Severe acute bovine viral diarrhea in Ontario, 1993–1995

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Abstract. In 1993, noncytopathic bovine viral diarrhea virus (BVDV) strains with enhanced virulence caused unprecedented outbreaks of severe acute bovine viral diarrhea (BVD) in dairy, beef, and veal herds in Ontario (Canada). Fever, pneumonia, diarrhea, and sudden death occurred in all age groups of cattle. Abortions often occurred in pregnant animals. Gross lesions in the alimentary tract were similar to those associated with mucosal disease, especially in animals >6 months of age. Cattle of all age groups had microscopic lesions in the alimentary tract similar to those seen with mucosal disease. The epidemic peaked in the summer of 1993, with 15% of all bovine accessions from diseased cattle presented to the diagnostic laboratory being associated with BVDV. The virus strains involved in the outbreak were analyzed using monoclonal and polyclonal antibodies and the polymerase chain reaction. The virus isolates from these outbreaks of severe disease were determined to be type 2 BVDV. Type 2 BVDV has been present in Ontario at least since 1981 without causing widespread outbreaks of severe acute BVD, which suggests that type 2 designation in itself does not imply enhanced virulence. Cattle properly vaccinated with type 1 BVDV vaccines appear to be protected from clinical disease.

Bovine viral diarrhea (BVD) was originally described in 1946 in New York State as an acute, rarely fatal, highly contagious disease with fever, diarrhea, mucosal lesions, and leukopenia. Although initial reports described a clinically severe disease, acute BVD has since been considered a mild or subclinical disease of a few days duration, with negligible mortality at any age. Occasionally, scattered outbreaks of severe disease do occur. The emergence of virulent viral strains in the USA and reports of severe acute BVD in Great Britain in 1992 preceded the occurrence of widespread outbreaks of severe acute BVD in both Quebec and Ontario (Canada) in 1993.

Mucosal disease is another clinical manifestation of bovine viral diarrhea virus (BVDV). It occurs in specifically immunotolerant carrier animals that are persistently infected with noncytopathic (NCP) BVDV and that become superinfected with a cytopathic (CP) BVDV strain. Mucosal disease usually affects animals 6–24 months of age. A naturally occurring postnatal infection of BVDV alone will not cause mucosal disease in an immunocompetent animal.

In Ontario in 1993, NCP-BVDV strains with enhanced virulence caused unprecedented outbreaks of severe acute BVD, with deaths in all age groups, including adult animals. Surveys of provincial diagnostic data and extension work estimated that 150 dairy, 600 beef, and 100 milk- and grain-fed veal herds were affected. Economic losses in severely affected dairy herds were estimated to be $40,000–$100,000 per herd due to lost animals, decreased milk production, and abortion. In 1993, outbreaks of disease primarily occurred in the southeastern and southwestern regions of the province, which are the major cattle raising areas. Disease spread to the central region of the southern part of the province in 1994, with the epidemic declining in 1995.

The objective of this report is to describe the 1993–1995 BVD outbreak in Ontario. Clinical history of 7 intensively studied herds from eastern Ontario that became affected in 1993 and 1994 is also provided. For a separate group of cattle from southwestern Ontario, mucosal disease-like lesions were studied in the carcasses of 73 calves and adult cattle dying of BVD. This report also includes the antigenic and genomic characterization of 64 BVDV isolates recovered from cattle across Ontario during the outbreak and identifies both type 1 and type 2 BVDV strains in Ontario since at least 1981.

Material and methods

Clinical presentation of severe acute BVD. To describe the severity and variation in clinical presentation of this form of BVD, 7 herds with outbreaks of severe, acute BVD and from which NCP-BVDV was recovered were studied intensively. Information on clinical disease was collected by re-
view of farm records and interview of both farm managers and attending veterinarians. These 7 herds were from eastern Ontario and had been identified through submissions sent to the diagnostic laboratory between August 1993 and December 1994. A crude abortion rate, not a specific BVD rate of abortion, was calculated based on the number of breeding cattle in the herd at the beginning of the outbreak, because not all aborted fetuses were submitted for diagnosis. The length of each outbreak was defined as the number of weeks between the first clinical case and the last death or abortion.

