Papers

Risk factors for seropositivity to bovine coronavirus and bovine respiratory syncytial virus in dairy herds

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A cross-sectional study was conducted to evaluate the association between herd-level characteristics, management routines and farm-level antibody status of bovine coronavirus (BCV) and bovine respiratory syncytial virus (BRSV) in 257 Swedish dairy herds. The possible spatial clustering of positive herds compared with negative herds was also investigated. For each herd, a pooled milk sample from five primiparous cows was analysed for the presence of antibodies to BCV and BRSV. Herd-level information was obtained by a questionnaire. Logistic regression was used to evaluate the association between predictors and antibody status to BCV and BRSV. Large herd size, being located in southern Sweden, and not providing boots for visitors were found to be associated with being antibody-positive to BCV and BRSV. A short distance to the nearest cattle herd was an additional risk factor for BCV. One of the studied areas was suitable for spatial analysis. Positive herds were not spatially autocorrelated when compared with negative herds as estimated by the K-function regarding both BCV and BRSV. This indicates that local factors such as daily visiting milk trucks and wild animals were unlikely to be important sources of infection in this area. Moran's *I* statistics and semi-variogram showed no evidence of spatial autocorrelation in the residuals, indicating that remaining unidentified factors are not spatially dependent in the areas under study.

BOVINE coronavirus (BCV) and bovine respiratory syncytial virus (BRSV) are two contagious viruses affecting beef and dairy cattle worldwide (Clark 1993, Paton and others 1998, Valarcher and Taylor 2007). The prevalence of antibodies to BCV and BRSV in bulk tank milk was 100 per cent in a nationwide study in England and Wales (Paton and others 1998). BCV has tropism for both enteric and respiratory tract epithelium, causing winter dysentery in adult cattle, diarrhoea in calves, and various degrees of respiratory tract disease (Stair and others 1972, Saif and others 1986, Saif 1990, Alenius and others 1991). BRSV replicates in the respiratory tract epithelium and can cause respiratory signs, fever and emphysema, and can lead to secondary bacterial pneumonia and death (Verhoeff and others 1984, Viuff and others 1996). In endemic areas the infections mainly affect young animals, whereas in non-endemic areas adult cattle may also be affected (Alenius and others 1991, Elvander 1996). Once these viruses are introduced into susceptible herds, within-herd transmission is generally rapid (Verhoeff and others 1984, Alenius and others 1991, Hägglund and others 2007). It has been shown that acquired antibodies remain detectable for years, even without reinfection (Alenius and others 1991, Elvander 1996), whereas maternal antibodies are detectable for only a few months. To the authors' knowledge, there is no evidence that chronic shedders are involved or that BCV and BRSV circulate within a herd for longer periods (Van der Poel and others 1993).

Little is known about the transmission routes of BCV and BRSV between cattle herds in Sweden or worldwide. Given the contagious nature of these viruses, it is important to identify factors that may increase herd-level exposure as well as between-farm spread of these viruses in order to target control or prevention measures properly. Such measures may also assist with preventing other infectious diseases from entering the herd. Previous studies, conducted in Norway and Sweden, have evaluated the association between herd-level characteristics and BCV and BRSV infections in dairy herds. The identified risk factors were similar for both viruses. Large herd size was found to be a risk factor compared with small herd size (Tråvén and others 1999, Norström and others 2000), as was artificial insemination (AI) by farm personnel compared with AI by external technicians, conventional compared with organic management, and the use of free stalls compared with tie stalls (Bidokhti and others 2009).

To the authors' knowledge, no other studies have been conducted regarding management practices as risk factors for the introduction of BCV and BRSV into dairy herds. Additionally, there have been no spatial analyses conducted regarding BCV and BRSV infections. Spatial analyses are useful for exploring mechanisms of geographical clustering and between-herd spreading of these diseases.

