J Vet Diagn Invest 14:308–313 (2002)

Detection of respiratory and enteric shedding of bovine coronaviruses in cattle in an Ohio feedlot

Mustafa Hasoksuz, Armando E. Hoet, Steven C. Loerch, Thomas E. Wittum, Paul R. Nielsen, Linda J. Saif

Abstract. Recently, bovine coronavirus (BCV) has been isolated from new cattle arrivals to feedlots, but the association between respiratory and enteric infections with BCV in feedlot cattle remains uncertain. Fecal and nasal swab samples from 85 Ohio Agricultural Research and Development Center (OARDC) feedlot cattle averaging 7 months of age were collected at arrival (0) and at 4, 7, 14, and 21 days postarrival (DPA). An antigen capture enzyme-linked immunosorbent assay (ELISA) was used to detect concurrent shedding of BCV in fecal and nasal samples. All samples ELISA positive for BCV were matched with an equal number of BCV ELISA-negative samples and analyzed by reverse transcription-polymerase chain reaction (RT-PCR) of the N gene. Paired sera were collected at arrival and 21 DPA and tested for antibodies to BCV using an indirect ELISA. Information on clinical signs, treatments provided, and cattle weights were collected. The overall rates of BCV nasal and fecal shedding were 48% (41/85) and 53% (45/85) by ELISA and 84% (71/85) and 96% (82/85) by RT-PCR, respectively. The peak of BCV nasal and fecal shedding occurred at 4 DPA. Thirty-two cattle (38%) showed concurrent enteric and nasal shedding detected by both tests. Eleven percent of cattle had antibody titers against BCV at 0 DPA and 91% of cattle seroconverted to BCV by 21 DPA. The BCV fecal and nasal shedding detected by ELISA and RT-PCR were statistically correlated with ELISA antibody seroconversion ($P < 0.0001$); however, BCV fecal and nasal shedding were not significantly related to clinical signs. Seroconversion to BCV was inversely related to average daily weight gains ($P < 0.06$). Twenty-eight respiratory and 7 enteric BCV strains were isolated from nasal and fecal samples of 32 cattle in HRT-18 cell cultures. These findings confirm the presence of enteric and respiratory BCV infections in feedlot calves. Further studies are needed to elucidate the differences between enteric and respiratory strains of BCV and their role in the bovine respiratory disease complex of feedlot cattle.

Bovine coronavirus (BCV), a member of the family Coronaviridae, order Nidovirales,[4,11] is associated with severe diarrhea in newborn calves (CD), winter dysentery (WD) in adult cattle, and respiratory tract infections in calves and feedlot cattle.[3,17] The BCV is enveloped and possesses a single-stranded, nonsegmented RNA genome of positive polarity.[4] The BCV was first recognized as a cause of potentially fatal diarrhea of neonatal calves in 1972.[18] Economically important CD and WD BCV outbreaks were reported by many investigators.[7,17,22,29,30] The first isolation of BCV from lung washes and nasopharyngeal swabs from calves occurred in 1982 and involved 2 outbreaks of pneumonia.[26] Subsequently, respiratory bovine coronavirus strains were frequently detected by enzyme-linked immunosorbent assay (ELISA) and were isolated from nasal swab samples of feedlot cattle with respiratory tract disease after shipping.[2,10,21,25] Cattle shedding BCV nasally after entering the feedlot were at increased risk for respiratory disease[12] and had high mortality to BCV infection.[25] Thus, BCV infections may contribute to the bovine respiratory disease complex (BRDC), which is the single most important syndrome affecting 6- to 10-month-old beef cattle after entry into feedlots in North America.[14] The BRDC is a multifactorial disease arising from a combination of environmental, host, management, viral, and bacterial factors. The viruses involved include bovine viral diarrhea virus (BVDV), bovine respiratory synctial virus (BRSV), infectious bovine rhinotracheitis virus (IBRV), parainfluenza-3 virus (PI-3V), and the main bacterial component, *Mannheimia* (*Pasteurella*) *haemolytica* serotype A1.[25,27] Although evidence of BCV infections in feedlot cattle exists, the role of BCV in the BRDC and its association with respiratory and/or enteric tract infections and the rate of seroconversion to BCV in feedlot cattle are largely undefined.

