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The increase in seroprevalence of bluetongue virus (BTV) serotype 8 infections and associated risk factors in Dutch dairy herds, in 2007

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Abstract

Bluetongue virus serotype 8 (BTV-8) emerged in the Netherlands in August 2006 for the first time. In the winter of 2006/2007, during the transmission free period, the government decided to establish a sentinel network to monitor the re-emergence of BTV-8 in 2007.

Between June and December 2007, a sentinel network of 275 dairy herds with 8,901 seronegative cows at start, was in place for BTV-8 testing in milk samples. Besides estimates of the monthly BTV-8 within-herd prevalence per region (south, central and north), this sentinel was used to determine BTV-8 associated risk factors. Information on management and housing practices that were hypothesized to be related to the increase in BTV-8 prevalence (risk factors) were used. Complete information on BTV-8 testing and management was obtained for 234 herds. At herd level, the increase in seroprevalence was defined as the total increase in seroprevalence among sentinel cows per herd during the sampling period (in months) divided by the number of sampling months in which the spread of BTV-8 occurred within the particular compartment in which the herd was located. This parameter was used as dependent variable in the linear regression analysis. The final model revealed four risk

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factors remained in the final model. Herds in the central and southern region of the Netherlands had a higher monthly increase in seroprevalence (6.4% (95% CI: 3.1-9.9) and (10.1% (95% CI: 6.2-14.3)), respectively compared to herds in the northern region. Furthermore, there was a strong association with grazing. The monthly increase in seroprevalence in cattle pastured a few hours per day or throughout the day was 5.6% (95% CI: 1.4-10.2) to 11.4% (95% CI: 6.0-17.3) higher, relative to that for cattle kept indoors. For cattle that grazed outdoors throughout the day and the night, the monthly increase in seroprevalence was 13.6% (95% CI: 7.2-20.8). In addition, an association was found between the monthly increase in seroprevalence and some factors relating to stable design. Keeping the stable doors closed during the day was linked to a higher seroprevalence rate compared to that in stables with the door left open (3.6% (95 CI: 0.3-7.1). Furthermore, a horizontal ventilation opening (>30 cm) along the walls of the stable, and with a wind break curtain, appeared to offer some protection (-3.0% per month (95% CI: -6.0-0.2) as compared to stables that had no or, only a small, ventilation opening (<30 cm). By the second half of 2007, bluetongue had spread over throughout the Netherlands. Our study indicated that there were some management factors that may help limit exposure to BTV-8 and its consequences.

Keywords: bluetongue virus serotype 8; cattle; prevalence; risk factors; sentinel herds

1. Introduction

In August 2006, bluetongue virus serotype 8 (BTV-8) was diagnosed in the Netherlands for the first time (van Wuijkhuise et al., 2006). A few days later, Belgium and Germany also reported cases of BTV-8. This discovery was remarkable because, prior to its unexpected

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appearance in northern Europe, BTV-8 was known only from Africa, Central America and parts of south-east Asia (Mo et al., 1994; Daniels et al., 2004; Gerdes et al., 2004).

In December 2006, the assumed BTV-8 transmission free period commenced. By this point, outbreaks of BTV-8 had been reported from five European countries: Belgium (695), France (7), Germany (952), Luxemburg (8) and the Netherlands with 460 outbreaks (260 in sheep flocks and 200 in cattle herds) (EFSA, 2007).

In the winter of 2006/2007, the European Union enforced a monitoring and surveillance program in all affected countries to detect new infections and to monitor disease status, in the hope of regaining its BTV-8 free status within the shorter term. The Dutch government decided to conduct a program using a network of sentinel dairy herds.

The monthly test results from the sentinel network made it possible to pinpoint the moment of introduction of BTV-8 and to predict the rate at which it would spread. Furthermore, by analyzing, the diagnostic results from the ELISA in milk in combination with the management and housing practices on the sentinel farms, risk factors for BTV-8 at herd level could be determined.

