Comparative virulence of isolates of bovine viral diarrhea virus type II in experimentally inoculated six- to nine-month-old calves

Clayton L. Kelling, DVM, PhD; David J. Steffen, DVM, PhD; Christina L. Topliff, DVM, MS; Kent M. Eskridge, PhD; Ruben O. Donis, MV, PhD; Deborrah S. Higuchi, BS

Objective—To determine the comparative virulence of 5 isolates of bovine viral diarrhea virus (BVDV) type II by inoculating 6- to 9-month-old beef calves with isolates originating from the tissues of cattle affected with naturally occurring, transient, acute, nonfatal infections or naturally occurring, peracute, fatal infections.

Animals—22 calves that were 6 to 9 months old.

Procedure—The study used BVDV isolates 17011, 713, and 5521 that originated from fetuses aborted from cows with transient, nonfatal, acute BVDV infections and isolates 23025 and 17583 that originated from the tissues of cattle with peracute, fatal BVDV infections. Calves were allotted to 6 groups (1, mock-infected control calves [n = 2]; 2, inoculated with BVDV 17011 [4]; 3, inoculated with BVDV 713 [4]; 4, inoculated with BVDV 5521 [4]; 5, inoculated with BVDV 23025 [4]; and 6, inoculated with BVDV 17583 [4]). Rectal temperatures and clinical signs of disease were recorded daily. Total and differential WBC and platelet counts were performed. Histologic examination and immunohistochemical analysis were conducted to detect lesions and distribution of viral antigens, respectively.

Results—Calves inoculated with BVDV 23025 or 17583 developed more severe clinical signs of disease (fever and diarrhea), more severe lymphopenia, and more severe lesions (alimentary epithelial necrosis, lymphoid depletion, and BVDV antigen deposition in lymphatic tissues), compared with calves inoculated with BVDV 713, 5521, or 17011.

Conclusions and Clinical Relevance—Relative severity of experimentally induced infections corresponded to severity of clinical signs of naturally occurring infections with respective BVDV isolates. (*Am J Vet Res* 2002;63:1379–1384)

Bovine viral diarrhea virus (BVDV) causes acute and persistent infections. Prenatal acute BVDV infections cause substantial reproductive losses[1,2] and adverse effects on fetuses.[3-5] Fetuses infected with noncytopathic BVDV during the first 4 months of gestation are born persistently infected with the virus and are specifically immunotolerant to it.[6] Mucosal disease develops in persistently infected cattle when they are superinfected with a cytopathic biotype of BVDV that is antigenically and genetically closely related to the persisting noncytopathic BVDV isolate.[7,8]

Outbreaks caused by acute infections with BVDV type II in the United States[9] and Canada[10] have resulted in high mortality, which has increased awareness of the importance of postnatal acute BVDV infections. Variability in the virulence potential of specific isolates of BVDV type II is likely, because there is wide variation in clinical signs of cattle with BVDV infection. Transient, subclinical infections with BVDV type II are common. Severe acute disease characterized by thrombocytopenia, hemorrhage, leukopenia, fever, diarrhea, and death has resulted from naturally occurring infections.[9,10] Most reported episodes of naturally occurring fatal acute infections have been reported in Holstein cattle.[11] Severe acute infections have been reproduced experimentally in dairy calves in several studies by inoculation of young calves (5 to 65 days,[12] 14 to 54 days,[13] 14 to 63 days,[11] and 31 to 35 days[14,15]) with various high-virulence isolates of BVDV type II. Confirmation of the effects of a low-virulence isolate of BVDV type II in 1- to 2-day-old gnotobiotic calves[16] and of an isolate of BVDV type I, a high-virulence isolate of BVDV type II, and a low-virulence isolate of BVDV type II in 3-day-old dairy calves[17] has been reported.

We are not aware of reports of simultaneous experimental comparison of the comparative virulence of North American isolates of BVDV type II with varying virulence in older beef calves. Specifically, there is a paucity of published information regarding the comparative virulence of isolates of BVDV type II in beef calves that are ≥ 6 months old. Six months is a common age for weaning of calves from their dams in typical beef cow-calf production systems; this corresponds to the age when beef calves are susceptible to BVDV infection, because antibody titers are low in calves of this age as a result of waning colostrum derived antibody titers and lack of active stimulation of the immune system. Furthermore, prevalence of **bovine respiratory tract disease complex (BRDC)** in beef feeder calves is greatest at this time. It is at the time of weaning that the greatest potential exists for acute BVDV infection to contribute to BRDC[18] in beef calf production systems in North America. Consequently, an understanding of the comparative virulence of vari-