The purpose of this study was to examine respiratory and enteric BCV shedding patterns in 7-month-

From the Food Animal Health Research Program, Department of Veterinary Preventive Medicine, (Hasoksuz, Hoet, Nielsen, Saif), and the Department of Animal Sciences (Loerch), Ohio Agricultural Research and Development Center, The Ohio State University, Wooster, OH 44691-4096, and the Department of Veterinary Preventive Medicine (Wittum), The Ohio State University, Columbus, OH 44691-4096. Permanent address (Hasoksuz): Istanbul University, Veterinary Faculty, Department of Microbiology, Avcilar, 34850, Istanbul, Turkey. Permanent address (Hoet): Universidad del Zulia, Facultad de Ciencias Veterinarias, Dept. Enfermedades Transmisibles, Maracaibo, Venezuela.

Received for publication September 12, 2001.

old cattle within 3 weeks after arrival in a feedlot by using an antigen capture ELISA and reverse transcription-polymerase chain reaction (RT-PCR). Relationships between BCV shedding, seroconversion, clinical signs (respiratory disease and diarrhea), and weight gains in feedlot cattle were also analyzed.

Materials and methods

Sample collections. A total of 85 crossbred beef steers at an average age of 7 mo were purchased in October 2000 from a mixed-source livestock auction market in West Virginia and transported to the Ohio Agricultural Research and Development Center (OARDC) feedlot in Wooster, Ohio. Fecal and nasal swab specimens were collected at arrival (0) and 4, 7, 14, and 21 days post arrival (DPA) as previously described.[2] Paired serum samples were collected at arrival (0) and 21 DPA to test for antibody seroconversion to BCV by ELISA. All cattle were vaccinated at arrival, using multivalent inactivated vaccines against IBRV, BVDV, BRSV, PI-3V, *Leptospira*,[a,b] *Clostridium* spp., and *Mannheimia* (*Pasteurella*) *hemolytica*.[c]

Clinical signs, treatment, and weight gains. The fecal consistencies at collection were recorded on a scale of 0 to 4, with 0 representing normal; 1, pasty; 2, semiliquid; 3, liquid with some solid material; and 4, totally liquid. Cattle with scores ≥2 were considered to be clinical cases of diarrhea. Respiratory signs were scored on a scale of 0–4, with 0 being normal; 1, mucopurulent or small serous nasal discharge; 2, moderate serous or mucopurulent nasal discharge with mild to moderate coughing; 3, severe mucopurulent nasal discharge with moderate to severe coughing; and 4, clear signs of respiratory distress and dyspnea. Scores ≥2 were considered to be clinical respiratory disease. Body weights were measured at each sample-collection time. The steers received treatment based on disease status with either amprolium,[d] oxytetracycline,[e] sulphamethazine,[f] Flunixin Meglumine,[g] and/or Tilmicosin.[h]

ELISA for BCV antigen. An indirect antigen-capture ELISA using a pool of 3 monoclonal antibodies directed against the S, N, and HE structural proteins of the BCV strain DB2 was used to detect BCV in nasal swab fluids and fecal suspensions as previously described.[10,22] Briefly, 96-well plates[i] were coated overnight at 4 C with the pooled antibodies or unrelated ascitic fluids, then blocked for 2 hr at 23 C with 5% nonfat dried milk. Specimens were applied for 1 hr at 23 C, and a secondary antibody (guinea pig hyperimmune serum against the DB2 strain of BCV) was then applied for 1 hr at 23 C. Goat anti-guinea pig IgG (H+L) conjugated to horseradish peroxidase[j] was added and the plates were incubated for 1 hr at 23 C. Reactions were developed using 2,2-azino-bis-3-ethybenzthiazoline-6-sulfonic acid applied for 20 min at 23 C and then stopped with 5% sodium dodecyl sulfate. The absorbance value of each well was read with a computer-linked ELISA plate reader,[k] and the readings were saved as ASCII files. Specimens that had an absorbance >0.1 after subtraction of background absorbances were considered positive for BCV.[10,22]

ELISA for BCV antibody. An antibody-detection ELISA, previously described by Lathrop et al.[13] was used to detect IgG antibodies to BCV in the serum samples. Briefly, 96-well plates[l] were coated with a mixture of the same 3 monoclonal antibodies as used in the antigen capture ELISA described above. After blocking, clarified semipurified human rectal tumor (HRT)-18 cell-culture supernatants containing BCV were added to each well and incubated for 1 hr at 23 C. Serial twofold dilutions in phosphate buffered saline (PBS) (range 1:100 to >1:51,200) of serum samples were applied to 2 rows of wells and then plates were incubated for 1 hr at 23 C. Goat anti-bovine IgG (H+L) conjugated to horseradish peroxidase[l] was added and the plates were incubated for 1 hr at 23 C. The same chromogen described for the antigen capture ELISA was applied to each well for 20 min at 23 C. The color reaction was stopped using 5% sodium dodecyl sulfate and the plates were read with a computer-linked ELISA plate reader. The titer was defined as the inverse of the serum dilution at which the positive-coated wells had an absorbance value of 0.1 or greater above the mean absorbance of the negative wells. Antibody seroconversion was defined as a four-fold increase in antibody titer to BCV.[13]

