cows, the fetal infection was significantly delayed or the fetal infection occurred later
184 with respect to that of the cow. Alternatively, these calves could be infected by uptake of
185 colostrum contaminated with BTV-8 like this has been shown experimentally (Backx et al.,
186 2009). There is no evidence that colostrum was contaminated by BTV-8, although colostrum can

187 easily contain blood due to collapsed veins in the udder of freshly calved cows. Furthermore,
188 nine calves from 229 seropositive cows were negative in both tests. Probably, these calves did
189 not receive antibodies by insufficient uptake of colostrum with antibodies or by uptake of
190 colostrum from a seronegative cow.

191 In total, 37 calves out of the 229 seropositive cows tested PCR positive in our study, indicating
192 that the transplacental transmission rate in our study was 16.2% (95% CI:11.4-21.0). This rate is
193 lower than the transplacental transmission rates found in the study of Galleau et al. (2009) and
194 Batten et al. (2009). Galleau et al. (2009) tested 10 out of 24 calves (41.7%), born from
195 seropositive dams, PCR positive within the first four days after birth. Batten et al. (2009) found
196 21 out of 61 calves (34.4%), born from seropositive dams, PCR positive within the first three
197 days after birth. The difference between our study and theirs was that Batten et al. (2009) and
198 Galleau et al. (2009) tested the calves within three or four days after birth, whereas the calves in
199 our study could be up to three months old at the moment of sampling. Our study and the study of
200 Batten et al. (2009) showed that the PCR positive calves became PCR negative during the time
201 period of the study. Thus the 16.2% PCR positive calves we found in April 2008 is probably an
202 underestimation of the real transplacental transmission rate, because calves born PCR positive,
203 could have become PCR negative at the moment of first testing.

204 From the 156 PCR- and seronegative cows, 14 calves tested seropositive. The most logical
205 explanation is that these calves were fed colostrum of a seropositive cow.

206 In our study, no acute infections were detected in the first sampling at the end of March, as there
207 were neither cows nor calves PCR positive and seronegative. This is in agreement with the
208 absence of *Culicoides* activity in the *Culicoides*-free period.

209

210 Fetal infection in the first three months of gestation has been assumed to result in abortion (De
211 Clercq et al., 2008; MacLachlan and Osburn, 2008; Wouda et al., 2008; MacLachlan and Osburn,
212 1983). Fetal infection later in gestation could result in the birth of PCR positive calves up to six
213 months after infection of the cow. At the time of birth, a new generation of *Culicoides* could be
214 infected by feeding on these newborn viraemic calves and subsequently start spread of BTV-8
215 again after a *Culicoides*-free period. However, we were not able to cultivate virus from the
216 samples of these calves with standard procedures, probably due to the presence of maternal
217 neutralizing antibodies (data not shown). Nevertheless, the blood from the calves may be
218 infectious to *Culicoides*. Mertens et al. (1996) showed that pretreatment of BTV-containing blood
219 samples with serum or protease, increased the infectivity for *Culicoides* cells. We speculate that
220 partial digestion in the gut of *Culicoides* could result in increased infectivity and thus in BTV-
221 replication. In addition, in Dutch dairy herds about 10% to 25% of the newborn calves do not
222 drink (sufficient) colostrum from their dams. In the absence of maternal antibodies, it was shown
223 that BTV-8 could be easily cultivated from a viraemic newborn calf (Backx et al., 2009).

224
225 Our study also showed that calves could remain PCR positive up to five months of age. Although
226 it is unknown whether PCR-positivity is related to infectivity of the midge and although the
227 majority of the viraemic calves are PCR positive for only a short period of time, we emphasize
228 that one or a few viraemic calves could induce the (re)start of BTV-8 circulation. Studies with
229 species of *Culicoides* are needed to proof whether *Culicoides* can be infected by bites on these
230 PCR positive calves. If so, BTV-8 could easily overcome a *Culicoides*-free period of 4-5 months
231 by transmission to the fetus.

