Nationwide survey of antibodies to bovine coronavirus in bulk milk from Swedish dairy herds

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Bulk milk samples from 2236 dairy herds randomly selected throughout Sweden in proportion to region and herd size were analysed for antibodies to bovine coronavirus (BCV) in an ELISA. The results were expressed as optical density (OD) values and an OD>0·04 was considered positive. Eighty-nine per cent of the samples were antibody-positive and 52 per cent had high levels of antibodies to BCV (an OD>0·70). There were significantly higher OD values (P<0·001) and fewer antibody-negative samples (P<0·001) from larger herds than from smaller herds. There were also significantly higher OD values and fewer antibody-negative samples from herds in southern Sweden than from herds in northern Sweden (P<0·001 and P<0·001, respectively). These results indicate a higher frequency of BCV infections in larger herds and in herds in southern Sweden.

BOVINE coronavirus (BCV) has been shown to be involved in several disease syndromes: winter dysentery of adult cattle (Saif 1990, Alenius and others 1991), calf diarrhoea (Stair and others 1972, Mebus and others 1973) and calf respiratory disease (Thomas and others 1982, McNulty and others 1984, Möstl and Bürki 1987). In studies of winter dysentery diagnosed by farmers an incidence of 28·5 per cent was recorded in central Sweden in one year (Tråvén and others 1993) and an incidence of 33 per cent was recorded during nine months in an area on the east coast of the USA (White and others 1989). The seroprevalence of BCV in Swedish heifers was 61 per cent (Alenius and others 1991). In more limited surveys of adult cattle a seroprevalence of 61 per cent was recorded in Germany (Storz and Rott 1980) and 93 per cent in Switzerland (Battaglia and others 1986). However, no nationwide surveys for BCV antibodies have been reported.

Winter dysentery is economically important because of the marked reduction in milk yield that often occurs in affected herds (Roberts 1957, Campbell and Cookingham 1978). The aim of this study was, therefore, to estimate the infection rate of Swedish dairy cattle with BCV by measuring the level of antibodies in bulk milk.

MATERIALS AND METHODS

Sampling procedures

All Swedish dairy herds delivering milk to dairies (about 24,000 in 1991) are listed in the database of the Swedish Association for Livestock Breeding and Production (SALBP). The herds were grouped into 17 geographical regions and divided into three herd size groups on the basis of annual milk delivery: small herds producing less than 50,000 kg, medium size herds producing 50,000 to 199,000 kg, and large herds producing 200,000 kg or more. Ten per cent of the herds in each size group were randomly selected from each of the regions. The data processing for the selection procedure was done by Farmdata at the SALBP in Hållsta. Bulk milk samples from the 2408 randomly selected herds were then requested from the local milk classification laboratories, and 2236 samples were obtained for BCV antibody analysis, a 92·9 per cent participation rate.

Antibody detection

IgG antibodies to BCV were detected in an indirect ELISA (Alenius and others 1991). The bulk milk samples were analysed undiluted in duplicate wells. Samples with a mean optical density (OD) at 450 nm of more than 0·04 were considered positive.

Statistical analyses

The distribution of OD values among the three herd size groups and four major geographical areas were compared by Friedman's non-parametric two-way analysis of variance. An examination of the residuals showed that the data did not meet the requirement for a parametric analysis of variance with equal variance in each group whatever transformation was applied. The major areas were derived by grouping the 17 regions from south to north (Table 1, Fig 1). Logistic regression was used specifically to evaluate the distribution of antibody-negative herds (Table 2) and the participation rates of herds of different sizes in the four major areas. An examination of the residuals showed that these two data sets did satisfy the requirement for logistic regression with a normal distribution of the residuals. The approximate Wilk-Shapiro normality statistic was 0·97 for the analysis of the distribution of antibody-negative herds, and 0·90 for the participation rates of herds.

RESULTS

Of the 2236 bulk milk samples, 1994 were BCV-antibody positive, giving a prevalence of 89·2 per cent. A majority of the samples (51·7 per cent) had an OD over 0·70 (Fig 2). Of the total, 18·5 per cent were small herds, 63·0 per cent were medium size herds, and 18·5 per cent were large herds (Table 1). There was a significant difference between the OD values of the herds of different sizes (P<0·001). Adjusted mean ranks in the Friedman's analysis were 232, 288 and 307 for the small, medium and large herds, respectively. Thus, the lowest OD values were among the small herds and the highest were among the large herds. There was also a significant difference between the proportions of antibody-negative herds among the herds of different sizes (P<0·001, Table 2), with the small herds having a higher proportion of antibody-negative herds.

