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

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Abstract

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

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

Keywords: bluetongue, BTV-8; calves; vertical transmission

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1. Introduction

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

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

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

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

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

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

2. Materials and Methods

Study population and data collection

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

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

(Figure 1)

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

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(Figure 2)

Three combinations were excluded from the study because of improper samples. Eventually, 385 cow-calf combinations remained for analysis. Sera from cows and calves were tested for antibodies to BTV-8 with a competitive VP7 ELISA (Institut Pourquier, Montpellier, France). EDTA-blood samples were tested for BTV-8 with an in-house developed real time reverse transcriptase polymerase chain reaction assay (PCR test) (van Rijn et al., 2009). If possible, PCR positive calves in the first sampling (Figure 2) were monthly resampled for serum and EDTA-blood, starting in June (Figure 2).

Statistical analysis

Proportion tests were used to determine the significance in difference between the number of cows and calves positive in ELISA or PCR test.

3. Results

Results of the first sampling

From the 385 available combinations, 229 (8+221) cows (59.5%) and 234 (37+197) calves (60.8%) were seropositive (Table 1). Of these 229 seropositive cows, eight cows were PCR positive (3.5%), of which three had a relatively weak PCR-signal. Of the 234 seropositive calves, 37 calves were PCR positive (15.8%).

There was no significant difference between the number of seropositive calves and cows at first testing (proportion test; *P*-value: 0.77). However, the number of PCR positive calves was

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

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

(Table 1)

From 229 seropositive cows (59.5%; 95% CI: 54.6-64.4%), 37 (3+34) calves (16.2%; 95% CI: 11.4-21.0) were both PCR positive and seropositive. Eight of these seropositive cows were PCR positive. Their respective calves were all seropositive and three out of eight were also PCR positive (Table 1). The remaining 221 seropositive cows (57.4%; 95% CI: 52.5-62.3%) were PCR negative. From their 221 respective calves, 178 calves (80.5%; 95% CI: 75.3-85.8%) were seropositive and PCR negative, however, 34 (15.4%; 95% CI: 10.6-20.1%) calves were positive in both tests and nine (4.1%; 95% CI: (1.5-6.8%) calves were negative in both tests (Table 1).

Results of the follow-up period

At first sampling, 37 out of 385 calves (9.6%; 95% CI: 6.6-12.6) were PCR positive (Table 1). These PCR positive calves were resampled monthly from June 2008 until the end of the study period in September 2008.

At second sampling in the first week of June, 34 calves were available for resampling; two had died for unknown reasons and one was no longer present on the farm. Three out of 34 initially PCR positive calves were still PCR positive (8.8%; 95% CI: 1.9-23.7) (Figure 3). From these three, one calf was born in January 2008 and the other two calves were born in March 2008.

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(Figure 3)

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

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

4. Discussion

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

A small scale study performed in the Netherlands in the spring of 2007 (van Wuijckhuise and Vellema, 2008), demonstrated that there was no or a small risk on transplacental transmission. Hence, it was unclear whether the findings of Menzies et al. (2008) were a common or rare

