

Bovine Coronavirus as the Causative Agent of Winter Dysentery: Serological Evidence

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Alenius, S., R. Niskanen, N. Juntti and B. Larsson: Bovine coronavirus as the causative agent of winter dysentery: Serological evidence. Acta vet. scand. 1991, 32, 163–170. – Sera from 9 dairy herds with epizootic enteritis (winter dysentery) were examined for antibodies to bovine coronavirus (BCV) and bovine virus diarrhoea virus (BVDV). Cows in 8 of the 9 herds seroconverted to BCV alone, while the animals in the ninth herd, which showed severe symptoms of the disease, seroconverted both to BCV and BVDV. The BCV antibodies, which were present in high titres 1 year postinfection, were transferred to the offspring via the colostrum and were then detectable in sera of calves until these were approximately 5 months old.

A serological survey of 549 Swedish heifers showed that 61 % of the animals were reactors to BCV. The prevalence of seroreactors to BCV was equally distributed over Sweden but was commonly either high or low in herds.

In conclusion, BCV is commonly detected in animals suffering from winter dysentery. A co-infection with BVDV appears to aggravate the disease.

bovine virus diarrhoea virus; indirect ELISA; antibodies.

Introduction

Winter dysentery or epizootic enteritis was first described by *Hedström & Isaksson* (1951) in Swedish dairy herds. The disease still causes economic losses not only in Sweden but in many other countries as well. Winter dysentery is mainly characterized by severe diarrhoea and decreased milk production. The disease is highly contagious and occur predominantly in the adult cattle population. The mortality rate is low and an individual cow commonly recovers within a few days, although its milk production may not reach the preinfection level (for a review, see *Fox* 1980).

The causative agent of winter dysentery is still not fully known. Infection with bovine virus diarrhoea virus (BVDV) was initially described as a disease with a high morbidity rate, causing severe diarrhoea and coughing

(*Olafson et al.* 1946). However, *Takahashi et al.* (1983) found no significant increase in antibody titre to BVDV in cattle with winter dysentery. *Campylobacter* spp. have been isolated from outbreaks of winter dysentery and were considered as aetiological agents (*Jones & Little* 1931). The rapid spread of the disease resembles more a virus infection. During the last decade bovine coronavirus (BCV) has been detected in faeces from cows with winter dysentery and has also been serologically related to this disease (*Takahashi et al.* 1980, *Espinasse et al.* 1982, *Takahashi et al.* 1983, *Saif et al.* 1988). Bovine coronavirus occurs with both enteric and respiratory strains but, apparently, as a single serotype (*Reynolds et al.* 1985, *El-Ghorr et al.* 1989). This circumstance facilitates serodiagnosis of BCV infection in winter dysentery.

The aims of the present study were (a) to serologically relate BCV and BVDV to outbreaks of winter dysentery, (b) to examine the titre of antibody to the agent during 1 year after infection and determine whether antibodies to BCV are transferred to the offspring via the colostrum, and (c) to examine the distribution of seroreactors to BCV in Sweden.

Materials and methods

Dairy herds and blood samples

During the winter and spring of 1986/87, outbreaks of winter dysentery occurred in a large number of dairy herds in the central and northern parts of Sweden. Nine of the dairy herds (no. 1 to 9) with winter dysentery were serologically examined. All cows in these herds were affected and the main symptoms were watery to haemorrhagic diarrhoea and a decreased milk production. The cows in herds no. 3 to 9 also showed moderate to severe respiratory symptoms. Only a few animals succumbed to the disease or had to be emergency slaughtered. The body temperature was elevated in the few animals examined before they showed any symptoms, but was commonly normal during the phase of diarrhoea. Calves were usually asymptomatic but the calves in herd no. 2 had mild to severe diarrhoea and 1 calf succumbed to the disease.

Four to 7 cows per herd were bled in the acute phase of diarrhoea and 3 to 4 weeks later during the convalescence period. A third sample was collected from the animals approximately 1 year after the episode of diarrhoea. In addition, 6 calves born within 5 to 8 months after the disease in herd no. 1 were bled before they received colostrum and then at 4 to 6 days, and 1, 2, 3, 4, 5, 6 and 7 months of age.

