Coronavirus-associated diarrhea (winter dysentery) in adult cattle

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Outbreaks of acute diarrhea in adult cattle during the winter season have been reported from a number of countries (1-5), the disease often being referred to as winter dysentery. Early investigations attributed the disease to *Campylobacter fetus* subspecies *jejuni* (3), but the disease was subsequently thought to have a viral etiology (1,3,6,7). It is considered unlikely that the disease is caused by infectious bovine rhinotracheitis (IBR) or bovine viral diarrhea (BVD) viruses, parvoviruses, or enteroviruses (8-10). In recent years there have been several reports associating the disease with infection by coronaviruses (4,5,11-14).

We document herein two outbreaks of winter dysentery in Saskatchewan herds associated with enteric infection by coronavirus-like agents.

On *property A*, an outbreak of diarrhea occurred in a dairy herd of 35 cows, ten heifers, one bull, one steer and 13 calves during March 1988. The animals had all been housed in a dairy barn since late November 1987, and there had been no additions to the herd during the previous six months. The herd had a history of a similar but much milder outbreak about five years previously.

The disease began in three breeding age heifers and an 18-month-old bull that were held together in one area of the barn. The disease quickly spread to animals in all areas of the barn, affecting all ages of stock. Eventually, 27 cows, eight heifers, one bull and five calves were affected (68% of the herd). Fourteen cows and heifers developed severe diarrhea and dysentery, while moderate and mild disease was seen in 12 and 10 animals respectively.

Most animals developed mild diarrhea on the first day. More severe diarrhea and dysentery then developed, and lasted for two to three days before fecal consistency returned to normal. Many animals also developed a mild cough. Feed consumption dropped considerably, two cows being completely anorexic for two days. During the outbreak, herd milk production dropped to less than half normal. Treatment for coccidiosis using sustained release tablets and liquid preparations of sulfamethazine was unsuccessful.

Affected animals were bright and alert. All had normal rumenal motility, and rectal temperatures were normal (except in one cow which also had mastitis). Numerous small oral erosions were seen in one cow.

Following a provisional diagnosis of winter dysentery, fecal samples were collected from ten severely affected animals and serum samples from six animals. Further serum samples were collected three weeks later.

The feces were subjected to routine bacteriological, parasitological and virological examinations. Small numbers of *Campylobacter*-like organisms were seen in fecal smears from six of ten animals, but no *Campylobacter* or *Salmonella* organisms were isolated. Small numbers of oocysts of *Eimeria zuernii* and *E. bovis* were seen in two of the samples. Indirect fluorescent antibody (FA) tests for rotavirus were negative, but FA reactions for coronavirus were suspicious in two of ten fecal samples.

The fecal suspensions were prepared for electron microscopy by low speed centrifugation followed by ultracentrifugation through 20% sucrose (Airfuge, Beckman Instruments, Palo Alto, California) at 122,000 *g* for 12 min. Examination of the resultant pellets stained with phosphotungstic acid revealed coronavirus particles in all ten fecal specimens, though generally in low numbers (Figure 1). Attempts to culture the virus in embryonic bovine kidney and trachea cells in the presence of 10 μg/mL of trypsin were unsuccessful. Several fecal samples were tested and found to agglutinate rat erythrocytes at 4°C, 24°C and 37°C. A high titered extract was therefore used to test acute and convalescent sera for antibodies using a standard hemagglutination inhibition test. Although antibody titers of up to 1:64 were present, no patterns of seroconversion could be demonstrated. Examination of six paired sera by ELISA showed no pattern of seroconversion to IBR, BVD or parainfluenza 3 (PI3) viruses.

On *property B*, an outbreak of diarrhea occurred during February 1988 in a herd containing 60 adult Simmental cows and 18 yearling heifers. The two groups of animals were held in separate corrals. Four heifers and several adult cows developed bloody diarrhea and weight loss over a period of one to two

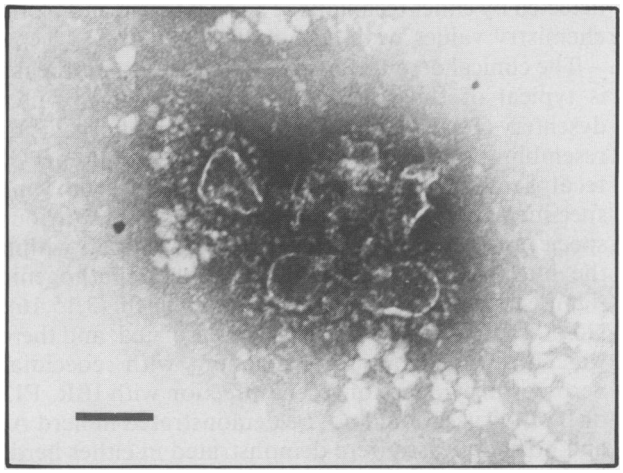

Figure 1. Electron micrograph showing a cluster of coronavirus particles from a bovine fecal sample from property A. Bar = 100 nm.

