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Experimental reproduction of winter dysentery in lactating cows using BCV — comparison with BCV infection in milk-fed calves

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Abstract

Infection models were developed for adult cows and for young calves using the same strain of bovine coronavirus (BCV), which for the first time allows experimental reproduction of winter dysentery (WD) in seronegative lactating cows. The cattle were infected through direct contact with an experimentally inoculated calf. All experimental cattle shed faecal BCV with development of diarrhoea, being profusely watery with small amounts of blood in the most severely affected animals, including both cows and calves. The cows, in contrast to the calves, showed depressed general condition and appetite leading to a marked decrease in milk yield. Further age-associated differences were a shorter incubation period in the two youngest calves, but with milder fever and milder decrease in white blood cell counts. These findings shed light on the apparent epidemiological differences between WD and calf BCV diarrhoea suggesting that, (1) the same strains of BCV cause natural outbreaks of calf diarrhoea and WD, (2) seronegative cows are more severely affected by the infection than seronegative conventionally reared calves, and (3) unaffected
general condition in diarrhoeic calves may lead to underestimation of the occurrence of calf diarrhoea in WD outbreaks.

In response to infection, all cattle produced early interferon type 1 in serum and, except for one calf, in nasal secretions. A finding not previously reported is the detection of interferon type 1 responses in bovine milk. All cattle developed high IgM antibody responses and long-lasting IgA antibody responses both systemically and locally. The serum IgM antibody responses came earlier in most of the calves than in the cows. Prolonged IgM antibody responses were detected in serum and milk, while those in nasal secretions were much shorter. BCV-specific IgA was present in nasal secretions from all cattle throughout the 6 months follow-up. The IgA antibody response in serum was detected up to 17 months post-infection and the duration showed an age-related variation indicating a more prominent IgA memory in the adult cattle, and in the older calves than in the younger ones. BCV-specific IgG was detected in all cattle during the experimental period of up to 22 months.

In conclusion, WD was reproduced in seronegative lactating cows. The cows showed a more severe general diseases than seronegative calves infected concurrently. Very long-lasting IgA antibody responses were detected both systemically and locally. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Cattle-bacteria; Winter dysentery; Bovine coronavirus; Experimental infection; IgM; IgA; Interferon type 1

1. Introduction

Experimental reproduction of winter dysentery (WD) in adult cattle has met with difficulties in the past. The early experiments (reviewed by Kruijningen et al., 1985) were performed before the etiological role of bovine coronavirus (BCV) was elucidated, and since the BCV antibody status of the animals could not be determined it is conceivable that clinical disease was inconsistently produced. Recently, WD reproduction was attempted in BCV seropositive cows, but diarrhoea could only be produced by inoculation through a duodenal catheter after immunosuppression using serial dexamethasone injections and/or large amounts of iced water orally (Tsunemitsu et al., 1999).

BCV has been recognised for 30 years as one of the important calf diarrhoea agents (Stair et al., 1972; Mebus et al., 1973). Yet, in WD outbreaks calves often are clinically unaffected (Rollinson, 1948; Hedström and Isaksson, 1951; Roberts, 1957; Trávén et al., 1993). Antigenic differences between BCV strains isolated from diarrhoeic calves and from cows with WD have been sought in experimental cross-protection studies (Reynolds et al., 1985; El-Kanawati et al., 1996), as well as in vitro by use of panels of antisera and monoclonal antibodies applied in functional and cross-reactivity tests (Tsunemitsu and Saif, 1995; Dea et al., 1995), complemented with sequence analysis of the haemagglutinin esterase gene (Milane et al., 1997). However, no consistent differences between cow- and calf-derived BCV strains have been found so far. The objective of this experiment was to reproduce WD in BCV-seronegative lactating cows and to infect milk-fed BCV-seronegative calves with the same strain of the virus and to compare the outcome between the cows and the calves with respect to clinical disease, faecal excretion
of the virus, isotype-specific antibody and interferon type 1 responses in serum, milk and nasal secretions.

2. Materials and methods

2.1. Animals and sampling

The experimental design of the study was approved by the Ethics Committee for Animal Experiments, Uppsala, Sweden (protocol C270/91).

Four lactating cows and five conventionally reared calves were used in the experiment. All cattle were of the Swedish Red and White breed (SRB) and were confirmed to be seronegative to BCV, bovine virus diarrhoea virus (BVDV) and bovine leukemia virus (BLV) before the trial started. All donor herds were also tested to be free from antibodies to BVDV and BLV in bulk milk. The experimental design, including the BCV source, herd of origin and ages of the cattle are summarised in Table 1.

The experimental animals were housed in individual pens, but within the same stable compartment. No other cattle were kept on the premises during the trial, but sheep and horses were housed in another stable compartment. The sheep were BCV and BVDV seronegative. Clinical examinations were made on several occasions before the trial and daily during the first 18 days post-infection (dpi).

The cows were fed a ration of hay ad libitum and a 50/50 mixture of a pelleted concentrate and crushed barley corresponding to their milk yield. Concentrate was offered even on days with watery diarrhoea. During the clinical study period, the calves were fed hay ad libitum and 2 l of fresh milk 3 times daily, except the oldest calf (107) that was fed 2.5 l twice daily and one meal of a calf starter concentrate. The milk fed

| ID  | Age | Sex | Herd of origin | Installed before trial | Source of BCV | Milk yield (kg) | Lowest yield | Lowest yield at dpi |
|-----|-----|-----|----------------|------------------------|--------------|----------------|--------------|---------------------|
| 134 | 6 years | F | UJ | 4w | Contact with 306 | 18.2 | 6.4 (35) | 6 |
| 297 | 4 years | F | KT | 4w | Contact with 306 | 15.7 | 3.1 (20) | 6 |
| 302 | 4 years | F | KT | 4w | Contact with 306 | 26.4 | 4.9 (19) | 7 |
| 310 | 4 years | F | KT | 4w | Contact with 306 | 15.9 | 8.9 (56) | 5 |
| 33  | 6 days | M | IR | 6d | Contact with 306 |  |  |  |
| 42  | 4 weeks | M | OS | 7d | Contact with 306 |  |  |  |
| 43  | 10 days | M | OS | 7d | Contact with 306 |  |  |  |
| 107 | 9 weeks | F | OS | 7d | Contact with 306 |  |  |  |
| 306 | 3 months | M | ME | 4w<sup>b</sup> | Faecal sample PO/IN<sup>c</sup> |  |  |  |

<sup>a</sup> Daily milk yield as mean of 5 days prior to infection except for cow 134 with mean of 2 days.

<sup>b</sup> One day before the BCV inoculation of calf 306 it was removed from the premises, then reintroduced on day 0.

<sup>c</sup> PO = oral administration, IN = intranasal administration.
during the entire clinical study period was BCV antibody negative, derived from the experimental cows before infection and stored at \(-20^\circ\text{C}\) until needed. The milk was served at body temperature. On days with moderate to watery diarrhoea, depending on the severity of the diarrhoea, 1–3 meals of milk were replaced by an electrolyte solution, but no calf was completely without milk for more than 1 day.

