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Serologic evidence of coronavirus infection in New York and New England dairy cattle with winter dysentery

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Abstract. Acute and convalescent sera were collected from 8 dairy herds with classic clinical features of winter dysentery. An enzyme-linked immunosorbent assay was used to measure coronavirus antibody titers, employing calf diarrhea coronavirus as antigen. Twenty-two of the 35 animals tested (63%) showed a ≥4-fold seroconversion. Adult cattle in all 8 herds seroconverted. These findings complement previously reported immunoperoxidase and electron microscopic evidence, suggesting an etiologic role for an enteric coronavirus in this disease.

Winter dysentery is an acute intestinal disorder of adult cattle, particularly dairy cattle, in the northern United States.[11] The disease occurs in epizootic proportions from November to March and is highly contagious within a farm or community. In susceptible herds, all of the adults are affected within 48 hours, periparturient heifers 2-4 years of age are the most susceptible, and calves <9 months old are unaffected. The illness is characterized by severe diarrhea, which is often blood tinged or black and has a characteristic odor, severe decrease in milk production, variable depression and anorexia, and sometimes a mild cough. Fever, leukocytosis, and leukopenia are notably absent at the time of diarrhea. The disease is worldwide in distribution and has been transmitted by feces or fecal filtrates in Sweden, Canada, Israel, Australia, New York, and Minnesota.[11] Cattle that have had the disease cannot be reinfected for several years.

In 1985, the histologic lesions were characterized for the first time.[11] The lesions consist of focal colonic crypt cell degeneration and necrosis, with hyaline intracytoplasmic inclusion bodies, and focal loss of mucosal macrophages, creating a moth-eaten appearance in the lamina propria. These features are suggestive of "virus-induced enterocolitis," and in 1987, using immunoperoxidase and electron microscopic methods, bovine coronavirus was demonstrated in epithelial cells and macrophages of diseased colonic mucosa.[12]

From 1980 to 1984, investigators from Japan, France, and Belgium reported coronavirus-like agents or coronavirus in the feces of adult cattle with "epizootic diarrhea" and "winter dysentery."[2,6,10] Canadian workers, however, reported finding coronavirus antigen, mostly in the form of immune complexes, in the feces of 70% of normal cows.[4] A report from Colorado documented a high incidence of shedding of coronavirus particles in the feces of normal dairy cattle during the winter stabling season.[3] Japanese investigators isolated a coronavirus-like agent from the feces of 1 cow with diarrhea and demonstrated hemagglutination-inhibition seroconversion to reference strains of bovine coronavirus in 59% of animals with epizootic diarrhea.[10] Over the past several winters, we have documented rises in serum enzyme-linked immunosorbent assay (ELISA) antibody titers to bovine coronavirus in herds with spontaneous winter dysentery, and tissues from animals that have died of spontaneous disease routinely contain the virus in the lesions (Van Kruiningen HJ, Khairallah LH, Grumprecht LA, et al.: 1988, Proc 39th Annu Meet, Am Coll Vet Pathol).

Coronavirus particles were recently demonstrated by immunoelectron microscopy in 8 of 9 fecal samples from an Ohio herd with acute winter dysentery, and elevated neutralizing antibody titers to the Nebraska calf diarrhea coronavirus were recorded in 5 of 6 animals.[9] Rises in antibody titers were 2-14-fold. Inoculation of 3 gnotobiotic calves with pooled feces from the sick cows resulted in diarrhea and fecal coronavirus shedding in all of the calves (Saif LJ, Redman DR, Brock KV, et al.: 1988, Proc 69th Annu Meet, Conf Res Workers Anim Dis). Coronavirus immunofluorescence was demonstrated in the intestine of 1 calf that was sacrificed, and the other 2 seroconverted. Subsequently, a coronavirus antigenically related to the Mebus strain calf diarrhea virus (CDCV) was isolated from the feces of each calf.[1]

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Canadian workers were successful in finding coronavirus particles in the feces of cattle from 2 herds with winter dysentery; however, no virus was recovered in tissue culture, and a hemagglutination-inhibition test, using fecal extract as antigen, failed to demonstrate seroconversion.[5] British workers, using an ELISA technique and electron microscopy on feces, demonstrated bovine coronavirus in 3 of 9 herds with winter dysentery, and in 1 herd tested serologically by latex agglutination inhibition, 3 of 5 affected cattle had seroconverted.[7]

The present study documents seroconversion by naturally affected adult cattle to bovine coronavirus (CDCV), as determined by ELISA, in 8 dairy herds that experienced winter dysentery.

Materials and methods

A number of veterinarians engaged in bovine practice in Connecticut, Massachusetts, and New York were contacted by letter and telephone and invited to collaborate in a study of spontaneous winter dysentery. Reprints of a published review[11] were distributed, and clinical criteria were established for the diagnosis of winter dysentery, including occurrence during the winter stabling season; negative history of winter dysentery in the previous 2-3 yr; present history of acute onset profuse diarrhea in adults, with bloody diarrhea in some; rapid spread through the herd; severe reduction in milk production; sparing of calves and young stock <9 mo of age; and absence of fever or oral lesions (of bovine virus diarrhea). Cooperating veterinarians were asked to collect acute- and convalescent-phase sera (21 days later) from 5 or 6 of the sick adult cattle in each herd selected for study and to freeze these sera for later shipment and serology.