232 In seasons and/or in areas with a high level of *Culicoides* activity, vertical transmission will be of
233 less or no importance for spread of BTV-8. However, in periods and/or areas without or with a

234 very low level of *Culicoides* activity, vertical transmission could become relatively important by
235 lack of other routes of transmission. Further research will be needed to proof the significance of
236 potential routes of transmission of BTV-8 in order to survive the winter in north-western Europe.
237 Here we have shown that vertical transmission of BTV-8 in cattle is not rare and that, in the
238 period without *Culicoides* activity, this mechanism could play an important role for the vector-
239 borne disease bluetongue.

240

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242

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245

246

247 **References**

248

249 Backx, A., Heutink, C.G., Van Rooij, E.M.A., van Rijn P.A., 2009. Transplacental and oral
250 transmission of wild-type bluetongue virus serotype 8 in cattle after experimental
251 infection. Accepted for publication in Vet. Microbiol.

252 Batten, C., Darpel, K., Veronesi, E., Williamson, S., Anderseon, P., Dennison, M., Clifford, S.,
253 Smith, C., Phillips, L., Bachanek-Bankowska, K., Sanders, A., Bin-Tarif, A., Wilson, A.,
254 Gubbins, S., Mertens, P., Oura, C., Mellor, P., 2009. Transplacental transmission of
255 bluetongue serotype 8 (BTV-8) in the United Kingdom (2007-2008). In proceedings of
256 Epizone, 3rd edition. 12-15 May 2009 Antalya, Turkey.

257 EFSA, 2007. Scientific Report of the scientific Panel on Animal Health and Welfare on request

- 258 from the Commission (EFSA-Q-2006-311) and EFSA self mandate (EFSA-Q-2007-063)
259 on bluetongue. EFSA J 479, 1-29 & 480, 1-20.
- 260 Elbers, A.R.W., Backx, A., Meroc, E., Gerbier, G., Staubach, C., Hendrickx, G., Spek van
261 der, A., Mintiens, K., 2008. Field observations during the bluetongue serotype 8 epidemic
262 in 2006. I. Detection of first outbreaks and clinical signs in sheep and cattle in Belgium,
263 France and the Netherlands. *Prev. Vet. Med.* 87, 21-30
- 264 De Clercq, K., Vandebussche, F., Vandemeulebroucke, E., Vanbinst, T., De Leeuw, I.,
265 Verheyden, B., Goris, N., Mintiens, K., Méroc, E., Herr, C., Hooybergs, J., Houdart, P.,
266 Sustronck, B., De Deken, R., Maquet, G., Bughin, J., Saulmont, M., Lebrun, M., Bertels,
267 G., Miry, C., 2008. Transplacental bluetongue infection in cattle. *Vet. Rec.*, 162, 564.
- 268 Galleau, S., Hamers, C., Blossé, A., Bolon, A., Blanchet, M., Goutebroze, S., 2009. Can
269 vaccination prevent transplacental transmission of BTV-8? In proceedings of Epizone, 3rd
270 edition. 12-15 May 2009 Antalya, Turkey.
- 271 Jochim, M.M., Luedke, A.J., Chow, T.L., 1974. Bluetongue in cattle: immunogenic and clinical
272 responses in calves inoculated in utero and after birth. *Am. J. Vet. Res.* 35, 517-522.
- 273 Maan, S., Maan, N. S., Ross-Smith, N., Batten, C. A., Shaw, A.E., Anthony, S.J., Samuel, A.R.,
274 Darpel, K.E., Veronesi, E., Chris A.L. Oura, Karam P. Singh, Kyriaki Nomikou, Abraham
275 C. Potgieter, Houssam Attoui, van Rooij, E., van Rijn, P., De Clercq, K., Vandebussche,
276 F., Zientara, S. Bréard, E. Sailleau, C., Beer, M., Hoffman, B., Mellor P.S., Mertens.
277 P.P.C., 2008. Sequence analysis of bluetongue virus serotype 8 from the Netherlands 2006
278 and comparisons to other European BTV strains. *Virology* 377, 308-318.
- 279 MacLachlan, N.J., Osburn, B.I., 2008. Induced brain lesions in calves infected with bluetongue
280 virus. *Vet. Rec.* 162, 490-491.
- 281 MacLachlan, N.J., Osburn, B.I., 1983. Bluetongue virus-induced hydranencephaly in cattle. *Vet.*

