Enteric and nasal shedding of bovine torovirus (Breda virus) in feedlot cattle

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Objective—To assess fecal and nasal shedding patterns of bovine torovirus (BoTV) in cattle at time of arrival and periodically throughout the first 21 days after arrival at a feedlot.

Animals—57 steers.

Procedure—Fecal and nasal-swab samples collected on days 0, 4, 14, and 21 after arrival were tested for BoTV, using ELISA. A subset of samples from calves testing positive and negative for BoTV was analyzed, using reverse transcriptase-polymerase chain reaction (RT-PCR). Paired serum samples were collected on days 0 and 21 and tested for BoTV antibodies, using a hemagglutination inhibition assay.

Results—Overall rate of fecal shedding of BoTV was 21 of 57 (37%) by ELISA and 40 of 42 (95%) by RT-PCR with peak shedding on day 4. Diarrhea was more common in calves shedding BoTV than those not shedding the virus (odds ratio, 1.72). Overall rate of nasal shedding of BoTV was 15 of 57 (26%) by ELISA and 42 of 42 (100%) by RT-PCR, with peak shedding on day 0. Specificity of the RT-PCR product was confirmed by sequence analysis. Approximately 93% of the calves seroconverted to BoTV (> 4-fold increase in titer). Differences were not detected between calves shedding BoTV and nonshedders in relation to disease and treatments, perhaps because of the low number of cattle in the study.

Conclusions and Clinical Relevance—This study confirmed BoTV infections in feedlot cattle, including BoTV antigen and viral RNA in nasal secretions, and the shedding pattern during the first 21 days after arrival in a feedlot. (*Am J Vet Res* 2002;63:342–348).

Diarrhea and respiratory tract disease are important health problems in beef cattle during the first days after arrival at feedlots. Major economic losses are attributable to treatment costs, labor costs, poor growth rates, and mortality.[1,2] It is estimated that these losses cost the US cattle industry between $168 million and $624 million each year.[3,4] Infectious diarrhea and respiratory tract disease can be caused by a large number of pathogens (bacteria, viruses, and parasites), some of which cause disease in healthy feedlot cattle by themselves, whereas others require interactions with other pathogens or environmental, nutritional, and management factors.[1,5-7] Therefore, a key element to establish measures for prevention and control of diarrhea and respiratory tract disease in feedlot cattle is to recognize and detect new or emerging pathogens that may affect the health and performance of recently arrived cattle and to understand their epidemiologic characteristics.

In 1981, Saif et al[a] reported the detection of **bovine torovirus (BoTV**; Breda virus) in feces of 5- to 6-month-old beef calves arriving at feedlots from sale barns. This enteric pathogen produces mild to moderate diarrhea in calves in experimental and field conditions.[8-16,a] Bovine torovirus is an enveloped single-stranded RNA virus in the *Torovirus* genus within the family Coronaviridae.[17-19] Other members of the Coronaviridae family such as **bovine coronavirus (BCV)** have a dual tropism for epithelial cells lining the respiratory and intestinal tracts and cause respiratory and enteric infections of calves and feedlot cattle, including winter dysentery in feedlot cattle.[3,4,20,21] The BoTV cause a cytolytic infection of the villous and crypt enterocytes of the mid-jejunum, ileum, colon, and cecum, inducing villous atrophy and necrosis of the crypts that results in diarrhea.[10,11,22,23] To our knowledge, respiratory tract infections of cattle attributable to infection with BoTV have not been reported.

Since its discovery in the early 1980s, few epidemiologic studies have been conducted on BoTV because of its failure to grow in cell culture and a lack of diagnostic reagents. Nevertheless, researchers have reported[13,24,25] a high prevalence of antibodies to BoTV in the cattle population, ranging from 55 to 95%, which suggests this virus is endemic. The BoTV also have been detected in fecal samples by use of **electron microscopy (EM)** and ELISA in many countries, including the United States,[8,9,22,a] Canada,[26,27] Costa Rica,[28] the Netherlands,[15] Germany,[b] and South Africa.[29] However, nasal and fecal shedding patterns as well as general epidemiologic characteristics of BoTV infections in feedlot cattle have not been studied.