Received Nov 29, 2001
Accepted May 16, 2002.
From the Departments of Veterinary and Biomedical Sciences (Kelling, Steffen, Topliff, Donis, Higuchi) and Biometry (Eskridge), Institute of Agriculture and Natural Resources, University of Nebraska, Lincoln, NE 68583-0905.
Supported by the Nebraska Agricultural Experiment Station. Presented in part at the annual meeting of the Conference of Research Workers in Animal Diseases, Chicago, Ill, November 14, 2000.
This manuscript has been assigned Journal Series No. 13569 Agricultural Research Division, University of Nebraska.
Address correspondence to Dr. Kelling.

ous isolates of BVDV type II that can infect calves of this age and contribute to BRDC is important. Moreover, additional isolates of BVDV type II with experimentally determined virulence characteristics are needed for pathogenesis studies that are coupled with functional studies at the molecular level. The objective of the study reported here was to determine the comparative virulence of 5 isolates of BVDV type II by experimentally inoculating 6- to 9-month-old beef calves. The isolates originated from the tissues of cattle with naturally occurring, fatal, peracute infections or naturally occurring, transient, nonfatal, acute infections.

Materials and Methods

Animals—Twenty-two crossbred beef calves that were 6 to 9 months old and that weighed approximately 273 kg were used in the study. Calves were free of BVDV infection, BVDV neutralizing antibodies, and neutralizing antibodies against bovine respiratory syncytial virus. Calves were housed in biosecurity level-2 isolation rooms and were allowed to acclimate for up to 4 days before experiments were initiated. This project was reviewed and approved by the University of Nebraska Institutional Animal Care and Use Committee. The committee's guidelines for humane use and treatment of animals were strictly adhered to at all times.

Cell cultures and viruses—Bovine turbinate cells[a] that were free of BVDV were used to propagate virus isolates. All cells used in this study were tested for extraneous BVDV, bacteria (including mycoplasmal organisms), and fungi. Cells were grown as monolayers in **Dulbecco modified essential medium (DMEM)** supplemented with equine serum (10%) in a humidified incubator with 5% CO_2. The 5 isolates originated from 5 separate herds. Isolates 23025 and 17583 originated from the tissues of cattle with peracute, fatal BVDV infections. Isolate 23025 originated from tissues of an adult pregnant cow that was 1 of 4 adult cows that died as a result of peracute BVDV infection in a herd of 55 adult cattle. Isolate 17583 originated from tissues of an adult pregnant cow with a near-term fetus that was 1 of 4 cows that died as a result of peracute BVDV infection in a herd of 130 adult cows. Isolates 17011, 713, and 5521 were derived from fetuses aborted from cows with transient, nonfatal, acute BVDV infection. The 3 low-virulence isolates originated from tissues of aborted fetuses from pregnant cows that did not have clinical signs of disease other than abortion. Moreover, herdmates in those 3 herds did not have clinical signs of disease. Each isolate was cloned as described elsewhere[19] and used at the sixth cell culture passage.

Inoculation—Calves were allotted to 6 groups as follows: group 1, mock-infected control calves (n = 2); group 2, inoculated with BVDV 17011 (4); group 3, inoculated with BVDV 713 (4); group 4, inoculated with BVDV 5521 (4); group 5, inoculated with BVDV 23025 (4); and group 6, inoculated with BVDV 17583 (4). Calves were inoculated (day 0) with 10 mL of cell culture fluid or 10 mL of BVDV-infected cell culture fluid by use of an intranasal aerosolizer.[b] Each calf received 10 mL of DMEM containing 10^6 $TCID_{50}$ of BVDV. Control inoculum consisted of noninfected cultures of bovine turbinate cells that had been frozen at –80°C, thawed, apportioned into aliquots, and stored at –80°C until used.

Antemortem observations—Beginning on day 0, rectal temperature and clinical signs of infection were recorded daily for each calf. In addition, nasal swab specimens were collected from each calf for use in BVDV isolation. Calves were observed twice each day to ensure that they did not have adverse clinical responses and that appropriate treatment was instituted when warranted; thus, calves were exposed to minimal intensity and duration of discomfort. Clinical signs of disease were assigned numeric values on the basis of a scoring system.[18] Respiratory rate and dyspnea were scored by use of a 3-point scale (0, normal; 1, rapid shallow breathing; and 2, dyspnea), and nasal secretions were scored by use of a 3-point scale (0, normal; 1, excessive serous; and 2, excessive mucopurulent). Cough was recorded as a binary variable (0, not detected; 1, detected). Lethargy was determined by observing whether calves were responsive to the observer each time an isolation room was entered. Lethargy was scored by use of a 4-point scale (0, responsive; 1, mild signs of depression and inactivity; 2, severe signs of depression and inactivity; and 3, recumbent and unresponsive). Fecal characteristics were scored by use of a 3-point scale (0, normal; 1, nonformed feces; and 2, watery feces).