- 282 Pathol. 20, 563-573.
- 283 Menzies, F.D., McCullough, S.J., McKeown, I.M., Forster, J.L., Jess, S., Batten, C., Murchie,
284 A.K., Gloster, J., Fallows, J.G., Pelgrim, W., Mellor, P.S., Oura, C.A.L., 2008.
285 Evidence for transplacental and contact transmission of bluetongue virus in cattle. Vet.
286 Rec. 163, 203-209.
- 287 Mertens, P.P.C., Burroughs, J.N., Walton, A., Wellby, M.P., Fu, H., O'Hara, R.S., Brookes,
288 S.M., Mellor, P.S., 1996. Enhanced infectivity of modified bluetongue virus particles for
289 two insect cell lines and for two *Culicoides* vector species. Virol. 217, 582-593
- 290 Osburn, B.I., Silverstein, A.M., Prendergast, R.A., Johnson, R.T., Parshall, C.J., 1971.
291 Experimental viral-induced congenital encephalopathies. I. Pathology of hydranencephaly
292 and porencephaly caused by bluetongue vaccine virus. Lab. Invest. n 25, 197-205.
- 293 Santman-Berends, I.M.G.A., Bartels, C.J.M., Van Schaik, G., Vellema P., 2009. The monthly
294 increase of the BTV-8 within-herd prevalence and associated risk factors in Dutch dairy
295 herds, during the second episode in 2007. Submitted
- 296 Van Rijn., P.A., Heutink, C.G., Van Gennip, H.G.P., 2009. High throughput RRT-PCR diagnosis
297 for Bluetongue base don genome segment 10. Manuscript in prep.
- 298 Van Schaik, G., Berends, I.M.G.A., Langen van, H., Elbers, A.R.W., Vellema, P., 2008.
299 Seroprevalence of bluetongue serotype 8 in cattle in the Netherlands in spring 2007 and
300 its consequence. Vet. Rec. 163, 441-444.
- 301 Van Wuijckhuise, L., Dercksen, D., Muskens, J., Bruijn de , J., Scheepers, M., Vrouwenraets,
302 R., 2006. Bluetongue in the Netherlands; description of the first clinical cases and
303 differential diagnosis. Common symptoms just a little different and in too many herds.
304 Tijdschr. Diergeneeskd. 131, 649-654.

- 305 Van Wuijckhuise, L., Vellema, P., 2008. The role of pregnant cows in the overwintering of
306 bluetongue (in Dutch). Report number 2080005 GD Deventer, the netherlands
- 307 Waldvogel, A.S., Anderson, G.A., Phillips, D.L., Osburn, B.I., 1992. Association
308 of virulent and avirulent strains of bluetongue virus serotype 11 with premature births of
309 late-term bovine fetuses. *J. Comp. Pat.* 106, 333–340.
- 310 Wouda, W., Roumen, M.P.H.M., Peperkamp, N.H.M.T., Vos, J.H., Van Garderen, E.,
311 Muskens, J., 2008. Hydranencephaly in calves following the bluetongue serotype 8
312 epidemic in the Netherlands. *Vet. Rec.* 162, 422-423.
- 313

314 Figure 1. The Netherlands has been divided in 20 compartments. The within-herd prevalence per
315 compartment in 2007 is indicated. The dairy herds selected for this study are located in the area
316 indicated in blue.

317
318 Figure 2. The timeline of the study in 43 Dutch dairy herds from January until September 2008.
319 The period without proposed *Culicoides* activity (midge free period), the period of calving (birth
320 of the calves) and the time points of sampling (arrows) are indicated.

