

Detection and isolation of coronavirus from feces of three herds of feedlot cattle during outbreaks of winter dysentery-like disease

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- ▶ Clinical signs of a winter dysentery-like syndrome in 6- to 9-month-old cattle in 3 feedlots included acute onset of diarrhea with high morbidity and low mortality, respiratory tract problems that included dyspnea, coughing, and nasal discharge, and high rectal temperatures.
- ▶ Bovine coronavirus was detected by use of an ELISA and immune electron microscopy in fecal and nasal swab samples and by immunohistochemical analysis of intestinal sections collected from calves during necropsy.
- ▶ Bovine coronavirus should be considered in the differential diagnoses for diseases that cause acute onset of bloody diarrhea in feedlot cattle.

During September and November 1999, 6- to 9-month-old feedlot cattle weighing 227 to 364 kg (500 to 800 lb) in 3 herds in Iowa (herds 1 and 2 in central Iowa, herd 3 in northwest Iowa) had acute onset of diarrhea that sometimes contained blood. During a 3-day period, all calves in herds 1 (120 calves) and 2 (50) and 6 of 27 (22%) calves in herd 3 had acute onset of diarrhea. Feces were fluid and dark (brown-black) and sometimes contained frank blood. Diarrhea typically lasted for 7 to 14 days in each herd. Two calves in herd 1 and 1 calf in herd 3 had severe bloody diarrhea that resulted in death. Rectal temperatures were high (40 to 41.4 C [104 to 106.5 F]) in the most severely affected cattle. Ten of 50 (20%) calves in herd 2 and almost all calves in herds 1 and 3 developed transient respiratory tract problems characterized by dyspnea, coughing, and nasal discharge. During the fall of the preceding 3 years, calves in herd 3 that were of similar weight (approx 364 kg) had nonfatal bloody diarrhea, with the first episode coinciding with the introduction of a new bull into the herd.

All calves in these 3 herds had been vaccinated with killed vaccines against infectious bovine rhinotracheitis virus, bovine viral diarrhea virus (BVDV) types

1 and 2, parainfluenza virus 3, bovine respiratory syncytial virus, *Haemophilus somnus*, and 7 species of clostridial organisms. Vaccinations were performed 2 weeks prior to weaning, and calves were given booster vaccinations at weaning. Cows and calves in these herds were not given vaccines against diseases that typically cause diarrhea in calves. Twenty-five fecal samples and 3 nasal swab specimens were collected from calves in these herds and submitted to the Iowa State University Veterinary Diagnostic Laboratory and the Ohio Agricultural Research and Development Center.

Using standard flotation techniques, a low number of eggs of *Trichostrongylus* and *Moniezia* spp and coccidia oocysts were detected. In general, in feces of calves with diarrhea in the 3 herds, oocysts were consistent in size and morphologic characteristics to that of *Eimeria bovis* and *E auburnensis* (herd 1), *E zuernii* and *E canadensis* (herd 2), and *E cylindris* (herd 3).

To detect *Salmonella* spp, *Escherichia coli*, and *Clostridium perfringens*, intestinal swab specimens were cultured aerobically on a specific agar^a as well as Brilliant Green plates and aerobically and anaerobically on 10% blood agar plates. Intestinal swab specimens also were incubated in tetrathionate broth. High numbers of mixed-colony *E coli* were isolated from fecal samples obtained from calves in herd 2, but they were considered likely to be normal flora. Fecal samples from herds 1, 2, and 3 were negative for *Salmonella* spp.

Fecal suspensions (1:25 dilutions) and fluid from nasal swab specimens were analyzed for bovine coronavirus (BCV) antigen by use of an ELISA, using a pool of 3 monoclonal antibodies directed against the spike, nucleocapsid, and hemagglutinin-esterase components of BCV.¹ The ELISA detected BCV antigen in 4 of 12 fecal samples from herd 1, 9 of 10 fecal samples from herd 2, and 3 of 3 fecal samples from herd 3 (Table 1). The ELISA also detected BCV antigen in 2 of 3 nasal swab specimens from herd 2.