RT-PCR Total RNA was extracted from nasal and fecal samples by using TRIZOL LS[m] reagent according to the manufacturer's instructions. The RNA was extracted from uninfected cell culture media or uninfected gnotobiotic calf feces for negative controls, and supernatants from cell cultures infected with BCV respiratory strain 440 were used to extract RNA for positive controls. A one-step RT-PCR assay was performed as previously described.[1] Briefly, extracted RNA was treated with dimethyI sulfoxide for 10 min at 70 C. Then the treated RNA sample was mixed with the RT-PCR cocktail, which contained 10× commercial buffer,[n] $MgCl_2$ (25 nM), deoxynucleoside triphosphates (10 mM), upstream and downstream primers (200 ng), avian myeloblastosis virus reverse transcriptase[o] (5 U), RNasin[p] (20 U), Taq polymerase[q] (2.5 U), and enough distilled water to produce a final volume of 50 μl. The mixture was overlaid with mineral oil and subjected to 1 reverse transcription phase of 90 min at 42 C, an initial denaturation step of 5 min at 94 C, and 35 cycles of 1 min at 94 C, 2 min at 50 C, and 1 min at 72 C. The final extension step was 10 min at 72 C.[1]

The oligonucleotide primers used in the RT-PCR were designed from the published sequence of the N gene of the Mebus strain (GenBank accession No. M16620). The sequence of primers (positions calculated from the start codon of the nucleocapsid gene) were as follows: upstream primer, 5′-GCAATCCAGTAGTAGAGCGT-3′ (21–40); downstream primer, 5′-CTTAGTGGCATCCTTGCCAA-3′ (750–731). The predicted RT-PCR product size was 730 bp.

Virus isolation. Monolayers of human rectal tumor (HRT-18) cell cultures grown in 6-well plates were used for virus isolation, as previously described.[19] Briefly, duplicate wells previously washed with cell culture medium (Eagles minimal essential medium [EMEM][r] containing 1% antibiotics [penicillin, dihydrostreptomycin, and nystatin] and 1% $NaHCO_3$) were inoculated with selected ELISA-positive filtered (0.45 μm) fluids from nasal swab specimens and fecal suspensions. The filtered fluids from the positive specimens were absorbed for 1 hr with rocking, and EMEM containing pancreatin (5 μg/ml) was added. The plates were incubated

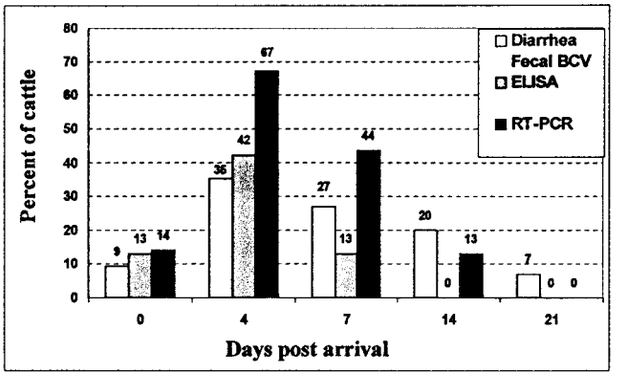

Figure 1. Percentage of cattle with diarrhea and BCV shedding in feces detected by ELISA and RT-PCR at arrival and 4, 7, 14, and 21 days postarrival in the feedlot.