Hematologic evaluation—Blood samples were collected daily from a jugular vein of each calf into sterile tubes containing EDTA. Samples were used for determination of total and differential WBC and platelet counts.[20] Blood samples also were collected daily into sterile tubes without coagulant; serum was harvested and used for virus isolation.

Virus isolation and titration—Specimens (5 g) obtained from the ileal Peyer's patches, mesenteric lymph nodes, and thymus during necropsy were homogenized in 20 mL of DMEM containing 100 µg of gentamicin/mL and 0.25 µg of amphotericin B/mL. Sera and tissue homogenates were stored at –80°C until tested for detection of BVDV. For BVDV isolation, specimens obtained from the mesenteric lymph nodes and thymus and serum samples were diluted to determine the number of **plaque-forming units (PFU)** per milliliter in cell monolayers with agarose overlays. Specimens also were stained by use of an indirect immunoperoxidase test that used **monoclonal antibody (MAb)** 15C5 directed against gp48 of BVDV,[21] as described elsewhere.[18]

Necropsy—All calves were euthanatized on day 9. Specimens were obtained from the mesenteric lymph nodes and thymus for use in virus isolation. Specimens obtained from the lungs, liver, kidneys, spleen, and ileum were submitted for aerobic culture of bacterial pathogens. Specimens obtained from the tonsils, thymus, trachea, esophagus, lungs, liver, kidneys, spleen, rumen, abomasum, duodenum, jejunum (approx 2 m proximal to the ileocecal valve), mesenteric lymph nodes, ileum (approx 15 cm proximal to the ileocecal valve), cecum, and colon were fixed in neutral-buffered 10% formalin. The day after necropsy, tissues were processed and embedded in paraffin, sectioned at a thickness of 5 µm, and stained with H&E.

Immunohistochemical analysis—All paraffin-embedded tissues were sectioned at a thickness of 5 µm and stained by use of an avidin-biotin-alkaline phosphatase method to detect BVDV antigens. Sections were deparaffinized, rehydrated in xylene and a series of graded alcohol solutions, and treated by use of protease XIV in 0.5M **Tris-buffered saline solution (TBSS)**, pH 7.6, for 15 minutes at 37°C. Sections were then blocked by incubation in TBSS with 4% horse serum for 30 minutes at 18 to 24°C. After blocking, primary antibody was added, and sections were incubated for 1 hour at 18 to 24°C. Anti-BVDV MAb 15C5 ascites fluid was diluted 1:1,000 in TBSS. Sections were then washed twice in TBSS containing 1mM EDTA and 0.05% Tween 20 (4 min/wash). Biotinylated horse anti-mouse immunoglobulin antiserum[c] diluted 1:200 in TBSS with 4% horse serum was applied, and slides were incubated for 30 minutes at 18 to 24°C. Slides were then washed as described previously, and an avidin-conjugated alkaline phosphatase complex[d] was added, followed

by incubation for 15 minutes. After washing, substrate[c] was applied to the tissue sections, which were then incubated for 10 minutes at 18 to 24°C in the dark. Slides were then washed in tap water for 2 minutes, counterstained with Mayer hematoxylin, and dehydrated. A coverslip was applied, and sections were examined microscopically.[18]

Statistical analysis—An ANOVA for a completely randomized design and comparison of least-squares means were used to test for significant differences among treatment groups for rectal temperature, total WBC count, differential WBC count, and platelet count. A value of $P \leq 0.05$ was considered significant.

Results

Antemortem observations—Calves did not manifest signs of pain and appeared to have only minimal discomfort during the study. Treatment of specific calves was not indicated, because the magnitude of their responses to viral inoculation, as determined on the basis of clinical scoring, did not warrant it. Calves in groups 5 and 6 that were inoculated with BVDV 23025 or 17583, respectively, became lethargic, whereas calves in the other 4 groups were not lethargic.

None of the control calves or calves in groups 2, 3, and 4, which were inoculated with BVDV 17011, 713, or 5521, respectively, developed diarrhea. Three of 4 calves in group 5 that were inoculated with BVDV 23025 developed diarrhea, and 3 of 4 calves in group 6 that were inoculated with BVDV 17583 developed diarrhea. For the 3 calves in group 5, 1 had watery diarrhea on days 7 and 8, another had watery diarrhea on days 7 and 8 and nonformed feces on day 9, and the third had nonformed feces on days 8 and 9. For the 3 calves in group 6, 1 had nonformed feces on day 7, and the other 2 had nonformed feces on days 8 and 9.

