Experimental Infection with Bovine Coronavirus (BCV) in Lactating Cows: Clinical Disease, Viral Excretion, Interferon-α and Antibody Response

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Introduction

No attempts have been made to reproduce winter dysentery (WD) experimentally in adult cattle since the viral etiology (BCV) of the disease was elucidated. In the early experiments[1] clinical disease was inconsistently produced, probably mainly because the BCV antibody status of the animals could not be determined. The object of this experiment was to reproduce WD in lactating cows and to study the clinical and immunological events and the excretion of virus.

Materials and Methods

Cows and sampling

Four lactating cows of the Swedish Red and White breed (SRB) were purchased from two different farms. All cows were devoid of antibodies to BCV and were housed for four weeks in the experimental unit before starting the trial. Clinical examinations were done on several occasions before the trial and daily during the first 18 days post infection (dpi).

Blood samples were drawn from the ventral tail vein using sterile evacuated tubes. Milk samples were collected from the same front teat on each cow every time. Nasal secretions were collected using cotton tampons that were inserted into one nare and left to suck for 10 to 30 minutes. No elusion fluid was used. Faecal samples were taken from freshly laid faeces, or from the rectum when the cow had diarrhoea. Samples were taken on two or three occasions before infection and 2, 4, 7, 9, 11, 14 days, 3, 4, 5, 6, 7, 8, 10, 12, 14, 16, 18 weeks, 5 and 6 months after infection.

Virus and method of infection

BCV-containing faeces collected from cows during a winter dysentery outbreak was stored in -70°C with 10% DMSO before inoculation. 50 ml of faeces suspended in 50 ml of phosfate buffered saline were given through a stomach tube to a three-month old conventionally reared SRB calf devoid of antibodies to BCV. Also, 3 ml of the faecal suspension were sprayed into each nostril. Two days after inoculation the calf was brought to the cows and kept for one hour in each cow's pen. Each cow had direct nose contact with the calf and each cow also was brought in nose contact with the calf's faeces.

Haematological analyses

Erythrocyte volume fraction, white blood cell count, differential leukocyte count, sodium, potassium and chloride concentrations were analyzed using routine methods in samples taken before and during the first three weeks after infection. Statistical analyses of the haemotological results were done in Student's t-test for paired samples, comparing the mean of the three sampling occasions before infection to the value on specific dpi.

Bioassay for Interferon-α (IFN)

Bovine IFN was assayed as previously described for porcine IFN[2] in microtiter plates using bovine kidney cells challenged with vesicular stomatitis virus. End-point titres were estimated as the reciprocal of the sample dilution protecting 50% of the cells from destruction, and expressed as units (U) per ml. IFN was analyzed in sera, milk and nasal fluid samples collected before infection and 2, 4 and 7 dpi.

Virus analysis

Viral excretion in faeces was measured in all faecal samples in a capture Elisa system. Briefly: microtitre plates were coated with a mouse anti-BCV monoclonal antibody (mab). An optimal dilution of the same mab conjugated to horseradish peroxidase and the faecal sample in 1:5 dilution were added simultaneously to the wells and incubated in a single step at 37°C for one hour. After washings, the substrate was added and the reaction stopped after 10 minutes. Absorbance values were read in an automatic microtitre plate reader.

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Antibody isotype-specific assays

IgG1 was measured in an indirect Elisa system,[3] and IgM and IgA in capture Elisa systems.[4] All sera and milk samples were analyzed.

Results

Clinical disease

A short fever peak (40.0-41.3°C) was detected in three cows two dpi. On that day all cows showed slightly loose faeces, but undisputable diarrhoea was not seen until 4 to 5 dpi. All cows developed diarrhoea, ranging from moderate on only one day to profuse watery for four days in the two cows most seriously affected. One of the cows had slightly blood-tinged faeces. All cows also developed mild to moderate respiratory symptoms: serous to mucopurulent nasal discharge and a dry cough. One cow also had moderate dry and moist rales on auscutation of the lungs for several days. All cows, however, had a period of dry cough one to three weeks before the BCV infection, but appeared without cough the last week before infection. There was no consistent change in respiratory frequency, but all cows showed a decreased pulse rate about 5 to 8 dpi. Average heart rate values before infection were between 62 and 76 beats per minute (bpm). The lowest values after infection were between 48 and 56 bpm, a 20 to 26% decrease for each cow. The cows were yielding 16 to 27 litres of milk per day before the BCV infection. The yield was reduced to 19 to 56% of preinfection levels at the lowest point, 5 to 7 dpi. The total milk loss per cow was equivalent to between 2.7 and 5.8 days production.

Haematology

There was a significant decrease in white blood cell count ($p<0.01$), the neutrophil granulocute count ($p<0.01$) and the lymphocyte count ($p<0.05$) at 2 dpi. One of the cows with watery diarrhoea showed a transient rise in the eryhtrocyte volume fraction 7 dpi, but there was no change among the others. There was no change in the electrolyte levels even in the two cows with watery diarrhoea.

IFN

All cows lacked detectable IFN in serum, milk and nasal fluid on the day of infection. There was an IFN peak in serum (9.2 to 37.0 U/ml) and nasal fluid (5.6 to 110.0 U/ml) 2 to 4 dpi. In milk a lower peak (2.8 to 5.6 U/ml) appeared 4 to 7 dpi.

Viral excretion

BCV was detected in faecal samples from all cows for 2 to 4 days. Almost all virus positive samples were collected on days with clinical diarrhoea. However, in three of the cows virus could not be detected during the last one or two days with diarrhoea. The two cows with watery diarrhoea showed the largest excretion of virus.

Antibodies

All cows were shown to seroconvert to BCV, IgM antibodies were detected in all cows within a week after infection. At present, the samples are in process, therefore the details of the antibody response will be presented at the poster session.

Conclusions

Four lactating cows seronegative for BCV developed a clinical disease closely resembling winter dysentery after contact with a calf experimentally infected with BCV. The transmission of BCV was indicated by the appearance of IFN and was confirmed both by the presence of BCV in faeces and the detection of seroconversion. This is to the authors' knowledge the first time that winter dysentery has been experimentally induced through infection with BCV. The symptoms observed in the cows were those commonly reported in WD outbreaks[1]: diarrhoea, a short fever before the diarrhoea, a decrease in the milk yield and rather mild respiratory symptoms. In addition a bradycardia was noted.

Summary

Four bovine coronavirus (BCV)-antibody negative lactating cows were infected with a Swedish field strain of BCV through one hour of contact with an experimentally infected calf. The cows developed a short febrile response, moderate to profuse watery diarrhoea, respiratory symptoms and a marked reduction in milk yield. The incubation period with respect to the diarrhoea was 4 to 5 days. There was a transient leukopenia and neutropenia two days post infection (dpi). A peak of interferon-α was detected using a viral inhibition bioassay in serum and nasal secretions 2 to 4 dpi and in milk 4 to 7 dpi. Using a capture Elisa, antigen was defected in faeces for 2 to 4 days in all cows, mainly during the diarrhoea. IgM, IgA and IgG1 antibody responses were detected after infection in serum and milk samples from all cows using isotype-specific Elisas.

References

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