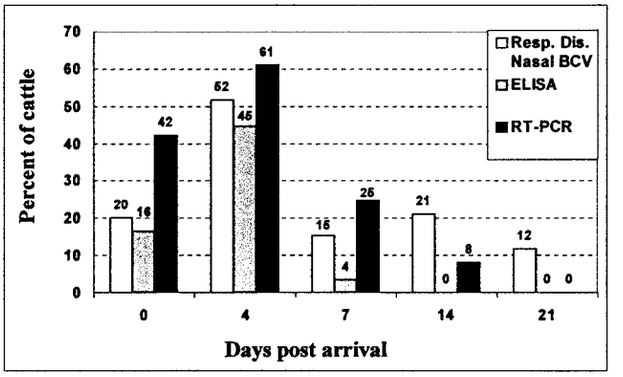

Figure 2. Percentage of cattle with respiratory disease and BCV shedding nasally detected by ELISA and RT-PCR at arrival and 4, 7, 14, and 21 days postarrival in the feedlot.

for 3–4 days under standard conditions at 5% CO_2, and cultures were examined daily for evidence of cytopathic effects. The BCV was confirmed by use of immunofluorescence tests, as described elsewhere.[20] Viruses were cloned by liquid-limiting dilution, and the highest dilution of virus that caused cytopathic effects was passaged an additional 3 times in HRT-18 cells.

Statistical analysis The data was analyzed using a computer software program.[s] The prevalence of BCV shedding in fecal and nasal samples from each collection day was calculated first to identify the shedding patterns (peak day of shedding and duration). Associations between nasal and fecal shedding of BCV and seroconversion were also compared. Then the association between BCV shedding (fecal or nasal) and diarrhea and respiratory disease in calves was evaluated using chi-square analysis and odds ratios (ORs) for each collection day and for the overall study period. Confidence intervals for the different odds ratios were calculated. The association between weight gains and BCV shedding (fecal or nasal) was measured using an analysis of variance (ANOVA). A multiple ANOVA model was used to study the relationship of seroconversion to weight gain. In this model, we controlled for initial weight, acute titer, and fecal or nasal shedding. In the final model, we included the initial weights and the acute antibody titers as independent variables.

Results

The percentages of cattle shedding BCV nasally and fecally during the first 21 days are shown in Figs. 1 and 2. The overall rate of cattle with BCV nasal or fecal shedding was 48% (41/85) and 53% (45/85) by ELISA and 84% (71/85) and 96% (82/85) by RT-PCR, respectively. Any calf with at least 1 positive fecal or nasal sample was included just once in the calculation of the overall rates. The peak of BCV nasal and fecal shedding occurred at 4 DPA, as detected by ELISA (45% and 42%) and RT-PCR (61% and 67%) (Figs. 1, 2). Thirty-two cattle (38%) showed concurrent fecal and nasal shedding detected by both tests at 4 DPA, the peak of the shedding period.

Respiratory illness, characterized by coughing and nasal discharge, along with diarrhea was observed in the feedlot cattle (Figs. 1, 2). Sixty-two percent (53/85) and 77% (66/85) of cattle had diarrhea and respiratory illness during the first 21 days (Table 1). No statistically significant association was detected between fecal and nasal shedding of BCV by ELISA and RT-PCR and clinical signs. However, by ELISA or RT-PCR, 37.6% and 47%, respectively, of calves with diarrhea shed BCV in feces, compared with only 4.1% and 8.2%, respectively, of calves without diarrhea. Similarly, 32.9% and 48.3%, respectively, of calves with respiratory disease shed BCV nasally by ELISA and RT-PCR, compared with only 3.1% and 5.8%, respectively, of calves with no respiratory disease.

The average weight of all cattle was 544 lb. at arrival and 582 lb. at 21 DPA. At arrival, 52% of cattle had low antibody titers (≤100) against BCV and 11% of cattle had high titers, ranging between 3,200 and 51,200. The majority of cattle (91%) seroconverted (a four-fold increase) to BCV, with antibody titers ranging between 3,200 to over 51,200 at 21 DPA (Fig. 3). Animals that had BCV geometric mean antibody titers (GMT) over 1,600 did not shed BCV fecally or nasally. Seroconversion was nearly statistically associ-

Table 1. Percent of calves with clinical signs and shedding BCV in feces or nasally by ELISA and RT-PCR.