Postmortem findings for BVD deaths. In a separate study, the postmortem reports of 73 cattle presented for necropsy from farms in southwestern Ontario during 1993 were evaluated. The 73 necropsied cattle selected for this study were divided by age into 51 calves <6 mo of age (from 26 herds) and 22 cattle ≥6 mo of age (from 18 herds). For 6 calves in the younger group, the age was indicated as “veal,” and these calves were assigned an age of 8 wk. The average age of the calves was 9 wk (SD = 4 wk, range = 2–18 wk). For 5 cows in the older group, the age was indicated only as “adult,” and these cattle were assigned an age of 36 mo. The average age of the older cattle was 23 mo (SD = 13 mo, range = 6–36 mo).

Sections of upper alimentary tract (oral cavity, esophagus, forestomachs, abomasum) and respiratory tract were taken for microscopic examination if gross lesions were seen. Sections of lower alimentary tract (small intestine, Peyer’s patches [PP], colon) were taken regardless of observable gross lesions. Tissues were fixed by immersion and gentle agitation in 10% neutral buffered formalin, processed in an automated tissue processor, embedded in paraffin, sectioned, and stained with hematoxylin and eosin. A histologic diagnosis of BVD was made on the basis of crypt epithelial cell necrosis in the intestine and lymphoid necrosis and depletion in the PP, regardless of the presence or absence of erosive/ulcerative lesions in the upper alimentary tract. Frozen sections for direct fluorescent antibody (FA) staining were prepared from PP and other areas of the alimentary tract with gross lesions suggestive of BVD. Portions of PP, spleen, and mesenteric lymph node were submitted for virus isolation.

Virus identification. Similar FA tests and procedures for virus isolation were used to detect BVDV for all bovine laboratory accessions for 1989–1995. Fluorescein-conjugated goat anti-BVDV reagents used for direct FA testing on postmortem tissues were prepared against type 1 BVDV strains. Virus isolation was performed on tissue supernatants, washed buffy coat cells, or serum using 2 passages in secondary bovine spleen cells. These cells were prepared in the laboratory using enzymatic disaggregation with dispase, stored frozen in liquid nitrogen, and used at the 3rd to 5th passage levels. These cells were free of BVDV in indirect FA assays using bovine polyclonal antiserum produced against type 1 strains of BVDV in association with secondary affinity purified fluorescein-conjugated rabbit anti-bovine IgG (H + L).6 Fetal bovine sera used for both growth and maintenance of these cells was determined to be free from BVDV using the same indirect FA assays and free from antibody to BVDV in standard microtiter virus neutralization assays using 100 CCID50 of Oregon strain BVDV. BVDV isolation from diagnostic specimens was confirmed using indirect FA assays.

BVDV-associated diagnoses. Pathology diagnostic records containing diagnostic codes for all bovine accessions (18,125) presented for histopathology to the provincial diagnostic laboratory for 1993–1995 were evaluated retrospectively. Each accession record contained 1 topography code indicating the body system involved and at least 1 etiology code indicating the causative agents suspected from histologic lesions and/or other laboratory tests. More than 1 pathology diagnostic record could be generated by a single submission if more than 1 animal was submitted or more than 1 diagnosis was given. For the purposes of this study, abortions were considered a body system.

A diagnosis was considered associated with BVDV if ≥1 of the following conditions were met: 1) FA testing was positive for BVDV, 2) BVDV was isolated, and/or 3) the pathologist had entered an etiology code for BVDV for the case. Records were tallied by month or by year, by body system, and by BVDV association status.

The proportion of BVDV-associated diagnoses (BVDV-AD) out of the total of all bovine diagnoses was determined on a yearly basis. For respiratory, gastrointestinal, abortion, and other diagnoses, the proportions of each of these diagnoses associated with BVDV were calculated on a yearly basis as a percentage of that category.

Proportional rates were also used as estimates of the monthly prevalence of BVDV-AD for cattle presented to the diagnostic lab for 1989–1995, with the epidemic curve plotted on a monthly basis using the number of BVDV-associated diagnoses as the numerator and all bovine diagnoses as the denominator.

Monoclonal and polyclonal antibody studies. One isolate of BVDV recovered in 1991 before the outbreak and 25 viral isolates recovered during the summer of 1993 (representing 21 different premises) were compared to Singer, NADL, CD-87, CD-89, and a 1993 Cornell isolate (NY-93) using 11 monoclonal antibodies (MAbs) as previously described. These antibodies, originally produced against the Singer strain of BVDV, were reactive to 3 different BVDV proteins: gp48/EOOrns, p80/NS3, and the highly variable envelope protein gp53/E2.