The aim of this study was to evaluate the relation between herd-level characteristics and management practices and antibody status of BCV and BRSV in Swedish cattle herds from April to May 2007. A

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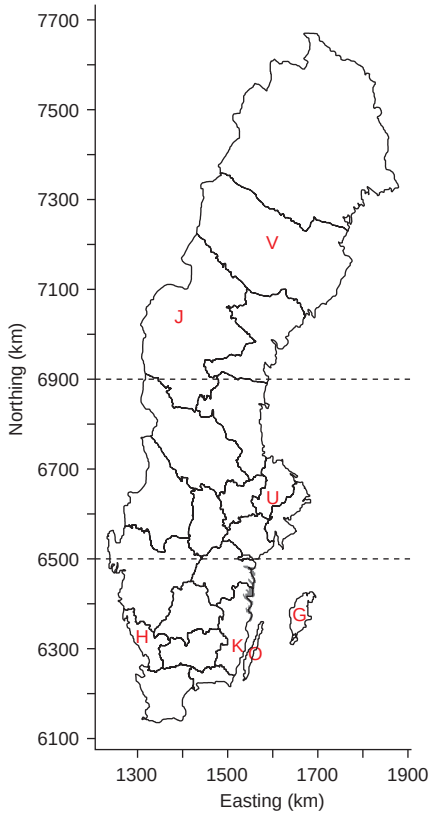

FIG 1: Map of Sweden showing the seven counties included in the study. Dashed lines delineate the northern, central and southern regions defined in the text. Counties: G Gotland, H Halland, J Jämtland, K Kalmar, O Öland, U Uppland, V Västerbotten

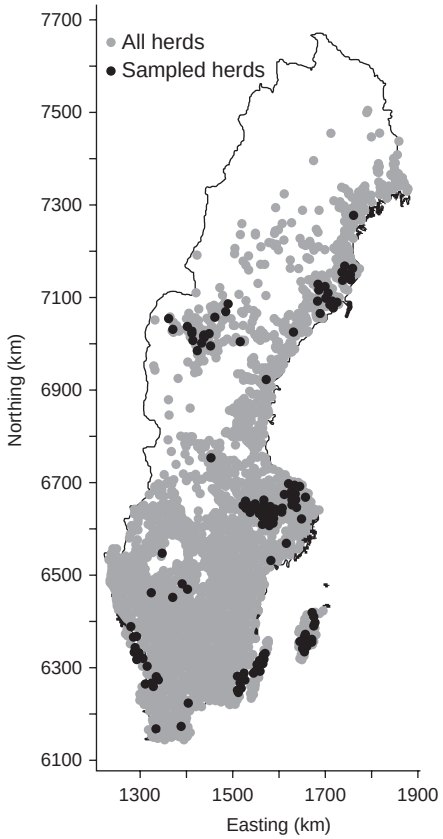

FIG 2: Map of Sweden showing the point location of the 257 study herds and the location of all 7240 dairy herds that were members of the Swedish Official Milk Recording Scheme in 2005

secondary objective was to investigate whether there were spatial patterns in the distribution of the two viruses in the study areas.

Materials and methods

Study population

This was a cross-sectional study of 280 dairy herds within seven counties in Sweden (Fig 1). The study was conducted from April 1 to May 31, 2007. Herds were eligible for inclusion in the study if they were members of the local livestock association and enrolled in the National Animal Disease Recording System (Emanuelson 1988) and the Swedish Official Milk Recording Scheme (Olsson and others 2001).

Herd selection varied by county for practical reasons. In Uppland, Kalmar and Öland the aim was to include the majority of the existing dairy herds within a selected sampling area. Herds were included if the farmers were willing to participate as they were routinely visited by personnel from the local livestock association. In Halland, Gotland, Jämtland and Västerbotten, a convenience sample of 20 herds as indicator herds was selected: 10 herds with 30 to 80 cows, and 10 herds with more than 80 cows. These herds were also sampled by personnel from the local livestock association. Finally, the authors included 16 large herds (>180 cows) that were considered as being managed by progressive farmers (farmers with very good herd management skills who were keen to apply new recommendations from veterinarians and other experts) according to the local veterinarian. These herds were distributed throughout Sweden. None of the herds had known antibody status to BCV and BRSV in advance of sampling. All herds under study were free from bovine viral diarrhoea virus, bovine leukaemia virus and bovine herpesvirus type 1 and were not vaccinated against BCV or BRSV. The point location of the 257 herds that provided data for analysis is shown in Fig 2.

