Evaluation of concurrent shedding of bovine coronavirus via the respiratory tract and enteric route in feedlot cattle

Kyoung-Oh Cho, DVM, PhD; Armando E. Hoet, DVM; Steven C. Loerch, PhD; Thomas E. Wittum, PhD; Linda J. Saif, PhD

Objective—To assess the relationship between shedding of bovine coronavirus (BCV) via the respiratory tract and enteric routes and the association with weight gain in feedlot cattle.

Animals—56 crossbred steers.

Procedures—Paired fecal samples and nasal swab specimens were obtained and were tested for BCV, using antigen-capture ELISA. Paired serum samples obtained were tested for antibodies to BCV, using antibody-detection ELISA. Information was collected on weight gain, clinical signs, and treatments for enteric and respiratory tract disease during the study period.

Results—Number of samples positive for bovine respiratory coronavirus (BRCV) or bovine enteric coronavirus (BECV) was 37/224 (17%) and 48/223 (22%), respectively. Some cattle (25/46, 45%) shed BECV and BRCV. There were 25/29 (86%) cattle positive for BECV that shed BRCV, but only 1/27 (4%) cattle negative to BECV shed BRCV. Twenty-seven of 48 (56%) paired nasal swab specimens and fecal samples positive for BRCV were positive for BRCV. In contrast, only 10/175 (6%) paired nasal swab specimens and fecal samples negative for BRCV were positive for BRCV. Only shedding of BECV was associated with significantly reduced weight gain. Seroconversion to BCV during the 21 days after arrival was detected in 95% of the cattle tested.

Conclusions and Clinical Implications—Feedlot cattle infected with BCV after transport shed BCV from the respiratory tract and in the feces. Fecal shedding of BCV was associated with significantly reduced weight gain. Developing appropriate control measures for BCV infections could help reduce the decreased weight gain observed among infected feedlot cattle. (Am J Vet Res 2001;62:1436–1441)

Bovine coronavirus (BCV) was first identified as a possible cause of diarrhea in calves in 1972 and is now recognized as a primary pathogen in diarrhea of neonatal calves and epizootic diarrhea of adult cattle. Bovine coronaviruses replicate in the differenti-
were diluted 1:25 in maintenance medium and centrifuged (850 X g for 20 minutes). Supernatants were saved for testing by use of an ELISA, immune electron microscopy, and virus isolation techniques. Serum samples were obtained at the time of arrival and again 21 days after arrival to test for seroconversion to BCV, using an ELISA. Ten to 15 ml of blood was obtained via jugular venipuncture. Blood samples were centrifuged at 2,000 X g for 20 minutes. Serum was removed, heat-inactivated at 56 C for 30 minutes, and stored in aliquots at –20 C.

Clinical signs, treatments, and weight gain—Rectal temperature, color and consistency of feces, and amount and characteristics of coughing and nasal exudate for each calf were recorded immediately prior to collection of samples or specimens at time of arrival and on days 4, 14, and 21 after arrival at the feedlot. Feces were scored on a scale of 0 to 3 (0, normal; 1, pasty; 2, semiliquid; 3, liquid). Signs of respiratory tract disease were scored on a scale of 0 to 3 (0, normal; 1, mild mucopurulent nasal discharge; 2, moderate mucopurulent nasal discharge with mild to moderate coughing; 3, severe mucopurulent nasal discharge with moderate to severe coughing). Cattle were weighed at each sample collection time, and changes in body weight were calculated. The cattle were treated with flunixin meglumine, cephalosporin, or tilmicosin when signs of respiratory tract or enteric disease were observed during sample collections or throughout the study.