Microtiter virus cross-neutralization assays were performed using the BVDV isolated before the outbreak and 3 representative isolates of BVDV from 1993. The polyclonal antibodies used for cross-neutralization were prepared against specific standard laboratory strains of BVDV: Singer, NADL, and NY-1 (representative of type 1 viruses) and against the virulent field viruses CD-89, CD-87, and NY-93 (representative of type 2 viruses).

Polymerase chain reaction. Sixty-four isolates recovered during the outbreak of 1993–1995 were typed using the polymerase chain reaction (PCR) as previously described. Three PCR primer pairs were used in the typing experiments: 1) primers for identification of pestiviruses in general, 2) type 2 primers for the genomic region encoding the p125/NS23 protein, and 3) type 2 primers for 5′ untranslated region. A primer pair was not available to directly identify type 1 isolates. Therefore, pestiviruses that were identified
as not being type 2 BVDV using the 2 type-specific primer pairs were considered to be type 1 BVDV.

In an attempt to gain further information on the history of BVDV isolates in Ontario and to possibly determine when type 2 BVDV first appeared in the province, an additional 104 isolates obtained from 1981 to 1994 were typed using PCR. These 104 isolates were selected from a large collection of CP-BVDV and NCP-BVDV isolates from Ontario.

Statistics. Using the necropsy data on cattle presented to the diagnostic laboratory during 1993, statistical tests of significance and epidemiologic strength of association were determined by chi-square analysis and by calculating odds ratios. Levels of significance and confidence limits were adjusted for clustering within the herd of origin.10

Analysis of trends of period prevalence estimates over time were conducted using smoothed data (3-mo rolling averages). Chi-square tests using $2 \times 2$ tables assessed trends using historic data for equivalent periods within epidemic periods and by comparing epidemic and nonepidemic periods.6

Mortality information indicated on laboratory submission forms (completed by owners or veterinarians) was compared for 60 BVD case herds and 474 other dairy herd submissions with completed mortality data. To help assess the impact of BVD in these herds, mortality data were compared using the Kruskal–Wallis test for nonparametric data.7

Results

Clinical presentation of severe, acute BVD. Six herds were tiestall dairies, and 1 was a beef feeder calf operation. The mean number of cattle in each herd was 93 (range, 40–191). There had been a recent purchase or introduction of cattle to each herd. These herds had either not been vaccinated against BVD or were not vaccinated according to the manufacturer’s recommendations.

The initial clinical complaints reported by the owners or their veterinarians were respiratory disease in calves or adults (4 of 6 dairies) or acute diarrhea in adult cattle (2 of 6 dairies). One of the 6 dairies also reported abortion as an initial clinical complaint. The beef herd reported acute BVD as the initial complaint. The disease spread slowly in herds, with only a few cattle clinically ill at a time. The mean duration of these outbreaks was 13 weeks (range, 4–32 weeks). The one outbreak lasting 4 weeks was prematurely ended by the elective slaughter of all cattle in the herd.

Once the outbreaks were established, the main clinical feature in 6 of 7 herds was acute BVD with fever, diarrhea, and oral ulceration. Abortion was also a prominent clinical presentation in 1 herd, and the birth of weak calves was observed in another. The thrombocytopenic form of BVD,6,7 with generalized hemorrhage, was not a prominent clinical feature in these herds but was sporadically reported in other outbreaks. The duration of clinical disease in individual animals was highly variable, lasting hours, days, or weeks before death or recovery. In 2 herds, sudden death was observed, in which cattle either were found dead without previous signs of illness or died within 24 hours of first being seen ill.

Abortions eventually occurred in each of the 5 dairy herds at risk. One herd was shipped to slaughter before abortions were expected to occur. Abortion rate was calculated based on the number of breeding age females in each herd at the onset of the outbreak. The mean abortion rate was 44% (SD = 33%, range = 3–83%). The crude mortality rate for all 7 herds was 25% (SD = 14%, range = 5–43%). Immature cattle were more severely affected. The average mortality rate in cattle <2 years of age was 53% (SD = 32%, range = 13–100%), which was significantly greater than ($P = 0.019$) the average mortality of 9% in mature cattle (SD = 12%, range = 2–26%).

BVDV was recovered from at least 1 animal in each of the 7 herds and was determined to be type 2 using PCR.