	ELISA Fecal shedding		RT-PCR Fecal shedding	
Clinical signs	Positive	Negative	Positive	Negative
Diarrhea	37.6	24.7	47	15.3
No diarrhea	4.1	33.6	8.2	29.5
	Nasal shedding		Nasal shedding	
	Positive	Negative	Positive	Negative
Respiratory signs	32.9	44.7	48.3	29.4
No respiratory signs	3.1	19.3	5.8	16.5

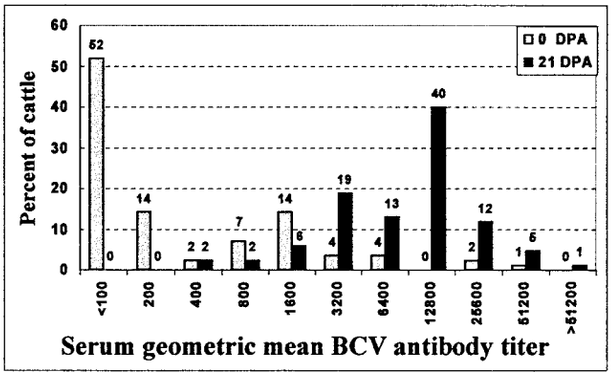

Figure 3. Distribution (percent) of geometric mean BCV antibody titers by ELISA among 85 feedlot cattle at arrival (day 0) and on day 21.

ated ($P < 0.06$) with reduction in weight gain but not with clinical signs. The analysis showed that there was nearly a significant difference ($P < 0.06$) in the weight gain between the seroconverted and the nonseroconverted groups. Calves that seroconverted to BCV as indicated above gained 13 lb. (26%) less than the nonseroconverted group during the 21 DPA.

When the ELISA and RT-PCR results were compared with weight gain, clinical signs, and seroconversion, BCV nasal and fecal shedding detected by RT-PCR concurred with a higher proportion of animals that seroconverted during the study. Ninety percent (77/85) of fecal and 80% (68/85) of nasal shedders by RT-PCR seroconverted during the study period ($P < 0.0001$) (Table 2). In comparison, 53% (45/85) of fecal and 48% (41/85) of nasal shedding-positive animals by ELISA seroconverted (Table 2). There were no significant differences between ELISA and RT-PCR detection of fecal (OR: 1.96 × SE [0.6746]; 95%CI [OR]: 0.73, 10.25) or nasal (OR: 1.96 × SE [0.5938]; 95%CI [OR]: 0.79, 8.14) shedding compared with clinical signs or weight gain. However, cattle shedding BCV nasally by ELISA were 2.7 times more likely to show respiratory signs, and those shedding BCV fecally were 2.5 times more likely to show diarrhea than calves that were BCV negative by ELISA or were positive by RT-PCR alone.

Twenty-eight respiratory and 7 enteric BCV strains were isolated from 32 nasal and 32 fecal samples of cattle, respectively (samples positive by ELISA and RT-PCR). Cytopathic effects (CPE), characterized by enlarged, rounded, detached, dark cells, were observed at approximately 72 hours postinoculation of HRT-18 cells following 2–6 blind passages. Single plaques from CPE-positive strains were isolated in agar and stored at −70 C. The BCV infection of HRT-18 cells was confirmed by immunofluorescence for all 35 BCV isolates using FITC-conjugated bovine anti-Mebus BCV serum.

Table 2. Association between BCV shedding status for respiratory or enteric and seroconvertion to BCV in 85 feedlot cattle.

Test	BCV shedding status	Seroconversion status*		
		Positive	Negative	Total
ELISA				
Positive	respiratory	41	0	41
	enteric	45	0	45
	both	30	2	32
Negative	respiratory	36	8	44
	enteric	32	8	40
	both	47	6	53
RT-PCR				
Positive	respiratory	68	2	70
	enteric	77	5	82
	both	39	4	43
Negative	respiratory	9	6	15
	enteric	0	3	3
	both	38	4	42

* Seroconversion classified as a four-fold increase in BCV antibody titer from DPA 0 to 21.

Discussion

The BCV and other pathogens have been detected in fecal samples from cattle of various ages and in feedlot cattle in the USA[17,25] and other countries.[6,7,15,28] In these studies, investigators reported that BCV and other pathogens, including BVDV, rotavirus, bovine torovirus (Breda virus), *Salmonella* spp., *E.coli*, coccidia, and *Cryptosporidium parvum* were associated with diarrhea in cattle. In addition, although previous investigators described BCV infection of the respiratory tract of feedlot cattle after shipping,[24,25] the association between respiratory and enteric BCV infections in feedlot cattle is unclear. Investigators have suggested that stress is a predisposing factor for BRDC.[5] Animals are subjected to many stressors including weaning, shipping, comingling, dietary changes, and long holding times in the sale barn.[5,23]