None of the control calves (group 1) developed signs of respiratory tract disease. Calves in groups 2, 3 ,and 4 had signs of only slight respiratory tract disease. In contrast, calves in groups 5 and 6 had signs of severe respiratory tract disease (**Table 1**).

Calves inoculated with BVDV had a biphasic pattern of increased rectal temperature with peaks on days 3 and 8 (**Fig 1**). Calves in groups 5 and 6, which were inoculated with BVDV 23205 or 17583, respectively, had significantly higher rectal temperatures on day 6, compared with rectal temperatures for calves in groups 1 to 4, which were inoculated with control medium or BVDV 17011, 713, or 5521.

Hematologic findings—Beginning on day 4 and continuing through day 7, all calves of groups 2 to 6, which were inoculated with virus, had reduced mean total leukocyte counts, compared with counts for control calves. Calves in groups 5 and 6 were inoculated with BVDV 23025 and 17583, respectively; these calves had significantly more severe lymphopenia on days 3 ($P < 0.05$), 5 ($P = 0.02$), 6 ($P = 0.03$), and 7 ($P = 0.008$), compared with calves in groups 2 to 4, which were inoculated with BVDV 17011, 713, and 5521, respectively (**Fig 2**). Significant differences in platelet counts were not observed among treatment groups.

Table 1—Differences in clinical respiratory scores among groups of 6- to 9-month-old calves inoculated with media (control group) or isolates of bovine viral diarrhea virus (BVDV) type II

Group/BVDV Isolate	Calf No.	Time after inoculation (d)*									
		0	1	2	3	4	5	6	7	8	9
1/Control											
	50	–/–	–/–	–/–	–/–	–/–	–/–	–/–	–/–	–/–	–/–
	137	–/–	–/–	–/–	–/–	–/–	–/–	–/–	–/–	–/–	–/–
2/17011											
	72	–/–	–/–	+/–	+/–	–/–	–/–	–/–	–/–	–/–	–/–
	101	–	–/+	+/+	+/–	–/+	–/+	–/+	–/–	–/–	+/–
	15	–/–	–/–	–/–	–/–	–/+	–/+	–/–	–/+	–/–	–/–
	130	–/–	–/–	–/–	–/–	–/+	–/–	–/–	–/+	–/+	–/–
3/713											
	7	–/–	–/+	–/+	+/++	–/–	–/–	–/–	–/–	–/–	–/–
	61	–/–	–/–	–/–	–/–	–/–	–/–	–/–	–/–	–/–	–/–
	135	–/–	–/–	–/–	–/–	–/–	–/–	–/–	–/–	–/+	–/–
	140	–/–	–/–	–/+	–/–	–/–	–/–	–/–	–/–	–/+	–/–
4/5521											
	56	–/–	–/–	–/–	–/–	–/–	–/–	–/–	–/–	–/++	–/+
	Y56	–/–	–/–	–/–	–/–	–/–	–/–	–/–	–/–	–/–	–/–
	105	–/–	–/–	–/–	–/+	–/–	–/–	–/–	–/–	–/–	–/–
	169	–/–	–/–	–/+	+/+	+/+	–/–	–/–	–/+	–/+	–/–
5/23025											
	52	–/–	–/–	+/+	+/+	++/++	–/–	+/+	+/+	+/+	++/++
	83	–/–	–/–	+/+	+/+	+/++	+/+	+/+	+/+	+/+	+/+
	132	–/–	–/–	+/+	+/+	+/+	+/+	+/+	+/+	+/+	–/–
	141	–/–	–/–	+/+	+/+	+/+	+/+	+/+	+/+	+/+	–/–
6/17583											
	J5-7	–/–	–/–	–/–	–/+	–/–	–/–	–/+	+/++	+/+	+/+
	71	–/–	–/–	–/–	–/+	–/–	–/–	–/–	+/++	+/+	+/+
	32	–/–	–/–	–/–	–/–	++/–	+/+	+/+	+/+	+/–	–/+
	122	–/–	–/–	–/–	–/–	+/+	+/+	+/+	+/–	–/–	–/–

*Day 0 = Day of inoculation.

Results respresent respiratory character/nasal secretions. Respiratory character was classified as follows: – = normal, + = rapid shallow breathing, and ++ = dyspneic. Nasal secretions were classified as follows: – = normal, + = excessive serous, and ++ = excessive mucopurulent.