Postmortem findings. Lesions seen on postmortem for 73 cattle dying of BVD were generally as described previously for mucosal disease,1 with gross lesions varying from mild and local to severe and diffuse. Gross lesions (Fig. 1) included mild to moderate erosions and ulcers in the upper alimentary tract, with necrosis of intestinal mucosa (especially PP), that were more prominent in animals >6 months of age. Lesions were uncommonly recognized on gross postmortem in younger animals, where no or only very mild gross lesions were seen in the upper alimentary tract.

Necrosis of intestinal crypt epithelial cells with dramatic lymphocytolysis and lymphoid depletion of PP were consistent microscopic lesions (Fig. 2) in cattle of all ages. Necrosis of crypt epithelial cells was often segmental. Other characteristic lesions consisted of necrosis of squamous epithelial cells and focal erosions in the esophagus and rumen. Lesions in the rumen were occasionally diffuse and could not always be recognized grossly. Thymic necrosis was also seen in calves, with lymphocytolysis being most marked in animals that died in the very early stages of illness. Pneumonia was the most common concurrent diagnosis in all age groups.

Among the cattle necropsied, the older group was 18 times more likely to have gross oral lesions (95% confidence limits [CL] = 4, 66; $P < 0.001$), 9 times more likely to have gross esophageal lesions (95% CL = 3, 28; $P < 0.001$), 5 times more likely to have gross lesions of the small intestine (95% CL = 2, 16; $P = 0.005$), and 41 times more likely to have gross lesions in PP (95% CL = 5, 34; $P < 0.001$) than younger cattle (<6 months of age). There was no association between age and lesions in the large intestine and mes-
enteric lymph nodes or with other diagnoses noted at necropsy ($P > 0.05$) (Fig. 1).

For microscopic lesions, older animals were 13 times more likely to have oral lesions (95% CL = 4, 48; $P < 0.001$), and 7 times more likely to have esophageal lesions (95% CL = 2, 22; $P < 0.001$) than younger cattle. There was no association with age and microscopic lesions in the other areas examined at necropsy ($P > 0.05$) (Fig. 2).

For this section of the affected population, where BVD was diagnosed on the basis of microscopic lesions, direct FA tests were positive for BVDV on selected tissues from 64% of 28 animals <6 months and 65% of 20 animals >6 months of age. BVDV was isolated from 67.5% of animals <6 months of age and 69% of animals >6 months of age. For these animals, 93.8% of the isolates were NCP-BVDV, with only 3 CP-BVDV recovered. For the 30 BVDV isolates recovered from this group, all but 3 (2 NCP, 1 CP) were identified as type 2 using PCR.

**Virus identification.** The overall virus isolation rates for all specimens presented to the diagnostic laboratory for 1989–1995 are presented in Table 1. During 1993, the virus isolation rate of BVDV from all bovine specimens submitted from across the province was 15.4%. This represents a significant increase ($P < 0.001$) over the mean for BVDV recovery for the previous 4 years. In 1994, the rate of recovery decreased to 11.7%, with 8.5% recovery in 1995. These levels in 1994–1995 represented a significant decrease ($P < 0.001$) from that of 1993. Of the 422 BVDV isolates recovered in 1993, 87.7% of isolates were NCP-BVDV. Significantly more CP-BVDV isolates ($P < 0.001$) were recovered in 1994 and 1995.
**Severe acute BVD**

**Table 1.** Bovine viral diarrhea virus (BVDV) isolation for all bovine specimens sent to the diagnostic laboratory for virus isolation attempts, 1989–1995.

<table>
<thead>
<tr>
<th>Year</th>
<th>No. isolation attempts</th>
<th>No. BVDV isolates</th>
<th>% positive BVDV</th>
<th>BVDV type (%)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1989</td>
<td>893</td>
<td>118</td>
<td>13.2</td>
<td>66.1</td>
</tr>
<tr>
<td>1990</td>
<td>1,025</td>
<td>140</td>
<td>13.6</td>
<td>72.9</td>
</tr>
<tr>
<td>1991</td>
<td>1,123</td>
<td>145</td>
<td>12.9</td>
<td>67.6</td>
</tr>
<tr>
<td>1992</td>
<td>1,408</td>
<td>147</td>
<td>10.4</td>
<td>74.1</td>
</tr>
<tr>
<td>1993</td>
<td>2,749</td>
<td>422</td>
<td>15.4</td>
<td>87.7</td>
</tr>
<tr>
<td>1994</td>
<td>3,751</td>
<td>439</td>
<td>11.7</td>
<td>80.2</td>
</tr>
<tr>
<td>1995</td>
<td>3,380</td>
<td>286</td>
<td>8.5</td>
<td>79.0</td>
</tr>
</tbody>
</table>

* NCP = noncytopathic; CP = cytopathic.