In this study, the spread of BTV-8 across the Netherlands, and its monthly increase in seroprevalence in dairy herds is quantified based on results obtained from the 2007 sentinel network program which has enabled us to identify risk factors down to herd level.

2. Material and Methods

2.1 Study population

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The Netherlands is divided into 20 compartments based on geographic boundaries as proposed in Commission Decision 2005/393/EC (Figure 1). Compartment 1 was divided in two sub-compartments (1a and 1b), because of its large size.

For the sentinel network, a sample size of 150 sentinel cows per compartment was needed to achieve 95% confidence and a precision of 2% (Dutch Ministry of Agriculture, Nature and Food Quality, 2006). It was determined that in each compartment at least 10 randomly selected herds had to be sampled (with at least 15 cows per herd) to obtain the required sample size. Herds were not necessarily completely BTV-8 seronegative, but cows designated for the sentinel program had to be BTV-8 seronegative at moment of selection in May 2007. Dairy herds included in the program had to have at least 50 cows and had to participate in the dairy herd improvement program of the Cattle Improvement Cooperation (CRV) with test-day intervals monthly.

Thirteen or 14 herds per compartment, with at least 16 BTV-8 seronegative cattle per herd, were selected for sampling.

The 16 BTV-8 seronegative cattle were identified by collecting milk samples taken from 26 lactating cows per selected herd in compartments 1 to 13. In compartments14 to 20, milk samples were taken from all lactating cattle. All the cattle that tested negative were included in the sentinel network. In total, 8,901 initially seronegative cows housed in 275 Dutch dairy herds were selected for the BTV-8 sentinel program in 2007.

This method of selection was used because van Schaik et al. (2008) found that seroprevalence rates in compartment 1 to 13 did not exceed 1% between January and June 2007 whereas prevalence rates in compartments 14 to 20 (in the south of the Netherlands) were higher. This selection method increased the likelihood of obtaining at least 16 seronegative cattle per herd for inclusion in the network.

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All cattle that tested negative at first sampling in May 2007 were included in the study. Each month thereafter, and from this selected group, 16 randomly chosen cows were sampled in each herd.

2.2 Study period

The first round of monthly testing of sentinel cows was done in June 2007. The BTV-8 epidemic started in the south of the Netherlands and spread to the north. Sampling was halted in September in compartments 10 to 20 and in October in compartments 7 to 9. Because the prevalence of BTV-8 in these compartments was increasing rapidly, rendering further sampling was unnecessary. Initially, the sentinel study would end in October, but at that point a low number of cattle in the northern compartments of the Netherlands had seroconverted and it was decided to follow the herds in these compartments (1-6) up to and including December. Thus, cows located in the south were sampled for four months, those in the central region for four, five or seven months and those in the north for seven months (Figure 1 and 2). For the analyses, the test-results of the sentinel cows in the months in which BTV-8 did not spread on compartment level were excluded because risk-factors could not be determined in case there was no spread of BTV-8. Therefore, observations from sentinel cows located in central or northern compartments in June were excluded and observations from sentinel cows located in compartment 6 in June to August were also excluded.

2.3 Data collection and management

The monthly milk samples were tested at the Animal Health Service (GD) for antibodies to BTV-8 using a commercial ELISA (sELISA; ID.VET, Montpellier, France). The result of the

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ELISA is expressed in a S/P ratio. This ratio quantifies the amount of colouring (extinction) caused by the sample as compared to the amount of colouring (extinction) caused by the standard positive sample (extinction sample/extinction positive control x 100%). When the S/P ratio of the ELISA was >100 the sample was considered BTV-positive; those with an S/P ratio of <100 negative. The diagnostic test used is described in detail by Kramps *et al*. (2008). Between March and May 2008, among the farmers of the sentinel herds, a short questionnaire was administered by telephone with questions concerning the type of housing and the grazing patterns of their cattle during 2007. All 275 sentinel herd owners were contacted of which 245 (88%) responded. The remaining 30 herds could not be reached by telephone or did not want to cooperate. Answers of the questionnaire were submitted in Net Q (Net Q, 2008), a program for preparation of questionnaire data. After validation, data from 241 herds remained for analysis. Of these, six herds were only tested once, and in one herd sampling errors were made. These seven herds were excluded from further analysis.