Data collection

A pooled milk sample from five primiparous cows that were homebred and had calved since the start of January 2007 was obtained from each herd as described by Ohlson and others (2009). The sampling was performed by veterinarians and technicians from the local livestock association; 10 ml test tubes containing 1.5 mg of the preservative agent bronopol were used to collect the samples, which were not diluted or centrifuged and were stored at –20°C until analysis. The milk samples were analysed for the presence of IgG antibodies to BCV (Alenius and others 1991) and BRSV (Elvander and others 1995) by commercially available indirect ELISAs (SVANOVA Biotech). The same batch was used for all analyses. The optical density (OD) at 450 nm was corrected by subtraction of the negative control antigen OD. To adjust between day-to-day variations, the authors calculated the per cent positivity (PP) as: (corrected OD/positive control corrected OD) x 100. Cut-off was set to PP <20, corresponding to a corrected OD of 0.20, which is the cut-off recommended by the manufacturer for individual milk samples. Sensitivity is estimated to be 84.6 per cent for BCV and 94.6 per cent for BRSV, and specificity 100 per cent for both (individual samples). The lower sensitivity of the ELISA for BCV antibodies is due to difficulty in detecting weakly positive samples (Alenius and others 1991), that is, a risk of false-negative herds. Herds that have had an outbreak during the past two years, reflected by the primiparous cows sampled for this study, should not be false negative, however, because recently infected cows are expected to have a high antibody titre.

A questionnaire (available in Swedish from AO) was administered to farmers by veterinarians or technicians from the local livestock association during the routine farm visits when milk samples were collected. In the event that questionnaires were not administered during sampling, they were sent to the farmers by post. To facilitate spatial analysis, the X and Y coordinates of the herds were obtained from the Swedish Board of Agriculture.

Spatial analyses of point data

The spatial distribution of BCV- and BRSV-positive farms were examined for broad patterns and local dependencies in the distribution across the studied areas. The Poisson inhomogeneous K-function (Ripley 1977, Diggle and Chetwynd 1991) was used to assess statistically whether antibody-positive herds were spatially aggregated over antibody-negative herds (ie, local dependencies). The analysis was repeated for BCV and BRSV, respectively, in all herds and also separately for the herds located within the county of Uppland. Spatial aggregation at the national level might not give a true reflection of possible clustering because the sampled herds were only a small and non-

TABLE 1: Number of herds in levels of categorical variables and means of continuous variables (95 per cent confidence interval) of herd characteristics and herd routines as risk factors in 257 Swedish dairy herds that were antibody positive/negative to bovine coronavirus (BCV) or bovine respiratory syncytial virus (BRSV) in May 2007

Variable	Level	Number of herds				
		BCV-negative	BCV-positive	BRSV-negative	BRSV-positive	All herds
Abortions, past six months	Yes	20	86	18	88	106
	No	29	121	22	128	150
	If yes, number	1.4 (1.1 to 1.7)	1.9 (1.6 to 2.1)	1.6 (1.1 to 2.0)	1.8 (1.6 to 2.1)	1.8 (1.6 to 2.0)
Artificial insemination	Technicians	33	114	24	123	147
	Farm personnel	16	94	16	94	110
Biosecurity, importance (1 Not at all, 5 Very important)	1	1	0	1	0	1
	2	0	1	1	0	1
	3	5	14	3	16	19
	4	20	90	16	94	110
	5	23	102	19	106	125
Biosecurity, do visitors respect the routines of the farm? (1 Never, 5 Always)	1	0	1	0	1	1
	2	1	10	2	9	11
	3	9	32	4	37	41
	4	26	127	24	129	153
	5	13	36	10	39	49
Boots provided for visitors	No or never used	29	136	21	144	165
	Seldom used	1	13	2	12	14
	Sometimes used	5	23	5	23	28
	Often used	7	24	5	26	31
	Always used	7	11	7	11	18
Coats provided for visitors	No or never used	9	55	11	53	64
	Seldom used	2	3	1	4	5
	Sometimes used	8	21	4	25	29
	Often used	11	54	8	57	65
	Always used	19	71	16	74	90
Commercial breeders used for calves	Yes	3	7	3	7	10
	No	46	201	37	210	247
If yes, quarantine	Yes	0	0	0	0	0
	No	3	7	3	7	10
Distance to nearest cattle herd	km	2.4 (1.6 to 3.2)	1.2 (1.0 to 1.4)	2.5 (1.4 to 3.6)	1.2 (1.1 to 1.4)	1.4 (1.2 to 1.7)
Equipment shared (past two years)	Yes	27	119	26	120	146
	No	21	89	13	97	110
Herd size	Number of cows	70 (53 to 88)	82 (74 to 91)	76 (55 to 97)	81 (72 to 89)	80 (72 to 88)
Lent or borrowed animals past two years	Yes	2	13	1	14	15
	No	47	195	39	203	242
If yes, quarantine	Yes	0	1	0	1	1
	No	2	12	1	13	14
Organic management	Yes	5	6	3	8	11
	No	44	202	37	209	246
Pasture, animals can reach other herds over fence	Yes	8	83	5	86	91
	No	41	123	35	129	164
Purchased animals, past two years	Yes	21	115	17	119	136
	No	28	93	23	98	121
If yes, season	Pasture	3	16	1	18	19
	In-stable	6	27	5	28	33
	Both	10	59	11	58	69
If yes, quarantine	Yes	1	9	0	10	10
	No	48	106	40	109	126
Visitors, past two weeks (numbers)	Veterinarians	1.0 (0.7 to 1.4)	1.3 (1.1 to 1.6)	1.0 (0.6 to 1.4)	1.3 (1.1 to 1.5)	1.3 (1.1 to 1.5)
	Technicians	2.3 (1.7 to 3.0)	2.5 (2.1 to 2.9)	2.0 (1.2 to 2.7)	2.5 (2.2 to 2.9)	2.4 (2.1 to 2.8)