Blood samples were drawn from the ventral tail vein of the cows and from the jugular vein of the calves using sterile evacuated tubes. Milk samples were collected from a front quarter showing a low cell count level. On each cow the same quarter was sampled every time. Nasal secretions were collected using cotton tampons that were inserted into one nostril and allowed to absorb for 10–30 min to obtain at least 1.5–2 ml. The fluid was then pressed out of the tampon using a sterile 20 ml syringe. Faecal samples were taken from the rectum when the cattle had diarrhoea and when faeces were not passed during the examination, and otherwise from the freshly laid faeces. Samples were collected on three occasions before infection for the cows and two for the calves, and then 2, 4, 7, 9, 11, 14 days, 3, 4, 5, 6, 7, 8, 10, 12, 14, 16, 18 weeks and 5 and 6 months post-infection (mpi). Milk samples were not available after the 18 weeks sampling. Cow 134 was slaughtered after the 6 months sampling, but further serum samples were obtained 7 mpi from calf 42 and 7, 8 and 9 mpi from the other calves and cows. Calf 43 was also sampled 10 mpi, calf 33 at 10 and 17 mpi and calf 107 at 10, 17, 19 and 22 mpi. Serum from the transmitter calf (306) was also obtained up to 17 mpi.

2.2. BCV inoculum and experimental design

The inoculum consisted of BCV-containing faeces collected from three cows during a field WD outbreak, pooled and stored at \(-70^\circ\text{C}\) with 10% DMSO for 3 months before use. The inoculum was controlled for presence of BCV using the antigen ELISA described below. A 3-month-old BCV-seronegative conventionally reared SRB calf (306) was used as source of virus (transmitter calf) for the experimental animals (Table 1). At a location 5 km from the experimental unit, calf 306 was orally and intranasally inoculated with a 50% faecal suspension in phosphate buffered saline. 100 ml of the suspension were given through a stomach tube and 3 ml instilled in each nostril. Two days after inoculation, calf 306 was brought to the experimental unit and kept for 1 h in each of the cows’ pens. Then the four experimental calves were kept together with calf 306 for 1 h in a cow-size pen. Each experimental animal had direct nose contact with calf 306 and each animal also had nose contact with faeces from calf 306. After the contact the calves were put back into single pens and calf 306 was removed from the experimental unit. Clinical examinations were performed daily from the day of infection to 18 dpi and on several occasions before and after this period. Rectal temperatures were measured once daily (morning). Clinical signs and body temperature were also monitored daily in the transmitter calf 306, taking place after the examinations and sampling of the experimental group.

2.3. BCV detection

BCV excretion in faeces was measured in all faecal samples in an antigen capture ELISA system. Briefly, microtitre plates (Polysorp, Nunc, Denmark) were coated with a
mouse monoclonal antibody (MAb) to BCV (BCV59:8) in coating buffer, pH 9.6 at 4°C over night. The faecal sample in 1:5 dilution and an optimal dilution of an anti-BCV MAb (BCV15:11) conjugated to horseradish peroxidase (HRP) were added simultane-
ously to the wells and incubated in a single step at 37°C for 1 h. The production and characterisation of the MAb has been described (Näslund et al., 2000). After washings, the TMB/H₂O₂ substrate was added and the reaction stopped after 10 min. Optical density (OD) values at 450 nm were read in a microtitre plate reader. Three preinfection faecal samples per animal were analysed and 2 × mean of these samples was used as cut off level (OD 0.15).

The performance of the BCV antigen capture ELISA was compared with that of a BCV ELISA developed at the Moredun Research Institute (MRI), Edinburgh, Scotland (Clark, 1991), available through Svanova Biotech (Uppsala, Sweden), on 47 faecal samples.

2.4. Isotype-specific antibody assays

BCV-specific IgG was measured in an indirect ELISA system (Alenius et al., 1991) using the HRP-conjugated MAb anti-bovine IgG 22:26 on sera and nasal secretion samples and anti-bovine IgG1 2:2 on milk samples. Cut off levels for serum and milk were 0.20 and 0.04, respectively (Näslund et al., 2000). For nasal secretions, 2 × mean of the preinfection samples was used as cut off (OD 0.15). BCV-specific IgM and IgA were analysed in isotype-specific capture ELISA systems as previously described (Näslund et al., 2000). Briefly: microtitre plates (Polysorp, Nunc, Denmark) were coated with an optimal dilution of a MAb to bovine IgM (M69:2) or IgA (A12:2) in coating buffer, pH 9.6, at 4°C over night. The test samples including positive and negative reference samples, a BCV antigen preparation and a HRP-conjugated MAb to BCV (BCV15:11) were added with intermediate washings. All samples were analysed with negative control antigen in 1:25, 1:2 and 1:50 dilution for sera, milk and nasal secretions, respectively. Samples with an OD value > 2 × the value with the negative control antigen were regarded as positive. Positive sera and milk samples were reanalysed in five-fold dilution series to determine the end-point titres.

2.5. Haematological analyses

Erythrocyte volume fraction, white blood cell count, differential leucocyte count, sodium, potassium and chloride concentrations were analysed using routine methods (Department of Clinical Chemistry, Swedish University of Agricultural Sciences, Uppsala, Sweden) in samples taken before infection and during the first 3 weeks after infection.

Statistical analyses of the haematological results were done using Student’s t-test for paired samples, comparing the mean of the three sampling occasions before infection with the value on specific dpi.

2.6. Bioassay for Interferon type 1 (IFN)

Bovine IFN was detected in a cytopathic effect inhibitory bioassay as previously described (Trävén et al., 1991) using bovine kidney (MDBK) cells challenged with
vesicular stomatitis virus (VSV). In brief, MDBK cells were grown to confluence in 96 well microtitre plates and incubated over night at 37°C with serial dilutions of the test samples. The samples were replaced by medium containing VSV in a sufficient amount to cause complete destruction of the cells in unprotected wells within 24 h. Antiviral activity in samples was compared to that of a laboratory porcine IFN-α standard after staining of residual cells with crystal violet and is expressed as units (U) per ml. IFN was analysed in sera, nasal secretions and milk collected before infection and on 2, 4, 7, 9, 11 and 14 dpi. The samples were diluted 1:4 in medium and the standard was diluted in 25% serum, nasal secretion or milk collected from cows 302 and 310 before infection. Appropriate dilutions of 20 serum samples with positive reactions in the bioassay were retested after preincubation of 240 µl of sample for 1 h at 37°C with 5 µl of a polyclonal ovine antibody to natural human IFN-α. This pretreatment neutralised in mean 46.2 ± 21.7% of the antiviral activity in the samples.

2.7. Dry matter content in faeces

Determination of the dry matter content (DMC) in faeces was performed as described (Bridger et al., 1984). Briefly: 5 g (1–8) of calf faeces and 20 g (13–30) of cow faeces was desiccated in an incubator at 55°C to constant weight (overnight). The accuracy of the clinical scoring of the faecal consistency as normal, mild, moderate or severe diarrhoea (including half steps between these levels) was evaluated against the DMC of the faeces.