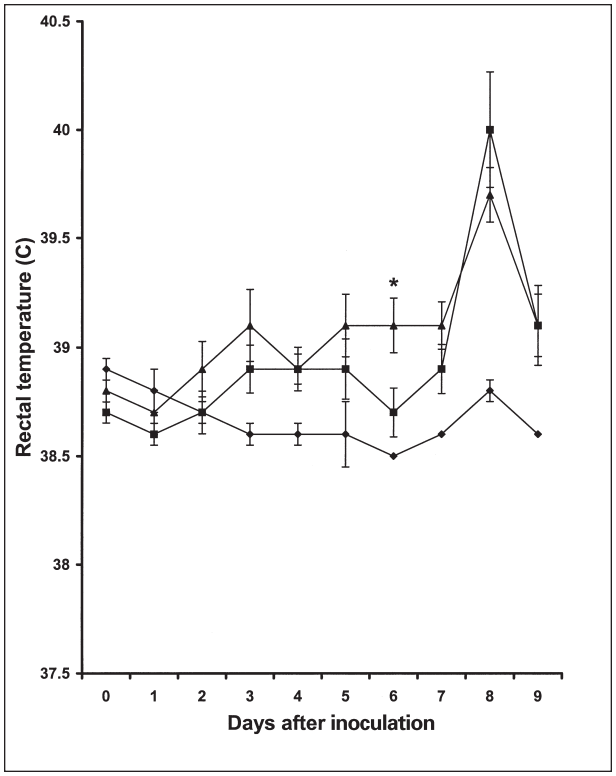

Figure 1—Mean ± SEM rectal temperatures for calves inoculated with media alone (control group; n = 2 [diamond]) or isolates of bovine viral diarrhea virus (BVDV) type II (4 calves/BVDV isolate). Day of inoculation was designated as day 0. *Rectal temperature of calves inoculated with either of 2 high-virulence isolates (BVDV 23025 or 17583 [triangle]) was significantly ($P < 0.05$) higher, compared with rectal temperatures of control calves or calves inoculated with any of 3 low-virulence isolates (BVDV 713, 5521, or 17011 [square]).

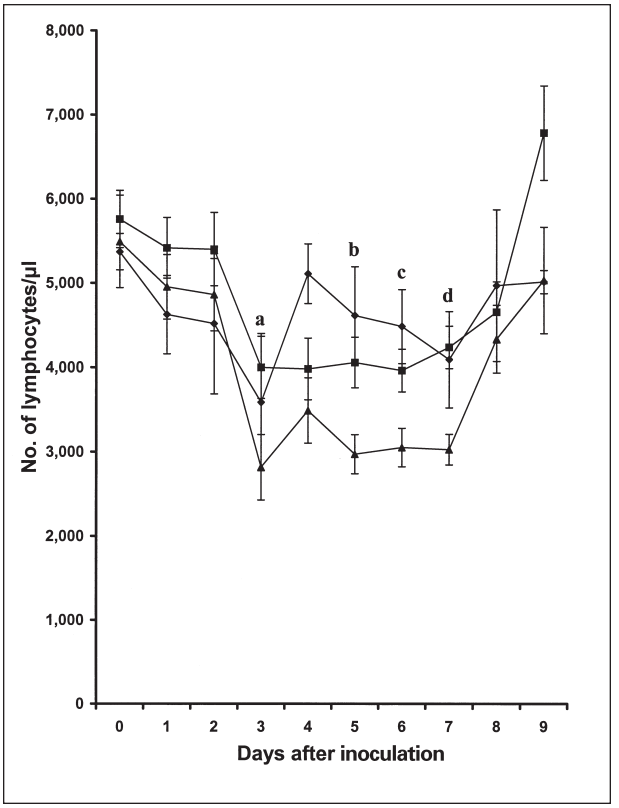

Figure 2—Mean ± SEM total lymphocyte counts for 2 control calves and calves inoculated with isolates of BVDV type II (4 calves/BVDV isolate). Calves inoculated with either of 2 high-virulence isolates had more severe lymphopenia, compared with values for calves inoculated with any of 3 low-virulence isolates. [a-d]Values for calves inoculated with the high-virulence isolates differ significantly (a, $P < 0.05$; b, $P = 0.02$; c, $P = 0.03$; d, $P = 0.008$), compared with values for control calves or calves inoculated with any of 3 low-virulence isolates. *See* Figure 1 for key.