**Table 2.** Various bovine diagnoses associated with bovine viral diarrhea (BVD) in Ontario, Canada, 1989–1995.

<table>
<thead>
<tr>
<th>Year</th>
<th>Total</th>
<th>Respiratory</th>
<th>Gastrointestinal</th>
<th>Abortion</th>
<th>Other</th>
</tr>
</thead>
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<tr>
<td>1989</td>
<td>1,764</td>
<td>5.5</td>
<td>5.7</td>
<td>17.9</td>
<td>2.8</td>
</tr>
<tr>
<td>1990</td>
<td>2,606</td>
<td>4.8</td>
<td>6.1</td>
<td>14.2</td>
<td>1.3</td>
</tr>
<tr>
<td>1991</td>
<td>2,339</td>
<td>5.0</td>
<td>6.0</td>
<td>15.4</td>
<td>3.5</td>
</tr>
<tr>
<td>1992</td>
<td>2,436</td>
<td>5.3</td>
<td>7.0</td>
<td>11.9</td>
<td>4.9</td>
</tr>
<tr>
<td>1993</td>
<td>3,240</td>
<td>15.2</td>
<td>19.1</td>
<td>34.8</td>
<td>7.4</td>
</tr>
<tr>
<td>1994</td>
<td>3,242</td>
<td>14.4</td>
<td>15.1</td>
<td>33.7</td>
<td>5.6</td>
</tr>
<tr>
<td>1995</td>
<td>2,498</td>
<td>8.6</td>
<td>10.7</td>
<td>20.7</td>
<td>3.2</td>
</tr>
</tbody>
</table>

**BVDV-associated diagnoses.** Total annual bovine diagnoses for all bovine laboratory accessions from 1989 to 1995 are shown in Table 2. The proportion of BVDV-AD increased significantly \((P < 0.001)\) in 1993 (15.2%) over the mean of the previous 4 years (5.1%) (Table 2). Similarly, the total annual bovine respiratory tract diagnoses associated with BVDV tripled to 19.1% \((P < 0.001)\) for 1993 as a result of the epidemic. The specific proportional rate of bovine gastrointestinal tract diagnoses associated with BVDV increased 2.3 times \((P < 0.001)\) over the mean of the previous 4 years to 34.8%. Similarly, the total annual bovine abortions submitted for histopathology and attributed to BVDV increased 2.4 times \((P < 0.001)\) to 7.4% in 1993. In 1994 and 1995, the number of BVDV-AD declined significantly \((P < 0.001)\), suggesting the waning of the disease outbreak.

Using 3-month rolling averages, the monthly proportional rate of total diagnoses associated with BVDV was consistent from 1989 to 1991 (Fig. 3). A rise in BVDV-AD occurred regularly each fall, followed by a significant decline in January–March \((P < 0.01)\). In the fall of 1992, the usual rise in BVDV-AD occurred but was significantly higher than that in previous years \((P < 0.01)\). In January–March 1993, BVD-AD were 1.9 times higher than in previous years \((P < 0.001)\) and demonstrated a significant change from the previous years in temporal pattern. The epidemic peaked in June–August 1993, with the BVDV-AD 4 times higher than the mean for the same months of the previous 4 years \((P < 0.001)\). July–August 1993 was determined as the peak of the epidemic; BVDV-AD were >5 standard deviations from the expected rate from 1989 to 1992 (5.1%), and the 95% CL of the expected levels from 1989 to 1992 did not overlap with that of 1993 (4.3, 6.0 vs. 11.2, 19.8). Although BVDV-AD dropped in January 1994, they were still 2 times higher than before the outbreak \((P < 0.01)\). The outbreak continued to decline in 1995. However, the BVDV-AD were still 1.6 times higher than in the 4 years before the outbreak \((P < 0.001)\).