The prevalence of antibody in each region is shown in Fig 1. There was a significant geographical difference in the distribution of OD values between the four major areas (P<0·001), with higher OD values in the southern areas than in the northern areas. The adjusted mean ranks for areas A, B, C and D were 294, 407, 243 and 159, respectively. There was also a significant difference between the frequency of antibody-negative herds in the major areas (P<0·001). The proportions of antibody-negative herds were 6·0 per cent in area A, 6·5 per cent in area B, 15·5 per cent in area C, and 22·2 per cent in area D.

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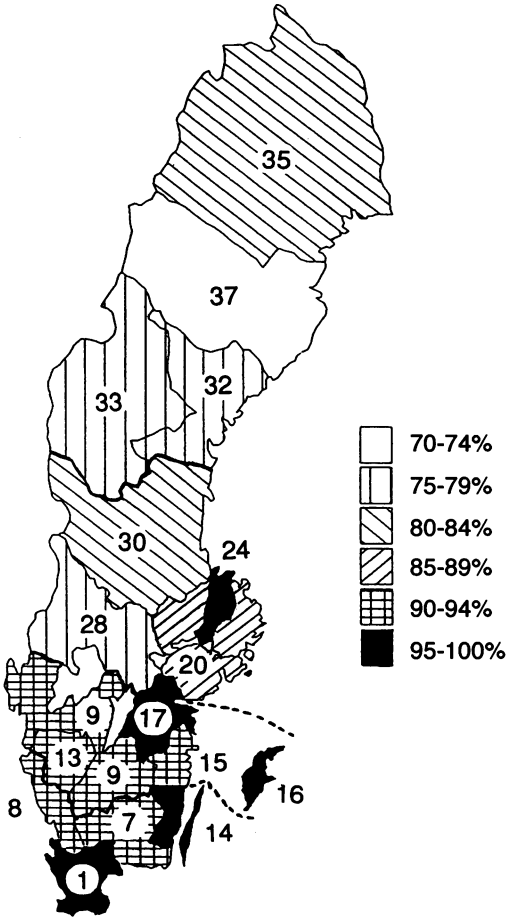

FIG 1: Prevalence of bovine coronavirus antibody-positive dairy herds observed by screening bulk milk samples from 2236 farms in 17 regions of Sweden, indicated by the numbers. The herds sampled represented 10 per cent of the dairy herds in the country. Bold and dotted lines show the grouping of regions into four major areas

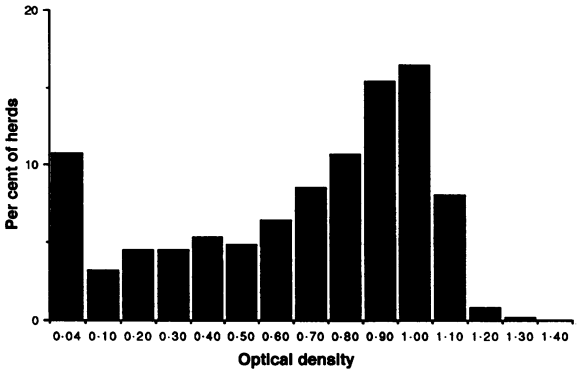

FIG 2: Distribution of antibodies to bovine coronavirus in bulk milk from 2236 Swedish dairy herds. Figures on the X-axis indicate the upper limit of the optical density (OD) interval represented by each bar; OD values >0·04 are regarded as positive

The participation rates of the herds of different sizes are shown in Table 1. There was a significantly lower participation ($P<0·001$) among the small herds than among the larger herds, 87·2 per cent, 94·4 per cent and 94·1 per cent, respectively, for the small, medium and large herds, but there was no significant difference in participation rate between the four major areas, 93·2 per cent, 93·0 per cent, 94·1 per cent and 90·1 per cent, respectively, in areas A, B, C and D.

DISCUSSION

The prevalence of BCV antibodies in bulk milk from the 2236 herds in this study was 89·2 per cent, and corresponded with the high incidence of winter dysentery recorded by Tråvén and others (1993). There was a higher prevalence of BCV antibodies ($P<0·001$) and higher OD levels ($P<0·001$) among the larger herds than among the smaller herds, indicating that the larger herds had a higher infection rate; winter dysentery is more common among large herds than smaller herds (White and others 1989), probably as a result of the greater number of contacts with potential virus carriers or vectors (human beings, vehicles or equipment). In the study by White and others (1989) large herds had more than 60 cows, whereas in this study 'large herds' had more than approximately 30 milking cows.