Additional data were obtained on net return of milk, herd size, and the purchase of cattle in the BTV-8 period (between May and December 2007).

2.4 Statistical analysis

Monthly within herd prevalences of the sentinel cows for each sentinel herd were calculated and aggregated at compartmental and regional level. These results were displayed geographically and depict infection levels to BTV-8 in 2007 (Figure 1). Univariate descriptive analyses were performed to obtain means and medians and to compare herd characteristics (i.e. herd size, net return on milk) and the spread of BTV-8 between the regions south, central and north. Median tests (Kruskal-Wallis test: Kruskal and Wallis, 1952) were performed on

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the parameters that were not normally distributed. A Fisher's Exact test (Fisher, 1922) was used to compare proportions.

For the analysis of the risk factors, for each herd the diagnostic results were aggregated to one outcome variable which was calculated as:

$$MI_i = \frac{prev_{t_n} - prev_{t_0}}{N}$$

Where:

MI_i = the mean monthly increase in seroprevalence amongst sentinel cows in each herd (i; i=1 to 234)

$Prev_{tn}$ = the within herd seroprevalence between the sentinel cows in the last month (t_n) in which the herd was sampled

$Prev_{t0}$ = the within herd seroprevalence between the sentinel cows in the first month (t_0) in which spread of BTV-8 occurred within the particular compartment in which the herd was located

N = the number of months between t_0 and t_n

For the risk factor analyses, the outcome variables on herd level, which were based on the diagnostic results, were combined with the results of the questionnaire.

The outcome variable, the monthly increase in seroprevalence of BTV-8 was not normally distributed and a log transformation was used to normalize it. The log monthly increase in

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seroprevalence was used as a dependent variable and a general linear model in SAS 9.1 (SAS, 2006) was used to analyze risk factors related to the monthly increase in seroprevalence.

First, all variables derived from the questionnaire, and demographic data on the herds, were subjected to univariate analysis. When the *P*-value was below 0.20, the variable was used in the multivariate model. The multivariate analysis was done using a backward and a forward elimination procedure. After each run, the variable with the highest *P*-value was excluded from the model until all variables had a *P*-value <0.05. Confounding was monitored by the change in the coefficient of a variable after removing another variable. If the change of the estimates exceeded 25% or 0.1 when the value of the estimate was between -0.4 and 0.4, the removed variable was considered a potential confounder and was re-entered in the model. In the final model, all possible two-way interactions were tested singly. The residuals of the final model were tested for normality and the r^2 was calculated to measure the proportion of variance explained by the model.

3. Results

3.1 Descriptive results

Of the 275 sentinel herds that initially entered the Dutch sentinel program, 78 herds were located in the south, 114 in the central region and 83 in the north; respectively, 63, 100 and 71 of these herds, i.e. 85.1% overall, remained for final analysis.

Due to a lower dairy herd density, dairy herds in the southern region (2.0%) were significantly more often included in the sentinel study, than herds in the central (1.1%) or northern (0.9%) part of the Netherlands (Fisher's Exact test, P-value<0.001) (Table 1).

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(Table 1)

The monthly increase in whining herd seroprevalence and herd size differed significantly between regions (Kruskal-Wallis *P*-value=0.001 and *P*-value=0.02).

The percentage of herds in which all cattle tested negative in the last test round was not significantly different between regions (Kruskal- Wallis, *P*-value=0.59) (Table 1). However, we found that in 48% of the herds kept indoors all summer, all cattle tested negative in the last test round, as opposed to 14% for herds maintained outdoors (Kruskal-Wallis *P*-value<0.0001).

Nevertheless, the percentage of herds in which all cattle tested positive, and the average within-herd prevalence in the last test round, did not significantly differ between regions (Kruskal-Wallis, *P*-value=0.14 and *P*-value=0.64, respectively).