Therefore, the objective of the prospective longitudinal study reported here was to assess the fecal and nasal shedding patterns of BoTV in a herd of beef cattle at time of arrival and periodically throughout the first 21 days after arrival in a feedlot. Relevant epidemiologic data such as clinical signs (diarrhea and

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respiratory tract disease), treatments, and body weight were collected. For our study, an indirect double-sandwich antigen-capture ELISA developed in our laboratory was used to detect BoTV. A **reverse transcriptase-polymerase chain reaction (RT-PCR)** assay for BoTV also was developed and used in conjunction with **immune EM (IEM)** to confirm the detection of BoTV in these feedlot cattle. Concurrent shedding of BCV in fecal and nasal-swab samples also was assessed, using an ELISA.[30]

Materials and Methods

Animals—During fall 1999, 57 crossbred steers between 6 and 7 months old were selected from an incoming group of 216 feedlot cattle. The 57 steers were randomly selected without discrimination between those with apparent respiratory tract and enteric disease and those that appeared to be healthy. The source of the cattle was a mixed-provider livestock auction market in West Virginia. They were transported to a feedlot at the **Ohio Agricultural Research and Development Center (OARDC)** in Wooster, Ohio. Cattle were vaccinated at time of arrival (day 0), using multivalent inactivated vaccines against infectious bovine rhinotracheitis virus, bovine viral diarrhea virus, bovine respiratory syncytial virus, bovine parainfluenza-3 virus, *Leptospira* organisms, *Pasteurella multocida*, and *Mannheimia haemolytica*.

Collection of samples—Paired nasal-swab specimens and fecal samples were collected from the cattle on days 0, 4, 14, and 21 after arrival at the feedlot. Using sterile cotton-tipped 6-inch-long applicators, swab specimens were obtained from both nostrils of each calf, and the swabs were placed in 4 ml of transport medium (minimum essential medium with 1% sodium bicarbonate and 1% penicillin and streptomycin, pH 7.4). At the laboratory, tubes were immediately vortexed, swabs were removed, and nasal fluids were centrifuged (1,000 × g for 10 minutes). Supernatants were collected and frozen at –70 C until subsequent testing.[20] Fecal samples were diluted 1:10 in transport medium, vortexed for 30 seconds, and centrifuged (1,200 × g for 20 minutes); supernatants were collected and stored at –70 C until analyzed by use of ELISA and RT-PCR.

Blood samples were obtained on days 0 and 21 to test for seroconversion to BoTV, using the **hemagglutination inhibition (HI)** test. Blood samples (10 to 15 ml) were obtained via jugular venipuncture. Blood samples were centrifuged (1,200 × g for 15 minutes). Serum was collected, heat-inactivated at 56 C for 30 minutes, divided into aliquots, and stored at –20 C.[3] All samples were coded to prevent investigators from having prior knowledge of clinical signs of the calves.

Clinical signs, treatments, and weight gains—At each collection point, rectal temperatures were measured, and fecal consistency and signs of respiratory tract disease were scored for each steer. The same investigator (AEH) performed all scoring. Feces were scored on a scale of 0 to 4 (0, normal feces; 1, pasty feces; 2, semiliquid feces; 3, liquid feces with some solid material; and 4, totally liquid feces). Cattle with scores ≥ 2 were classified as having diarrhea. Respiratory tract signs were scored on a scale of 0 to 4 (0, normal; 1, little serous or mucopurulent nasal discharge; 2, moderate amounts of serous or mucopurulent nasal discharge with mild to moderate coughing; 3, severe mucopurulent nasal discharge with moderate to severe coughing; and 4, obvious signs of respiratory distress and dyspnea). Cattle with scores ≥ 2 were considered as having respiratory tract disease. Cattle were weighed at each sample collection, and changes in individual body weight were calculated. Steers were treated with florfenicol,[c] cephalosporin,[d] flunixin meglumine,[e] and tilmicosin,[f] alone or in combination, when a calf was febrile, had lost weight, or had clinical signs of severe respiratory tract or enteric disease.

Antigen-capture ELISA for BoTV—An indirect double-sandwich antigen-capture ELISA was used for detection of BoTV in the nasal swabs and fecal samples. Briefly, paired rows of a 96-well microtiter plate[g] were coated (100 µl/well) with a 1:1,500 dilution of polyclonal guinea pig hyperimmune anti-BoTV serum (positive coating) or a 1:1,500 dilution of BoTV antibody-negative serum (negative coating) in carbonate-bicarbonate buffer (pH 9.6) and incubated overnight at 4 C. Following incubation, plates were rinsed 4 times (250 µl/well for each rinse) with PBS solution-0.05% Tween 20. After coating, plates were blocked to minimize nonspecific binding, using 200 µl of 5% nonfat dry milk in PBS solution (pH 7.2) and incubation for 2 hours at 25 C or overnight at 4 C. Then, 100 µl of each sample (nasal-swab supernatant or fecal suspensions) was placed in paired wells coated with BoTV antibody-positive or -negative serum and incubated for 1.5 hours at room temperature (23 C). Secondary antibody (100 µl of purified gnotobiotic calf hyperimmune anti-BoTV serum, produced in our laboratory, diluted 1:3,000 in PBS solution-0.05% Tween 20-2% **bovine serum albumin [BSA]**) was added, and plates were incubated for 1 hour at 25 C. Next, 100 µl of a commercial goat anti-bovine IgG conjugated to horseradish peroxidase[h] (diluted 1:1,000 to 1:2,500 in PBS solution-0.05% Tween 20-2% BSA) was added, and the plates were incubated for 1 hour at 25 C. Reactions were then developed, using 100 µl of the chromogen substrate 2,2'-azino-bis(3-ethyl-benzthiazoline) sulfonic acid in 0.1*M* sodium citrate plus H_2O_2 (1:1,000). After 20 minutes, absorbance value of each well was measured, using a computer-linked ELISA plate reader.[i] Six internal controls (1 positive and 5 negative reference fecal samples) were included on each plate. A spreadsheet program[j] was used to calculate the ELISA value (ie, mean absorbance of the paired BoTV antibody-positive-coated wells minus mean absorbance of the paired BoTV antibody-negative-coated wells) for each corresponding sample. A cutoff value was determined for each microtiter plate, as defined by Frey et al.[31]