A 20% suspension of each fecal sample was processed for immune electron microscopy (IEM), as described elsewhere,² and examined by use of hyperimmune bovine anti-Mebus BCV serum.² Aggregated coronavirus particles were observed in 7 of 12, 10 of 10, and 3 of 3 fecal samples from herds 1 to 3, respectively (Table 1). Coronavirus particles were 80 to 150 nm in diameter and had typical coronavirus surface spikes (Fig 1). The aggregated coronavirus particles were surrounded by an antibody-like fringe, indicative of a specific reaction with the coronavirus antiserum.

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Table 1—Summary of incidence of diarrhea, morbidity, mortality, and detection of bovine coronavirus (BCV) from fecal samples and fluids of nasal swab specimens obtained from 6- to 9-month-old calves in 3 feedlots in Iowa

Herd	No. of calves	Morbidity†	Mortality‡	Detection of BCV*		
				Feces		Nasal swab specimens
				ELISA	IEM	ELISA
1	120	120/120	2/120	4/12	7/12	SNA
2	50	50/50	0/50	9/10	10/10	2/3
3	27	6/27	1/27	3/3	3/3	SNA

*No. of samples with positive results/No. of samples tested. †No. of affected calves/total No. of calves. ‡No. of calves that died/total No. of calves.
IEM = Immune electron microscopy. SNA = Samples not available.

Monolayers of human rectal tumor (HRT)-18 cell cultures grown in 6-well plates were used for virus isolation, as described elsewhere.³ Of the 6 fecal samples that had positive results when tested by use of the ELISA and IEM, 2 BCV strains were isolated and adapted to serial passage in HRT-18 cells. After 3 to 5 initial blind passages, cytopathic effects, characterized by enlarged, rounded, detached, dark cells, were usually observed approximately 72 hours after inoculation. Immunofluorescence, using fluorescein isothiocyanate-conjugated bovine anti-Mebus BCV serum, was observed following inoculation of HRT-18 cells with the

2 BCV isolates after 2 and 5 cell passages, respectively. Attempts to isolate additional BCV strains, including strains from the fluids of the nasal swab specimens, are in progress. Using monolayers of bovine nasal turbinate cells, BVDV was isolated from pooled lung and lymph nodes from 1 of the 2 calves that died in herd 1. Efforts to isolate BVDV from lymphoid tissues of the calf that died in herd 3 were unsuccessful.

During necropsy, the 2 calves from herd 1 and the calf from herd 3 had similar lesions. Large blood clots and frank blood were evident in the lumen of the spiral colon and rectum. Ulcers were not detected, but moderate numbers of petechial hemorrhages were evident on the mucosa of the colon of the calves from herd 1. Other organs and tissues, including Peyer's patches, were grossly normal.

Histologic lesions in the 3 calves were predominantly in the large intestine. Approximately a third of

