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A serologic investigation for coronavirus and Breda virus antibody in winter dysentery of dairy cattle in the northeastern United States

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Since 1980, the amount of evidence implicating a bovine coronavirus as the cause of winter dysentery has been increasing.[13] Japanese workers were the first to recover a coronavirus from the feces of a cow with "epizootic diarrhea";[15] this discovery was followed by similar reports from Belgium[2] and the United States. [1] Because coronavirus-like particles and coronavirus antigen can be found in the feces of a high proportion of normal dairy cows in some herds during the winter stabling season,[3,4] the significance of these isolations has been interpreted with some reservation. Serologic studies revealed hemagglutination-inhibition seroconversion to reference strains of bovine coronavirus in 59% of affected Japanese cattle; [15] workers from Ohio reported 4-fold or greater rises in serum neutralization (SN) titers in 19 of 26 animals (73%);[14] and the British, using a latex agglutination inhibition test, found seroconversion in 3 of 5 affected cattle? We recently reported 63% seroconversion by an enzyme-linked immunosorbent assay (ELISA) method, in 35 sick animals from 8 herds with winter dysentery.[16] Additional evidence, and perhaps the most convincing, that a coronavirus is responsible for winter dysentery, is the demonstration by immunoperoxidase and electron microscopy methods of coronavirus in damaged colonic epithelial cells and mucosal macrophages of both spontaneous and experimentally induced cases. [18]

In the Netherlands, however, there has been a serologic association of Breda virus infection with the occurrence of winter dysentery.[7,10] Sera from 149 cows from 19 farms were tested by a blocking ELISA method, and 4-fold or greater seroconversion was found in 7-60% of cattle tested from 10 of the farms; cattle from 9 farms showed no seroconversion. In view of questions raised about the respective roles of Breda virus and coronavirus, previously assembled and some newly acquired sera were tested (retested in the case of previously assembled sera) for the presence of antibodies to these 2 viruses. The procedure used to obtain and select the sera used in this study was described in the prior report.[16]

Two serotypes of Breda virus are recognized; Breda virus serotype 1 (BRVl) represents the original isolate from Breda, Iowa, and serotype 2 (BRV2) comprises an isolate from Ohio and the second Iowa isolate. Thus far, Breda virus has not been successfully propagated in tissue culture; therefore all studies conducted with this agent employ density-gradient-purified virus particles obtained from an experimentally infected calf.[9] BRV2 is used for routine diagnostic tests because it is more stable than BRVl .[8] A direct blocking ELISA that has been previously described was used to detect antibody to Breda virus.[9,11]

Serum neutralization anti-coronavirus antibody titers were determined by a method similar to that employed for rotaviruses.[6] Nebraska strain calfhood diarrhea coronavirus (No. 874 from the American type culture collection) was grown in Madin Darby bovine kidney cells in the presence of 0.1% pancreatin and in the absence of serum. Four-fold serial dilutions of test sera were challenged with 200-500 $TCID_{50}$ for 90 minutes at 37 C. Four- or 5-day-old cell monolayers in microplate wells (grown in Eagle's minimum essential medium [EMEM] with 10% fetal calf serum) were washed free of serum, inoculated with 200 µ1 of the serum-virus mixtures (adsorbed at 37 C for 120 minutes), then rinsed and incubated further (with EMEM and pancreatin). The test was read after 2-4 days, and the antibody titer was expressed as the reciprocal of the highest serum dilution that completely inhibited cytopathic effect.

Acute and convalescent titers, the latter taken 14-26 days after the onset of illness, were determined for 37 cattle with winter dysentery. Results are summarized in Table 1. Of 36 cattle tested for serum neutralizing antibodies to bovine coronavirus, 22 (61%) showed a ≥ 4-fold seroconversion; of 37 tested for Breda virus antibody, only 3 (8%) had a ≥ 4-fold seroconversion. Seven of the 8 herds tested had seroconversion to bovine coronavirus during recovery from winter dysentery.

The 61% seroconversion to bovine coronavirus of affected cattle in herds with winter dysentery agrees very well with results reported previously from Japan (59%), Great Britain (60%) and the United States (63%).[5,15,16] When a comparison was made of serologic findings for 28 cattle that were studied by both SN and ELISA, SN recognized a 4-fold change in anti-coronavirus titer in 17 of 28 cattle, versus 20 of 28 for the ELISA. [16] The Dutch workers compared serologic findings from 149 affected cows from 19 farms with those of 67 healthy cows from 8 farms without dysentery, a comparison we have not made. They found no statistical difference in the numbers of seroconversions to coronavirus between the two groups.[10] In view of the low number of seroconversions recorded (13 in 149 tested), coronaviruses appear to be less important in winter dysentery in The Netherlands than they are in the United States and several other countries. Baseline studies are needed to determine how many dairy cattle in the United States seroconvert during winter stabling without showing signs of winter dysentery.