random part of the total population; therefore, Uppland was selected as the study herds represented approximately 85 per cent of all herds in this area. For each virus, separate K-functions were generated for positive herds (K_{pos}) and negative herds (K_{neg}). The difference between the two metrics as a function of distance was calculated as $D(s)=K_{pos}(s)-K_{neg}(s)$. The observed difference can be interpreted as a measure of the aggregation of antibody-positive herds over and above that observed for the antibody-negative herds at relatively small distances (up to 100 km). To test the hypothesis that there was no aggregation, the location of antibody-positive herds was randomly permuted (n=99 times) and the observed difference function for each permutation was calculated (Chetwynd and Diggle 1998). The upper and lower limits of the simulations were subsequently plotted to determine whether the observed difference function fell outside the limits of the permutation envelope. This would indicate significant spatial aggregation of antibody-positive herds relative to antibody-negative herds.

Risk factor analyses

The outcomes being evaluated were the presence of antibodies to BCV or BRSV in the study herds. Herds were defined as antibody-positive if the pooled milk samples obtained from primiparous cows were positive to BCV and BRSV, respectively. Previous studies show that BRSV and BCV infections are effectively spread within the herd (Verhoeff and others 1984, Alenius and others 1991, Hägglund and others 2007). A five-cow pooled milk sample therefore increases the power to discriminate antibody-positive from negative herds compared with a single sample. The predictor variables included a number of herd-level management and routine practices, listed in Table 1. Given that none of the continuous variables was linearly related to the logit of the outcome, these were categorised according to biological plausibility or equal sized groups. Herd size was divided according to quartiles: up to 38, 39 to 59, 60 to 99, and 100 or more. Distance to the nearest cattle herd in kilometres was categorised into four groups: up to 0.2 km, over 0.2 to 1 km, over 1 to 2 km, and 2 km or more. Number of visits during the past two weeks by technicians and veterinarians were grouped into three categories: one, one to three, and four or more. The two variables 'providing boots for visitors' and 'providing coats for visitors' were dichotomised as 0 No or yes but not always used, and 1 Yes and always used. Farmers' attitude regarding biosecurity and visitors respecting the biosecurity routines of the herd were scored 1 to 5 (5 Very important/always respecting) and were both grouped into three categories: 1 to 3, 4, and 5. The different study areas were allocated into three regions: south, central, and north (Table 2). Collinearity between the predictors was assessed by calculating the Spearman rank correlation, for which $r < 0.60$ was considered as little or no collinearity.

Separate logistic regression models were used to quantify the effects of predictors associated with antibody status (positive v negative) for BCV and BRSV, respectively.

$$\text{logit}(p_i)=\beta_0+\beta_1 x_{1i}+\ldots+\beta_m x_{mi}$$

In this equation, the logit of the observed probability of the i^{th} herd being antibody-positive, p_i, was modelled as a function of m herd-level predictor variables.