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

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

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

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

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

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

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

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

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

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

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

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

†: dead

na: not available

++: PCR positive and seropositive

-+: PCR negative and seropositive

--: PCR negative and seronegative

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

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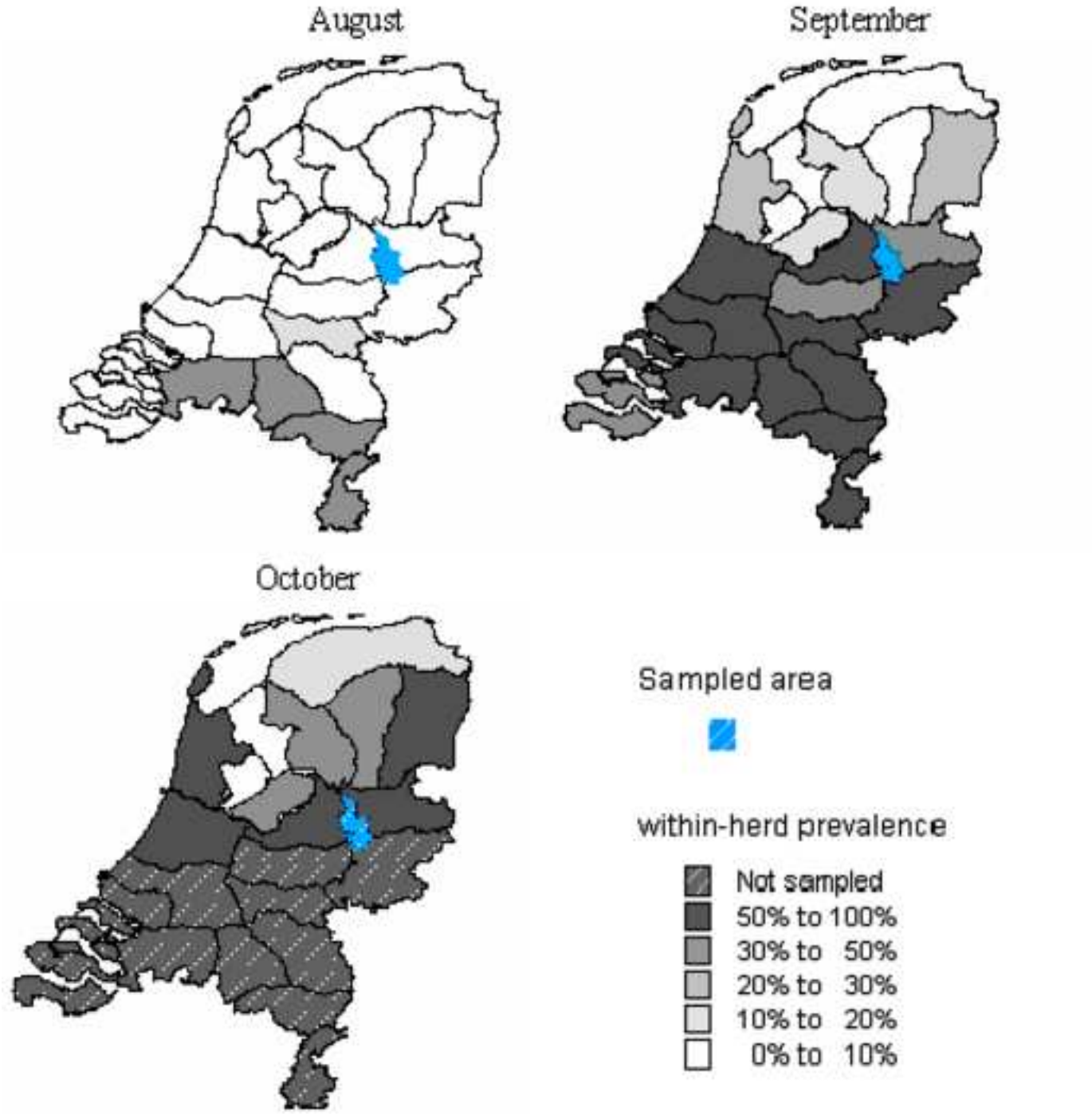

Figure 2

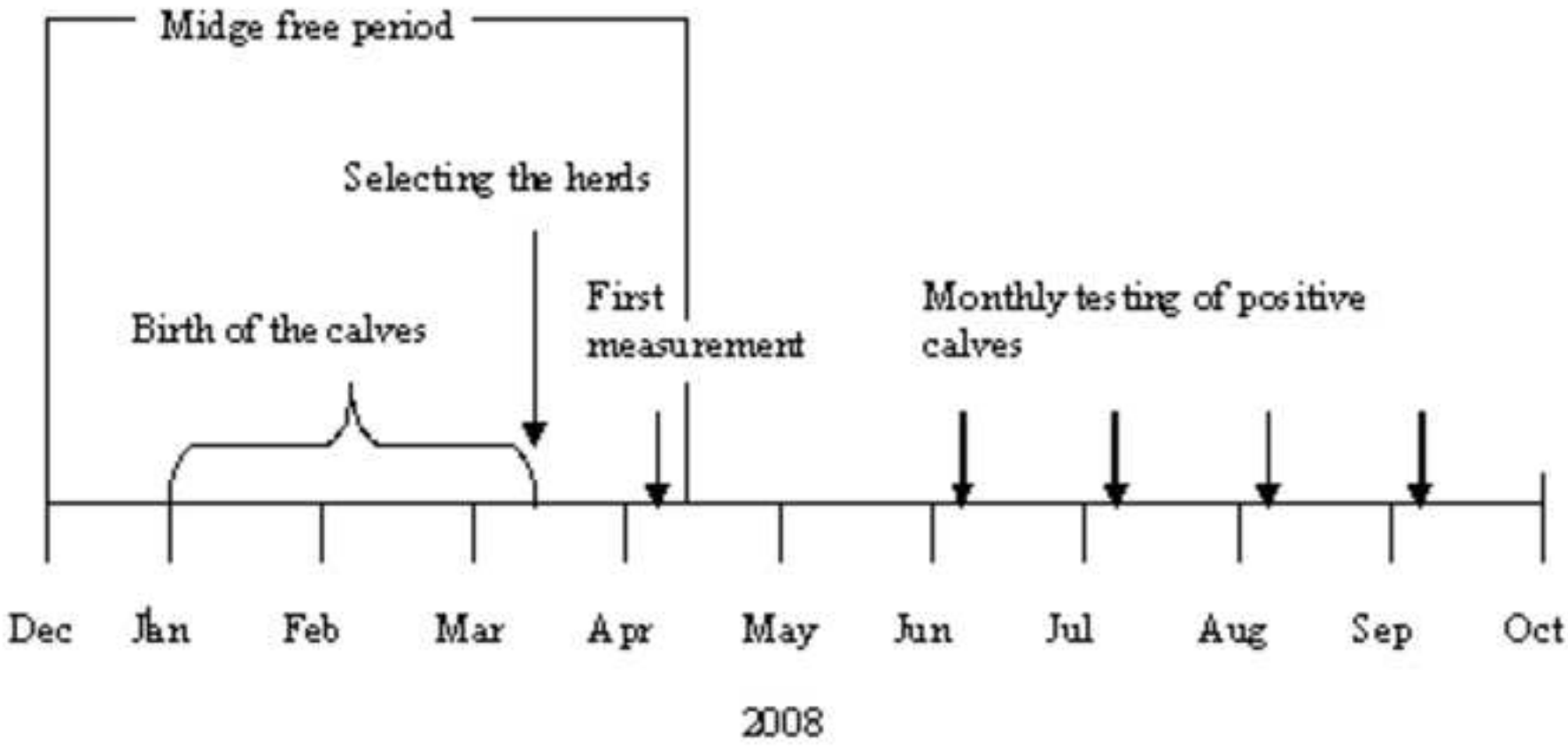
Midge free period
Selecting the herds
Birth of the calves
First measurement
Monthly testing of positive calves
Dec
Jan
Feb
Mar
Apr
May
Jun
Jul
Aug
Sep
Oct
2008

