Bovine coronavirus infection in Ontario, 1990–1991

P. Suzanne Carman, Murray J. Hazlett

Bovine coronavirus (BCV) is a common cause of neonatal diarrhea in calves 3–21 days old (1). The virus replicates in the epithelium of the villi of the small intestine, and in superficial and cryptal colonic epithelium. Viral destruction of cells results in villus atrophy, dilated crypts, decreased numbers of goblet cells, and profuse watery diarrhea. This can result in dehydration, acidemia, and death of severely affected calves in four to five days (1). As with bovine rotavirus, lactogenic immunity is required to protect calves from infection (1).

Infection with BCV has also been associated with outbreaks of winter dysentery in adult cattle (2,3). Histological lesions, similar to those occurring in calves, are present in crypts of the spiral colon (2,4); BCV has been demonstrated in these lesions using immunohistochemical techniques (4). Infected cows develop profuse watery diarrhea, become listless, lose weight, have decreased milk production, and may cough (2).

Many calves and most adult cattle within an infected herd have serum antibodies to BCV (1). Bovine coronavirus can remain in infected herds within persistently infected calves or cows. Five to 30% of clinically normal cows may shed coronavirus in their feces (5,6), with higher numbers of animals shedding during the stress of winter (6). The incidence of shedding can increase to 70% at parturition (6) and can result in increased clinical enteric disease in neonatal calves born into infected herds (7). Recently, BCV has been shown to replicate in the respiratory tract of calves, with viral antigen present in the epithelium of the lung, trachea, and nasal turbinates. Calves may show no clinical disease or may have a cough, nasal discharge, and increased respiratory rate (8–10). Some strains of virus have been shown to cause both enteritis and respiratory infections in susceptible calves (8).

Over the past two years (1990–1991) the Veterinary Laboratory Services Branch, Ontario Ministry of Agriculture and Food, has used three tests for the investigation of bovine coronavirus infection:

1) Beldico Eli-Vet bovine coronavirus antigen detection ELISA (BCE) assay (ConPharma Vaccines, Markham, Ontario). This commercially available, 96-well, plate assay, was used to demonstrate BCV proteins in the feces/gut scrapings of calves with neonatal diarrhea or cows with winter dysentery. Feces and intestines from animals reported to have diarrhea, respiratory disease, or both were submitted frozen.

2) Bovine coronavirus direct immunofluorescent (BCVF) assay on frozen tissue using commercially available fluorescein-conjugated antiserum (VMRD, Inc., Pullman, Washington, USA). Specimens from animals, submitted alive for necropsy to one of the six provincial diagnostic laboratories, were tested. The test was used to identify viral antigen in the distal small intestine/spiral colon of scouring calves, the spiral colon of adult cattle with winter dysentery, and the lungs of animals with pneumonia.

3) Bovine coronavirus microtiter virus neutralization (BCV-VN) assay. Because most herds have detectible levels of antibody to BCV (1), paired sera collected two to three weeks apart were used in BCV-VN antibody tests. All sera were from groups with clinically ill animals, being described by the submitting veterinarian as calves, cows, or herds suffering from respiratory disease, diarrhea, or both. The number of pairs of sera (1–10) submitted from each herd was variable and depended on what was forwarded by the veterinary practitioner attending the herd. A microtiter BCV-VN assay was performed using fourfold serial dilutions of heat inactivated (56°C, 30 min) test sera, mixed with equal volumes of BCV (Dr. L. Babiuk, VIDO, Saskatoon, Saskatchewan), containing 100 $CCID_{50}$ infectious virus units. The mixtures were incubated for one hour at 4°C, Madin-Darby bovine kidney cells were added, and plates were further incubated for four days at 37°C in a CO_2 humidified incubator. Sera were tested in duplicate, with known positive and negative BCV control antisera included for each assay. The titer was the 50% endpoint of the dilution of sera which completely inhibited the virus replication in 50% of the wells. Seroconversion (negative to positive) or a fourfold (or greater) rise/fall in concentration of antibody to BCV in one or more animals, were considered significant and were the criteria for a serological diagnosis of BCV infection.

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The Beldico BCV antigen detection ELISA test has been a useful assay for the diagnosis of BCV-induced neonatal diarrhea. Using this assay, 54 of 312 (17.3%)

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Veterinary Laboratory Services Branch, Ontario Ministry of Agriculture and Food, Box 3612, Guelph, Ontario N1H 6R8.

Table 1. Number of animal groups with bovine coronavirus (BCV)-associated clinical disease identified by seroconversion or fourfold (or greater) changes in antibody titer in paired sera using a microtiter BCV virus neutralization assay, 1990–1991

History/Age	Calves only affected	Adults only affected	Entire herd affected	Total from all groups affected
Respiratory disease only	2/5/8[a]	9/10/27	6/11/6	17/26/41
Diarrhea only	2/0/3	4/10/8	0/0/3	6/10/14
Respiratory disease and diarrhea	0/2/5	1/2/3	2/2/5	3/6/13
No history and/or age given				3/1/3

[a]Number of groups with seroconversion or fourfold (or greater) changes in antibody to BCV alone/to BCV as well as other viruses/groups with stable BCV titers
In total, 143 animal groups were tested

specimens submitted from calves with diarrhea were positive for BCV antigens. Feces from two of 50 (4%) adult groups tested were diagnosed as being positive for BCV. Where the entire herd was reported to be scouring, specimens from four of 15 (26.7%) groups tested were positive with the BCE test.

The BCV fluorescent antibody assay was not requested frequently enough to be useful for the diagnosis of BCV-related diarrhea or pneumonia. A single positive test, of the 34 attempted, was from the intestine of a scouring calf submitted alive for necropsy.