Virus isolation and viral titers—Bovine viral diarrhea virus was isolated from serum and tissues of calves from groups 2 to 6 but not from serum or tissues from calves of group 1. Calves of groups 5 and 6 developed viremia of longer duration (9 days), compared with the duration of viremia of groups 2, 3, and 4 (8, 6, and 6 days, respectively). Viral titers in mesenteric lymph nodes and thymus were comparable among groups and ranged from 100 to 1,000 PFU/mL of tissue homogenate.

Necropsy—Lesions of the digestive and lymphatic systems were clearly more severe for groups 5 and 6 than for groups 2, 3, or 4. Lesions of the digestive tract were evident only in calves of groups 5 and 6. One calf in each group developed mild esophageal ulcers, and 1 calf in group 6 developed abomasal ulcers. Severe lesions developed in the lymphatic system of calves only in groups 5 and 6. Lesions were not observed in specimens obtained from the duodenum, liver, and kidneys of any calves. Relevant bacterial pathogens were not isolated from tissues of any of the calves.

Gross or microscopic lesions were not evident in tissues of calves of group 1. Gross lesions were not evident in calves of groups 2 or 3; however, slight thymic atrophy was microscopically evident in 1 calf of group 2, and mild thymic atrophy was microscopically evident in 1 calf of group 3. In group 4, slight thymic atrophy was grossly evident in 2 calves, and mild thymic atrophy and slight thymic atrophy were microscopically evident in 2 other calves.

Gross and microscopic evidence of a mild epithelial ulcer was detected in the distal portion of the esophagus in 1 calf of group 5. All calves in group 5 had gross and microscopic evidence of thymic atrophy (moderate in 3 calves, slight in 1 calf). The thymus of each of the 3 calves with moderate lymphoid depletion consisted of a mixture of adipose and loose connective tissue with multifocal coalescing hemorrhages, degenerated collagen, and collapsed stroma with a few scattered lymphocytes. Lymphoid depletion also was evident in Peyer's patches and mesenteric lymph nodes.

Gross evidence of an ulcer in the esophageal epithelium was observed in 1 calf of group 6. Microscopically, sections of esophagus from that calf had foci of epithelial necrosis. Another calf in group 6 had gross and microscopic evidence of abomasal mucosal ulcers. All calves in group 6 had gross evidence of thymic atrophy (moderate atrophy in 3 calves and severe atrophy in 1 calf). Microscopically, lymphoid depletion was mild to severe in the thymus of each calf evaluated. Lymphoid depletion was evident in jejunal and ileal Peyer's patches and mesenteric lymph nodes.

In calves of groups 5 and 6, lymphoid depletion in the thymus, Peyer's patches, and mesenteric lymph nodes was characterized primarily by depletion of the mantle zones of germinal centers, which were atrophied and depleted of lymphocytes to leave only a network of stromal cells. A few lymphatic nodules in ileal Peyer's patches remained unaffected. Moderate to severe cortical lymphoid depletion was observed in the thymus of these calves.

Immunohistochemical findings—Bovine viral diarrhea viral antigen was most often detected in lymphatic tissues, particularly in areas of germinal centers of Peyer's patches, tonsils, and mesenteric lymph nodes as well as the thymic cortex. Cell types containing BVDV antigen were large stellate-shaped interdigitating cells and macrophage-like cells. Distribution and intensity of staining correlated with lesions in the tissues, with the most intense staining evident in Peyer's patches.

Discussion

In the study reported here, the comparative virulence of 5 isolates of BVDV type II was determined experimentally by inoculating beef calves, and it correlated with the severity of naturally occurring infections for each isolate. Experimentally induced infection of 6- to 9-month-old calves with 2 BVDV isolates that originated from tissues of cattle affected with peracute, fatal BVDV caused more severe disease than did experimentally induced infections attributabale to any of 3 BVDV isolates derived from fetuses aborted from cows with transient, nonfatal, acute BVDV infections.