In this study, the shedding of respiratory and enteric BCV using both ELISA and very sensitive RT-PCR assays[1] and the prevalence of seroconversion to BCV were investigated. Some calves had respiratory and enteric disease signs and seroconverted to BCV at arrival. The peak (4 DPA) of BCV nasal and fecal shedding by ELISA and RT-PCR was similar to that reported previously for BCV and other viruses of the same family[2] (Hoet A, Cho K, Chang K, et al.: 2000, Enteric and nasal shedding of bovine torovirus [Breda virus] by feedlot cattle. Presented at the Conference of Research Workers in Animal Diseases, November 12–14, 2000, Chicago, IL. Abstract 77). No statistical association between clinical signs and virus shedding was

identified in this study. This may be due to the relatively small number of animals ($n = 85$), which influences the statistical power to detect such differences. Although there was no statistical association between clinical signs and virus shedding, high proportions of animals with respiratory and enteric disease were more likely to shed virus nasally and fecally, respectively, detected by both ELISA and RT-PCR. In the present study, we report variable detection of concurrent viral shedding from feces and nasal swabs. This variation may be the result of differing virus incubation periods for respiratory and enteric infections and different shedding rates between the respiratory and enteric BCV infections.

In the feedlot cattle of this study, a seroconversion rate of 91% was observed, indicating an active immune response against BCV. In previous studies of feedlot cattle, active immunity was reported as indicated by moderate to high seroconversion to BCV with enteric and respiratory signs in 61% of 604 cattle,[14] 95% of 57 cattle,[2] and 58% of 814 cattle.[13] In a recent study of Ohio feedlots, seroconversion rates of 58% to BCV were reported, whereas respiratory shedding of BCV was 7.2% over a 28-day period (fecal shedding was not studied).[13] In that study, some feedlots had a lower rate of nasal shedding of BCV and nasal samples were obtained only once each week. Therefore, greater rates of nasal shedding may have gone undetected. In experimental infection studies[1] of colostrum-deprived calves, nasal shedding of BCV was detected between the third and fourth postinoculation days and shedding persisted for 5 or 6 days and 2–10 days as detected by ELISA and RT-PCR, respectively. In the present study, there was a statistical association between BCV detection by ELISA and RT-PCR and seroconversion of calves to BCV, in agreement with a similar previously reported correlation.[12] The observation that the number of nasal samples that were positive by RT-PCR was higher than those positive by ELISA from the group of animals that seroconverted suggests that RT-PCR may be a better predictor of seroconversion. Because animals subclinicaly infected with BCV are a potential source of exposure to uninfected animals, the more sensitive RT-PCR assay may detect BCV-positive animals that might otherwise be classified as BCV negative by ELISA or other methods.

The BCV infections may have a potential negative impact on the performance of feedlot cattle, reflected by an increase in production costs. Others[2] reported a 17.9 lb. decrease from starting weights over a 21-day period, which is corroborated by findings of the present study (loss of 13 lb. over 21 days). Therefore, BCV infections may contribute directly to economic losses in feedlot cattle by impacting weight gains or, like other respiratory viruses, by predisposing cattle to secondary bacterial infections. In the future, BCV vaccines may be needed to decrease such economic losses due to BCV infection in feedlot cattle.

Respiratory BCV strains have been detected by ELISA and isolated from nasal swab samples of feedlot cattle with respiratory tract disease after shipping.[2,12,21,25] It is still unclear whether respiratory and enteric BCV strains are distinctive in biological, antigenic, and genetic properties and in their ability to replicate in the enteric and respiratory tracts. Previous researchers[8–10,29,30] demonstrated that some BCV strains isolated from the respiratory tract had different biological and antigenic properties from enteric BCV strains isolated from the gastrointestinal tract, whereas others[16,31] did not detect any consistent differences between enteric and respiratory BCV strains. Interpretation of these results is difficult because of widely different years of isolation of the respiratory versus the enteric BCV strains and few comparisons of isolates from the same herds or same animals. The BCV strains isolated concurrently from the respiratory and enteric tracts of the same animals should provide unique samples to investigate differences between respiratory and enteric BCV strains. Therefore, further studies to characterize the biological, antigenic, and genetic properties of the BCV strains isolated from the enteric and respiratory tracts of the same feedlot cattle are warranted.