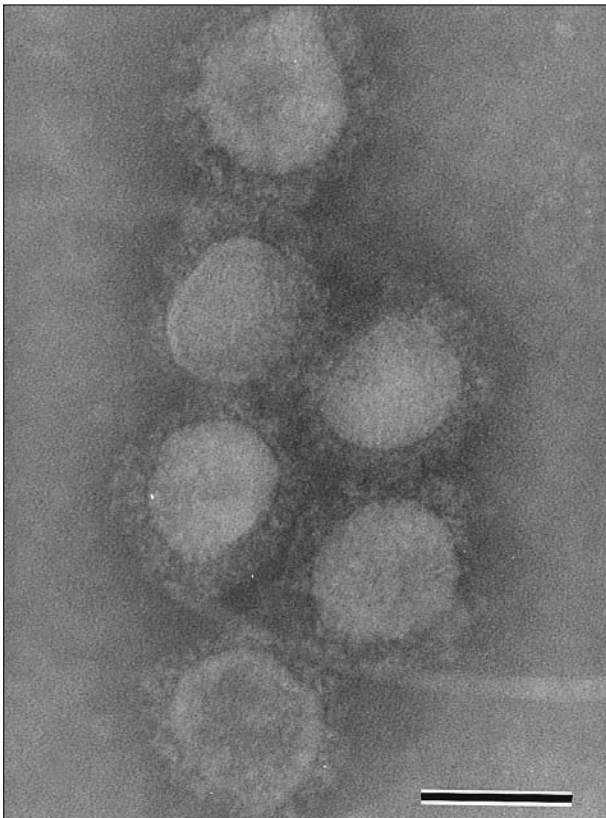


Figure 1—Photomicrograph of immune electron microscopy of coronavirus in a fecal sample obtained from a calf with clinical signs consistent with winter dysentery. The fecal sample was incubated with gnotobiotic calf hyperimmune anti-Mebus bovine coronavirus serum. Notice the aggregation of the viral particles and the antibody-like fringe coating the surface spikes. Phosphotungstic acid stain, pH 7.0. Bar = 100 nm.

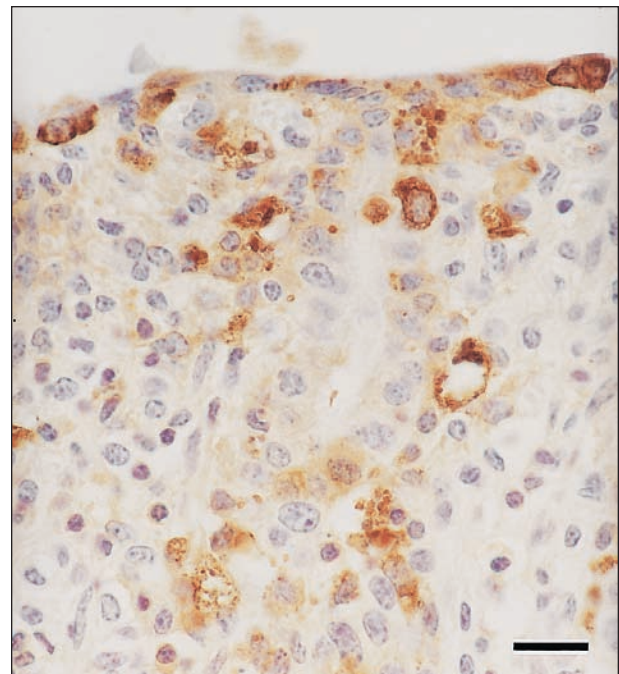


Figure 2—Photomicrograph of a section of spiral colon obtained from a calf that died as a result of diarrhea. The bovine coronavirus (BCV) antigen is detectable immunohistochemically in crypt epithelium and scattered macrophages. Avidin-biotin peroxidase immunohistochemistry, using a monoclonal antibody specific for the spike protein of BCV. Bar = 15 µm.

the crypts in the colon and rectum were dilated and lined by necrotic and metaplastic epithelium. Lamina propria was moderately expanded by neutrophils, macrophages, and increased numbers of lymphocytes and plasma cells. Small intestinal villi were of typical length, crypts were considered normal, and there was mild mixed inflammation in the lamina propria. Lesions were not observed in the lungs, kidneys, and mesenteric lymph nodes. There was moderate hepatic lipidosis with a slightly increased number of leukocytes within hepatic sinusoids. Using a monoclonal antibody-based immunohistochemical test for BVDV⁴ and BCV⁵ only BCV antigen was detected in the intestines in all 3 calves. In the colon, BCV antigen was associated with necrotic epithelium in the crypts (Fig 2).