For 1993, 18% (534/3,038) of dairy herd owners and veterinarians provided data on the crude mortality rate in their herds, representing data from 60 dairy herds diagnosed with BVD and 474 other dairy herds in the diagnostic database. Significantly higher median mortality \((P < 0.001)\) was reported among adult dairy cattle in herds diagnosed with BVD (10%) as compared with other dairy herds (2%) in 1993.

**Monoclonal and polyclonal antibody studies.** When viruses recovered in 1993 from animals dying of severe acute BVD were studied using MAbs (Fig. 4), all 25 viral isolates tested reacted with MAb 15c5 directed to the gp48/EOrns protein. MAb 20.10.6, which is directed to the highly conserved p80/NS3 protein, also reacted with all viruses. For the remaining 9 MAbs directed to the highly antigenically variable gp53/E2 protein located in the viral envelope, 1 (17.4.9) reacted with all viral isolates. The viral isolates reacted variably with the remaining 8 MAbs.

**Figure 3.** Proportion of total bovine diagnoses associated with bovine viral diarrhea virus for each 3-month rolling average period for 1989–1995 in Ontario, Canada.
Microtiter virus cross-neutralization assays using polyclonal antibody (Fig. 5) segregated the viruses into 2 groups. The standard type 1 laboratory strains and the 1991 Ontario virus (91.884) were representative of type 1 viruses, and the thrombocytopenic (CD-89, CD-87), the NY-93, and the 1993 Ontario viruses (93.1236, 93.1279, 93.1336) were representative of type 2 viruses.

PCR typing. Sixty-one of 64 BVDV isolates recovered during the outbreak were determined to be type 2 using PCR technology. These type 2 BVDV were recovered from the 7 intensively studied herds (7/7), from the 73 necropsied animals dying of severe acute BVD (27/30), and from provincial submissions (27/27).

Type 2 virus was present in Ontario as early as 1981 (Table 3); 4 of 14 BVDV isolates recovered during 1981 were type 2. The first type 2 BVDV previously identified in North America using these methods was from a calf born in the USA in 1984 (data not shown). Type 2 viruses were found for almost all subsequent years tested; 24 of 104 (23%) isolates were type 2 BVDV. The recovery of a type 2 isolate was just as likely in the 1981–1985 (nonepidemic) period as in the 1992–1994 (epidemic) period ($P = 0.17$) based on the samples tested.

Discussion

The clinical information gathered during this outbreak shows that acute BVD can result in major economic losses to the cattle industry, with death of animals of all ages due to pneumonia, diarrhea and abortion, even after only brief periods of illness. Alimentary lesions in this group of animals were generally as described for mucosal disease, ranging from mild and focal to severe and diffuse erosions or ulcers of the alimentary tract. Gross lesions in these acute BVD cases varied in distribution and extent and were minimal in animals <6 months of age. The lack of gross lesions especially in animals <6 months of age or in older animals with a history of only 24 hours of illness made a diagnosis of BVD by gross postmortem difficult. The crypt epithelial cell necrosis was often segmental in distribution, making it important to routinely collect multiple sections of small intestine, including PP, rumen, abomasum, and colon, for histopathology.

Disease attributed to type 2 BVDV was readily diagnosed using standard reagents for type 1 BVDV. FA testing was helpful as a rapid confirmatory test in 65% of the animals, where gross lesions were consistent with BVD. Without gross lesions to aid in specimen selection, direct FA tests were less often requested in animals <6 months of age. The periphery of the lesions in the upper alimentary tract (especially the esophagus and rumen) and PP were the most useful specimens for FA testing. FA tests should be followed by virus isolation, which is more sensitive and provides isolates for further characterization.

Failure to isolate virus from 30% of the carcasses with lesions consistent with BVD may have been due
to a number of factors, including quality of the sample determined by length of time from death to sample collection or presence of antibody in tissue. It was not unusual to isolate virus from 1 carcass but not from another with similar lesions when several animals from the same herd were submitted at the same time.

It was not possible to differentiate severe acute BVD from mucosal disease in individual animals using gross and microscopic lesions alone. The epidemiology of the outbreak needed to be considered. Where the clinical history indicated that large numbers of animals and animals of different age groups were affected, with isolation of only NCP-BVDV, clinical recovery, and seroconversion in some animals, the herd diagnosis was presumed to be severe acute BVD. CP-BVDV was isolated from 3 individual animals, and these were considered to have mucosal disease.\(^\text{15}\) NCP-BVDV was likely also present in these animals but was masked by CP-BVDV in indirect immunofluorescent assays on cell cultures. Further studies to demonstrate NCP-BVDV, in addition to CP-BVDV, were not performed for these 3 animals.