There was a higher prevalence of BCV antibodies and higher OD levels in the southern than in the northern parts of Sweden ($P<0·001$ and $P<0·001$, respectively), indicating that there was a higher infection rate in southern Sweden. One possible reason for the lower infection rate in the northern parts of Sweden is the much larger distances between herds, as indicated approximately by the numbers of herds sampled

TABLE 1: Percentage distribution of small, medium and large herds and their (percentage) participation in the survey of antibodies to bovine coronavirus in bulk milk samples from dairy herds in 17 regions of Sweden

Region (Fig 1)	Small	Medium	Large	Total number	Area
1 Skåne	13·6 (81·6)	63·2 (96·0)	23·2 (98·1)	228 (94·7)	A
7 Blekinge Kronob	19·9 (84·4)	66·9 (96·8)	13·2 (94·7)	136 (93·8)	(S. Götaland)
8 Halland	15·6 (87·5)	65·2 (96·7)	19·3 (83·9)	135 (92·5)	
14 Kalmar	11·5 (92·3)	63·5 (93·0)	25·0 (86·7)	104 (91·2)	
9 Skara	18·5 (86·4)	63·9 (94·5)	17·6 (94·4)	482 (92·9)	B
13 S. Älvsborg	23·3 (87·5)	63·3 (89·1)	13·3 (100·0)	90 (90·0)	(N. Götaland)
15 Tjustbygden	10·5 (75·0)	66·7 (97·4)	22·8 (100·0)	57 (95·0)	
16 Gotland	14·5 (92·3)	71·1 (98·3)	14·5 (100·0)	83 (97·6)	
17 Östergötland	7·1 (80·0)	63·7 (90·0)	29·2 (100·0)	113 (91·9)	
20 Malmen	8·9 (93·3)	63·3 (96·2)	27·8 (93·6)	158 (95·2)	C
24 Uppsala	15·3 (100·0)	66·1 (92·9)	18·6 (84·6)	59 (92·2)	(Svealand)
28 Örebro Värml	23·1 (91·2)	57·5 (93·9)	19·4 (96·3)	134 (93·7)	
30 Dala Gävleborg	26·0 (97·4)	58·2 (91·4)	15·8 (100·0)	146 (94·2)	
32 Ångermanland	40·3 (92·6)	54·8 (97·1)	4·8 (75·0)	62 (93·9)	D
33 NNP	28·7 (84·4)	60·6 (98·3)	10·6 (100·0)	94 (94·0)	(Norrland)
35 NLP	23·4 (68·8)	59·6 (82·4)	17·0 (72·7)	47 (77·0)	
37 Västerbotten	29·6 (84·2)	61·1 (94·3)	9·3 (100·0)	108 (91·5)	
All regions	18·5 (87·2)	63·0 (94·4)	18·5 (94·1)	2236 (92·9)	

TABLE 2: Antibodies to bovine coronavirus in bulk milk from 2236 randomly selected Swedish dairy herds. Herds grouped by herd size and antibody status shown as optical density (OD) of undiluted samples in an indirect ELISA

Herd size (yearly milk output)	Number of herds (%) in OD category 0·0-0·040	0·041-0·700	0·701-1·400	Total number of herds
Small (≤49,000 kg)	106 (25·6)	150 (36·2)	158 (38·1)	414
Medium (50,000–199,000 kg)	128 (9·1)	524 (37·2)	757 (53·7)	1409
Large (≥200,000 kg)	8 (1·9)	163 (39·5)	242 (58·6)	413

in each region (Table 1) compared with the areas on the map (Fig 1). Long distances reduce the risk of spread of the virus between herds through local contacts with infected cattle or vectors. The statistical analysis revealed no geographical differences in the participation rate of herds in the different areas corrected for herdsize, but the prevalence of antibody might have been overestimated marginally owing to the significantly lower participation rate among the small herds ($P<0.001$).

The results show that BCV antibodies are present in a large proportion of the Swedish dairy cow population. The prevalence is higher among larger herds and higher among herds in the southern part of the country.