Antigen-capture ELISA for BCV—An indirect double-sandwich antigen-capture ELISA[32] was used for detection of BCV in fecal samples and nasal-swab specimens.

Hemagglutination inhibition assay—Antibody titers against BoTV were determined by use of the HI test, modified from techniques described elsewhere.[33] Briefly, 2-fold dilutions of serum that had been treated with kaolin (1:5) and 50% mouse RBC were prepared in 25 µl of veronal buffer in 96-well U-bottom plates. Then, 25 µl containing 8 hemagglutination units of purified (sucrose gradient) BoTV from feces of an experimentally inoculated calf were added to each well and incubated for 1 hour at room temperature (23 C). Immediately thereafter, 50 µl of a 0.5% suspension of mouse RBC (washed 3 times with veronal buffer) was added to each well and incubated for 2 hours at 4 C before the test results were determined. A standard positive-control serum with known HI antibody titer to BoTV and a negative-control standard (serum without antibodies to BoTV obtained from a gnotobiotic calf) were included in each test. Mouse RBC control and virus control (back titration) samples were included on 1 plate. The arrival (day 0) and convalescent (day 21) sera from the same steer were included on the same plate in duplicate wells. The HI titers were expressed as the reciprocal of the highest serum dilution that caused complete inhibition of hemagglutination. Titers > 1:10 were considered positive.

Immune electron microscopy—A subset of ELISA-positive (10/57) and negative (18/57) fecal samples obtained on

day 4 (peak of fecal shedding of BoTV) was examined, using IEM as described by Saif et al,[34] to detect BoTV particles or BCV. Briefly, fecal samples were diluted 1:5 in PBS solution and centrifuged (1,200 × g for 20 minutes). Supernatants were filtered through 0.45-µm filters and then incubated with hyperimmune gnotobiotic calf anti-BCV serum (1:500) or anti-BoTV serum (1:500) overnight at 4 C. Immune complexes were pelleted, using ultracentrifugation (69,000 × g for 35 minutes at 4 C), resuspended in distilled water, and pelleted again by use of ultracentrifugation. The final pellets were suspended in 25 µl of distilled water. The suspension was mixed with an equal volume of 3% phosphotungstic acid (pH 7.0), placed on formvar-coated carbonized copper grids, and evaluated by use of an electron microscope.[k]

Reverse transcriptase-polymerase chain reaction—All nasal-swab specimens and fecal samples obtained throughout the study period from 21 steers with positive results on the BoTV ELISA and an equal number of randomly selected steers with negative results on the BoTV ELISA were selected as a subset of samples for testing by RT-PCR. A commercial total RNA isolation reagent for liquid samples[l] was used for RNA extraction from all fecal samples and nasal-swab fluids obtained from the 42 steers on days 0, 4, 14, and 21. Primers for BoTV were designed in our laboratory from the published sequence of the spike gene of BoTV-II.[35] Sequences were as follows: upstream primer (spike 5'), 5'-GTG TTA AGT TTG TGC AAA AAT G-3' (positions 37 to 57); and downstream primer (spike 3'), 5'-TGC ATG AAC TCT ATA TGG TGT-3'(positions 758 to 777). The predicted RT-PCR product was 741 base pairs from the region of the 5' end of the spike gene.