2.8. Other infections

Bovine rotavirus (BRV) excretion in faeces was examined using an ELISA for group A rotavirus antigen (Svensson et al., 1983, 1986), before the experimental infection and in all diarrhoeic samples. The presence of non-group A rotavirus was checked using RNA-PAGE (Svensson et al., 1986) during 2 days with diarrhoea in all cows and calves. The inoculum and faecal samples taken at the beginning of the most severe diarrhoeal phase for each experimental animal were analysed by electron microscopy (EM) for the presence of other viruses, including a “centrifugation-on-grid” step to increase the sensitivity for small viruses as previously described (Hedlund et al., 2000). Faecal samples from the calves with watery diarrhoea were analysed for the presence of Cryptosporidium, and cultured for the presence of Salmonella and enterotoxigenic Escherichia coli K99+ and verotoxin+ using established methods at the National Veterinary Institute, Uppsala, Sweden. Faecal samples from the cows with watery diarrhoea were cultured for the presence of Salmonella and Campylobacter. The inoculum was analysed for, and found free from, BRV and Cryptosporidium.

Serum antibody responses (IgG) to BVDV, bovine respiratory syncytial virus (BRSV), parainfluenza virus type 3 (PIV3), bovine adenovirus type 3 (BAV3) and BLV were examined using commercial indirect ELISAs (Svanova Biotech) on day 0 of the BCV infection and for BVDV and PIV3 on day 21, for BLV 6 months after the BCV infection and for BRSV on all sampling occasions up to 5 weeks after the BCV infection.
3. Results

3.1. Clinical disease

3.1.1. All cows and calves developed diarrhoea

All four cows developed diarrhoea at 4–5 dpi (Fig. 1a), but already at 2 dpi slightly loose faeces was observed. Severity of the diarrhoea ranged from moderate on 1 day only to profuse watery for 4 days in the two cows most seriously affected (297 and 302). One of these cows (302) had a mild blood admixture in faeces in the late phase of the diarrhoea. Faeces from both cows that showed watery diarrhoea also developed the sweet stinking smell characteristic for WD. This smell appeared 1–2 days after the diarrhoea started and was maintained for a few days after the diarrhoea had resolved. Mucus was present in the faeces of cow 297 and 302 in the late diarrhoeal phase.

The two youngest calves (33 and 43) developed diarrhoea already at 1–2 dpi (Fig. 1b), being at first mild to moderate for 2–3 days, then watery profuse for the three following days after which diarrhoea resolved abruptly. The two older calves showed only mild to moderate diarrhoea which started 5–6 dpi and continued for a further 2–7 days. Calf 33 had blood in the faeces on the first day with watery diarrhoea and calf 43 had small amounts of blood in the faeces after the diarrhoea had resolved. The faeces of these two calves had a moderate mucus admixture during the first days of diarrhoea, and in calf 43 also after the diarrhoea. Mucus was also observed in the faeces of calf 107 in the late diarrhoeal phase. The transmitter calf developed softened faeces 2 days post-inoculation (dpi), and showed a mild diarrhoea on 3 dpi only.

3.1.2. High correlation between diarrhoea score and dry matter content of the faeces

The DMC of normal faeces ranged from 12 to 19% for the cows and from 18 to 36% for the calves. The DMC of faeces during watery diarrhoea ranged from 2 to 7% for both cows and calves. The overall correlation between DMC and the degree of diarrhoea (Pearson’s coefficient of correlation) was \(-0.65\) \((P < 0.001, n = 136)\). Separating the age categories gave a correlation of \(-0.91\) \((P < 0.001, n = 70)\) for the cows and \(-0.93\) \((P < 0.001, n = 29)\) for the youngest calves (33 and 43). The lowest correlation was found for the older calves (42 and 107) with a correlation coefficient of \(-0.54\) \((P < 0.001, n = 37)\), reflecting a larger range of DMC values among the normal samples from this group.

3.1.3. Fever peaks in the cows and in the older calves

A short fever peak (40.0–41.3°C) was detected in three of four cows at 2 dpi. On 3 dpi all cows had normal body temperatures except cow 134 that had a moderate fever (40.2–40.6°C) during 4 days. This extended fever was probably caused by a relapsing sinusitis, indicated by a muco-purulent single-sided nasal discharge 3–5 dpi. This cow appeared healthy during the 7 days prior to the start of the trial, but had a period of dullness and a mild increase in body temperature (39.0–39.9°C) 8–11 days before the trial, indicating a mild sinusitis after dehorning 17 days before the trial. The cow recovered without antibiotics treatment.
Fig. 1. BCV excretion in faeces of (a) four cows and (b) four calves measured by an antigen-capture ELISA developed at our laboratory. Occurrence of diarrhoea is shown in the lower part of the figure. Symbols within parenthesis signify mild diarrhoea and symbols without parenthesis signify moderate to severe diarrhoea. Clearly looser faeces than normal for the respective age group was scored as mild diarrhoea, fluid faeces as moderate diarrhoea and watery faeces as severe diarrhoea.
The two oldest calves (42 and 107) showed short fever peaks at 2–3 dpi (40.2–40.5°C), whereas the two youngest showed very mild increases in body temperature at 1–2 dpi (39.6–39.7°C). The transmitter calf showed fever (40.0–40.2°C) on 3 and 5 dpi.

3.1.4. Depressed general condition and appetite in cows but not in calves

The appetite of the cows was decreased for 5–10 days, all four cows leaving most of the concentrate and/or hay for 2–6 days, mainly during the diarrhoea period. The two cows with watery diarrhoea showed a mild depression (dull appearance, slow to rise) for 1–2 days during the diarrhoea. Cow 302 had a mild increase in dermal turgor, but only at 7 dpi. A calmer temper, but without obvious depression, was observed for 7 days in cow 134 and for 2–3 days in the other cows. Cow 310 had the shortest period of changed temper and decreased appetite, together with 3 days of mild to moderate diarrhoea. Cow 134 had the longest period of changed temper and decreased appetite, but the sinusitis probably contributed to this, since she had the mildest diarrhoea of the cows.

The appetite was not markedly decreased in any of the calves on any day. The milk ration was decreased for 3 days per calf, but they immediately drank the electrolyte solution when offered in spite of the continuous access to fresh water. A mild depression was only observed in calf 42 at 2 dpi. Dermal turgor was mildly increased at 4 dpi in calf 42 and at 5 dpi in calf 33. A calmer temper without obvious depression was observed for 2–5 days in the calves except in calf 43 which was not visibly affected. The lack of depression in the calves with watery diarrhoea was surprising. The appetite of the calves was much better retained than that of the cows both with and without watery diarrhoea. The transmitter calf showed a mild depression (dull appearance, lying down more than usual) on 3 dpino, the day after the transmission contacts, but the appetite was not decreased.