Sources and manufacturers

a. TSV-2™, bovine rhinotracheitis and parainfluenza 3, modified live virus vaccine, Pfizer Animal Health, Exton, PA.
b. Bovi-Shield™ 4+L5, bovine rhinotracheitis, virus diarrhea, parainfluenza$_3$, respiratory syncytial virus modified live virus vaccine and *Leptospira canicola-grippotyphosa-hardjo-icterohaemorrhagiae-pomona* bacterin, Pfizer Animal Health, Exton, PA.
c. One Shot Ultra™ 8, *Clostridium chauvoei-septicum-novyi-sordellii-perfringens* types C and D-*Pasteurella haemolytica* bacterin-toxoid, Pfizer Animal Health, Exton, PA.
d. CORID™ (amprolium) 9.6% oral solution, Merial Limited, Iselin, NJ.
e. Oxytetracycline, Fermenta Animal Health Co., Kansas City, MO.
f. Sulphamethazine, RX Vet Products, Porterville, CA.
g. Banamine™, Schering-Plough Animal Health Corp., Union, NJ.
h. Micotil™, Elanco Animal Health, Indianapolis, IN.
i. Nunc-Immuno Plate Maxisorp Surface, Nunc, Roskilde, Denmark.
j. Goat anti-guinea pig IgG (H+L), Kirkegaard and Perry Lab, Gaithersburg, MD.
k. Titertek Multiskan MCC/340, Labsystems and Row Lab, Helsinki, Finland.
l. Goat anti-bovine IgG (H+L), Kirkegaard and Perry Lab, Gaithersburg, MD.
m. Gibco BRL, Life Tech, Grand Island, NY.
n. Thermophilic DNA Poly. 10× buffer, Promega, Madison, WI.
o. AVM-RT, Promega, Madison, WI.
p. RNasin, Promega, Madison, WI.
q. *Taq* polymerase, Promega, Madison, WI.
r. Eagles minimal essential medium, Gibco, Grand Island, NY.

s. SAS Institute Inc., Cary, NC.

Acknowledgements

The authors thank P. Tirabosso, G. Lowe, J. Schoonmaker, and J. Rossi for assistance with sampling cattle. This study was supported in part by the American Association of Bovine Practitioners and by State and Federal funds appropriated to OARDC, including an OARDC Competitive Research Enhancement-Matching Grant.