The 1-step RT-PCR for the fecal samples was conducted as follows: 4 µl of extracted RNA was treated with 1 µl of dimethyl sulfoxide for 10 minutes at 70 to 75 C. Then, 5 µl of the treated RNA sample was mixed with the RT-PCR mixture (5 µl of 10× commercial buffer),[m] 5 µl of $MgCL_2$ (25 n*M*), 1 µl of each **deoxynucleoside triphosphate (dNTP**; 10 m*M*), 1 µl of upstream primer (200 ng/µl), 1 µl of downstream primer (200 ng/µl), 0.5 µl of a commercial reverse transcriptase (10 U/µl),[n] 0.5 µl of RNasin (40 U/µl),[o] 0.5 µl of *Taq* polymerase (5 U/µl),[p] and 31.5 µl of distilled water in a final volume of 50 µl. The mixture was overlaid with mineral oil and subjected to 1 RT phase of 90 minutes at 42 C, an initial denaturation step of 5 minutes at 94 C, and 30 cycles of 1 minute at 94 C, 2 minutes at 55 C, and 2 minutes at 72 C. The final extension step was 10 minutes at 72 C.

A more sensitive set of RT enzymes and a commercially available DNA polymerase system[q] were used with the RNA from nasal-swab fluids to detect the expected smaller amounts of BoTV RNA in these specimens. In accordance with the manufacturer's instructions, principal modifications from the previously described procedure were performed during the preparation of the RT-PCR mixture and by the addition of an extra step during the RT-PCR cycle. The master mix contained 10 µl of 5× commercial buffer containing $MgCl_2$,[r] 2 µl of each dNTP,[r] 1 µl of upstream primer (200 ng/µl), 1 µl of downstream primer (200 ng/µl), 0.5 µl of RNasin (40 U/µl),[o] 2 µl of RT-PCR enzyme mix,[s] and distilled water for a final volume of reaction mixture of 50 µl. The mixture was overlaid with mineral oil and subjected to 1 RT phase of 30 minutes at 50 C. Then, the temperature was increased to 95 C for 15 minutes to activate the DNA polymerase.[q] The number of cycles and temperatures were the same as described for the 1-step conventional RT-PCR. The RT-PCR products were developed on 1.5% agarose gels and stained with ethidium bromide, and size of the products was determined by comparison with DNA molecular weight markers.

Disposable aerosol-resistant pipette tips with advance micropore filters[t] were used throughout RNA extractions and RT-PCR to prevent cross-contamination. A sample of distilled water was included with every 6 to 7 samples of feces or nasal secretions as a negative-control sample for possible cross-contamination during the RNA extraction and RT-PCR as well as for possible carryover from previous RT-PCR reactions. Also, in every RNA extraction or RT-PCR, a positive-control sample for BoTV was included to verify the reaction. As an additional precaution, the RNA extraction was performed in a location different from the one used for preparation of the RT-PCR mixture, which was prepared in a clean workstation.[u] The thermocycler also was located in a separate room, and development of the RT-PCR products was accomplished in another location. Separate pipettes were used for each step.

Eight RT-PCR products from nasal and fecal samples were submitted for sequencing to the OARDC, The Ohio State University Molecular and Cellular Imaging Center to confirm if specificity of the nucleotide sequence was consistent with that for BoTV. Sequence analysis of the RT-PCR products was performed, using a software program,[v] and results were compared with data published in GenBank.

Statistical analysis—Data were analyzed, using a statistical program.[w] The prevalence of BoTV shedding in fecal and nasal samples from each collection day was calculated to identify the shedding patterns (peak day of shedding, duration) and any associations between nasal and fecal shedding of BoTV. Then, the association between BoTV shedding (fecal or nasal) and diarrhea, respiratory tract disease, and treatments in calves was evaluated, using χ^2 analysis and **odds ratios (OR)** for each collection day and for the entire study period. The OR was used in this study, rather than relative risk, because infection and disease were measured at the same time. **Confidence intervals (CI)** were calculated for the various OR.

Results

Fecal shedding—Frequency and percentage for fecal shedding of BoTV on each day of sample collection was calculated (**Fig 1**; **Table 1**). Overall enteric shedding of BoTV were detected in 21 of 57 (36.8%) steers on the basis of ELISA results and 40 of 42 (95.3%) steers on the basis of RT-PCR results, with peak shedding rates on day 4. None of the steers that shed BoTV on day 4, as determined by results of the ELISA, shed BoTV on day 14. Only 3 steers shed BoTV in their feces on the day of arrival, as determined by results of RT-PCR; none of these steers had positive results for the ELISA. All the fecal samples that had

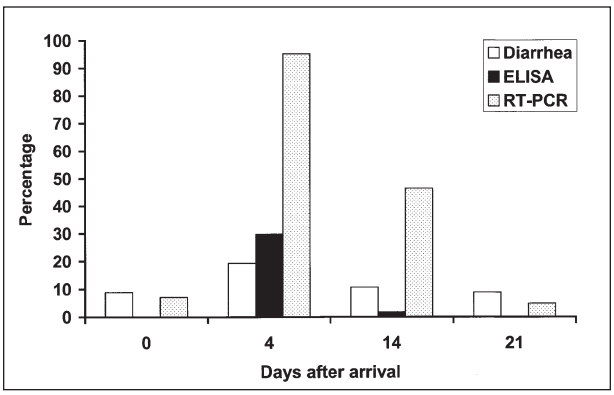

Figure 1—Percentages of steers that had diarrhea and that were shedding bovine torovirus (BoTV) in feces on various days after arrival in a feedlot, as determined by results of ELISA and reverse transcriptase-polymerase chain reaction (RT-PCR). Day 0 = Day of arrival.