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References

ALENIUS, S., NISKANEN, R., JUNTTI, N. & LARSSON, B. (1991) Bovine coronavirus as the causative agent of winter dysentery: serological evidence. *Acta Veterinaria Scandinavica* **32**, 163-170

BATTAGLIA, M., LUTZ, H. & WYLER, R. (1986) Serologische Übersichtsuntersuchung über die Verbreitung des bovinen Coronavirus in der Schweiz. *Schweizer Archiv für Tierheilkunde* **128**, 213-218

CAMPBELL, S. G. & COOKINGHAM, C. A. (1978) The enigma of winter dysentery. *Cornell Veterinarian* **68**, 423-441

MCNULTY, M. S., BRYSON, D. G., ALLAN, G. M. & LOGAN, E. F. (1984) Coronavirus infection of the bovine respiratory tract. *Veterinary Microbiology* **9**, 425-434

MEBUS, C. A., STAIR, E. L., RHODES, M. B. & TWIEHAUS, M. J. (1973) Pathology of neonatal calf diarrhea induced by a coronavirus-like agent. *Veterinary Pathology* **10**, 45-64

MÖSTL, K. V. & BÜRKI, F. (1987) Ursächliche Beteiligung boviner Coronaviren an respiratorischen Krankheitsausbrüchen bei Kälbern und pathologisch-immunologischer Überlegungen hierzu. *Deutsche Tierärztliche Wochenschrift* **95**, 19-22

ROBERTS, S. J. (1957) Winter dysentery in dairy cattle. *Cornell Veterinarian* **47**, 372-388

SAIF, L. J. (1990) A review of evidence implicating bovine coronavirus in the etiology of winter dysentery in cows: an enigma resolved? *Cornell Veterinarian* **80**, 303-311

STAIR, E. L., RHODES, M. B., WHITE, R. G. & MEBUS, C. A. (1972) Neonatal calf diarrhea: purification and electron microscopy of a coronavirus-like agent. *American Journal of Veterinary Research* **33**, 1147-1156

STORZ, J. V. & ROTT, R. (1980) Über die Verbreitung der Coronavirusinfektion bei Rindern in ausgewählten Gebieten Deutschlands: Antikörpernachweis durch Mikroimmundiffusion und Neutralisation. *Deutsche Tierärtzliche Wochenschrift* **87**, 252-254

THOMAS, L. H., GOURLAY, R. N., STOTT, E. J., HOWARD, C. J. & BRIDGER, J. C. (1982) A search for new microorganisms in calf pneumonia by the inoculation of gnotobiotic calves. *Research in Veterinary Science* **33**, 170-182

TRÅVÉN, M., SUNDBERG, J., LARSSON, B. & NISKANEN, R. (1993) Winter dysentery diagnosed by farmers in dairy herds in central Sweden: incidence, clinical signs and protective immunity. *Veterinary Record* **133**, 315-318

WHITE, M. E., SCHUKKEN, Y. H. & TANKSLEY, B. (1989) Space-time clustering of, and risk factors for, farmer-diagnosed winter dysentery in dairy cattle. *Canadian Veterinary Journal* **30**, 948-951

Hypocalcaemia in 23 ataxic/recumbent ewes: clinical signs and likelihood ratios

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Twenty-three ewes in a flock of 2000 were identified as having acute onset ataxia and/or having become recumbent in late pregnancy and early lactation. The presence or absence of 15 clinical signs were recorded. Thirteen of the ewes (57 per cent) were hypocalcaemic and 10 (43 per cent) were normocalcaemic. In the hypocalcaemic group, loss of anal reflex, constipation, tachycardia, hyposensitivity, ruminal stasis, ruminal tympany, salivation and tachypnoea were recorded in 50 per cent or more of the cases. In the normocalcaemic group, tachycardia, tachypnoea and ataxia were recorded in 50 per cent or more of the cases. Constipation, ruminal stasis, salivation and hyposensitivity had likelihood ratios of 3 and above for being associated with hypocalcaemia. Ruminal stasis and hyposensitivity had the likelihood ratios of 0·10 and 0·11 respectively for not being associated with hypocalcaemia.

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HYPOCALCAEMIA is an economically important, common and sometimes fatal disease of adult ewes during late pregnancy and early lactation. The diagnosis of hypocalcaemia is confounded by a number of differential diagnoses which are also associated with acute – onset, ataxia and/or recumbency, including pregnancy toxaemia, hypomagnesaemia, toxic endometritis, acute mastitis, listeriosis and scrapie. Biochemical analysers which can be used in the field for measuring serum calcium levels are expensive and are not usually available. A presumptive diagnosis of hypocalcaemia

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Notes