References

1. Cho KO, Hasoksuz M, Nielsen PR, et al.: 2001, Cross-protection studies between respiratory and calf diarrhea and winter dysentery coronavirus strains in calves and RT-PCR and nested PCR for their detection. Arch Virol 146:2401–2419.
2. Cho KO, Hoet A, Loerch SC, et al.: 2001, Evaluation of concurrent shedding of bovine coronavirus via the respiratory tract and enteric route in feedlot cattle. Am J Vet Res 62:1436–1441.
3. Clark MA: 1993, Bovine coronavirus. Br Vet J 149:51–70.
4. De Vries AAF, Horzinek MC, Rottier PJM, De Groot RJ: 1997, The genome organization of the Nidovirales: similarities and differences between arteri-, toro-, and coronaviruses. Sem Virol 8:33–47.
5. Filion LG, Willson PJ, Bielefeldt-Ohmann H, et al.: 1984, The possible role of stress in the induction of pneumonic pasteurellosis. Can J Comp Med 48:268–274.
6. Fuente F, Luzon M, Ruiz-Santa-Quiteria JA, et al.: 1999, Cryptosporidium and concurrent infections with other major enterophathogens in 1 to 30 day old diarrheic dairy calves in central Spain. Vet Parasitiol 80:179–185.
7. Ganaba R, Belanger D, Dea S, Poulin MB: 1995, A seroepidemiological study of the importance in cow-calf pairs of respiratory and enteric viruses in beef operations from northwestern Quebec. Can J Vet Res 59:26–33.
8. Gelinas AM, Boutin M, Sasseville AMJ, Dea S: 2001, Bovine coronaviruses associated with enteric and respiratory diseases in Canadian dairy cattle display different reactivities to anti-HE monoclonal antibodies and distinct amino acid changes in their HE, S and ns4.9 protein. Virus Res 76:43–57.
9. Hasoksuz M, Lathrop SL, Al-dubaib MA, et al.: 1999, Antigenic variation among bovine enteric coronaviruses (BECV) and bovine respiratory coronaviruses (BRCV) detected using monoclonal antibodies. Arch Virol 144:2441–2447.
10. Hasoksuz M, Lathrop SL, Gadfield KL, Saif LJ: 1999, Isolation of bovine respiratory coronaviruses from feedlot cattle and comparison of their biological and antigenic properties with bovine enteric coronaviruses. Am J Vet Res 60:1227–1233.
11. Lai MMC, Cavanagh D: 1997, The molecular biology of coronaviruses. Adv Virus Res 48:1–100.
12. Lathrop SL, Wittum TE, Brock KV, Saif LJ: 2000, Association between bovine coronavirus respiratory tract attributable to bovine coronavirus and health and growth performance of feedlot cattle. Am J Vet Res 61:1062–1066.
13. Lathrop SL, Wittum TE, Loerch SC, Saif LJ: 2000, Antibody titers against bovine coronavirus and shedding of the virus via the respiratory tract in feedlot cattle. Am J Vet Res 61:1057–1061.
14. Martin SW, Nagy E, Shewen PE, Harland RJ: 1998, The association of titer to bovine coronavirus with treatment for bovine respiratory disease and weight gain in feedlot calves. Can J Vet Res 62:257–261.
15. Naciri M, Lefay MP, Mancassola R, et al.: 1999, Role of *Cryptosporidium parvum* as a pathogen in neonatal diarrhea complex in suckling and dairy calves in France. Vet Parasitol 85:245–257.
16. Reynolds DJ, Debney TG, Hall GA, et al.: 1985, Studies on the relationship between coronaviruses from the intestinal and respiratory tracts of calves. Arch Virol 85:71–83.
17. Saif LJ, Brock KV, Redman DR, Kohler EM: 1991, Winter dysentery in dairy herds: electron microscopic and serological evidence for an association with coronavirus infection. Vet Rec 128:447–449.
18. Saif LJ, Heckert RA: 1990, Enteropathogenic coronaviruses. *In:* Viral diarrheas of man and animals, ed. Saif LJ, Theil KW, 1st ed., pp. 185–252. CRC Press, Boca Raton, FL.
19. Saif LJ, Heckert RA, Miller KL, Tarek MM: 1988, Cell culture propagation of bovine coronaviruses. J Tiss Cult Methods 11: 139–145.
20. Saif LJ, Redman DR, Moorhead PD, et al.: 1986, Experimentally induced coronavirus infections in calves: viral replication in the respiratory and intestinal tracts. Am J Vet Res 47:1426–1432.
21. Silva MR, O'Reilly KL, Lin X, et al.: 1999, Sensitivity comparison for detection of respiratory bovine coronaviruses in nasal samples from feedlot cattle by ELISA and isolation with the G clone of HRT-18 cells. J Vet Diagn Invest 11:15–19.
22. Smith DR, Tsunemitsu H, Heckert RA, Saif LJ: 1996, Evaluation of two antigen-capture ELISAs using polyclonal or monoclonal antibodies for the detection of bovine coronavirus. J Vet Diagn Invest 8:99–105.
23. Stephens DB: 1980, Stress and its measurement in domestic animals: a review of behavioral and physiological studies under field and laboratory situations. Adv Vet Sci Comp Med 24:179–210.
24. Storz J, Lin X, Purdy CW, et al.: 2000, Coronavirus and *Pasteurella* infections in bovine shipping fever pneumonia and Evan's criteria for causation. J Clin Microbiol 38:3291–3298.
25. Storz J, Purdy CW, Lin X, et al.: 2000, Isolation of respiratory bovine coronavirus, other cytocidal viruses, and *Pasteurella* spp. from cattle involved in two natural outbreaks of shipping fever. J Am Vet Med Assoc 216:1599–1604.
26. Thomas LH, Gourlay RN, Stott EJ, et al.: 1982, A search for new microorganisms in calf pneumonia by the inoculation of gnotobiotic calves. Res Vet Sci 33:170–182.
27. Thomson RG: 1974, Pathology and pathogenesis of the common diseases of the respiratory tract of cattle. Can Vet J 15:249–251.
28. Traven M, Sundberg J, Larsson B, Niskanen R: 1993, Winter dysentery diagnosed by farmers in dairy herds in central Sweden: incidence, clinical signs and protective immunity. Vet Rec 133:315–318.
29. Tsunemitsu H, Saif LJ: 1995, Antigenic and biological comparisons of bovine coronaviruses derived from neonatal calf diarrhea and winter dysentery of adult cattle. Arch Virol 140:1303–1311.
30. Tsunemitsu H, Yonemichi H, Hirai T, et al.: 1991, Isolation of bovine coronavirus from feces and nasal swabs of calves with diarrhea. J Vet Med Sci 53:433–437.
31. Zhang X, Herbst W, Kousoulas KG, Storz J: 1994, Comparison of the S genes and the biological properties of respiratory and enteropathogenic bovine coronaviruses. Arch Virol 134:421–426.