In The Netherlands, seroconversion to Breda virus occurred 3.5 times more frequently in cattle with winter dysentery than in healthy controls.[10] Cattle seroconverted at 10 farms studied but not at all at 9 farms with disease (6-10 animals were tested per farm). Among the 10 farms with

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Table 1. Bovine Coronavirus and Breda Virus Antibody Titers in Cattle from 8 US herds.

Herd no.	Location	Sampling dates*	Animal I.D.	Coronavirus		Breda virus	
				Acute	Convalescent	Acute	Convalescent
1	Brooklyn, CT	4/14/88, 5/5/88	Oreo	256	5,786	40	40
			Miranda	ND†	16,384	40	40
			Mia	724	1,445	<40	<40
			Dawn	724	2,902	40	<40
2	Cobleskill, NY	11/3/88, 11/17/88	Camille	4,096	11,481	160	160
			Collene	16,384	11,481	40	40
3	S. Woodstock, CT	11/9/88, 12/1/88	6	181	4,096	40	80
			44	64	2,902	40	320
			59	64	ND	40	ND
4	Storrs, CT	11/12/88, 12/3/88	32	22	256	320	80
			33	1,445	2,902	80	320
			34	11	256	320	80
			35	724	4,096	<40	40
			36	16	256	160	80
			37	22	64	>1,280	>1,280
			212	361	361	80	160
5	Cobleskill, NY	11/14/88, 12/5/88	431	724	5,786	40	320
			289	1,024	4,096	40	40
			463	5,786	11,481	<40	<40
			438	361	5,786	<40	<40
6	Cobleskill, NY	12/16/88, 1/6/89	1	22	1,024	320	640
			2	1,445	2,902	160	80
			3	1,024	11,481	40	40
			4	181	4,096	40	40
			5	1,024	5,786	40	80
			6	1,024	ND	80	ND
7	Cherry Valley, NY	3/2/89, 3/28/89	Joy	1,024	5,786	80	80
			Joyce	4,096	2,902	80	80
			Foxy	4,096	5,786	80	80
			Canary	5,786	2,902	80	80
			Ida	2,902	11,481	40	40
8	Cobleskill, NY	11/27/89, 12/19/89	3	724	4,096	40	40
			29	256	1,445	40	<40
			33	1,024	2,902	40	80
			36	361	5,786	80	80
			63	1,024	2,902	40	40
			78	361	11,481	80	80
			83	724	4,096	40	<40
			87	4,096	11,481	40	40

* Acute sample, convalescent sample.
† ND = Not determined.

winter dysentery at which seroconversion occurred, 6- 14 cattle per farm were sampled, and seroconversion occurred in 60%, 50%, 43%, 30%, 17% (4 farms), 10%, and 7% of the cattle tested. In the northeastern United States, there was 8% seroconversion to Breda virus.

Although these findings suggest that Breda virus was not important in the herds we studied, we have recently documented significant seroconversion to both Breda virus and coronavirus in 1 New York herd with winter dysentery. Both viruses were demonstrated in a pooled fecal specimen (by immunoelectron microscopy), and dual infection was reproduced in an experimental herd. In 4 of the 10 Dutch herds that seroconverted to Breda virus, there were also seroconversions to coronavirus, [10] suggesting that both viruses were active at the time of winter dysentery. Our experience with adult cattle suggests that there may be clinical differences between Breda virus diarrhea and coronavirus winter dysentery. However, these differences must be defined more extensively by experimental inoculations. Thus far, only calves have been experimentally infected with Breda virus, and the lesions in this age group have been described. [12] The frequency and distribution of Breda virus infection in adult cattle in the United States have not been defined.

This serology extends our observation that winter dysentery is associated with coronavirus infection. Workers in Ohio reported that they had recovered the coronavirus responsible for winter dysentery;[1] however, their organism was isolated from calves that had been given a mixed fecal inoculum from a diseased herd, and it is equally possible that they merely recovered an endemic calf diarrhea coronavirus. The high

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incidence of coronavirus shedding in normal stabled animals is well established.[3,4] Winter dysentery and calfhood coronavirus diarrhea do not coexist on susceptible farms. In fact, absence of disease in calves and youngstock below a given age, 9 months in some herds, up to 18 months in others, constitutes a criterion for the diagnosis of winter dysentery.[17] The coronavirus of winter dysentery may be antigenically related to, but different from, the calf diarrhea coronaviruses. There may be sufficient antigenic variation to preclude adults from infection with the calf diarrhea coronaviruses and to preclude calves from the winter dysentery coronavirus. Koch's postulates remain to be fulfilled.

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Detection of infectious bursal disease virus in digested formalin-fixed paraffin-embedded tissue sections by polymerase chain reaction

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Infectious bursal disease (IBD) affects the bursa of Fabricius of young chickens, resulting in immunosuppression, reduced weight gain, and reduced feed efficiency[9] Immunosuppression may result in poor vaccination response to other infectious agents. Despite recent advances in vaccination programs, outbreaks of this disease still occur. To help control the disease, a rapid and sensitive method for identifying IBD virus (IBDV) is essential

Viral isolation, electron microscopy, immunofluorescence, immunodiffusion, viral neutralization, enzyme-linked im-

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