3.1.5. Respiratory symptoms in both cows and calves

All cows had a period of dry cough 1–3 weeks before the BCV infection. Cow 302 and 310 appeared without symptoms at the start of the trial. These cows developed a mild dry cough and mildly increased breathing sounds upon auscultation on 2 dpi. Cow 134 and 297 had a mild cough at the start of the trial, but breathing sounds were normal on auscultation. The cough of these cows increased to moderate on 6 and 7 dpi. Cow 297 developed moderately to markedly increased breathing sounds with moderate wheezes and mild crackling sounds on 8–13 dpi, whereas cow 134 only showed mildly increased breathing sounds. These two cows retained a mild dry cough throughout 18 dpi. Cow 310 had weak wheezing sounds over the ventral lung field 10–13 dpi. Mildly elevated respiratory frequencies were recorded in cow 297, 302 and 310 in the period after the diarrhoea had resolved.

Calves 42 and 43 were without symptoms at the start or the trial. These calves developed a mild cough on 2–5 dpi. Calves 33 and 107 had a mild dry cough at the start of the trial and the cough of calf 107 increased to moderate on 4 dpi. Mildly to moderately increased breathing sounds were recorded in all calves, and wheezes were detected in small areas of the lung fields of calves 33, 42 and 43 on 12–15 dpi. The respiratory symptoms showed a biphasic appearance in both the cows and the calves, with
cough and nasal discharge appearing within a few days of the trial start and adventitious breathing sounds over the lung field appearing later. The adventitious breathing sounds of the calves were, however, much milder than those recorded in cow 297. Breathing sounds of calf 107 were not fully normalised at 18 dpi. Respiratory frequencies were never elevated in the calves. The transmitter calf had a mild cough before the inoculation. Mildly to moderately increased breathing sounds on auscultation were observed in this calf on 3–6 dpino.

3.1.6. Nasal and eye symptoms in both cows and calves

A mild to moderate, serous to muco-purulent nasal discharge was seen in all cattle starting at 2–6 dpi and continuing throughout 18 dpi. A mild to moderate conjunctival hyperaemia was observed starting 2–4 dpi and continuing through 18 dpi. Petechial haemorrhages were observed in the conjunctiva of all four cows, first in cow 297 at 8 dpi, but not in any of the calves. The calves showed a mild to moderate serous conjunctival discharge on 2–5 dpi and again 10–18 dpi. Conjunctival discharge was only observed in one of the cows (302) at 3 dpi. The transmitter calf showed a mild to moderate serous to muco-purulent nasal discharge on 3–11 dpino.

3.1.7. Decreased milk yield

The cows were yielding 16–26 kg of milk per day before the BCV infection (Table 1). A decrease in milk yield was evident on 3 dpi, thus before the diarrhoea had started (Fig. 2). Production was normalised about 14 days later, but herd yield stabilised at a level 5% lower than before infection. At the lowest point, the individual yield was 19–56% of the preinfection level. The milk loss per cow during the period was equivalent to 2.6 and 5.4 days of normal production. The total production of the group on 7 dpi was 36% of the preinfection level and the total milk loss up to 18 dpi was 316 kg, equivalent to 4.2 days of normal yield.

![Graph of milk yield over days post infection](image_url)
3.2. Faecal excretion of BCV

BCV was detected in faecal samples for 2–4 days in the cows and for 3–6 days in the calves. Faecal shedding of BCV in relation to diarrhoea is shown in Fig. 1. For the cows all virus positive samples were collected on days with clinical diarrhoea, except for cow 310 that showed her peak excretion before the diarrhoea started. Faecal BCV shedding was not detected during the last 1 or 2 days with diarrhoea in three of the cows. The two cows with less severe diarrhoea excreted BCV at low levels, while the two cows with watery diarrhoea showed the largest excretion of virus. For the two youngest calves (33 and 43) virus excretion occurred during the diarrhoea period, but the peak excretion took place before the diarrhoea turned watery. No virus shedding was detected in faeces of these calves during the first and the 1–2 last days with diarrhoea. For the two older calves diarrhoea was first seen on the last day of viral excretion, and for one of them the diarrhoea continued for 6 days after the detectable excretion period. The transmitter calf 306 excreted BCV in faeces (OD 0.24–0.33) 3–5 days after inoculation. This calf did not show detectable BCV in faeces 2 days after inoculation when it transmitted the virus to all in-contact cattle.

The agreement between the BCV antigen capture ELISA developed in our laboratory and the MRI ELISA was 87%, 23 samples being positive and 18 negative in both tests. Four samples were positive in the Uppsala ELISA only and two samples in the MRI ELISA only. Heterogeneity in the viral distribution within the faeces may account for these differences, since the ELISAs were run on separate dilution series of the original samples.

3.3. BCV-specific antibody responses in milk and serum

All cows were BCV antibody negative in milk and serum and all calves in serum at the start of the trial. All cattle developed BCV-specific antibody responses in all isotypes tested. The details of the isotype-specific antibody responses are shown in Tables 2–4 and examples of individual response kinetics are shown in Fig. 3. The most prominent difference between the antibody responses in the cows and the calves was that three out of four calves (42, 43 and 107) showed early weak IgM antibody responses in serum (titre 25) already 2 dpi (Table 2). In calf 33, serum IgM antibodies were first detected 7 days post-infection (dpi) as for all four cows in both serum and milk. Peak titres of BCV-specific IgM were high in both milk and serum and IgM antibodies were detectable for up to 43 days in serum and even longer in milk from three of the cows. The two cows (297 and 302) with the most severe WD symptoms still showed BCV-specific IgM end-point titres of 50 and 2, respectively, in the last samples before drying off at 126 days after the infection.

BCV-specific IgA was first detected at 7 dpi in serum and milk. The duration of the serum IgA antibody response was clearly shorter in the three youngest calves (3–4 months) than in the cows. The serum IgA antibody response of the oldest experimental calf (107) was also shorter (8 months) than that of the three cows that were followed up to 9 months and still were positive in the last samples (titres 25–125). The cows were not followed long enough to determine the exact duration of the IgA antibody response. The transmitter calf (306) was followed up to 17 mpi when BCV-specific IgA still was detectable in serum (titre 25). Peak levels of both IgM and IgA antibodies were reached a
Table 2
IgM antibody responses measured by capture ELISA in milk and serum of four cows and in serum of five calves experimentally infected with BCV, including the transmitter calf (306)

| ID | Sample | 1st IgM (dpi) | IgM detected (days) | IgM peak |
|----|--------|---------------|---------------------|----------|
|    |        |               |                     | Titre    | At day  |
| 134 | Serum  | 7             | 28                  | 78125    | 11      |
|     | Milk   | 7             | 28                  | 1250     | 9–17    |
| 297 | Serum  | 7             | 36                  | 15625    | 7–21    |
|     | Milk   | 7             | 120<sup>b</sup>     | ≥6250    | 9–14    |
| 302 | Serum  | 7             | 43                  | 15625    | 9–14    |
|     | Milk   | 7             | 120<sup>b</sup>     | ≥6250    | 11      |
| 310 | Serum  | 7             | 22                  | 78125    | 7–9     |
|     | Milk   | 7             | 50                  | 1250     | 9–11    |
| 33  | Serum  | 7             | 28                  | 78125    | 9       |
| 42  | Serum  | 2             | 33                  | 78125    | 7–11    |
| 43  | Serum  | 2             | 29                  | 78125    | 7–9     |
| 107 | Serum  | 2             | 41                  | 15625    | 9–11    |
| 306 | Serum  | 11<sup>c</sup> | 13<sup>c</sup>      | 15625    | 11<sup>c</sup> |

<sup>a</sup> dpi = days post-infection, mpi = months post-infection.