To estimate the prevalence of seroreactors to BCV, blood samples were collected from

549 heifers in 94 farms in 11 counties in different parts of Sweden. The herds and the cattle were selected by the local health veterinarians and the number of animals tested per herd varied between 1 and 14. From these 94 farms 58 herds with 5 to 14 tested animals were selected in order to evaluate the herd immunity. Herd selection was done by purposive and by convenience sampling. The blood samples were collected in vacutainer tubes and transported to the laboratory at ambient temperature. Serum was removed after centrifugation and stored at -20°C until analysis.

Virus and assays

The BCV was represented by the cytopathic strain Munich W 270/83, and the BVDV by the Danish cytopathic strain Ug59 (Borgen 1963). An indirect enzyme-linked immunosorbent assay (ELISA) was developed for detection of antibodies to BCV. Initially, the results of the ELISA were compared with those of a virus neutralization test (VNT). For detection of antibodies to BVDV, only ELISA has been used.

Preparation of BCV antigen for ELISA

The coating antigen was prepared as follows: Primary embryonic bovine lung (BL) cells were grown in Eagle's minimum essential medium (MEM) supplemented with 5% foetal calf serum (FCS) tested to be free from detectable IgG antibodies, as previously described by Juntti et al. (1987). The BL cells were grown at 37°C to confluence in 120-cm² bottles, the medium was removed and the cell monolayers were washed twice with Eagle's MEM (without FCS). Each bottle was inoculated with 5 ml of the BCV suspension, diluted 1:100 and containing 100 TCID₅₀ per 0.1 ml. After absorption for 1 h at 37°C , 40 ml of Eagle's MEM with 2% of IgG-free FCS were added. After 3 to 4

days of incubation when 90 to 95 % of the cells were destroyed, the bottles were frozen at -70°C . BCV antigen was prepared by thawing and freezing the virus suspension twice and then pelleting the virus and cell debris by centrifugation for 60 min at 100 000 g. The pellet from 400 ml of the virus suspension was dissolved in 1 ml of phosphate buffered saline (PBS) containing 1 % of the detergent MEGA-10 and incubated for 1 h at room temperature. The lysate was sonicated for 30 sec and clarified by low-speed centrifugation. The detergent-solubilized antigens were gel-filtered on a Sephadex G-50 column (Pharmacia Fine Chemicals, Uppsala, Sweden) to remove the detergent. The filtrate was used as BCV antigen for ELISA.

Detection of antibodies to BCV

In ELISA. The BCV antigen was diluted 1:1000 in coating buffer (0.05 M sodium carbonate buffer, pH 9.6, containing 0.1 % NaN_3) and 100 μl was added per well in microtitre plates (Nunc, Denmark). The sera to be tested for presence of antibodies to BCV were diluted 1:10, 1:50, 1:250 and 1:1250 in PBS, containing 0.05 % Tween (PBS-T). One hundred μl of the serum dilutions was added to the BCV-coated wells and incubated for 1 h at 37°C . After 3 washings with PBS-T, 100 μl of an optimum dilution of the horseradish peroxidase (HRP)-conjugated monoclonal antibody to bovine IgG was added per well and incubated for 1 h at 37°C . The plates were then washed and a substrate solution (0.1 mg tetramethylbenzidine/ml, 0.1 M sodium acetate and 0.05 % H_2O_2) was added in volumes of 200 μl per well. After 10 min at room temperature, 50 μl H_2SO_4 was added to stop the reaction. The absorbance value at 450 nm was measured on a Titertec microplate reader (Flow Labs., Irvine, Scotland). A se-

rum titre was estimated as the highest serum dilution giving an absorbance value above 0.10 (0.10 = mean $A_{450} \pm 3$ S.D. of 46 sera negative for VNT antibodies to BCV).

In VNT. Sera, preheated for 30 min at 56°C were diluted by 2-fold dilution steps from 1:4 to 1:256. Each dilution was mixed with an equal volume of the virus suspension containing 100 TCID₅₀ per 0.1 ml.