In the southern (compartment 15 to 20) and central (compartment 6 to 14) region of the Netherlands, the seroprevalence in the sentinel groups started to increase rapidly from August or September on (Figure 1 and 2) while the rapid increase in the northern region (compartment 1 to 5) of the Netherlands did not commence until October.

(Figure 1)

In compartment 6 (central region), the seroprevalence rate started to increase from September on. However, this increase was slower compared to that of other central compartments, where the seroprevalence rates remained at a low level for several months.

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(Figure 2)

The increase in seroprevalence between months where highest in September for the central (mean 44.8%; 95% CI: 37.9-51.7) and southern region (mean 48.2%; 95% CI: 38.2-58.2) (Figure 2). This increase in seroprevalence in September was significantly higher than the increase in seroprevalence in the other months (*P*-value<0.0001 in the central region and *P*-value<0.0001 in the southern region). For region north the highest increase in seroprevalence was found in October (mean 20.9%; 95% CI: 15.0-26.9) and November (mean 19.5%; 95% CI: 14.5-24.4) (Figure 2). These increases in seroprevalence were significantly higher compared to the other months (*P*-value<0.0001 in October and *P*-value<0.0001 in November, respectively).

3.2 Multivariate analyses

Nine of the 13 variables in the univariate analyses remained for multivariate analysis (*P*-value<0.20) (Table 2). The variables that were excluded based on the results of the univariate analyses, were purchase of cattle (*P*-value=0.37), stable doors open during the night (*P*-value=0.87), type of grazing management (rotation grazing, restricted grazing, siesta grazing, restricted grazing, grazing multiple weeks in the same field; *P*-value=0.99), and type of summer feed (maize, grass, both feeds; *P*-value=0.63).

(Table 2)

In the final multivariate model, the four factors 'region', 'grazing management between the end of June and October', 'keeping stable doors open during the day' and 'horizontal

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ventilation openings along the walls of the stable', were significantly associated with the monthly increase in seroprevalence of BTV-8 (Table 3).

(Table 3)

In herds in the central (6.4% (95% CI: 3.1-9.9)) and southern (10.1% (95% CI: 6.2-14.3)) parts of the Netherlands, BTV-8 spread more rapidly amongst cattle than in herds located in the north. Furthermore, it appeared that longer grazing hours were associated with a higher monthly increase in seroprevalence of BTV-8. Cattle that grazed outdoors for only a few hours a day (totaling between 1 to 1500 hours in summer and autumn), grazed outside all day (1501 to 2500 hours) or grazed outside day and night (>2500 hours), showed significantly higher monthly increases in seroprevalence of BTV-8 compared to cattle that remained indoors.

Keeping the stable door closed during the day was associated with a higher monthly increase in seroprevalence when compared to keeping the stable door open (3.6% (95% CI: 0.3-7.1). In addition, herds with horizontal ventilation openings along the side walls of the stable (>30 cm) in combination with a windbreak curtain, tended to a lower monthly increase in seroprevalence, when compared to herds with no or only small horizontal ventilation openings (<30 cm) (-3.0% (95% CI: -6.0-0.2)). However, large horizontal openings along the side walls, and without windbreak curtain, did not reduce the monthly increase in seroprevalence, when compared to herds in stables with no or with only small horizontal openings.

The final model explained 21% of the variation in the monthly increase in seroprevalence.

The variables in the final model were not significantly correlated amongst each other (Spearmann's correlation test). In the model one two-way interaction term was found to be

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significant: “grazing management” with “stable doors open at daytime”. However, this interaction term did not improve the amount of variance explained by the model nor the fit of the model. In addition, the result did not seem biologically relevant and the interaction was not included in the final model. The residuals of the final multivariate model were normally distributed.

4. Discussion

The sentinel study was originally developed to establish whether the Netherlands could regain its BTV-8 free status in 2007. In July 2007, the first seronegative cattle in the sentinel herds seroconverted. At that point it became clear that BTV-8 had over wintered and re-emerged. Subsequent to this the sentinel network was used to determine the spread of BTV-8 across the Netherlands.