The association between the herd-level BCV/BRSV status and each of the predictors was first evaluated by chi-squared and Fisher's exact tests. Variables with an α level of less than 0.25 were selected for inclusion in the logistic regression model. A backward stepwise approach was used for model building, with backward elimination of non-significant variables at $P>0.05$. The presence of confounding was assessed by examining the effect of each predictor variable on the coefficient of other variables in the model by simultaneously adding and removing them into and out of the model and examining the change in the coefficients of the remaining model variables.

TABLE 2: Prevalence and 95 per cent confidence intervals (CI of herds antibody-positive to bovine coronavirus (BCV) and bovine respiratory syncytial virus (BRSV) among primiparous cows in 257 Swedish dairy herds for each sampled region

Region	County	Number of herds	BCV (95% CI)	BRSV (95% CI)
South	Halland	18	100 (NA*)	89 (74 to 100)
	Kalmar	44	91 (82 to 100)	89 (79 to 98)
	Öland	50	96 (90 to 100)	98 (94 to 100)
	Gotland	20	90 (76 to 100)	90 (76 to 100)
Central	Uppland	69	67 (56 to 78)	83 (74 to 92)
North	Jämtland	20	70 (49 to 91)	55 (33 to 77)
	Västerbotten	20	55 (32 to 77)	75 (55 to 95)
Special†		16	81 (61 to 100)	75 (53 to 97)

* Not applicable, all herds antibody positive
† Herds considered as progressive according to the local veterinarian, distributed throughout Sweden

If there was a change of 25 per cent or more the predictor variable was included in the final model. Interactions between the variables included in the model process were also evaluated, but no evidence of interactions was found. Statistical significance of the model was assessed using the Hosmer-Lemeshow goodness-of-fit test and a likelihood ratio test at a significance level of $P<0.05$. Plots of Pearson residuals versus the predicted values were constructed and evaluated for eventual outliers.

The residuals of the two final models were tested for first- and second-order spatial patterns using Moran's *I* statistic (Moran 1950) and plots of semi-variograms (Isaacs and Srivistava 1989).

Moran's *I* statistic was used to determine whether there was evidence for dependency (autocorrelation) in the residuals between neighbouring herds. First-order neighbours were the farms that shared a common border with a farm of interest. Second-order neighbours were those of the immediate neighbours of a farm of interest. Moran's *I* was computed using first- to eighth-order neighbourhood definitions. The relationship between each pair of herds was defined as 1 Neighbours and 0 Otherwise. In the presence of positive spatial autocorrelation in model residuals the computed Moran's *I* statistic will be close to 1 and in its absence its value will be close to zero. The statistical significance of the observed Moran's *I* statistic was assessed using a Monte Carlo permutation approach in which all the residual values from the final model were randomly assigned to each herd and Moran's *I* calculated on each occasion. The observed Moran's *I* statistic was then ranked among the simulated values. If the observed statistic ranked kth among the 999 simulated values the one-sided significance level was k/999. A Moran scatter plot (Anselin 1995) provided a visual assessment of spatial autocorrelation among herds. Since the distance between farms varied throughout the studied areas, it was the authors' opinion that neighbourhood definitions based on adjacency (rather than distance) provided a measure of spatial dependence consistent across the entire study area.

Semi-variograms were produced for the herds located in Uppland because the study herds represented approximately 85 per cent of the existing dairy herds in the restricted sampling area. This plots the semi-variance as a function of distance in kilometres between pairs of herds (Isaacs and Srivistava 1989). If herds with more similar residuals were closer in space than those with less similar residuals, the semi-variance would be expected to increase as a function of distance. This would indicate the distances at which the residuals were no longer correlated. Significance was assessed using Monte Carlo permutation tests. If autocorrelation was present in the model residuals, this would indicate the presence of local factors involved in the dynamics of herd-level antibody status to BCV and BRSV, which is not accounted for by the variables included in the model.

The data analyses were either conducted in the R statistical package (R Development Core Team 2008) or in Intercooled Stata (Stata Statistical Software Release 10.0; StataCorp).