Following incubation for 60 min at 20°C , 0.2 ml of the serum-virus mixture was inoculated into each of 2 tube cultures of BL cells. The cultures were checked for cytopathic effect (CPE) and final readings were made 3 to 5 days after inoculation when in the control tubes the monolayers showed complete degeneration. The highest dilution of serum inhibiting CPE was taken as the end-point titre.

Detection of antibodies to BVDV

Antibodies to BVDV were detected by the indirect ELISA, as described by *Juntti et al.* (1987). In brief, serum antibodies which bound to the wells of microtitre plates coated with BVDV were detected by HRP-conjugated monoclonal antibody to bovine IgG.

Results

Comparison between VNT and ELISA for detection of antibodies to BCV

A total of 91 cattle sera were assayed in VNT and ELISA (Fig. 1). All sera with a VNT titre of 4 or less ($n = 26$) were negative in ELISA. Nine out of 19 sera with a VNT titre of 8 to 16, and all sera with a VNT titre of 32 or more were positive in ELISA, which was then used throughout this study for detection of antibodies to BCV.

Antibodies to BVDV and BCV during and after the episode of winter dysentery

The antibody titres to BCV and BVDV

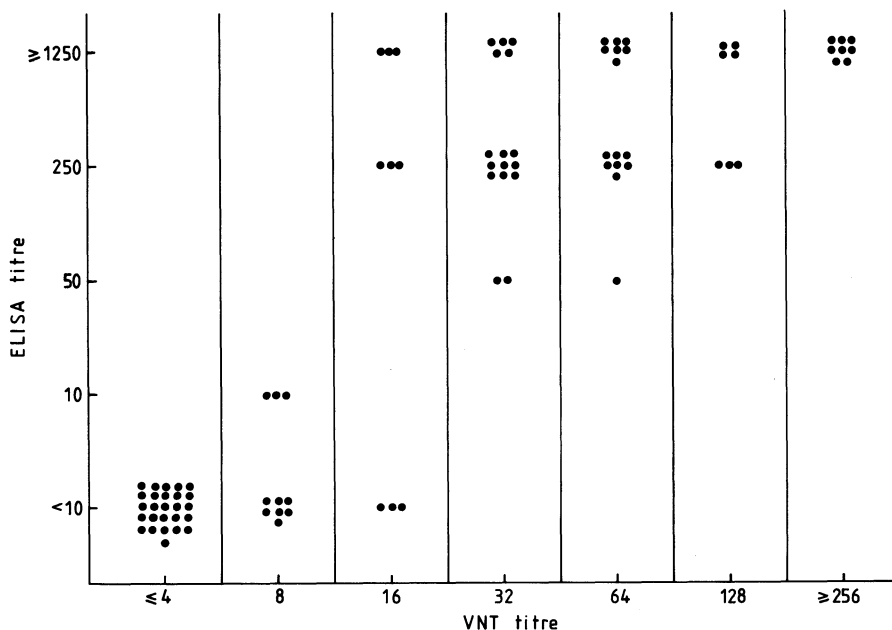


Figure 1. Comparison of antibody titre to bovine coronavirus, detected by ELISA and VNT, in 91 bovine sera.

Table 1. Antibody titres to bovine coronavirus (BCV) and bovine virus diarrhoea virus (BVDV) measured by ELISA in cattle sera during (A) the acute phase of winter dysentery, (B) 3 to 4 weeks later (convalescence phase), and (C) 1 year postinfection.

Herd (no.)	Main symptom	Number of cows tested	Titre to BCV			Titre to BVDV	
			A	B	C	A	B
1	D	7	< 10	≥ 1250	250– ≥ 1250	< 10	< 10
2	D	5	< 10	250– $\geq 1250^a$	n.d.	< 10	< 10
3	D+R	7	< 10	250– ≥ 1250	250– $\geq 1250^a$	< 10	< 10 ^a
4	D+R	4	< 10	250– ≥ 1250	50– ≥ 1250	50–250	50–250
5	D+R	5	< 10	250– ≥ 1250	250– ≥ 1250	< 10	< 10
6	D+R	5	< 10	250– ≥ 1250	250– $\geq 1250^b$	< 10	< 10
7	D+R	4	< 10	250 ^b	250– $\geq 1250^c$	< 10	< 10 ^b
8	D+R	4	< 10	250	250– ≥ 1250	< 10	< 10

D: diarrhoea; R: respiratory symptoms; n.d.: not done. a) 4 cows, b) 3 cows, c) 2 cows.

during and after an episode of winter dysentery are listed in Tables 1 and 2. All those animals tested in herds 1 through 8 were seronegative to BCV (titre < 10) in the acute phase of winter dysentery but seroconverted and showed high titres (250 to

> 1250) both 3 to 4 weeks and 1 year later. The animals showed no rise in antibody titre to BVDV.