Herds in different regions were sampled for different periods. This could have caused some bias in our data. However, within-herd prevalences in the south had increased to very high levels in September and further sampling would probably not provide additional information. At that point, BTV-8 within-herd prevalences were still very low in some central and northern compartments and for this reason it was decided to continue the testing in those compartments. To correct for the differences in sampling period for herds located in different compartments, the average monthly increase of seroprevalence within herds was determined. The median monthly increase in seroprevalence significantly differed between regions, with the highest increase in the south (18.8%) and the lowest in the north (7.5%). It is likely that the fraction of BTV-8 infected *Culicoides* in the southern and central region of the Netherlands was higher at the start of the *Culicoides* active period in 2007 compared to the fraction of infected *Culicoides* in the north, because BTV-8 had already spread in 2006 in the

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south and limited in the central region. Furthermore, these differences in the increase in the seroprevalence could be due to seasonal differences in the spread of BTV-8. In the south, BTV-8 spread in the summer months during which conditions were favourable and with temperatures high, ranging 15 and 25 degrees Celsius. In the north, BTV-8 started to spread from October to December. However, the conditions for the continued spread of BTV-8 deteriorated during these months due to declining temperatures.

Initially, we suspected that there would be a relationship between the purchase of cattle and the introduction of BTV-8 into a given herd. However, the results of the univariate analyses showed that there was no significant association between the purchase of cattle and increase in seroprevalence. To certify that the purchase (and movement) of cattle was not associated to the spread of BTV-8, we compared purchase of cattle (yes/ no) between June and December 2007 in herds that were BTV-8 positive with purchase of cattle between June and December 2007 in herds that had remained BTV-8 negative throughout the study period. Our data indicated no differences in purchase behavior between owners with herds that became BTV-8 positive and owners with herds that remained BTV-8 negative.

In compartment 6 (central region), the seroprevalence started to increase later than in the other central compartments and remained at a low level for a long time (Figure 1 and 2). The herd owners of only six of the 13 sentinel herds in compartment 6 responded to the questionnaire and so were included in our study. Of these six herds, four were kept stabled throughout the entire grazing season. Thus, the lower seroprevalence in this compartment may be linked by the specific management practices or a lower of herd density.

In the sentinel program, 275 sentinel herds were included; of these, 234 were included in the final model. A fixed number of 13 to 14 herds per compartment were selected for the study. Because the south of the Netherlands has a lower dairy herd density than the north, the south was slightly overrepresented in our study. Moreover, in the north, fewer farmers responded to

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the questionnaire, perhaps due to lower BTV-8 morbidity and mortality rates and due to its belated arrival in October 2007 (Santman-Berends et al., 2009). Although the sentinel herds were not fully representative of the national situation, we believe nevertheless that the range of management practices, barn types, and risk factors involved, are applicable to all dairy herds in the Netherlands.

It was found that more hours of grazing were related to a higher monthly increase in BTV-8 seroprevalence. Herds maintained indoors stayed significantly more often BTV-8 free (48%) than herds maintained outdoors (14%) and therefore the maintenance of cattle indoors may reduce the spread of infection. Meiswinkel *et al*. (2000) found that the ratio outdoors vs. indoors catches of *Culicoides* differed per subspecies. However, almost all subspecies were captured outside more often than indoors. In addition, Baylis *et al*. (2009) trapped *Culicoides* indoors and outside the stable in the United Kingdom and they also found lower numbers of trapped *Culicoides* indoors compared to outdoors (6.5 times less). These findings support our results of grazing as a risk factor. However, Baldet *et al*. (2008) found that in France certain species of *Culicoides* were captured indoors as much as outdoors. Based on the fact that a relatively high proportion of the *Culicoides* trapped indoors were freshly blood fed, they assumed that these had been actively feeding. Meiswinkel *et al*. (2008) obtained similar results in the Netherlands that included captures in which up to 33% of the *Culicoides* were freshly blood fed, and which likely had fed inside the stable.