<sup>b</sup> IgM still detectable in the last milk sample before drying off at 126 dpi.

<sup>c</sup> Transmitter calf 306 was only sampled before infection and on 11 and 23 dpi, and 3 and 4 mpi and then with the same frequency as the other cattle.

Table 3
IgA antibody responses measured by capture ELISA in milk and serum of four cows and in serum of five calves experimentally infected with BCV, including the transmitter calf (306)

| ID | Sample | 1st IgA (dpi)<sup>a</sup> | IgA detected (days) | IgA peak |
|----|--------|---------------------------|---------------------|----------|
|    |        |                           |                     | Titre    | At day  | IgA titre dpi |
|    |        |                           |                     | 112      | 126     | Followed to dpi (months) |
| 134 | Serum  | 7             | 176<sup>b</sup>    | 625      | 9–49    | 182 (6 m) |
|     | Milk   | 7             | 120<sup>b</sup>    | 1250     | 9–11    | 126 (4 m) |
| 297 | Serum  | 7             | 268<sup>b</sup>    | 3125     | 7–11    | 274 (9 m) |
|     | Milk   | 7             | 120<sup>b</sup>    | 6250     | 9–11    | 250 1250 126 |
| 302 | Serum  | 7             | 268<sup>b</sup>    | 3125     | 7–14    | 274 |
|     | Milk   | 9             | 118<sup>b</sup>    | 1250     | 9–11    | 250 1250 126 |
| 310 | Serum  | 7             | 268<sup>b</sup>    | 3125     | 7–11    | 274 |
|     | Milk   | 7             | 120<sup>b</sup>    | 1250     | 9–14    | 250 1250 126 |
| 33  | Serum  | 7             | 92                 | 3125     | 11      | 517 (17 m) |
| 42  | Serum  | 7             | 106                | 3125     | 9–11    | 219 (7 m) |
| 43  | Serum  | 7             | 120                | 15625    | 9–11    | 302 (10 m) |
| 107 | Serum  | 7             | 237                | 15625    | 9–14    | 667 (22 m) |
| 306 | Serum  | 11<sup>c</sup> | 509<sup>b</sup>    | 15625    | 11–23<sup>c</sup> | 519 (17 m) |

<sup>a</sup> dpi = days post-infection, mpi = months post-infection.

<sup>b</sup> IgA still detectable in the last milk or serum sample obtained.

<sup>c</sup> Transmitter calf 306 was only sampled before infection and on 11 and 23 dpi, and 3 and 4 mpi and then with the same frequency as the other cattle.
Table 4

IgG antibody responses measured by indirect ELISA in milk and serum of four cows and in serum of five calves experimentally infected with BCV, including the transmitter calf (306)

| ID | Sample | 1st IgG (dpi)a | IgG detected (days) | IgG peak Titre | IgG peak At dpi | IgG titre dpi 112 | IgG titre dpi 126 | Followed to dpi (months) |
|----|--------|----------------|---------------------|----------------|----------------|-----------------|-----------------|--------------------------|
| 134 | Serum  | 9              | 174b               | 625            | 11–98          |                 |                 | 182 (6 m)               |
|     | Milk   | 11             | 116b               | ≥250           | 17             | 10              | 50              | 126 (4 m)               |
| 297 | Serum  | 9              | 266b               | 3125           | 14–21          |                 |                 | 274 (9 m)               |
|     | Milk   | 9              | 118b               | 625            | 11–274         |                 |                 |                          |
| 302 | Serum  | 9              | 266b               | ≥250           | 14–17, 49      | 50              | 50              | 126                     |
|     | Milk   | 9              | 116b               | 625            | 14–70          |                 |                 | 274                     |
| 310 | Serum  | 9              | 266b               | 3125           | 56–70, 154–274 |                 |                 | 274                     |
|     | Milk   | 11             | 116b               | 625            | 14–274         |                 |                 |                          |
| 33  | Serum  | 11             | 507b               | 625            | 28, 49–56      |                 |                 | 517 (17 m)              |
| 42  | Serum  | 9              | 211b               | 625            | 21–183         |                 |                 | 219 (7 m)               |
| 43  | Serum  | 9              | 294b               | 625            | 28–155         |                 |                 | 302 (10 m)              |
| 107 | Serum  | 9              | 659b               | 625            | 21–667         |                 |                 | 667 (22 m)              |
| 306 | Serum  | 11c            | 509b               | 3125           | 23–519c        |                 |                 | 519 (17 m)              |

a IgG antibodies were measured in serum and IgG1 in milk using different conjugates in the indirect ELISA. dpi = days post-infection.
b IgG1 or IgG still detectable in the last milk or serum sample obtained.
c Transmitter calf 306 was only sampled before infection and on 11 and 23 dpi, and 3 and 4 mpi and then with the same frequency as the other cattle.

few days earlier in serum than in milk except for cow 134. In the last milk samples that were obtained just before drying off at 126 dpi the IgA antibody level in all cows was one titration step (five-fold) higher than the plateau level of the preceding samples (Table 3), probably an effect of the decrease in milk volume which was not measured during this phase of the study.

Three calves showed a serum IgA antibody plateau with titres of 25–125 after 28–34 dpi. Calf 107, however, had a second titre increase from 125 to 3125 between 70 and 98 dpi. This second IgA antibody peak was not accompanied by any increase in the IgG antibody titre.

BCV-specific IgG responses were first detected at 9 dpi in serum and milk (Table 4). Serum peak IgG antibody levels were reached 1–2 weeks later in the calves than in the cows and latest in the two youngest calves. BCV-specific IgG plateau titres of 50 in milk and 125–3125 in serum were retained throughout the study period for the cows. None of the calves became IgG seronegative to BCV during the extended follow-up period up to 22 mpi. An IgG antibody titre increase in milk of the same magnitude as that in IgA was recorded at 126 dpi before drying off.

3.4. Long-lasting IgA antibody responses in nasal secretions

All cattle showed BCV-specific IgM and IgA responses in nasal secretions (Fig. 4). The details of the individual antibody responses are shown in Table 5.
Fig. 3. Examples of individual isotype-specific antibody responses to BCV (end-point titres): (a) serum and (b) milk antibody responses of cow 297. (c) serum antibody response of calf 42 measured by isotype-capture ELISA (IgM, IgA) and indirect ELISA (IgG in serum, IgG1 in milk).

In nasal secretions, BCV-specific IgM was first detected 7 dpi, being detectable for at least 8 days in all cattle. In the cows, IgM antibodies were intermittently detected for a longer period, up to 5–12 weeks post-infection (wpi).