In herd no. 9, 2 cows had high antibody titres (> 1250) and 3 cows low titres (< 10) to BCV in the acute phase of winter dysen-

Table 2. Antibody titres to bovine coronavirus (BCV) and bovine virus diarrhoea virus (BVDV) measured by ELISA in sera from dairy herd no. 9 during (A) the acute phase of winter dysentery, (B) 3 to 4 weeks later (convalescence phase), and (C) 1 year postinfection.

Animal (no.)	Titre to BCV			Titre to BVDV		
	A	B	C	A	B	C
1	50	≥ 1250	≥ 1250	<10	50	250
3	50	≥ 1250	n.d.	<10	250	n.d.
12	≥ 1250	≥ 1250	≥ 1250	<10	250	250
100	10	≥ 1250	n.d.	<10	50	n.d.
113	≥ 1250	≥ 1250	250	<10	50	50

n.d.: not done.

tery. During the convalescence phase, all animals showed high titres to BCV (> 1250) as well as seroconversion to BVDV (Table 2).

Transfer of BCV antibodies to calves via colostrum

After the episode of winter dysentery in herd no. 1, sera collected from 6 newborn calves were analysed for presence of antibodies to BCV. Precolostral sera were found to be free from detectable antibodies to BCV. Sera collected from the same calves at the age of 4 to 6 days, *i.e.*, after they received colostrum,

showed all antibody titres to BCV. The antibody titre subsequently declined and was usually not detectable in sera from calves older than 5 months (Table 3). All 6 calves were seronegative to BCV at the age of 7 months.

Prevalence of Swedish heifers with antibodies to BCV

Serum antibodies to BCV were detected in 61 % of 549 Swedish heifers examined. The seroreactors to BCV were almost equally distributed in Sweden.

The herd immunity to BCV, evaluated in 58

Table 3. Antibody titre to bovine coronavirus (BCV) in sera from calves at various ages as measured in ELISA. The calves were born within 5 to 8 months after an episode of winter dysentery in herd no. 1.

Calf (no.)	Age and titre								
	0-1 db)	4-6 d	1 m	2 m	3 m	4 m	5 m	6 m	7 m
3	<10	50	50	10	10	10	<10	<10	<10
4	<10	nd	250	250	50	10	10	<10	<10
5	<10	≥ 1250	250	50	50	50	10	10	<10
7	<10	250	250	50	10	10	<10	<10	<10
16	<10	250	250	50	50	10	10	<10	<10
19	<10	≥ 1250	250	250	50	50	50	10	<10

b) Precolostral sera.

d: days; m: months; nd: not determined.

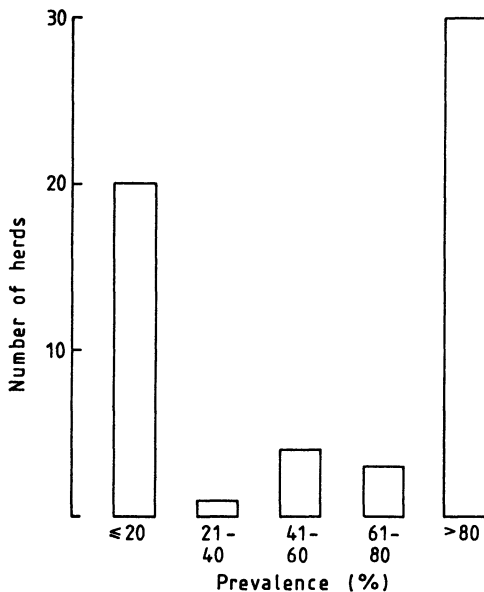


Figure 2. Prevalence of bovine coronavirus seropositive heifers in Swedish herds ($n = 58$), as measured in ELISA. Sera from 5 to 14 heifers per herd were examined.

herds, was commonly either high or low (Fig. 2). The prevalence of seroreactors was more than 80 % in 30 of the herds, and 20 % or less in 20 of them.