Compared to calves inoculated with BVDV 713, 5521, or 17011, calves inoculated with BVDV 23025 or 17583 developed more severe clinical signs of disease and more noticeable lesions. Calves inoculated with high-virulence isolates (BVDV 23025 or 17583) developed diarrhea, whereas diarrhea did not develop in calves infected with low-virulence isolates (BVDV 713, 5521, or 17011). Similarly, calves inoculated with BVDV 713, 5521, or 17011 developed signs of only mild respiratory tract disease. In contrast, calves inoculated with BVDV 23025 or 17583 developed signs of moderate respiratory tract disease. Lymphopenia also developed in calves inoculated with the 2 high-virulence isolates and was more severe and of longer duration than in calves inoculated with the 3 low-virulence isolates. Calves inoculated with the 2 high-virulence isolates also developed more extensive lesions in lymphatic organs and the digestive system than calves inoculated with low-virulence isolates. More severe lesions developed in lymphatic organs of calves inoculated with BVDV 23025 or 17583, compared with those of calves inoculated with the other 3 isolates. Lesions consisted of lymphoid depletion and atrophy of the thymus with corresponding deposition of BVDV antigen in the thymic cortex. Bovine viral diarrhea virus has affinity for leukocytes,[22] and lymphopenia[11] and depletion of lymphoid tissue[16,18] occur during primary acute infections with BVDV type I. Also, transient decreases in populations of T and B lymphocytes result from infection with BVDV type I.[23,24] An important potential consequence of the suppressive effects of naturally occurring acute BVDV infections on the immune system is the potentiation of the effects of other pathogens of the respiratory[18] or gastrointestinal system.[25]

Calves inoculated with BVDV 23025 or 17583 developed epithelial necrosis in the alimentary tract, whereas calves inoculated with the other 3 isolates did not. Characteristic lesions of the digestive tract, including esophageal and abomasal epithelial necrosis with intralesional BVDV antigens,[11,16-18,26] were detected. The fact that esophageal and abomasal ulcers developed only in calves infected with BVDV isolates 23025 or 17583 was further evidence that those 2 isolates were more virulent than the other 3 BVDV isolates. This finding is consistent with results of another study[26] that involved another high-virulence isolate of BVDV type II (ie, isolate NY93), which caused high temperatures, anorexia, and diarrhea in experimentally infected calves. In that study, prominent gross postmortem lesions consisted of linear ulcers in the esophagus. Consistent with the behavior of the 3 low-virulence isolates in the study reported here, isolate 7937 caused only mild disease in experimentally infected gnotobiotic calves, which was characterized by transient, low-grade fever, variable lethargy, anorexia, and lymphopenia.[16] Moreover, gross lesions were not detected in calves infected with low-virulence isolate 7937.[16]

Consistent with other reports,[12-14] experimental inoculation of calves with isolates of highly virulent BVDV type II in the study reported here did not result in development of lesions or clinical signs that were as severe as those for naturally occurring infections. Nonetheless, our findings are important, because a substantial relative difference in virulence was evident for the conditions of our study, which correlated with the comparative severity of naturally occurring infections for each isolate.

The relative virulence of the 5 isolates of BVDV type II, which was initially predicted by variability in the severity of clinical manifestations of disease in cattle with naturally occurring infection, corresponded with the comparative virulence of the isolates as confirmed in the controlled experiments reported here. To our knowledge, this is the first report of a direct experimental comparison in 6- to 9-month-old calves for the comparative virulence of North American isolates of BVDV type II with varying virulence. Taken together, results of the study reported here revealed that, with respect to the 5 isolates studied, severity of disease induced by isolates obtained from cattle with naturally occurring infections reflected the experimentally confirmed comparative virulence of each isolate.

[a]Bovine turbinate cells, National Veterinary Services Laboratory, USDA, Ames, Iowa.
[b]Atomizer No. 163, DeVilbiss Health Care Inc, Somerset, Pa.
[c]Biotinylated horse anti-mouse IgG (H&L), Vector Laboratories, Burlingame, Calif.
[d]Alkaline phosphatase substrate kit 1, Vector Laboratories, Burlingame, Calif.
[e]Vector Red, Vector Laboratories, Burlingame, Calif.

References

1. Virakul P, Fahning M, Joo H, et al. Fertility of cows challenged with a cytopathic strain of bovine viral diarrhea virus during an outbreak of spontaneous infection with a noncytopathic strain. *Theriogenology* 1988;29:441–449.

2. Liess B, Orban S, Frey H, et al. Studies on transplacental transmissibility of a bovine viral diarrhea (BVD) vaccine in cattle. II. Inoculation of pregnant cows without detectable neutralizing antibodies to BVD virus 90 to 229 days before parturition (51st to 190th day of gestation). *Zentralbl Veterinarmed [B]* 1984;31:669–681.

3. Kendrick J. Bovine viral-diarrhea-mucosal disease virus infection in pregnant cows. *Am J Vet Res* 1971;32:533–544.

4. Brown TT, de Lahunta A, Bistner SI, et al. Pathogenic studies of infection of the bovine fetus with bovine viral diarrhea virus. I. Cerebellar atrophy. *Vet Pathol* 1974;11:486–505.

5. Brown TT, Bistner SI, de Lahunta A, et al. Pathogenic studies of infection of the bovine fetus with bovine viral diarrhea virus. II. Ocular lesions. *Vet Pathol* 1975;12:394–404.