Viewed over the whole study period the calves generally showed higher OD levels of specific IgA in nasal secretions than the cows, the difference being most pronounced
Fig. 4. Isotype-specific antibody responses to BCV (end-point titres) in nasal secretions of (a) four cows and (b) four calves (mean ± S.E.) measured by isotype-capture ELISA (IgM, IgA) and indirect ELISA (IgG).

Table 5
Isotype-specific antibody responses in nasal secretions of four cows and four calves experimentally infected with BCV

| ID  | IgM detected (dpi) | IgM peak OD | IgM peak At day | IgA detected (dpi) | IgA peak (2nd peak) OD | IgA peak (2nd peak) At day | IgG detected (dpi) |
|-----|-------------------|-------------|----------------|-------------------|------------------------|--------------------------|-------------------|
| 134 | 7–14, 28–35, 70   | 2.37        | 9              | 7–182             | 2.10                   | 14                       | 11–14             |
| 297 | 7–14, 28, 42      | 2.28        | 9              | 7–182             | 1.05                   | 11                       | 11, 42            |
| 302 | 7–14, 35, 49, 84  | 1.56        | 9              | 9–182             | 0.81                   | 14                       | 56                |
| 310 | 7–14, 28–35       | 2.22        | 9              | 7–182             | 0.96                   | 14                       | 11, 21, 84, 112, 182 |
| 33  | 7–14              | 2.45        | 9              | 9–182             | 2.16                   | 11                       | Not detected       |
| 42  | 7–14              | 2.41        | 7              | 7–182             | 2.10                   | 11                       | 42                |
| 43  | 7–21              | 2.46        | 7              | 7–182             | 2.26                   | 9                        | 28                |
| 107 | 7–14, 49          | 2.44        | 7              | 7–182             | 2.11                   | 9                        | 28–56             |

*Samples were obtained during 6 months (182 days) after infection. Samples were analysed at 1:50 dilution in capture ELISA (IgM, IgA) and indirect ELISA (IgG).
during the first month of the response. Nasal IgA antibodies to BCV were first detected 7–9 dpi in all animals. IgA antibodies were detected in nasal secretions from all cattle throughout the 6-month study period. All calves and cow 134 reached peak IgA antibody levels at 9–14 dpi, but three cows showed their highest IgA OD values later, i.e. 6, 7. In most animals, IgA antibody levels started to decline after 10 wpi, but calf 43 maintained a high OD level of specific IgA in nasal secretions throughout 6 months.

BCV-specific IgG was only detected occasionally and at low levels in nasal secretions except for calf 107 that showed detectable IgG from 4 to 8 wpi.

3.5. Interferon type 1 responses in serum, nasal secretions and milk

All cows and calves lacked detectable IFN in serum, nasal fluid and milk on the day of infection except calf 33 which had a low level of IFN (3.5 U/ml) in serum. All cattle showed an IFN peak on 2–4 dpi in serum (9.2–75 U/ml) and all but the youngest calf in nasal fluid (5.6–110 U/ml, Fig. 5). In milk, a lower peak appeared on 4–7 dpi (2.8–5.6 U/ml). The calves showed higher serum IFN than the cows, but the cows showed higher nasal IFN. Calf 33 did not show detectable IFN in nasal secretions in spite of a peak of 70 U/ml in serum on 2 dpi. Second peaks of IFN detected in some animals were very low, 3.6 U/ml on 9 dpi in serum of cow 134, 1.0 and 1.4 U/ml in nasal fluid of calves 42 and 107 on 9 and 14 dpi, respectively. Cow 297, however, had a second peak of 45 U/ml in serum on 7 dpi.

3.6. Effect of BCV infection on haematological parameters

The cows showed a significant decrease in white blood cell counts (WBC, \( P < 0.01 \)) on 2 dpi, being below the normal range (4.0–12.0 × 10⁹/l) for three of them (Fig. 6). Both neutrophil granulocyte counts (NGC) and lymphocyte counts (LCC) were significantly decreased at 2 dpi \(( P < 0.01 \) and \( P < 0.05 \), respectively), LCC being below the normal range for three cows and NCC for two cows. Cows 134 and 302 showed a neutrophilia on 14 dpi and all cows showed a second lymphopenia on 14 or 21 dpi. The two oldest calves showed a decrease in WBC on 2 and 9 dpi, but in the younger calves the response was more heterogeneous. All calves showed a mild to moderate neutrophilia (4.2–8.4 × 10⁹/l) in the week before infection.

The highest EVF after infection, mean increase 13% (range 3–34%), was recorded on 7 dpi in most cattle, but calves 33 and 43 showed their highest EVF before infection. Not even in the cows and calves with watery diarrhoea were EVF values above the normal range detected.

Serum electrolyte levels only deviated slightly from the baseline in spite of probable losses in the cows and calves with watery diarrhoea, indicating that serum electrolytes were compensated by the intracellular pool. Potassium was slightly above the normal range in cow 302 during the diarrhoea (9 dpi) and in the two youngest calves (33 and 43) during or just after the diarrhoea. Calf 33 showed serum sodium on the lower border of the normal range during the diarrhoea. No changes in serum chloride were detected.
Fig. 5. Interferon type 1 responses in body fluids of (a) four cows and (c) four calves (mean ± S.E.) experimentally infected with BCV. (b) Individual milk interferon responses of the cows.

3.7. Other infections

Group A rotavirus infection was detected by ELISA in the two youngest calves: in faecal samples from calf 33 during the diarrhoea and in calf 43 before the start of the BCV experiment. The EM analysis confirmed the presence of rotavirus in faeces of calf 33 at 5 dpi. Calf 43 was without detectable diarrhoea during the days with BRV excretion, and BRV negative in faeces during all days with diarrhoea. Enteric pathogens other than BCV were not detected in any of the other faecal samples examined. The EM morphology of the inoculum was distorted, possibly due to the DMSO content, preventing proper EM examination.
Fig. 6. Total leukocyte counts of four cows experimentally infected with BCV.

No serological reactions were detected towards BVDV, PIV3, BAV3 or BLV. BRSV seroresponses were, however, detected in all cattle except calf 306, which was still seronegative on 37 dpi. All cattle were seronegative to BRSV on day 0, except cow 297 and calf 43. The BRSV antibodies of calf 43 were passive since the OD continuously declined through day 35. Cow 297 had a low level of serum antibodies to BRSV (Titre 25) on day −28 and 0. This cow showed an anamnestic BRSV antibody increase at day 4. Cow 310 showed a weak BRSV antibody response starting at day 9, while the other cattle showed weak antibody responses first detectable at 17–28 dpi.

4. Discussion

This report describes the establishment of infection models for both WD in adult cattle and calf diarrhoea using the same strain of BCV. Many similarities in the disease outcome and some differences were seen between the cows and the calves. Experimental evaluation of BCV infections is hampered by the difficulties to find seronegative cattle constituting an immunologically relatively homogeneous group. All cattle used in this experiment were seronegative to BCV. The contribution of predisposing factors to outbreaks of WD has been widely assumed (Campbell and Cookingham, 1978). In this experiment, however, there were no obvious predisposing events, e.g. adverse weather: the infection was done in the beginning of May; stress: the cows were installed and accustomed to the ration several weeks before the trial; preceding infections: the mild respiratory infection and the sinusitis occurred well in advance of the trial.