Their conclusions, in part, conflict with our results which, based on the significantly lower BTV-8 seroprevalence rates, indicate *Culicoides* attack rates to be higher outdoors than indoors. It is important to note that two of the potential BTV vectors in the Netherlands, i.e. *C. dewulfi* and *C. chiopterus,* breed exclusively in the fresh dung of cattle lying in the field outdoors; therefore, if all cattle at a dairy are being maintained indoors it will deprive *C.*

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dewulfi and *C. chiopterus* of their breeding habitat, leading to a decrease in their population levels, which, in turn, may impact on the dissemination of BTV locally.

We found an association between increase in seroprevalence and some factors relating to stable design. A horizontal air opening of more than 30 cm along the walls of the stable, combined with a windbreak curtain, seemed to be protective against BTV-8 infection; furthermore, keeping the stable doors open was associated with a significantly lower increase in seroprevalence compared to that found at stables where the doors were kept closed. Thus, it would appear that herds housed in stables with many air openings ("fresh stables") showed a lower increase in seroprevalence. It is possible that increased air circulation may inhibit *Culicoides*, resulting either in a reduced presence and/ or reduced biting frequency in fresh stables. However, stables with a large (>30 cm) horizontal opening but without windbreak curtain were not associated with a significantly lower increase in seroprevalence compared to that in herds maintained in stables with no or small horizontal openings in the walls. Windbreak curtains may in some way help to reduce entrance by *Culicoides* into stables, much as mosquito netting would do. Nevertheless, because of the fairly weak association, further research is required to prove causality.

In the second half of 2007, BTV-8 had spread over all regions in the Netherlands. Our study indicates that there are some management practices such as maintaining the herd indoors and characteristics relating to the stable design that may help limit exposure to BTV-8 and thus lead to a lower increase in BTV-8 prevalence.

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differential diagnosis. Common symptoms just a little different and in too many herds. Tijdschr. Diergeneesk. 131, 649-654.

Figure 1. Mean within-herd BTV-8 prevalence in 234 dairy herds in the Netherlands per compartment per month, from July to December 2007. Horizontal lines demarcate the southern, central and northern regions of the Netherlands: the numbering of the compartments is included in the map of July.

Figure 2. Mean within-herd prevalence per month of BTV-8 in the Netherlands in 2007: north (compartments 1-5: sampled June-December), central (shown by use of three separate lines: compartment 6: sampled June-December; compartment 7-9: sampled June-October, and compartments 10-14: sampled June-September) and in the south (compartments 15-20: sampled June-December).

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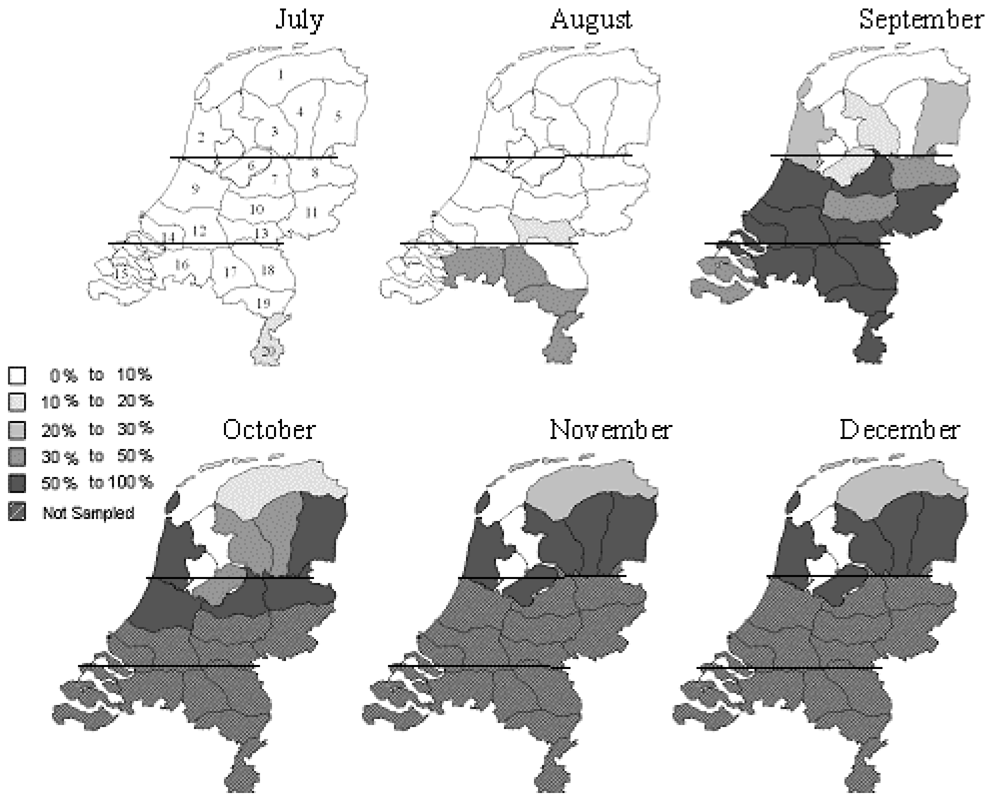