In 1990, 70 isolates from New York were evaluated using similar MAb studies.\(^\text{5}\) However, reactivity patterns were more variable, with 32 different patterns identified. Although the Ontario viral isolates were from 21 different premises, the majority of the virus isolates had very similar MAb binding patterns, although epitope conservation was incomplete. The cross-neutralization assays using polyclonal antisera for 3 representative isolates suggests that the viruses recovered from the outbreak were type 2 BVDV. The type 2 BVDV group also includes other virulent BVDV isolates that induce thrombocytopenia and vaccine escape mutants recovered from persistently infected animals.\(^\text{24}\)

PCR results also suggest the outbreak was due to type 2 BVDV; 61 of 64 isolates were type 2 BVDV. Because a primer pair was not available to directly identify type 1 BVDV isolates, mixed infections including both type 1 and type 2 BVDV cannot be excluded.

By reviewing diagnostic data on 18,125 herd accessions presented to the diagnostic laboratory for virus isolation and/or histopathology, the BVD epidemic was estimated to have begun by early 1993. For cases presented to the laboratory during 1993, there was a marked increase in the number related to BVDV, which resulted in an increase in the prevalence of BVDV-associated pneumonia (3 times), diarrhea (2.3 times), and abortion (2.4 times) for 1993. The epidemic peaked in the summer of 1993, as indicated by 3-month rolling averages, followed by a significant decline in the number of cases presented to the diagnostic laboratory by 1995. The significant increase in recovery of CP-BVDV during 1994–1995 suggests an increase in the birth of persistently infected calves fol-

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**Table 3.** Results of PCR typing for Ontario bovine viral diarrhea virus cytopathic (CP) and noncytopathic (NCP) isolates recovered from 1981 to 1994.

<table>
<thead>
<tr>
<th>Year</th>
<th>Total tested</th>
<th>CP</th>
<th>NCP</th>
<th>Type 1</th>
<th>Type 2</th>
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<td>14</td>
<td>0</td>
<td>10</td>
<td>4</td>
<td></td>
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<td>1984</td>
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<td>12</td>
<td>1</td>
<td></td>
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<tr>
<td>1985</td>
<td>22</td>
<td>0</td>
<td>19</td>
<td>3</td>
<td></td>
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<tr>
<td>1986</td>
<td>0</td>
<td>4</td>
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lowing the 1993 epidemic peak, with these calves subsequently succumbing to mucosal disease.

Herd information from diagnostic accessions in 1993 indicated an unusual and substantial excess mortality among adult dairy cattle in herds that experienced a BVD outbreak. In this epidemic, the prevalence and mortality rates derived from the diagnostic data were a useful indication of this significant loss to the dairy industry.

The results of this study show that both type 1 and type 2 BVDV have been present in Ontario since at least 1981, yet widespread, severe acute BVD was only recognized in the province beginning in 1993. This fact supports suggestions that BVDV type 2 designation is not synonymous with enhanced viral virulence. In addition, this study supports previous reports suggesting that type 2 BVDV is found across North America. The biological significance of subdivision using antigenic and genetic differences remains unclear, and as yet there are no laboratory markers for enhanced virulence for either type 1 or type 2 strains of BVDV.

During this outbreak, only cattle not properly vaccinated according to manufacturers’ instructions using type 1 BVDV vaccines succumbed to severe disease, suggesting that type 1 vaccines can provide protection against type 2 BVDV. Over the course of the outbreak, intensive efforts were made to encourage proper vaccination programs and increase herd biosecurity. The decline in the outbreak in 1995 may have been due in part to these control efforts and to a decline in the number of susceptible animals through natural infection of cattle in the province.

The antigenic segregation seen between type 1 and type 2 BVDV in cross-neutralizing tests using polyclonal antibodies illustrates the need for further studies to determine the significance of these antigenic variations as they pertain to the clinical expressions of BVDV infection.

Acknowledgements
We thank the Veterinary Laboratory Service Branch veterinarians, technologists, and clerical staff for their contributions to diagnostic reports. The support staff at Health Management and many private practitioners are gratefully acknowledged for their assistance in herd consultations and follow-up work.

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Severe acute BVD