Figure 3

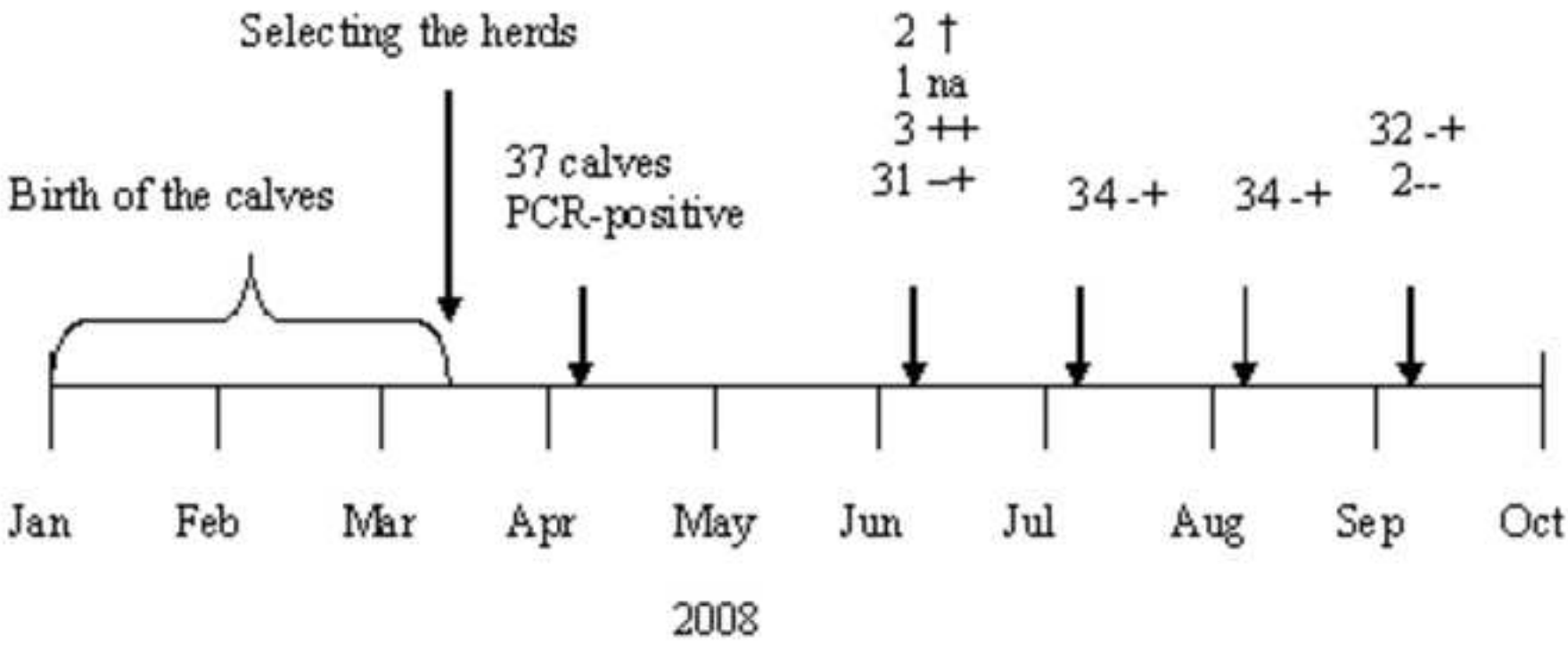
Selecting the herds
Birth of the calves
37 calves
PCR-positive
2 †
1 na
3 ++
31 –+
34 -+
34 -+
32 -+
2--
Jan
Feb
Mar
Apr
May
Jun
Jul
Aug
Sep
Oct
2008

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

			Cows				
PCR test			+	+	-	-	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

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