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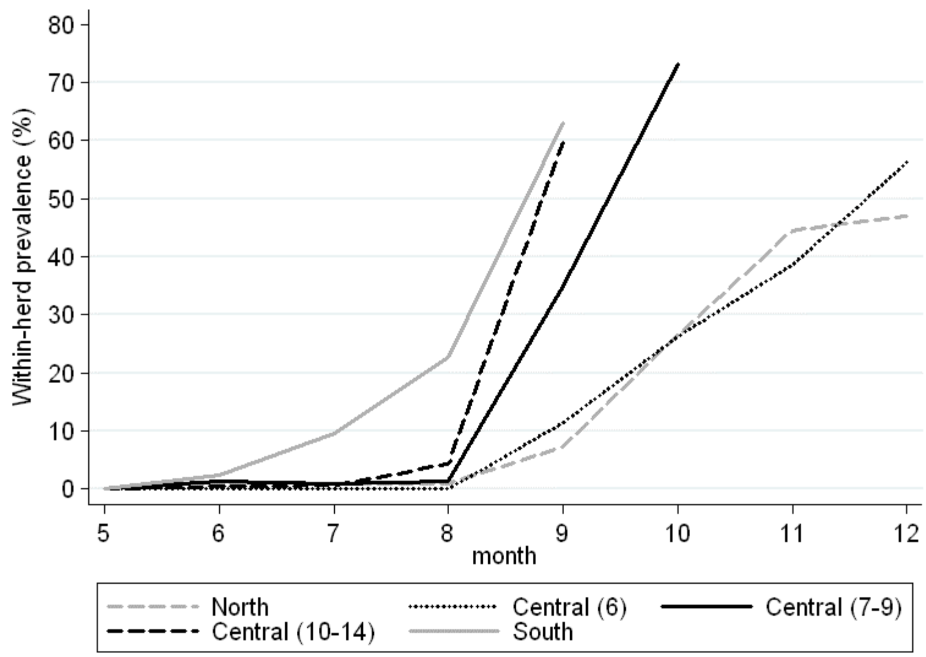

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Table 1. Median and interquartile range (25th and 75th percentile) of the dairy herds included in the Dutch BTV-8 sentinel study in 2007 (n=234).

	South (71 herds) Median (25th-75th percentile)	Central (100 herds) Median (25th-75th percentile)	North (63 herds) Median (25th-75th percentile)
Herd size: cows >2 years of age	67[a] (57-78)	65[a] (57-75)	59 (54-67)
Net return on milk in euros/ cow/	2,411	2,316	2,327