Discussion

In the present study, outbreaks of winter dysentery in dairy herds were related to seroconversion to BCV (Table 1). In addition, we have examined paired sera from Finnish cattle with winter dysentery and found that they also seroconverted to this virus (data not shown). These findings show that BCV is the aetiological agent of winter dysentery and thereby confirmed the results of other authors (Takahashi et al. 1980, Espinasse et al. 1982, Takahashi et al. 1983, Saif et al. 1988).

In 1 of the herds (no. 9), the winter dysentery was related to seroconversion to BVDV

and a rise in antibody titre to BCV (Table 2). An infection with BVDV is usually sub-clinical and a previous study indicates a rather slow spread of BVDV among cattle (Barber et al. 1985). In herd no. 9, all tested animals seroconverted to BVDV within a month, indicating that a mixed infection with BCV may increase the spread of BVDV. The cows in this herd were severely affected by the disease and 2 animals died. These findings indicate synergism between BCV and BVDV. The BVDV, which infects the cells of the immune system, is regarded as being an immunosuppressive agent. A concurrent infection with BVDV may therefore exacerbate the clinical signs caused by other pathogens such as BCV.

The BCV antibody titres remained high 1 year postinfection (Table 1) and were transferred to the offspring via the colostrum. The amount of the passively acquired antibodies subsequently declined and they were usually not detectable in sera from the calves in herd no. 1 when they reached an age over 5 months (Table 3). Also, calves born after the occurrence of the disease in herd no. 3 were seronegative to BCV at the age of 1 year (data not shown).

Following an episode of winter dysentery in a dairy herd, older seropositive animals will be slaughtered later because of old age or other reasons and they may be replaced with seronegative heifers. The herd immunity to BCV therefore decreases and the herd will again be susceptible to infection with BCV. This hypothesis is in agreement with the observation that winter dysentery seldom reappears in the same herd more often than every 3 to 4 years (Fox 1980). Winter dysentery only appears to occur in seronegative herds (Table 1). However, in 1 herd (no. 9) BCV antibodies were present in sera collected from cows during the acute phase of diarrhoea. The serum samples in this herd

were collected 5 days after the initial signs of infection and it is likely that the animals already had produced antibodies to BCV. The serological survey undertaken in the present study showed that about 35 % of Swedish herds had a low prevalence (< 20 %) of seroreactors to BCV among heifers, indicating that these herds are susceptible to winter dysentery. About 50 % of the herds had a high prevalence (> 80 %) of seroreactors to BCV among heifers and are probably protected against the disease.

Besides being possible aetiological agent of winter dysentery, BCV is also known to cause enteritis and respiratory disease in calves. Histopathological findings of BCV-induced severe enterocolitis in neonatal calves resemble those of winter dysentery in adult cattle (*Van Kruiningen et al.* 1987). In a herd with BCV-associated neonatal diarrhoea, BCV was detected in faeces from adult healthy cattle, particularly during the winter months and at parturition (*Collins et al.* 1987). In the present study, young calves were usually asymptomatic during the episode of winter dysentery in the adult cattle. More research in this field is obviously needed to achieve a better understanding of BCV infection and its epidemiology.

In conclusion, the present study provides serological evidence of BCV being the main causative agent of winter dysentery and shows that about 35 % of Swedish herds are at risk of getting the disease. It was also found that a co-infection with BVDV seems to increase the severity of the disease.

Acknowledgements

The BCV strain was a kind gift from Professor Anton Mayr, Munich, and the Finnish sera were kindly supplied by dr Erkki Neuvonen. Thanks to Eva Järnberg and Katarina Näslund for skilful laboratory help. Thanks also to Professor Zvonimir Dinter for helpful criticism. This work was

supported by grants from the Farmer's Research Council for Information and Development.