The efficiency of the BCV transmission model, and the homogeneity of the BCV exposure is shown by the highly homogenic appearance in all cattle of IFN, IgM, IgA and IgG responses locally and systemically, and for the cows by the concurrent fevers, leucopenias, diarrhoeas and faecal shedding of BCV. BCV faecal shedding also was detected in all the calves. Among the advantages of this virus transmission model is that
the virus infectivity and virulence are better retained by the use of a faecal sample instead of a cell cultured inoculum. Virus grown in cell culture goes through a selection and adaptation process where virulence and pathogenicity factors promoting the infection in vivo may be modified. For example, the cell culture passaged virus widely used in experimental BRSV infections have caused much milder symptoms than those described after natural infections (Kimman et al., 1987; Elvander et al., 1998). The second main advantage of this trial design is that the use of an experimentally inoculated transmitter calf mimics the natural way of transmission as closely as possible, ensuring that the virus reaches the proper host cell and that the host response to infection follows the scheme of a natural infection. This is of particular importance, since the mode of transmission in natural WD is not fully known. By keeping the experimental cattle as a herd in a shared stable unit, the model allows for circulation and recirculation of BCV, which may be an important feature of the natural BCV disease, since BCV is a highly contagious infection. The model, however, depends on the transmitter calf effectively propagating the virus. The use of a group of transmitter calves would compensate for individual differences and further improve the model.

The disadvantage of the model, that the viral dose received by the experimental cattle is not quantified, is of less importance than deviations in mode and virulence of an experimental infection from a natural one. The transmitter calf was much more physically active when inside the pens of the two cows that developed the most severe diarrhoea than when it was in contact with the two less affected cows. Physical activity of both carrier and recipient with frequent contacts is likely to increase the viral dose transmitted by aerosol and by oral/nasal contact with nasal secretions and faeces of the carrier calf. Differences in activity were not obvious in the calf group and differences in severity of the calf disease may, apart from viral dose, be linked to age, since severe BCV calf diarrhoea is usually described only during the first weeks of life (Torres-Medina et al., 1985; Möstl, 1990). The contribution by rotavirus to the diarrhoea in the youngest calves is discussed below.

The use of conventionally reared cattle and a faecal sample as viral source may impose difficulties in ensuring that no other infectious agents are present. The inoculum and faecal samples from the experimental animals were monitored for the presence of other enteric pathogens and sera were analysed for antibody responses to BVDV, BLV, PIV3, BAV3 and BRSV. No indication of involvement of other enteric pathogens was obtained, except for the group A rotavirus excreted by the two youngest calves. The serological reactions to BRSV detected in all cattle except the transmitter calf are discussed below.

There were striking similarities in disease between the cows and the calves. All cattle developed diarrhoea and respiratory symptoms and all cattle shed BCV in faeces. These results concur with previous reports of a BCV-strain isolated from adult cows with WD causing diarrhoea in colostrum-deprived and gnotobiotic calves (El-Kanawati et al., 1996). The calf diarrhoea seen in this experiment was more frequent and more severe than is usually reported from WD outbreaks. The BRV infection probably contributed to the severity of the diarrhoea in the youngest calf, which excreted BRV during the diarrhoea. Also, the preceding BRV infection, although subclinical, may have aggravated the diarrhoea elicited by the BCV infection in the other calf that developed watery diarrhoea. The BRV infection in these calves underlines the multifactorial pathogenesis of
neonatal calf diarrhoea, possibly explaining the variable diarrhoea frequency in young calves in natural WD outbreaks. The feeding of BCV antibody negative milk during the entire clinical study period may also have contributed to a more pronounced calf diarrhoea in this experiment than in natural WD outbreaks (Travén et al., 1993). Most Swedish dairy calves are raised on a milk replacer, usually containing a moderate level of BCV-specific IgG1 antibodies (Travén, unpublished observations). Frequently supplying such antibodies into the gut lumen may convey partial local immunity.

The major difference in clinical outcome between cows and calves was that while the calves were unaffected in general condition, the cows showed obvious depression with a surprisingly extended decrease in appetite even in the cows with less diarrhoea, leading to the marked decrease in milk yield. These results suggest that seronegative conventional calves, even without being fed specific milk antibodies, are less severely affected by a BCV infection than seronegative cows. The difference in disease possibly reflects differences in intestinal virus replication and cell destruction between lactating cows and calves, which may be influenced by the ration, a field for further research. Unaffected general condition in BCV-infected calves, as in this experiment, probably leads to an underestimate of the calf diarrhoea frequency in natural WD outbreaks reported (Rollinson, 1948; Hedström and Isaksson, 1951; Roberts, 1957; Travén et al., 1993). This study supports the view that the same strains of BCV cause WD and calf diarrhoea outbreaks. As a matter of fact, evidence for differences between WD and calf diarrhoea BCV isolates is lacking (Reynolds et al., 1985; El-Kanawati et al., 1996; Tsunemitsu and Saif, 1995; Dea et al., 1995; Milane et al., 1997).

For most of the experimental cows and calves, viral excretion at high levels was very short and for some the excretion peaked before diarrhoea started or became pronounced, explaining the high frequency of false negative results reported using both ELISA and immuno electron microscopy in diarrhoeic calves (Heckert et al., 1989) and in cows with WD (Smith et al., 1998). The sensitivity of our BCV antigen ELISA was comparable to that of the MRI ELISA implicating the necessity of frequent sampling over a period of time to detect the excretion of virus in individual animals.

Very short fever peaks as recorded in this experiment, except for the cow with sinusitis, are easily missed in field outbreaks, hence the low frequency of fever reported in WD outbreaks (Jones and Little, 1931; Hedström and Isaksson, 1951; Roberts, 1957; Travén et al., 1991). For the cows, the incubation period with respect to the diarrhoea concurs with that assumed in natural WD outbreaks (Hedström and Isaksson, 1951; Roberts, 1957). The shorter incubation period in the youngest calves than in the other cattle also agrees with previous reports (Mebus et al., 1973; Saif et al., 1986; Tsunemitsu et al., 1999).

The inadvertent BRSV infection makes it difficult to assess the contribution of BCV to the respiratory symptoms observed in this study. The lower respiratory tract symptoms shown by one of the cows were probably caused by BRSV, a well-known lower respiratory tract pathogen both in calves (Baker et al., 1986) and in cows (Elvander, 1996), while BCV replication mainly takes place in the upper respiratory tract epithelium (Reynolds et al., 1985; Saif et al., 1986). In two cows, the BRSV antibody response was detected early, indicating that these cows became infected with BRSV at about the same time as BCV was introduced. In the other cattle the BRSV antibody response appeared
later, indicating, together with the biphasic appearance of respiratory symptoms and lymphopenia in some of the animals, that the early mild respiratory symptoms may have been caused by BCV.