TABLE 3: Chi-squared and Fisher's exact statistic for the association between herd-level antibody status to bovine coronavirus (BCV) and bovine respiratory syncytial virus (BRSV) and each of the variables considered for inclusion in a logistic regression model

Exposure variable	Description	Number of farms	Positive farms (%)	P
BCV				
Artificial insemination*	0: by external technicians	147	77.6	0.10
	1: by farm personnel	110	85.5	
Boots provided for visitors†	0: no or yes but not always used	238	82.4	0.034
	1: yes and always used	18	61.1	
Distance to nearest cattle herd†	1: ≤0.2 km	51	94.1	0.002
	2: >0.2 to 1 km	107	83.2	
	3: >1 to 2 km	56	71.4	
	4: >2 km	36	66.7	
Herd size†	1: ≤38 cows	60	66.7	0.029
	2: 39 to 59 cows	62	83.9	
	3: 60 to 99 cows	65	86.2	
	4: ≥100 cows	66	84.9	
Management†	0: conventional	246	82.1	0.038
	1: organic	11	54.5	
Pasture†	0: cannot reach other herds	164	75	0.001
	1: can reach other herds	91	91.2	
Region†	1: south	140	94.3	<0.001
	2: central	73	67.0	
	3: north	44	63.6	
BRSV				
Boots provided for visitors†	0: no or yes but not always used	238	86.1	0.011
	1: yes and always used	18	61.1	
Commercial breeding†	0: no	10	70.0	0.19
	1: yes	247	85.4	
Equipment*	0: do not share with other herds	110	88.2	0.19
	1: share with other herds	146	82.2	
Herd size†	1: ≤38 cows	60	76.7	0.26
	2: 39 to 59 cows	62	87.1	
	3: 60 to 99 cows	65	89.2	
	4: ≥100 cows	66	83.3	
Pasture†	0: cannot reach other herds	164	78.7	0.001
	1: can reach other herds	91	94.5	
Region*	1: south	140	92.9	<0.001
	2: central	74	81.1	
	3: north	44	63.6	
Veterinary visits past two weeks†	1: 0 visits	88	78.4	0.13
	2: 1 to 2 visits	85	89.4	
	3: ≥3 visits	83	85.5	

* Chi-squared test
† Fisher's exact test

Results

Two hundred and fifty-seven of the 280 herds included provided data for analysis. Incomplete questionnaires were obtained from some farms, which resulted in data from 245 and 253 herds being available for analysis for the BCV and BRSV models, respectively. Twenty-one of the questionnaires were done by phone interview. All 257 farm coordinates were available for the spatial analyses. The overall prevalence of antibody-positive herds for the period April to May 2007 was 81 per cent (95 per cent confidence interval [CI] 76 to 86 per cent) for BCV and 84.5 per cent (95 per cent CI 80 to 89 per cent) for BRSV. Prevalence by region with 95 per cent CI is shown in Table 2. There was a significant ($P<0.001$) positive correlation between antibody status to BCV and antibody status to BRSV among the 257 herds included: 18 herds were antibody negative to both BCV and BRSV; 186 herds were antibody positive to both BCV and BRSV; 22 herds were positive to BCV but negative to BRSV; and 31 herds were negative to BCV but positive to BRSV.

TABLE 4: Regression coefficients with standard errors (se), odds ratios (OR) and 95 per cent confidence intervals (CI) of OR as estimated in logistic regression models evaluating herd-level risk factors for being antibody-positive to bovine coronavirus (BCV) and bovine respiratory syncytial virus (BRSV) in Swedish dairy herds in 2007