An ELISA was used to measure coronavirus serum antibody titers. One hundred microliters of purified CDCV antigen in a carbonate-bicarbonate buffer (pH 9.6) was applied to each well of a 96-well microtiter plate and incubated for 18 hr at 4 C. The antigen-coated plate was washed 3 times with phosphate-buffered saline (PBS)-Tween 20 buffer. Test serum was added in 3-fold (1 herd) or 2-fold (7 herds) dilutions to antigen-coated wells and to cell-control wells and was incubated for 1 hr at 37 C. After 3 washings in PBS-Tween 20 buffer, 100 µ1 of goat anti-bovine immunoglobulin conjugated to horseradish peroxidase was added to each well and incubated for 1 hr at 37 C. After washing the plate in PBS-Tween 20 buffer, 100 µ1 of an enzyme substrate was added to each well. The resulting color reaction was allowed to develop at 37 C until a positive control serum sample registered an optical density (OD) reading of 1.0 at 405 nm using a micro-ELISA reader. The mean OD values for sera in the cell-control wells were substracted from the mean OD values for same sera in the antigen-coated wells to determine the antibody value for each serum dilution. The serum endpoint antibody titer was determined as the dilution where the absolute OD reading was 10.2. This cutoff value was selected after testing many known positive and negative serum samples from cattle vaccinated and/or infected with CDCV and after taking into consideration inherent test (well-to-well and plate-to-plate) variability.

Results

Acute and convalescent (14-26 days after the illness) titers were determined for 35 cattle with winter dysentery (Table 1). Twenty-two of the 35 (63%) cattle showed a ≥ 4-fold seroconversion. Of the remaining cattle, 9 had ≥ 2-fold titers, and 4 showed no change. Adult cattle in 8 herds tested had significant seroconversion to CDCV during recovery from winter dysentery.

Discussion

This study provides serologic evidence that herds in New York and Connecticut expressing classic clinical features of winter dysentery experienced a coronavirus infection. Variation in the serologic responses of individual cattle is to be expected and may reflect previous exposure to coronavirus, idiosyncratic immune responsiveness, the influence of concurrent disease, and the time of sampling in relation to exposure. In the case of the herd with the weakest association, herd 1, clinical signs actually began March 6, 3 days prior to acute phase sampling. Data from 3 other herds were discarded when, in retrospect, it was discovered that in 2 herds the first samples had not been taken until the 10th day after onset, and in the third herd, the convalescent samples had not been taken until 64 days postexposure. In the first two herds, rises in titer had probably already occurred; whereas in the third herd, titers had probably already declined. The half-life of IgG, IgG_2, and IgM in infected cattle has been estimated as 17.4, 22.4, and 4.8 days, respectively.[8] Because fever occurs 2 days prior to clinical signs, and exposure occurs 3-5 days prior to fever, first day acute phase sampling is important in any serologic study dealing with winter dysentery.[11] Irrespective of these considerations, a few animals under careful scrutiny apparently do not seroconvert. Our finding of 63% seroconversion is in close agreement with the 59% reported in epizootic diarrhea of adult cattle in Japan and the 60% reported in winter dysentery in England.[7,10]

These results and those from transmission studies, immunocytochemistry, histopathology, and electron microscopy indicate that winter dysentery is caused by a coronavirus.[11,12] Continued study of this disease is necessary to determine 1) if the winter dysentery coronavirus and the calf diarrhea coronavirus are one and the same, 2) if there is serologic and clinical variation in winter dysentery from year to year or among locales, 3) the year-round habitat of the etiologic agent, 4) if winter dysentery exists in the summertime, 5) if there are carrier states, and 6) when during the course of disease or afterward virus shedding occurs.

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Table 1. Bovine coronavirus antibody titers (ELISA technique) for 35 cattle with winter dysentery.

Herd. no.	Location	Sampling dates*	Animal Id.	Titers†	
				Acute	Convalescent
1	Cobleskill, NY	3/9/87, 3/27/87	Chloe	1,620	4,860
			Tina	540	4,860
			Dottie	540	4,860
			Princess	1,620	4,860
			Cindy	1,620	4,860
			Dee	1,620	1,620
2	Brooklyn, CT	4/14/88, 5/5/88	Oreo	800	3,200
			Miranda	1,600	3,200
			Mia	800	3,200
			Dawn	800	3,200
3	Cobleskill, NY	11/3/88, 11/17/88	Camille	3,200	12,800
			Collene	12,800	25,600
4	So. Woodstock, CT	11/9/88, 12/1/88	6	200	12,800
			44	400	25,600
			59	800	ND
5	Storrs, CT	11/12/88, 12/3/88	32	400	1,600
			33	6,400	6,400
			34	100	1,600
			35	800	6,400
			36	<100	800
			37	800	3,200
			212	3,200	3,200
6	Cobbleskill, NY	11/14/88, 12/5/88	431	3,200	12,800
			289	1,600	6,400
			463	1,600	3,200
			438	800	12,800
7	Cobleskill, NY	12/16/88, 1/6/89	1	<100	800
			2	1,600	3,200
			3	800	3,200
			4	1,600	3,200
			5	800	12,800
			6	<100	ND
8	Cherry Valley, NY	3/2/89, 3/28/89	Joy	1,600	25,600
			Joyce	3,200	3,200
			Foxy	3,200	12,800
			Canary	3,200	6,400
			Ida	1,600	12,800

* Acute, convalescent.

† Antibody titers expressed as the reciprocal of the highest serum dilution with an absolute OD value of ≥0.2 at 405 nm. ND = not determined.

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