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lactation (in €)	(2,254-2,583)	(2,159-2,476)	(2,081-2,539)
Monthly increase in BTV-8 seroprevalence (%)	18.8[a] (3.1-31.3)	13.3 [a] (4.4-25)	7.5 (1.4-16.3)
Percentage of herds with all cattle testing BTV-8 negative in the last test round[b] (%)	19.7	15.0	20.6
Average within-herd prevalence of BTV-8 in the last test round[b] (%)	43.8 (6.3-93.8)	50.0 (12.5-93.8)	43.8 (7.1-81.3)
Herds with all cattle testing BTV-8 positive in the last test round[b] (%)	22.5	23.0	11.1

[a] significantly different from region north

[b] the month in which the cows were sampled for the last time

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Table 2. Univariate results of all farm management practices and housing variables with a *P*-value<0.20 in 234 Dutch dairy herds that participated in a testing program to estimate the monthly increase in seroprevalence of BTV-8 between June and September (compartments 16-20), June and October (compartments 7-15) or June and December (compartments 1-6).

Variable	Category	Frequency	Mean monthly increase in seroprevalence (%)	P-value (univariate)
Region	North (comp. 1-5)	63	10.3	0.001
	Central (comp. 6-14)	100	16.7	
	South (comp. 15-20)	71	18.7	
Herd size	10% smallest herds (less than 50 dairy cows)	23	19.0	0.08
	40% smaller herds (between 50 and 64 dairy cows)	92	16.9	
	40% larger herds (between 64 and 90 dairy cows)	96	14.9	
	10% largest herds (more than 90 dairy cows)	23	9.6	
Cattle were grazed in 2007	Yes	207	16.6	0.003
	No	27	8.3	

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Grazing practices between the end of June and October	No grazing (0 [a])	27	8.3	0.0005
	A few hours per day between milking (1-1500[a])	126	14.1	
	During the day (1501-2500[a])	55	19.7	
	Day and night (> 2500[a])	26	21.7	
Type of housing	Loose housed stable	212	15.1	0.19
	Different stable type	22	20.4	
Horizontal ventilation openings at the side of the stable	< 30 cm	72	18.3	0.02
	> 30 cm without windbreak curtain	103	16.4	
	> 30 cm with windbreak curtain	59	11.0	
Stable doors open through the day in summer	Yes	193	14.7	0.10
	No	41	20.0	
Roof with opening of at least 20 cm	Yes	182	14.7	0.13
	No	52	18.8	

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Net return on milk in euros/cow/ lactation	< € 1,838 (herds with 10% lowest net return)	18	21.8	0.15
	€1,838-€ 2,288 (herds with 40% lower net return)	73	16.9	
	€ 2,289-€ 2,619 (herds with 40% higher net return)	109	14.8	
	> 2,620 (herds with 10% highest net return)	34	12.0	

[a] The total number of hours was calculated as the number of hours per day times the number of days the cattle were in the field.

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Table 3. Variables in the final multivariate general linear model on the effect on the monthly increase in seroprevalence between June and December 2007 in the Netherlands and their categories, estimates (transformed from log transformation to real estimates), 95% confidence intervals and significance (P-value) (n=234).

Variable	Category	Estimated monthly increase in seroprevalence (%)	95% Confidence interval (%)		*P*-value
Intercept		0.73	-4.1	6.1	0.77
Region	North	Reference			
	Central	6.4	3.1	9.9	0.0001
	South	10.1	6.2	14.3	<0.0001
Grazing management between the end of June and October	No grazing (0 [a])	Reference			
	A few hours per day between milking (1-1500[a])	5.6	1.4	10.2	0.009
	During the day (1501-2500[a])	11.4	6.0	17.3	<0.0001
	Day and night (> 2500[a])	13.6	7.2	20.8	<0.0001
Stable doors	Yes	Reference			

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open throughout the day	No	3.6	0.3	7.1	0.03
Horizontal ventilation openings in the side walls the stable	None or smaller than 30 cm	Reference			
	Larger than 30 cm without windbreak curtain	-0.3	-3.2	2.7	0.82
	Larger than 30 cm with windbreak curtain	-3.0	-6.0	0.2	0.07

[a] Number of hours in the field during the pasturing period were calculated as the number of hours per day times the number of days the cattle were in the field.

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