Table 1—Nasal and fecal shedding of bovine torovirus in steers on various days after arrival at a feedlot, as determined on the basis or results of ELISA, reverse transcriptase-polymerase chain reaction (RT-PCR), and immune electron microscopy (IEM)

	Day 0				Day 4					Day 14				Day 21			
	Nasal secretions		Fecal samples		Nasal secretions		Fecal samples			Nasal secretions		Fecal samples		Nasal secretions		Fecal samples	
Steers	ELISA	RT-PCR	ELISA	RT-PCR	ELISA	RT-PCR	ELISA	RT-PCR	IEM	ELISA	RT-PCR	ELISA	RT-PCR	ELISA	RT-PCR	ELISA	RT-PCR
No. tested	57	42	57	42	57	42	57	42	28	57	42	57	43	57	42	57	42
No. positive*	7	24	0	3	6	42	17	40	9	5	36	1	20	1	5	0	2
No. negative†	50	18	57	39	51	0	40	2	19	52	6	56	23	56	37	57	40
Percentage positive	12.3	57.1	0.0	7.1	10.5	100.0	29.8	95.2	32.1	8.8	85.7	1.8	46.5	1.8	11.9	0.0	4.8

Day 0 = Day of arrival at feedlot.
*Number. of steers with positive results for the ELISA, RT-PCR, or IEM. †Number. of calves with negative results for the ELISA, RT-PCR, and IEM.

positive results when tested by use of the ELISA also had positive results when tested by use of RT-PCR. However, several fecal samples that had negative results when tested by use of the ELISA had positive results when tested by use of RT-PCR, including 3 of 42, 20 of 22, 19 of 41, and 2 of 42 samples on days 0, 4, 14, and 21, respectively, which suggested low numbers of BoTV particles that were not detectable by the ELISA. Diarrhea was observed in 20 of 57 (35%) steers; the peak of disease was on day 4 (11/57, 19%) and declined thereafter. However, only 5 of 20 (25%) of the steers shedding BoTV on day 4 as determined on the basis of ELISA results had diarrhea, compared with 6 of 37 (16.2%) steers that were not shedding BoTV.

On the basis of the ELISA results, there was not a significant (P = 0.49) difference for calves with diarrhea between calves that did or did not shed BoTV on day 4 (OR, 1.72; 95% CI, 0.35 to 8.0). Thirty of 57 (53%) calves were treated because of fever, weight loss, or clinical signs of severe respiratory tract or enteric disease. Of the 30 treated calves, 11 (37%) shed BoTV in feces, as determined on the basis of ELISA results, compared with 19 (63%) treated calves that did not shed BoTV. An association between fecal shedding of BoTV and treatment was not detected.

Nasal shedding—Frequency and percentage for nasal shedding of BoTV on each day of sample collection were calculated (**Fig 2**; Table 1). Overall, nasal shedding of BoTV was detected in 15 of 57 (26.3%) steers on the basis of results of the ELISA and 42 of 42 (100%) steers on the basis of results of RT-PCR. The peak of BoTV shedding as determined on the basis of ELISA results was on day 0 (7/57, 12%), which declined to 1 of 57 (1.8%) by day 21, whereas the peak of BoTV shedding as determined on the basis of results of RT-PCR was on day 4 (42/42, 100%), which similarly declined by day 21 (5/42, 11.9%). On the basis of ELISA results, 8 steers that shed BoTV on day 0 also shed detectable amounts of BoTV on days 4 (1/8) and 14 (3/8), but none of them shed detectable amounts of BoTV on day 21. Similarly, 24 of 42 (57%) steers had positive results for nasal shedding of BoTV on the basis of results of RT-PCR on day 0, but only 21 (87.5%) shed virus nasally on day 14, and only 3 (12.5%) shed BoTV on days 14 and 21. Similar patterns of shedding were observed in calves that had negative results for RT-PCR on day 0 but positive results from days 4 to 21.