321

322

323 †: dead

324 na: not available

325 ++: PCR positive and seropositive

326 -+: PCR negative and seropositive

327 --: PCR negative and seronegative

328

329 Figure 3. Results of the follow up period of PCR positive calves in Dutch dairy herds from April
330 to September 2008

331

332

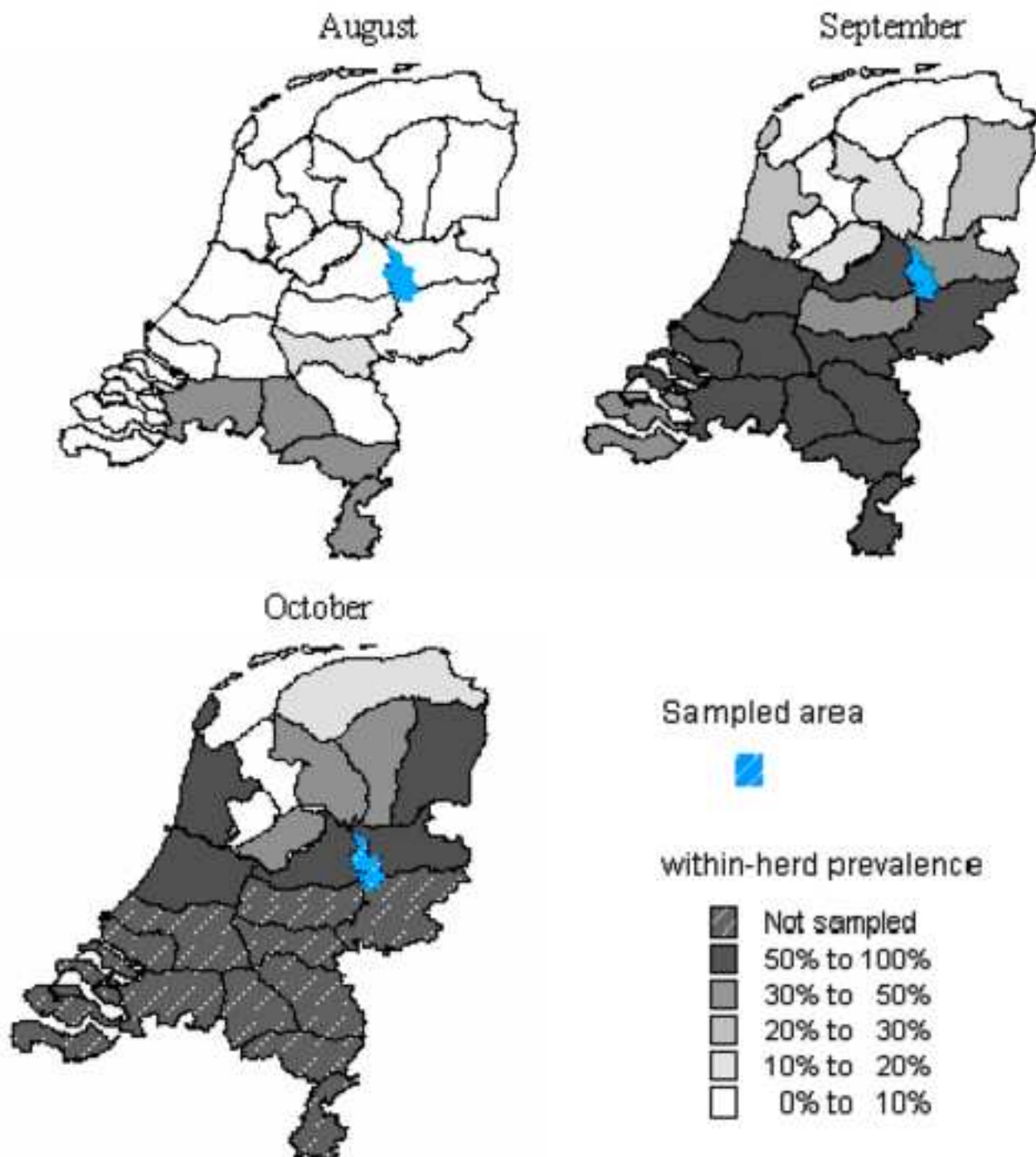


Figure 2

Manuscript

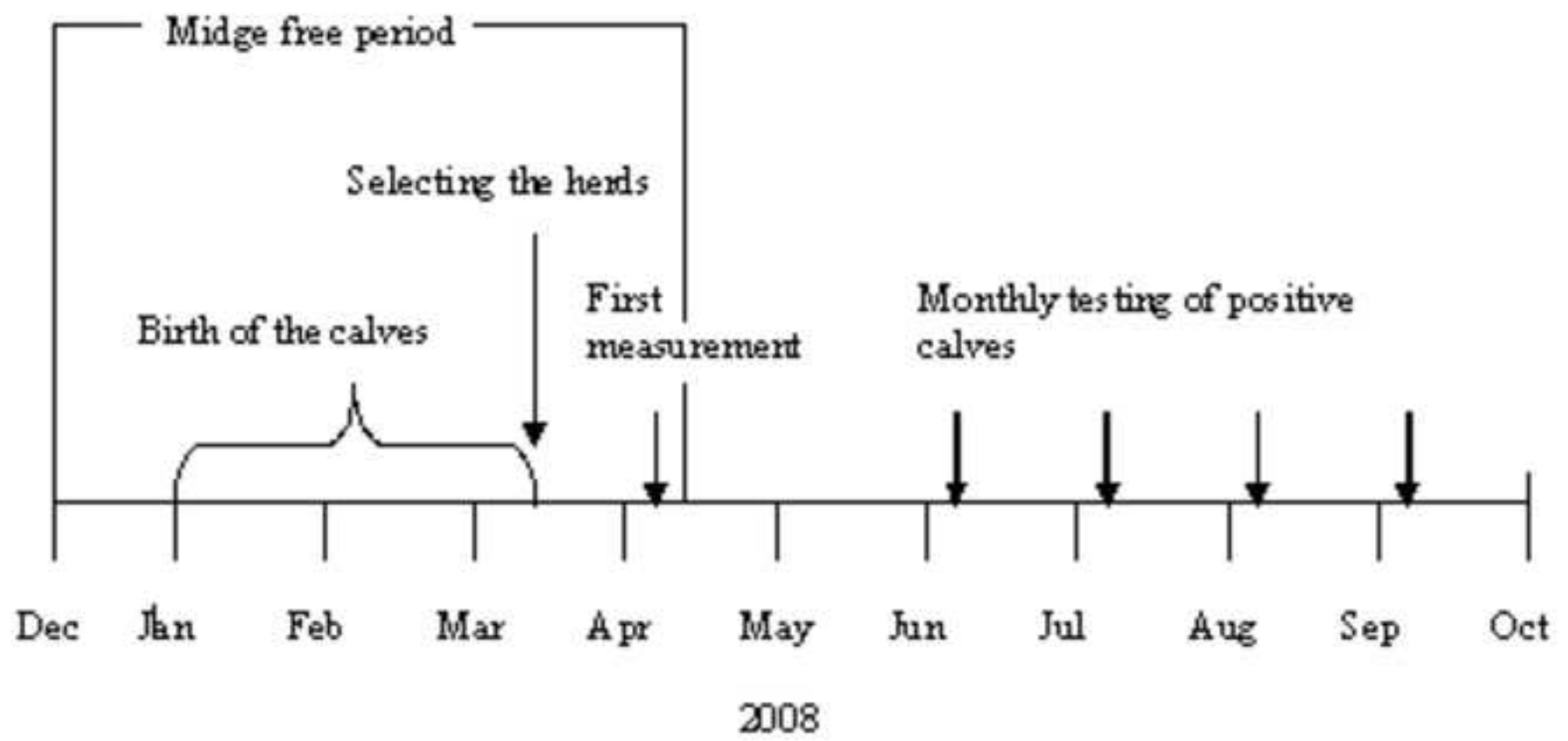


Figure 3

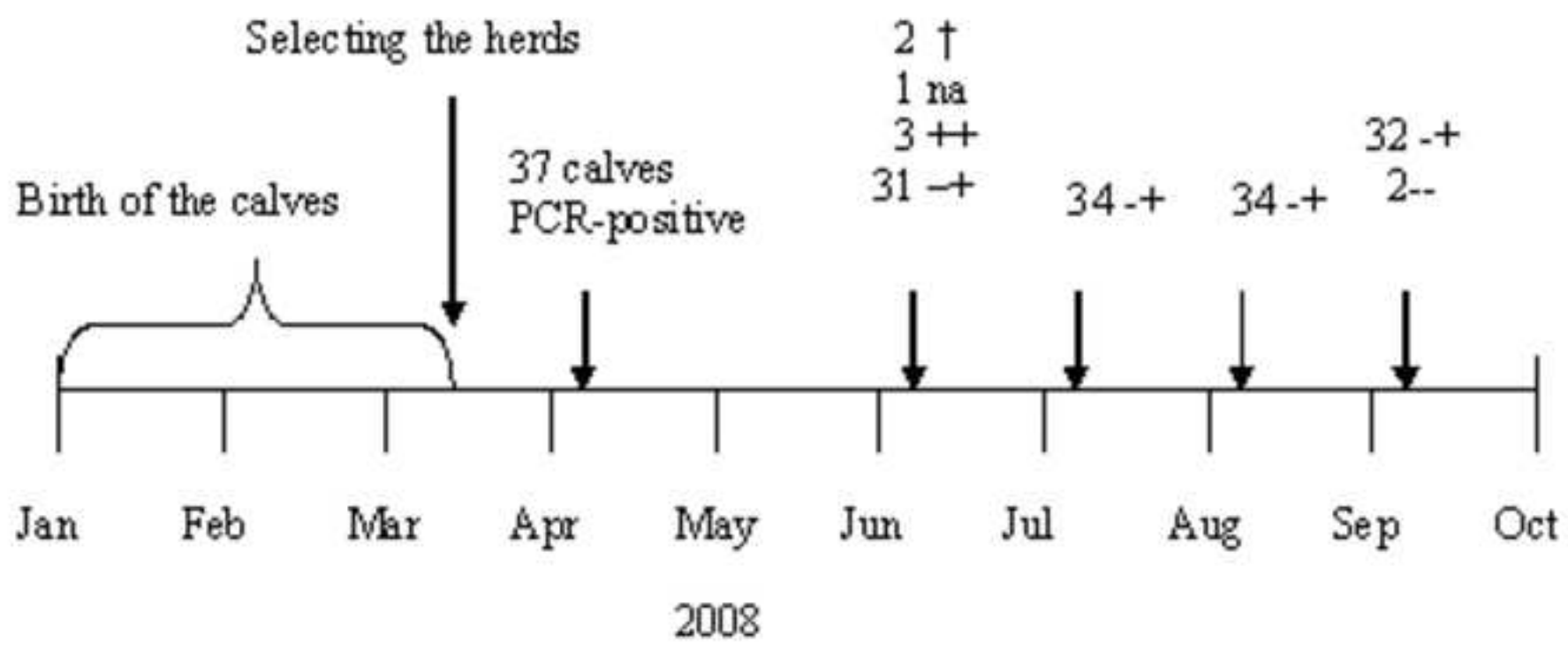


Table 1. Results of the PCR test and ELISA on 385 cow-calf combinations tested between March 27th and April 8th, 2008.

PCR test		Cows				Total	
		+	+	-	-		
ELISA		+	-	+	-		
Calves	+	+	3	0	34	0	37
	+	-	0	0	0	0	0
	-	+	5	0	178	14	197
	-	-	0	0	9	142	151
Total			8	0	221	156	385