Variable	Level	Coefficient	(se)	P	OR (95% CI)
BCV model					
Boots for visitors	0: no	ref		ref	1
	1: yes	−0.27	(0.11)	0.073	0.76 (0.56-1.03)
Distance to nearest cattle herd	1:≤0.2 km	ref		ref	1
	2: >0.2 to 1 km	−1.25	(0.15)	0.074	0.23 (0.07-1.13)
	3: >1 to 2 km	−1.94	(0.08)	0.008	0.14 (0.03-0.60)
	4: >2 km	−2.19	(0.06)	0.004	0.11 (0.02-0.50)
Herd size	1: ≤38	ref		ref	1
	2: 39 to 59	0.84	(1.65)	0.10	2.31 (0.84-6.29)
	3: 60 to 99	1.47	(2.67)	0.01	4.36 (1.41-13.44)
	4: ≥100	1.14	(1.81)	0.039	3.12 (0.85-6.29)
Region	1: south	ref		ref	1
	2: central	−1.63	(0.12)	0.001	0.19 (0.08-0.49)
	3: north	−2.30	(0.05)	<0.001	0.10 (0.37-0.28)
BRSV model					
Boots for visitors	0: no	ref		ref	1
	1: yes	−0.40	(0.14)	0.006	0.67 (0.51-0.89)
Herd size	1: ≤38	ref		ref	1
	2: 39 to 59	0.75	(0.54)	0.16	2.12 (0.74-6.09)
	3: 60 to 99	1.14	(0.53)	0.044	3.13 (1.03-9.52)
	4: ≥100	0.50	(0.47)	0.33	1.65 (0.60-4.55)
Region	1: south	ref		ref	1
	2: central	−0.81	(0.49)	0.094	0.45 (0.17-1.15)
	3: north	−2.02	(0.47)	< 0.001	0.13 (0.05-0.33)
Herd size	1: ≤38	ref		ref	1
	2: 39 to 59	0.75	(0.54)	0.16	2.12 (0.74-6.09)
	3: 60 to 99	1.14	(0.53)	0.044	3.13 (1.03-9.52)
	4: ≥100	0.50	(0.47)	0.33	1.65 (0.60-4.55)

ref Reference

K-functions for all study herds and herds within the Uppland region showed that antibody-positive farms were not spatially aggregated up to a distance of 100 km, indicating that local spread may not be an important factor for the transmission of the two viruses across the study herds within the Uppland region.

The results from the univariable screening for each of the predictor variables eligible for inclusion in the logistic regression models are shown in Table 3. Seven variables were available for BCV and seven for BRSV, with region, herd size and 'providing boots for visitors' being predictors common for both diseases. For herd size, P=0.26 for BRSV in the univariate analysis, but was kept for model building because it could be an important confounder. The odds ratios (ORs) were similar for the two logistic regression models (Table 4). Farms in northern Sweden were much less likely to be seropositive than those in the south (OR 0.10 and 0.13 for BCV and BRSV, respectively). Independent of region, the OR increased with herd size except for herds with more than 100 cows (upper quartile), which had slightly lower OR compared with the herds with 66 to 99 cows (third quartile). Providing boots for visitors was a protective factor that reduced the OR for BRSV to 0.67 compared with not providing boots, and for BCV the OR was decreased to 0.76 (P=0.07). Despite P=0.07, the authors chose to keep this biologically important variable in the model. The OR for BCV reduced as the distance to the nearest cattle herd increased.

The models showed good fit according to the Hosmer-Lemeshow goodness-of-fit test with chi-squared (df 8)=4.79, P=0.78 and chi-squared (df 6)=1.77, P=0.94 for BCV and BRSV, respectively. Pseudo R^2 was 23.7 per cent for BCV and 14 per cent for BRSV. Plots of Pearson residuals versus the predicted values showed no evidence of outliers.

Moran's *I* statistic for the model residuals was −0.7845 (P=0.78) and −0.048 (P=0.87) for BCV and BRSV, respectively, indicating that there was little or no spatial autocorrelation in the residuals of both diseases within the studied areas. The semi-variograms also confirmed the lack of spatial dependency in model residuals for the Uppland area, indicating a lack of evidence for spatial dependence. The authors repeated Moran's *I* statistic and semi-variogram using residuals from the BCV model after excluding the variable 'distance to nearest cattle herd' from the regression model. The result remained unchanged, demonstrating that distance had not accounted for a second-order effect in the spatial pattern for BCV.

Discussion

Region was strongly associated with antibody positivity to BCV and BRSV, with decreasing OR moving from south to north (Table 4). This agrees with previous nationwide prevalence surveys of BCV and BRSV (Elvander 1996, Tråvén and others 1999). The geographical differences might be because of a greater trade of animals between herds and/or higher animal density in the southern parts of Sweden, but other explanations may also be possible. The results from the semi-variograms show, however, that there was no evidence of spatial dependency (second-order) up to 100 km. This means that the unexplained variance, that is, the model residuals, is not due to factors that are spatially correlated.