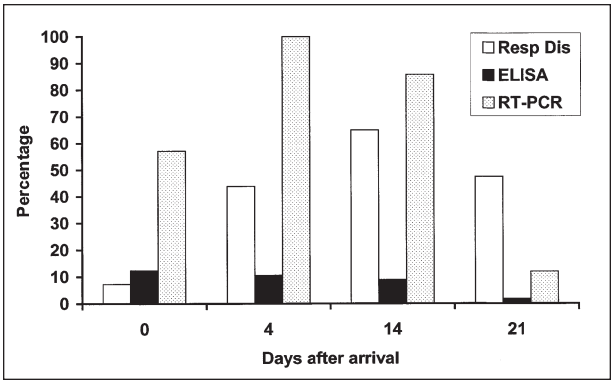

Figure 2—Percentages of steers that had respiratory tract disease and that were shedding BoTV in nasal secretions on various days after arrival in a feedlot, as determined by results of ELISA and RT-PCR.

This observation suggests that once a calf is positive for BoTV, it sheds virus for several days after the original detection. Of 20 nasal samples that had positive results as determined on the basis of ELISA results, 19 had positive results when tested by RT-PCR. However, a substantial number of nasal samples that had negative results when tested by ELISA had positive results when tested by RT-PCR, including 17 of 34, 36 of 36, 31 of 37, and 4 of 41 samples obtained on days 0, 4, 14, and 21, respectively, which again suggested low numbers of BoTV particles in the nasal-swab fluids that were not detectable by the ELISA. Respiratory tract disease was observed in 49 of 57 (86%) steers, and the peak disease was at day 14 after arrival (37/57) and declined after that (Fig 2). At day 14, 3 of 5 steers shedding BoTV (as determined by use of the ELISA) had signs of respiratory tract disease, compared with 34 of 52 calves not shedding BoTV.

Serologic analysis—Fifty-three of 57 (93%) steers seroconverted, as determined by a > 4-fold increase in antibody titers from day 0 to day 21 (**Fig 3**). Interestingly, 4 of 7 steers that had moderate titers (1:20 to 1:80) on the day of arrival did not seroconvert to BoTV and did not shed detectable amounts of viral antigen in the feces or nasal secretions, as determined on the basis of ELISA results. Eight (14%) steers had low to moderate titers against BoTV at the time of arrival. An association was not detected between titers or seroconversion and any of the other variables measured in this study.

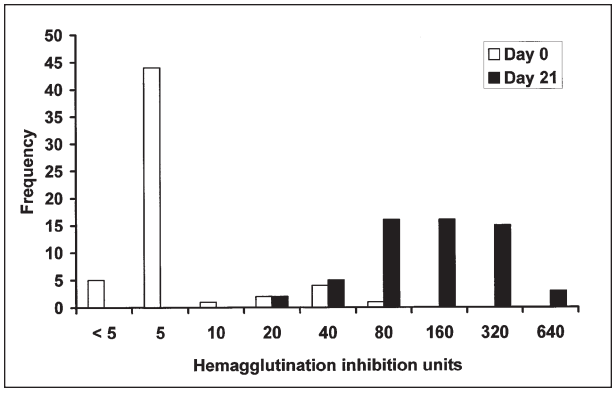

Figure 3—Number of steers seropositive for antibodies to BoTV on the basis of results of a hemagglutination inhibition test on days 0 and 21 after arrival in a feedlot.

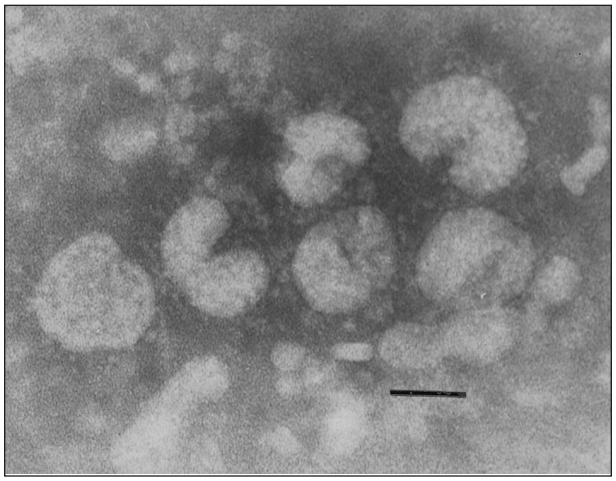

Figure 4—Electron micrograph of BoTV in feces obtained from a steer on day 4 after arrival at a feedlot. The feces had positive results when tested by use of ELISA and RT-PCR. A 20% fecal suspension was incubated with hyperimmune antisera to BoTV resulting in antibody-mediated aggregation of the viral particles. Bar = 100 nm.

Immune electron microscopy—From the subset of fecal samples analyzed by use of IEM, BoTV particles with typical torovirus morphology were observed in 9 of 28 steers (**Fig 4**). Seven of them also were positive on the basis of ELISA results, and all 9 of them were positive on the basis of results of RT-PCR. Other enteric viruses were not observed, except BCV, but aggregates of BCV were not observed with antibodies to BoTV. Control samples containing only BCV did not form viral aggregates when incubated with BoTV antiserum.