Can Vet J 1989; 30: 825-827

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days. One cow was presented to the Western College of Veterinary Medicine for examination. She was somewhat depressed, had poor body condition and slightly pale mucous membranes. Temperature and heart rate were normal, but the respiratory rate was elevated to 56 per minute. Rumenal movements occurred twice per minute, and were accompanied by splashing and gurgling sounds in the right paralumbar fossa. The feces were dark red and of watery consistency, and the tail and perineum showed considerable fecal staining. The cow had been examined one week previously because of the weight loss. At that time, her fecal consistency was normal, though analysis of a liver biopsy revealed a deficient hepatic copper status (0.04 μmol/g wet matter). Copper supplements were not given to the herd.

The cow was placed in isolation, treated with oral electrolytes, and given a subcutaneous injection of 100 mg of copper (as copper EDTA). Over the next two days the cow became progressively brighter, and the feces became soft and green with no evidence of blood. She continued to do well. The remaining animals recovered without treatment.

Fecal and blood samples were taken from the cow following development of diarrhea. Feces were also submitted by the owner from two of the affected heifers.

As coronavirus particles were detected in all samples examined in the present investigations, and no other agents could be incriminated, coronavirus infection is currently regarded as the most probable cause of the two outbreaks

Small numbers of *Campylobacter* spp. were isolated from one of the three fecal samples submitted, but no other significant bacteria were isolated or seen. Only small numbers of oocysts (not *Eimera zuernii* or *E. bovis*) were seen. FA tests demonstrated coronaviruses in all three specimens, being confirmed by electron microscopy in two. No rotaviruses were detected by either technique. The hemogram and blood chemistry values were essentially normal.

The clinical description given in these two outbreaks is typical of those previously described for winter dysentery (1,3). Although small numbers of organisms resembling *Campylobacter* were seen in a number of fecal samples, they were only isolated from one specimen and then only in very low numbers. Various species of this organism are commonly found within the intestinal tract of calves, but their pathogenic significance is currently regarded as doubtful (3,15,16). No other significant pathogens were isolated, and there was little evidence of infection with coccidia. Serological evidence of recent infection with IBR, PI3 or BVD viruses could not be demonstrated in herd A, and no rotaviruses were demonstrated in either herd. It was considered that the weight loss seen in the cow on property B was associated with copper deficiency, and was unrelated to the outbreak of diarrhea.

While there are numerous reports associating coronaviruses with diarrhea problems in calves, there have been relatively few reports of intestinal coronavirus infection in adult cattle. Most of these reports correlated the presence of coronavirus with outbreaks of acute diarrhea and dysentery (4,5,11-14). A recent report also demonstrated seroconversion to coronavirus in recovered cattle (4). Limited pathological data suggest that the lesions seen in winter dysentery (7) show some similarity to those seen in calfhood coronavirus infection (11), though possibly with greater damage to the colonic epithelium. This may account for the profuse watery diarrhea that is characteristic of the disease.

It is becoming increasingly probable therefore that coronaviruses may be involved in causing outbreaks of winter dysentery. Nevertheless, it should be appreciated that coronaviruses have been found in the feces of a proportion of normal cows (17,18), and have been demonstrated in cattle feces during the winter months in association with parturition (19).

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Acknowledgments

We wish to thank Dr. Douglas Bienia for his cooperation with the investigation into the outbreak on property A.

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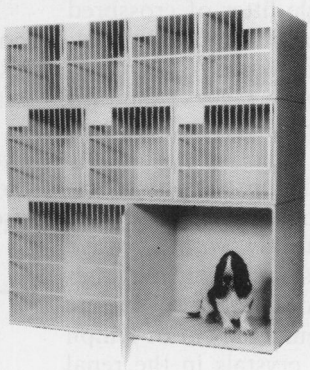

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