6. McClurkin A, Littledike E, Cutlip R, et al. Production of cattle immunotolerant to bovine viral diarrhea virus (BVDV). *Can J Comp Med* 1984;48:156–161.

7. Brownlie J, Clarke MC, Howard CJ. Experimental reproduction of fatal mucosal disease in cattle. *Vet Rec* 1984;114:535–536.

8. Bolin S, McClurkin A, Cutlip R, et al. Severe clinical disease induced in cattle persistently infected with noncytopathic bovine viral diarrhea virus by superinfection with cytopathic bovine viral diarrhea virus. *Am J Vet Res* 1985;46:573–576.

9. Rebhun WC, French TW, Perdrizet JA, et al. Thrombocytopenia associated with acute bovine virus diarrhea infection in cattle. *J Vet Intern Med* 1989;3:42–46.

10. Carmen S, van dreumel T, Ridpath J, et al. Severe acute bovine viral diarrhea in Ontario, 1993–1995. *J Vet Diagn Invest* 1998;1:27–35.

11. Bolin SR, Ridpath JF. Differences in virulence between two noncytopathic bovine viral diarrhea viruses in calves. *Am J Vet Res* 1992;53:2157–2163.

12. Corapi WV, French TW, Dubovi EJ. Severe thrombocytopenia in young calves experimentally infected with noncytopathic bovine viral diarrhea virus. *J Virol* 1989;63:3934–3943.

13. Bezek DM, Grohn YT, Dubovi EJ. Effect of acute infection with noncytopathic or cytopathic bovine viral diarrhea virus isolates on bovine platelets. *Am J Vet Res* 1994;55:1115–1119.

14. Ellis JA, West KH, Cortese VS, et al. Lesions and distribution of viral antigen following an experimental infection of young seronegative calves with virulent bovine virus diarrhea virus-type II. *Can J Vet Res* 1998;62:161–169.

15. Cortese VS, West KH, Hassard LE, et al. Clinical and immunologic responses of vaccinated and unvaccinated calves to infection with a virulent type-II isolate of bovine viral diarrhea virus. *J Am Vet Med Assoc* 1998;213:1312–1319.

16. Marshall DJ, Moxley RA, Kelling CL. Distribution of virus and viral antigen in gnotobiotic calves 10 days postinoculation with bovine viral diarrhea virus. *Vet Pathol* 1996;33:311–318.

17. Walz PH, Bell TG, Wells JL, et al. Relationship between degree of viremia and disease manifestation in calves with experimentally induced bovine viral diarrhea virus infection. *Am J Vet Res* 2001;62:1095–1103.

18. Brodersen BW, Kelling CL. Effect of concurrent experimentally induced bovine respiratory syncytial virus and bovine viral diarrhea virus infection on respiratory tract and enteric diseases. *Am J Vet Res* 1998;59:1423–1430.

19. Topliff CL, Kelling CL. Virulence markers in the 5' untranslated region of genotype 2 bovine viral diarrhea virus isolates. *Virology* 1998;250:164–172.

20. Schalm WO, Jain NC, Carroll EJ. Materials and methods for the study of blood including brief comments to consider in interpretation. *Veterinary hematology*. 3rd ed. Philadelphia: Lea & Febiger, 1975;22–81.

21. Donis RO, Corapi W, Dubovi EJ. Neutralizing monoclonal antibodies to bovine viral diarrhea virus bind to the 56k to 58k glycoprotein. *J Gen Virol* 1998;69:77–86.

22. Truitt RL, Schechmeister IL. The replication of bovine viral diarrhea-mucosal disease virus in bovine leukocytes in vitro. *Arch Gesamte Virusforsch* 1973;42:78–87.

23. Bolin SR, McClurkin AW, Coria MF. Effects of bovine viral diarrhea virus on the percentages and absolute numbers of circulating B and T lymphocytes in cattle. *Am J Vet Res* 1985;46:884–886.

24. Ellis JA, Davis WC, Beldin EL, et al. Flow cytofluorometric analysis of lymphocytic subset alterations in cattle infected with bovine viral diarrhea virus. *Vet Pathol* 1988;25:231–236.

25. Wray C, Roeder PL. Effects of bovine virus diarrhoea-mucosal disease infection on salmonella infection in calves. *Res Vet Sci* 1987;42:213–218.

26. Odeon AC, Kelling CL, Marshall DG, et al. Experimental infection of calves with bovine viral diarrhea virus genotype II NY 93. *J Vet Diagn Invest* 1999;11:221–228.