RT-PCR sequences—In our experience, the primers used will produce a 741-base pair RT-PCR product with serotypes I and II of BoTV from feces of gnotobiotic calves. Eight RT-PCR products from fecal (n = 4) and nasal (4) samples were sequenced. All of the sequences had 93 to 95% identity (data not shown) with the genomic sequence of BoTV-II contained in GenBank.

Discussion

Other than the initial report[a] of fecal shedding of BoTV in newly arrived feedlot calves, we are not aware of any reports on nasal shedding of BoTV or detailed observations of nasal and fecal shedding patterns of this enteric viral pathogen in feedlot cattle. Similarly, concurrent shedding of BoTV and BCV has not been examined. The prospective longitudinal study reported here revealed that feedlot calves between 6 and 7 months old became infected with BoTV soon after arrival and shed BoTV in feces and nasal secretions that was detectable by use of an ELISA. Furthermore, BoTV was confirmed by direct observation of viral particles in feces by use of IEM, detection of BoTV RNA by use of RT-PCR, nucleotide sequence analysis, and seroconversion to BoTV.

Most calves shed BoTV from the intestinal and respiratory tracts at various times throughout the first 21 days after arrival in the feedlot, as indicated by viral antigen detection by an ELISA with peak shedding on day 4 that declined by day 21. The extensive evidence of BoTV in those steers also was indicated by results of the RT-PCR, which revealed that almost every steer shed BoTV RNA in feces or nasal secretions on day 4. The widespread detection of BoTV shedding in this herd is in accordance with the high BoTV seroconversion rates (93%) observed. Similar patterns of fecal and nasal shedding for BCV have been described in feedlot cattle.[30] Bovine coronavirus also is a member of the *Coronaviridae* family; however, despite the fact it is morphologically similar to BoTV, it is antigenically distinct.[17,18]

Throughout the first 21 days after arrival at the feedlot, BoTV RNA was detected by RT-PCR in fecal and nasal samples in almost all (40 fecal and 42 nasal) samples obtained from the 42 calves, which may indicate a low titer that could not be detected by the ELISA. This low BoTV titer may be explained by low-grade subclinical infections with little active viral replication and shedding, pass-through virus attributable to a highly contaminated environment, or in the case of nasal samples, cross-contamination with feces at the time of collection of nasal-swab specimens. During collection of the nasal secretions, efforts were made to avoid contamination with feces or fecally contaminated material in or around the nostrils by inserting swabs deep into the nasal passages, using the 6-in applicators. On the basis of the high number of nasal samples with positive results, it is unlikely that all the collected specimens were contaminated with feces during their collection. However, experimental inoculation studies are needed to further analyze BoTV replication and shedding in the respiratory tract. To our knowledge, this is the first time that BoTV has been detected in nasal samples by ELISA and confirmed by RT-PCR. Our results suggest that the nasal route may be an additional portal of entry for BoTV, in addition to the oral route, as was hypothesized by Woode[9,13,22] and reported[3,4,20] for BCV, another member of the *Coronaviridae* family. Isolation of BCV in nasal fluids from clinically affected calves and from experimentally inoculated calves[30,36] has substantiated speculation that aerosol transmission and replication in the respiratory tract are part of the pathogenesis of BCV associated with bovine respiratory disease complex (ie, shipping fever).[37-40] In addition, some authors reported[21] that in some cases

under field conditions, respiratory tract infections attributable to BCV developed prior to enteric infections, which indicates the possible importance of this route of transmission in the spread and pathogenesis of BCV infections and the production of enteric and respiratory tract disease.

Furthermore, Vanopdenbosch et al[41,42] reported the detection of BoTV antigen, using immunofluorescence, in the larynx, trachea, and lungs of calves with respiratory tract disease. However, whether virus is detectable in nasal-swab fluids because of the fact it replicates in the nasal epithelium or whether the virus is only taken up nasally as a route of transmission to establish enteric infections is unclear. Therefore, additional studies are needed to determine whether BoTV replicates in nasal epithelial cells and other areas of the respiratory tract.

Serologic analysis revealed that the majority (53/57, 93%) of steers seroconverted to BoTV, indicating that these calves became infected with BoTV. These results are in agreement with similar findings of other studies,[16,24] which documented that calves between the ages of 6 and 10 months seroconverted to BoTV when they were under stressful conditions such as mixing with adult cattle. Eight (14%) calves had low to moderate antibody titers (1:10 to 1:80, as determined by HI testing) to BoTV at the time of arrival at the feedlot. The most likely source of these antibodies may have been contact with the virus prior to arrival, because it is unlikely that maternal antibodies would persist in 6- to 7-month-old calves. Maternal antibodies to BoTV decrease and disappear by the time calves are 2 to 3 months old.[13,24,x]

In the study reported here, we did not detect an association between BoTV shedding and clinical disease (diarrhea or respiratory tract disease). Although an OR of 1.72 suggests a possible association between fecal shedding of BoTV and diarrhea, the low sample size resulted in a wide CI, making the OR not different from 1. Nevertheless, this OR may indicate a possible pattern that should be studied by including a larger number of cattle. Because multiple infections with enteric pathogens (viruses, bacteria, and parasites) are frequently seen in calves with diarrhea,[6] it is rather difficult to establish the etiologic role of BoTV (or any other single agent) under field conditions.