The serological BCV-VN assay was valuable for the diagnosis of coronavirus-related pneumonia and winter dysentery. A total of 968 sera were tested. Endpoint titrations of antibody titers were commonly greater than 1/4096. The highest titer identified for animals with reported pneumonia was 1/32,768; the highest titer for animals with winter dysentery was 1/8,388,608. Of 143 groups tested (Table 1), 72 had one or more animals with fourfold or greater changes in antibody titer to BCV in paired sera; 41 of these 72 groups had eightfold or greater changes. Respiratory disease was associated with seroconversion or fourfold (or greater) changes in antibody titers in paired sera for BCV alone in 17 groups. In a further 26 groups with respiratory disease, one or more additional viral agents, as well as BCV, appeared to be involved in the outbreak of disease. These groups also had seroconversion or significant fourfold (or greater) changes in antibody titer to one or more of the following: bovine respiratory syncytial virus (BRSV)-18 groups, bovine virus diarrhea virus (BVDV)-8, parainfluenza virus-3 (PI3)-7, infectious bovine rhinotracheitis (IBR)-6, or bovine adenovirus serogroup I (BAV)-1. Antibody titers to BCV were stable in an additional 41 groups.

In the five groups where paired sera from calves with diarrhea were submitted, BCV alone was implicated serologically as a cause of disease in two. In the groups where only adults were recorded to have diarrhea suggestive of winter dysentery, seroconversion and significant fourfold (or greater) changes in antibody titers to only BCV were found in four groups, while 10 other groups showed significant changes in antibody to BCV as well as one or more of the following: BVD-10, bovine parvovirus (BPV)-2, and IBR-1 (Table 1). Bovine coronavirus was not implicated in an additional eight groups.

For animal groups reported to have both respiratory disease and diarrhea, three groups had significant changes in antibody to only BCV, while another six groups had significant changes in antibody titers to both BCV and BRSV (Table 1). Seven groups were tested where age and/or history were not reported.

It was not possible to correlate results of antigen detection BCE tests with these serological BCV-VN results. Paired sera were submitted several weeks after specimens for antigen identification, with the result that sera were most often given a separate laboratory accession number. This made tracking of named herds impossible. Several submissions may have been received from the same herd. For nine groups where antigen detection and serology results were recorded on the same record, there was agreement between the two methods for diagnosis of BCV infection in five. In the other four records, a serological diagnosis of BCV infection was made but antigen could not be demonstrated using the BCE test.

Bovine coronavirus field isolates cannot be readily recovered in cell culture using routine methods (1). Furthermore, few antigen detection BCVF tests were performed, for apparently few adult animals with pneumonia died, and diagnosticians did not routinely

request testing for calves. As a result we did not demonstrate BCV in the lungs of any animal with clinical pneumonia during this two year time period. Using the BCVF test, we have subsequently identified BCV in the lung of a six-week-old Holstein calf with bronchopneumonia, from a herd where calves were reported to scour and develop pneumonia between 6–12 weeks of age, as previously described by others (10).

This serological evidence collected over the past two years suggests that BCV can be associated with respiratory disease in adult cattle, as well as neonatal calf diarrhea (1), winter dysentery (2–4), and pneumonia in calves (8–10). Infections with multiple viral agents appeared to be common, and could be expected to result in more severe clinical disease. Bovine coronavirus, as well as BRSV, may be a significant cause of respiratory disease in cattle in Ontario. Because the diagnostic laboratory normally receives specimens from herds with outbreaks of disease, submissions from groups without diarrhea or pneumonia were not tested. Further testing of sera from animals not associated with diarrhea or pneumonia and larger planned studies would be helpful in deducing the role of BCV as a cause of respiratory disease in adult cattle.

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References

1. Saif LJ, Heckert RA. Enteropathogenic coronaviruses. In: Saif LJ, Theil KW, eds. Viral Diarrheas in Man and Animals. Boca Raton, Florida: CRC Press, 1990: 211–222.
2. Van Kruiningen HJ, Hiestand L, Hill DL, Tilton RC, Ryan RW. Winter dysentery in dairy cattle: recent findings. Compend Contin Educ Pract Vet 1985; 7: S591–S598.
3. Durham PJK, Hassard LE, Armstrong KR, Naylor JM. Coronavirus-associated diarrhea (winter dysentery) in adult cattle. Can Vet J 1989; 30: 825–827.
4. Van Kruiningen HJ, Khairallah LH, Sasseville VG, Wyand MS, Post JE. Calfhood coronavirus enterocolitis: a clue to the etiology of winter dysentery. Vet Pathol 1987; 24: 564–567.
5. Crouch CF, Raybould TJG, Acres SD. Monoclonal antibody capture enzyme-linked immunosorbent assay for detection of bovine enteric coronavirus. J Clin Microbiol 1984; 19: 388–393.
6. Collins JK, Riegel CA, Olson JD, Fountain A. Shedding of enteric coronavirus in adult cattle. Am J Vet Res 1987; 48: 361–365.
7. Bulgin MS, Ward ACS, Barrett DP, Lane VM. Detection of rotavirus and coronavirus shedding in two beef cow herds in Idaho. Can Vet J 1989; 30: 235–239.
8. Saif LJ, Redman DR, Moorhead PD, Theil KW. Experimentally induced coronavirus infections in calves: Viral replication in the respiratory and intestinal tracts. Am J Vet Res 1986; 47: 1426–1432.
9. Kapil S, Pomeroy KA, Goyal SM, Trent AM. Experimental infection with a virulent pneumoenteric isolate of bovine coronavirus. J Vet Diag Invest 1991; 3: 88–89.
10. McNulty MS, Bryson DG, Allan GM, Logan EF. Coronavirus infection of the bovine respiratory tract. Vet Microbiol 1984; 9: 425–434.