References

- Barber DML, Nettleton PF, Herring JA*: Disease in a dairy herd associated with the introduction and spread of bovine virus diarrhoea virus. *Vet. Rec.* 1985, *117*, 459-464.
- Borgen HC*: Mucosal Disease in Dänemark. II. Virologische und serologische Befunde in einem Bestand. (Mucosal disease in Denmark. II. Virological and serological findings in a herd). *Nord. Vet.-Med.* 1963, *15*, 346-356.
- El-Ghorr AA, Snodgrass DR, Scott FMM, Campbell I*: A serological comparison of bovine coronavirus in adult cattle. *Amer. J. vet. Res.* 1987, *48*, 361-365.
- Espinase J, Viso M, Laval A, Aavey M, le Layec Cl, Blot JP, l'Haridon R, Cohen J*: Winter dysentery: A coronavirus-like agent in the faeces of beef and dairy cattle with diarrhoea. *Vet. Rec.* 1982, *110*, 385.
- Fox FH*: Winter dysentery. In: *Bovine Medicine & Surgery*, 2nd edition, HE Amstutz (ed.), American Veterinary Publications, Inc., Santa Barbara, California, U.S.A. 1980, Vol. 2, pp. 679-681.
- Hedström H, Isaksson A*: Epizootic enteritis in cattle in Sweden. *Cornell Vet.* 1951, *41*, 251-253.
- Jones FS, Little RB*: The etiology of infectious diarrhea (winter scours) in cattle. *J. exp. Med.* 1931, *53*, 835-843.
- Juntti N, Larsson B, Fossum C*: The use of monoclonal antibodies in enzyme-linked immunosorbent assays for detection of antibodies to bovine viral diarrhoea virus. *J. Vet. Med. B.* 1987, *34*, 356-363.
- Olafson P, MacCallum AD, Fox H*: An apparently new transmissible disease of cattle. *Cornell Vet.* 1946, *36*, 205-213.
- Reynolds DJ, Debney TG, Hall GA, Thomas LH, Parsons KR*: Studies on the relationship between coronaviruses from the intestinal and respiratory tracts of calves. *Arch. Virol.* 1985, *85*, 71-83.

Saif LJ, Redman DR, Brock KV, Kohler EM, Heckert RA: Winter dysentery in adult dairy cattle: Detection of coronavirus in the faeces. *Vet. Rec.* 1988, *123*, 300–301.

Takahashi E, Akashi H, Inaba Y: Bovine epizootic diarrhoea resembling winter dysentery caused by bovine coronavirus. *Japan Agricult. Research Quart.* 1983, *17*, 185–190.

Takahashi E, Inaba Y, Sato K, Ito Y, Kurogi H, Akashi H, Satoda K, Omori T: Epizootic diarrhoea of adult cattle associated with a coronavirus-like agent. *Vet. Microbiol.* 1980, *5*, 151–154.

van Kruiningen HJ, Khairallah LH, Sasseville VG, Wyand MS, Post JE: Calfhood coronavirus enterocolitis: a clue to the etiology of winter dysentery. *Vet. Pathol.* 1987, *24*, 564–567.

Sammanfattning

Bovint coronavirus som orsak till vinterdysenteri: Serologiska bevis.

Sera från kor i 9 besättningar med vinterdysenteri undersöktes på förekomst av antikroppar mot bovint coronavirus (BCV) och bovint virusdiarré virus (BVDV). Kor i 8 av de 9 besättningarna serokonverterade mot BCV, men ej mot BVDV. I den nionde besättningen, där symptomen var grava, serokonverterade djuren både mot BCV och BVDV. Korna hade höga antikropstitrar mot BCV ännu 1 år efter genomgången infektion. Antikropparna överfördes från modern till kalven via råmjölken och kvartstod i kalvens serum tills dess att den var ungefär 5 månader gammal.

En serologisk undersökning av 549 kvigor i Sverige visade att 61 % hade antikroppar mot BCV. Prevalensen av antikropspositiva djur var likartad mellan olika områden i Sverige men var vanligen antingen hög eller låg i enskilda besättningar.

Konklusionen är att BCV är en vanlig orsak till vinterdysenteri och att en saminfektion med BVDV tycks förvärra symptomen.

(Received April 2, 1990; accepted May 16, 1990).

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