Winter dysentery (WD) is a sporadic acute intestinal disease of adult cattle associated with BCV infections³ that has been reported in cattle throughout the world.⁶ The clinical syndrome is characterized by an acute onset of dark bloody or liquid diarrhea in adult cows.⁶ It is most commonly seen in adult dairy cattle, with affected herds having a dramatic decrease in milk production⁷⁻⁹; however, WD also has been less frequently observed in adult beef cattle.¹⁰ To our knowledge, the results reported here are the first report of a coronavirus associated with WD in feedlot cattle. Bovine coronavirus was the only enteric pathogen detected in the feedlot cattle in herds 2 and 3. The clinical manifestation in the 3 groups of feedlot cattle mimics manifestations described in adult dairy and beef cows with WD.^{7,8,10-17} The consensus from those studies is that coronaviruses are commonly isolated from clinically typical cases of WD.^{3,12} Analysis of the IEM, ELISA, and immunofluorescence results also indicated that the coronaviruses detected were antigenically related to the Mebus BCV, similar to earlier reports.^{3,11,15,16,18}

Other pathogens, including BVDV, rotavirus, Breda virus, *Salmonella* spp, and coccidia, may be associated with infectious diarrhea in adult cows.⁶ Analysis of the IEM, ELISA, immunofluorescence, histologic, and immunohistochemical results confirmed the diagnosis of BCV infections in the feedlot cattle of our report. Other known viral enteropathogens were not identified in feces of the affected feedlot calves. The only other pathogens detected were small numbers of coccidia oocysts and *Trichostrongylus* and *Moniezia* eggs. Coccidia were not observed to be associated with necrotic crypts during histologic examination. Although BVDV was isolated from the lung and lymph nodes of 1 affected calf that died in herd 1, BVDV antigen was not detected in the intestines of that calf. However, the interactions of BVDV and BCV in cattle in herd 1 may have been contributory to the disease observed. In a study¹⁹ of WD in dairy cattle, BCV and BVDV were identified as potential risk factors for WD. Analysis of data and other findings suggests that BCV is highly associated with outbreaks of WD in dairy and beef cattle^{7,8,10,15,16} and played a role in the disease syndrome observed in cattle in the 3 feedlots reported here. However, experimental transmission of coronaviruses isolated from affected feedlot cattle or susceptible bovine hosts should be confirmed to define

more clearly the cause of diarrhea and definitively assign a causative role for coronavirus.

Feedlot cattle are susceptible to respiratory tract infections and disease induced by multiple pathogens such as bovine respiratory syncytial virus, BVDV, bovine herpesvirus 1, and *Mannheimia haemolytica* (formerly *Pasteurella haemolytica*), and it is likely that concurrent infections exacerbate clinical respiratory tract disease.²⁰ Some of the viruses are believed to act as predisposing agents for bacterial pathogens such as *M haemolytica* and *Pasteurella multocida*. The feedlot calves reported here developed respiratory tract disease (dyspnea, coughing, and nasal discharge) simultaneous with onset of diarrhea. The ELISA detected BCV antigen from 2 of 3 nasal swab specimens from calves in herd 2, but nasal swab specimens were not collected from calves of the other herds. Bovine coronaviruses replicate in the respiratory and intestinal tracts.²¹ Respiratory strains of BCV are frequently isolated and detected, using ELISA, from nasal swab specimens of feedlot cattle with respiratory tract disease after transportation.²²⁻²⁴ However, it is uncertain whether BCV alone induces the respiratory tract problems observed in affected feedlot cattle.²²⁻²⁴ El-Kanawati et al²⁵ and Tsunemitsu et al²⁶ reported that the DBA strain of WD caused diarrhea and viral shedding in nasal and fecal material, but signs of respiratory tract disease were not observed in experimentally inoculated gnotobiotic or adult dairy cows. However, Traven et al⁹ reported that BCV-containing feces collected from cows during an outbreak of WD induced mild to moderate signs of respiratory tract disease and WD when inoculated in BCV-seronegative lactating dairy cows. Therefore, studies of experimental inoculation of coronaviruses isolated from these affected feedlot calves into susceptible bovine hosts, including other susceptible feedlot calves, should be conducted to better define the role of BCV in the cause of respiratory tract disease.

⁴Bacto Tergitol 7 Agar, Difco Laboratories, Detroit, Mich.

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