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 central Iowa (herds 1 and 2) and in northwest Iowa (herd 3) 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. Oocysts could be surrounded by an antibody-like fringe, indicative of a specific reaction with the coronavirus antigen.

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Monolayers of human rectal tumor (HRT)-18 cell cultures grown in 6-well plates were used for virus isolation, as described elsewhere. 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
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 BVDV, 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 infections that has been reported in cattle throughout the world. The clinical syndrome is characterized by an acute onset of dark bloody or liquid diarrhea in adult cows. It is most commonly seen in adult dairy cattle with affected herds having a dramatic decrease in milk production; however, WD also has been less frequently observed in adult beef cattle. 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 cattle. The consensus from those studies is that coronaviruses are commonly isolated from clinically typical cases of WD. Analysis of data and other findings suggests that BCV infections in the feedlot cattle of our report.

Other pathogens, including BVDV, rotavirus, Breda virus, *Salmonella* spp, and coccidia, may be associated with infectious diarrhea in adult cows. 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 study, WD in dairy cattle, BVDV, 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 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 current infections exacerbate clinical respiratory tract disease. 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 tract and intestinal tracts. Respiratory strains of BCV are frequently isolated and detected, using ELISA, from nasal swab specimens of feedlot cattle with respiratory tract disease after transport infection. However, it is uncertain whether BCV alone induces the respiratory tract problems observed in affected feedlot cattle. El-Kanawati et al and Tsunemitsu et al 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 al 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.

Other correlations were noted with virus isolation. In 2 of the herds, 20 of 21 cattle had respiratory tract disease. Bovine coronaviruses were the only virus isolated from affected cattle. Bovine coronavirus was isolated from 2 of 3 nasal swab specimens from calves in herd 1, and the DBA strain of WD was isolated from 2 of 3 nasal swab specimens from calves in herd 2. 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.

**References**