It is important to highlight that the number of steers selected for inclusion in this study was achieved without previous knowledge of expected outcomes because of the lack of information on infections attributable to BoTV, especially in feedlot cattle. The number of steers used in this study could not be extremely large because of the costs associated with the special assays required to work with a fastidious virus that does not grow in cell culture. The only source of BoTV is that obtained through the continuous passage of virus in gnotobiotic and colostrum-deprived calves and its purification through sucrose gradients, all of which is a time-consuming, labor-intensive, and expensive process. Few reports have been published on this virus, presumably because of these technical difficulties.

We detected BoTV in fecal samples and nasal-swab specimens obtained from 57 cattle in a feedlot, using ELISA, and these results were confirmed by use of IEM or RT-PCR. Our results suggested that infections attributable to BoTV may be common in feedlot cattle but that these infections may be subclinical. However, the small number of steers in our study did not permit us to discriminate associations between BoTV shedding (fecal and nasal) and diarrhea, respiratory tract disease, or administration of treatments. Infections attributable to BoTV may be associated with diarrhea in cattle during the initial days after arrival at feedlots. Additional studies are needed to obtain more extensive information on BoTV shedding patterns in an infected herd and the impact on clinical disease and weight gains. In addition, epidemiologic data from the prospective longitudinal study reported here should be helpful when designing studies to determine the prevalence and role of BoTV in the performance of feedlot cattle in feedlots of various sizes, with differing management practices, and in various regions. Future studies should also determine the role that BoTV may play in respiratory tract infections and transmission of BoTV among susceptible animals as well as other interactions between concurrent infections of BoTV and BCV and performance of feedlot cattle.

[a]Saif LJ, Redman DR, Theil KW, et al. Studies on an enteric "Breda" virus in calves (abstr), in *Proceedings*. 62nd Annu Meet Conf Res Workers Anim Dis 1981;62:42.

[b]Kluver S. *Electron microscopical and serological study of the occurrence of Breda torovirus, a cause of calf diarrhoea (abstr)*. Thesis/Dissertation, Tierarztliche Hochschule, Hannover, Germany, 1991.

[c]Nuflor, Schering-Plough Animal Health Corp, Union, NJ.

[d]Excenel, Pharmacia & Upjohn Co, Kalamazoo, Mich.

[e]Anamine, Schering-Plough Animal Health Corp, Union, NJ.

[f]Micotil, Elanco Animal Health, Indianapolis, Ind.

[g]Nunc-Immuno plate Maxisorp Surface, Nunc, Roskilde, Denmark.

[h]Goat anti-bovine IgG(γ), Kirkegaard & Perry Lab, Gaithersburg, Md.

[i]Titertek Multiskan MCC/340, Labsystems & Row Lab, Helsinki, Finland.

[j]Microsoft Excel 97, Microsoft Corp, Redmond, Wash.

[k]Phillips 201, Norelco, Eindhoven, The Netherlands.

[l]TRIzol LS reagent, Life Technologies, Grand Island, NY.

[m]Thermophilic DNA poly 10X buffer, Promega, Madison, Wis.

[n]Avian myeloblastosis virus reverse transcriptase, Promega, Madison, Wis.

[o]RNasin, Promega, Madison, Wis.

[p]*Taq* polymerase, Promega, Madison, Wis.

[q]HotStart *Taq*, Quiagen Inc, Lanecia, Calif.

[r]Qiagen OneStep RT-PCR kit, Qiagen Inc, Lanecia, Calif.

[s]Qiagen OneStep RT-PCR enzyme mix, Qiagen Inc, Lanecia, Calif.

[t]Disposable pipette tips with advance micropore filters, Phenix Research Products, Hayward, Calif.

[u]Airclean 600 workstation, Phenix Research Products, Hayward, Calif.

[v]Lasergene, DNASTAR Inc, Madison, Wis.

[w]SAS, version 8.00, SAS Institute Inc, Cary, NC.

[x]Boom UVD. *Development of an ELISA for detecting toroviruses and torovirus antibodies in cattle; epidemiological studies on torovirus infections in cattle*. Theses/Dissertation, Tierarztliche Hochschule, Hannover, German Federal Republic, 1986.

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