Providing boots for visitors was found to be a protective factor for both BCV and BRSV antibody status. This was not surprising, as biosecurity is the key to preventing contagious diseases. The fact that the provision of protective coats for visitors was not significantly associated with antibody status for any of the infections can be explained by the fact that most Swedish veterinarians and technicians bring a change of coat for each herd visit, whereas boots are only cleaned. Herd size was also significant for both BCV and BRSV, with increasing risk as herd size increased. This may be explained by a higher frequency of visitors in larger herds than in the very small herds; the slightly lower prevalence in herds with more than 100 cows versus herds with 66 to 99 cows may simply be due to sampling variation in the high-risk herds, as prevalence approached 100 per cent in large herds. Another possible explanation may be more thorough implementation of biosecurity measures in the very large herds. Previous studies reported either positive (Tråvén and others 1999, Norström and others 2000) or no (Bidokhti and others 2009) correlations between herd size and BCV and BRSV status.

The similarity of the two final models is biologically plausible because BCV and BRSV are both highly contagious diseases and there was also a significant positive relationship between antibody status to BCV and BRSV. The additional significant factor for BCV, 'distance to nearest cattle herd', was not significant for BRSV. This could be explained by the fact that BCV is shed via faeces, which might be more easily spread between herds than nasal discharge, the primary means of transmission for BRSV (Clark 1993, Van der Poel and others 1994).

The results from analysing point data showed no evidence that antibody-positive herds were spatially aggregated over and above negative herds, either for BCV or BRSV. This result seems to contradict 'distance to nearest cattle herd' for BCV. In the logistic model, however, the variable 'distance to nearest cattle herd' is the distance to the closest herd, for which the antibody status is unknown (it could be a herd not included in the study, for example, a beef herd) whereas the K-function is based on the antibody status of the neighbours and includes up to the eighth neighbour of the study herd. Also, all herds under study were included in the logistic model, whereas the spatial analysis of point data included only herds from the county of Uppland. The absence of spatial autocorrelation regarding both BCV and BRSV in the Uppland area is an important finding, showing that local spread may not have a great effect on the herd-to-herd transmission of these viruses in areas with moderate

animal density. The authors believe that contacts through networks (for example, visitors, purchased replacements) could be more important transmission routes for BCV and BRSV in this area. A short distance to the nearest cattle herd (less than 200 m) may be related to other, unknown, factors. More research is needed, perhaps directed at the contact networks between herds, in order to draw further conclusions from this finding.

A potential weakness of the study is the sampling strategy, because the herds included were randomly selected. For Uppland, Kalmar and Öland this should not affect the results because the majority of the existing herds within limited areas were included. In the other counties the selection could lead to a bias, but because the antibody status of the herds was unknown before sampling and the selection was not based on management skills, the authors do not regard this as a major concern. The 16 herds selected to go into the special group of large herds, however, could be seriously biased by selection, but when running the model without these herds the results remained unchanged. There was a risk of information bias because farmers had to recall events from the past two years. However, most data were collected by interview and this probably reduced such a bias to an acceptable level. One strength of this study was the high participation rate: 92 per cent of the selected herds provided data, and only 5 per cent and 2 per cent of the herds were excluded from the final model because of missing values for BCV and BRSV, respectively.

The authors found only one management factor, providing boots for visitors, to be associated with antibody status to BCV and BRSV. The organic herds did not have a lower prevalence of antibodies to BCV and BRSV compared with conventional herds, as was found to be the case in the study by Bidokhti and others (2009). The present study included only 11 organic herds, whereas 20 of the 40 herds included in the study of Bidokhti and others (2009) were organic, and the power of the present study to identify differences was consequently lower. The absence of other significant management factors indicates that either non-infected farms were not managed differently from infected farms, or the questionnaire did not include the most critical factors for BRSV and BCV seropositivity. That the negative herds managed to stay negative, although being surrounded by positive herds, shows that it was possible to avoid infection despite the presence of virus in the area. Further investigations to elucidate transmission mechanisms, targeting the central and northern parts of Sweden, would be an interesting area for future research.

In conclusion, biosecurity measures in the form of providing boots for visitors were associated with a lower herd prevalence of both BCV and BRSV in Swedish dairy farms. The prevalence was higher in farms located in the southern part of the country than in central or northern areas, and increased with herd size. Whereas a short distance to the nearest cattle herd increased the probability of seropositivity to BCV, spatial analysis, considering the distance between farms as well as the infection status of each farm, did not provide any evidence that geographical proximity increased the risk of infection with either BCV or BRSV. This suggests that local factors such as daily visiting milk trucks, wild animals or airborne transmission were unlikely to be important sources of infection.

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Notes