A high and long-lasting IgM antibody response was detected in serum of all the experimental animals and in milk of all four cows, confirming a primary BCV infection. The early serum IgM antibody response of calves (2 dpi) detected in this study is consistent with results in mice where antigen-specific IgM producing cells were found in the spleen at 3 days after immunization, the earliest samples obtained (Buiting et al., 1996). In experimental infections of calves, weak faecal BRV-specific IgM was first detected on 5 dpi (BRV, Saif and Smith, 1985), and serum and local BRSV-specific IgM on 6 dpi (Kimman et al., 1987, 1989; Westenbrink and Kimman, 1987). Contributing to early detection is a sensitive test. These very early IgM antibodies, only detected in calves, may signify an initial T cell independent response. A potent innate immune system has been shown in neonatal rodents and humans in contrast to the low capacity for T cell dependent antibody responses (Marshall-Clarke et al., 2000). Further, viruses of several different types and families, and possessing repeated antigenic determinants, elicit T cell independent IgM responses in mice (Bachmann and Zinkernagel, 1996).

The very extended BCV-specific IgM responses in milk of two cows concur with previous results (Näslund et al., 2000). In nasal secretions, the IgM antibody peak was high and short (1 week), but for the cows this was followed by a period of intermittent detection, possibly indicating prolonged viral replication or recirculation of the virus. This concurs with the detection of BCV-positive nasal cells in naturally BCV infected calves up to >2 months after the first detection (Heckert et al., 1991), while experimentally infected calves only showed BCV antigen in nasal epithelial cells or intestinal tissues up to 18 and 21 dpi, respectively (Heckert et al., 1989; Kapil et al., 1990).

Long-lasting BCV-specific IgA responses were detected both locally and systemically in all experimental subjects, serum IgA antibodies being detectable up to 17 months post-infection. The correlation between age and the duration of the serum IgA antibody response (Table 3) confirms previous findings (Näslund et al., 2000), probably reflecting a more efficient induction of IgA memory in the cows compared with the calves and in the older calves compared with the younger ones. Interestingly, IgA antibodies were maintained in nasal secretions from all cattle throughout the 6-month study period, but the protective value is difficult to assess since such studies are lacking. The low and sporadic occurrence of BCV-specific IgG in nasal secretions indicates minimal serum transudation, caused by sampling or inflammation, confirming the local origin of the consistently detected IgA response. The late occurrence (6-7 wpi) of the highest specific IgA values (“2nd peak” in Table 5) in nasal secretions of three cows and one calf may indicate prolonged viral replication or recirculation of the virus within the experimental herd. The contribution to this finding by variations in total IgA content due to dilution through increasing amount of nasal secretions seems less likely, because nasal discharge during the early IgA antibody response was observed in both cows and calves. Also, the only cow showing markedly increased nasal secretions had the highest level of BCV-specific nasal IgA. Some investigators relate the level of specific IgA to total IgA for quantification and comparison of specific IgA responses in secretions (Hordnes et al.,
1997; Berstad et al., 1997) to circumvent an unknown dilution factor introduced by the sampling. In this study the tampon sampling technique was used, resulting in undiluted secretions, therefore the level of specific IgA should be more comparable between samples.

The second BCV-specific IgA peak in serum of calf 107 may indicate reinfection or, possibly, reactivation of the virus, even though no four-fold or greater IgG antibody response was detected. IgA responses in paired samples without significant IgG responses have been reported both in BCV-reinfected cows with WD (Alenius et al., 1991; Koopmans et al., 1991; Saif et al., 1991; Kruiningen, 1992) and in naturally BCV-reinfected calves (Heckert et al., 1990, 1991).

IFN type 1 detection is an early indicator of infection with IFN inducing viruses (Trávén et al., 1991). High titres of IFN were detected both locally, in the upper respiratory tract, and systemically in this study. These findings concur with the efficient IFN induction shown in vivo for another coronavirus, transmissible gastroenteritis virus (TGEV) of swine (Bonnadière and Laude, 1981) and in vitro for many coronaviruses, including BCV (Baudoux et al., 1998). Fever and leucopenia are rapidly occurring, reversible side-effects of IFN type 1 treatment in humans (Quíñada, 1992). In this experiment fever, leucopenia and the EVF decrease strikingly coincided with the serum IFN peak, indicating that these symptoms may be mediated by IFN. The low IFN level detected in serum of the youngest calf on the day of BCV infection was probably induced by the rotavirus infection (Broecke et al., 1984). The weak second IFN peak in serum or nasal secretions from some cattle may indicate recirculation of BCV within the group. IFN detection in bovine milk has not been reported previously, to the authors’ knowledge. Detectable milk IFN titres were retained for 6 days in three out of four cows, but the low level of the response, probably due to dilution, may limit utilisation of milk IFN as an infection marker.

It is unlikely that the non-specific responses to the BCV infection (IFN, fever, early leucopenia) were influenced by the BRSV infection, since the non-specific responses were highly synchronised in all cattle showing such responses, corresponding to the highly synchronised BCV-specific antibody isotype responses that indicated the establishment of a homogenous BCV infection. The BRSV seroresponses, on the other hand, were more divergent (seroconversions on days 9–28), indicating a slower transmission of BRSV within the experimental herd or a delayed replication of, or response to, BRSV caused by the BCV infection as previously shown with BVDV (Elvander et al., 1998). Furthermore, there was no difference in the non-specific responses (or in the clinical outcome) between the two cows that seroresponded early to BRSV and the two that responded more than 1 week later. Concerning the capacity of respiratory syncytial viruses to induce interferon type 1, Elvander et al. (1998) failed to detect IFN in serum of calves experimentally infected with BRSV, concurring with the poor IFN inducing capacity shown by human RSV in vitro (Roberts et al., 1992) and in vivo (Collins et al., 1996). The rotavirus infection may have contributed to the IFN response detected in the calf shedding BRV during the diarrhoea, but not in the other calf, since BRV shedding stopped before the IFN was detected. These two calves showed neither fever, nor leucopenia. The decrease in milk yield occurred simultaneously in all the cows, indicating that it was brought about by the BCV infection. The BRSV infection possibly
prolonged the subnormal yield level somewhat in the cow that showed more pronounced respiratory symptoms, but no obvious influence was seen comparing the individual yield recovery (Fig. 2).

In conclusion, both the cows and the calves clearly became infected by this strain of BCV and developed disease comparable to that seen in naturally BCV-infected cows (WD) and calves (neonatal diarrhoea), supporting the view that the BCV strains causing natural outbreaks of calf diarrhea and WD are identical. However, seronegative conventionally reared calves are less severely affected by BCV infection than seronegative, lactating cows. Co-infections or sequential infections with other enteric pathogens, like the rotavirus infection in some of the calves in this study, probably are important under field conditions for the development of severe calf diarrhoea from BCV infection. This assumption is in accord with the well-established multifactorial etiology of neonatal calf diarrhoea.

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