ELISA for BCV antigen—An indirect antigen-capture ELISA was used to detect BCV in fluids of nasal swab specimens and fecal suspensions, as described elsewhere. Three monoclonal antibodies (MAb; BC 22 F8.3 C for HE protein, BC 28 H1.1 C for N protein, and BC 29 G7.2 C for S protein) produced against the CD DB2 strain of BCV and hyperimmune MAb developed against BCV structural proteins (HE, N, and S) produced against the CD Mebus strain of BCV in 96-well microtiter plates were coated with a mixture of the 3 MAb used for antigen-coating and incubated for 1 hour at 4 C. After incubating wells overnight at 4 C, 5% normal mouse ascitic fluids (antibody-negative coating) or BCV antibody-negative mouse ascites. Fecal samples and nasal swab specimens from calves positive and negative for BCV and BCV antibody-negative ascites. Fecal samples and nasal swab specimens from calves positive and negative for BCV and unknown test samples were added to duplicate wells containing antibody-positive or -negative coating and incubated for 1 hour at 20 to 22 C. After washing plates with PBSS-0.05% Tween 20 (PBST), secondary antibody (ie, optimally diluted guinea pig anti-BCV hyperimmune serum) was added to each well. Plates were incubated for 1 hour at 25 C, and indicator antibody consisting of optimally diluted sheep anti-guinea pig IgG conjugated to horseradish peroxidase was added to each well. The chromogen substrate was 2,2′-azino-di-3-ethylbenz thiazoline sulfonic acid with a final concentration of 0.03% hydrogen peroxide. Plates were read at a setting of 414 nm, using an ELISA reader, and absorbances were saved as computer files. A computer spreadsheet program was used to calculate ELISA values for the samples by subtracting the average absorbance of a row of wells coated with mock-infected cell-culture supernatant from the average absorbance of BCV-coated wells at each dilution for each sample. The titer was defined as the dilution at which the mean absorbance of the positive wells was 0.1 greater than the mean absorbance of the negative wells.

Virus isolation—Monolayers of HRT-18 cell cultures grown in 6-well plates were used for virus isolation, as described previously. Brieﬂy, cells were washed with cell culture medium (Eagle minimal essential medium [EMEM] containing 1% antibiotics [penicillin, dihydrostreptomycin, and cycocstain] and 1% NaHCO3) and inoculated in duplicate wells along with selected ELISA-positive filtered (0.45-μm) fluids from nasal swab specimens and fecal suspensions. Fluids from nasal swab specimens and fecal suspensions were absorbed for 1 hour with rocking, and EMEM containing pancreatin (5 μg/ml) was added. Cultures were incubated for 3 to 4 days at 37 C in a 5% CO2 atmosphere. Cultures were examined daily for evidence of cytopathic effects, and BCV was conﬁrmed by use of immunofluorescence tests, as described elsewhere. 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 McNemar χ2 test was used to assess the relationship between shedding of BECV and BRCV. In addition, the k statistic to assess agreement beyond chance was calculated. A multivariable ANOVA was used to determine the adjusted effect of shedding of BECV and BRCV on total weight gain during the 21-day study period. Multivariable logistic regression was used to determine the effect of shedding of BECV and BRCV on signs of respiratory tract and enteric disease as well as treatment rate. Pen assignment and weight on day 0 were included in the multivariable models as potential confounders.

Results—Nasal swab specimens and fecal samples obtained from 36 cattle at time of arrival and on days 4, 14, and 21 after arrival yielded positive results when tested for BRCV (37/224, 17%) and BECV (48/223, 22%) by use of a BCV antigen-capture ELISA (Table 1). There were only 223 fecal samples, because 1 fecal sample was not collected at time of arrival. Shedding rate for BRCV at time of arrival and day 4 was 14 and 39%, respectively. For samples obtained on day 14, the percentage of nasal swab specimens that were positive for corona
ELISA, with seroconversion being defined as a cattle seroconverted to BCV, as determined by use of an ease on average daily weight gain.

ment or clinical signs of respiratory tract or enteric dis-

a significant difference in weight gain between calves that shed BECV and those that did not shed the virus. Calves that shed BECV gained 15.31 kg. There was not significant (\( P \geq 0.033 \)) difference in weight gain between calves that shed BECV and those that did not shed the virus. Calves that shed BECV gained 23.48 kg (adjusted for pen assignments). Calves that shed BECV gained 15.31 kg. There was not a significant difference in weight gain between calves that shed BRCV and those that did not shed the virus. In addition, there was not an apparent effect of treatment or clinical signs of respiratory tract or enteric disease on average daily weight gain.

During the 21 days in the feedlot, 53 of 56 (95%) cattle seroconverted to BCV, as determined by use of an ELISA, with seroconversion being defined as a ≥ 4-fold increase in BCV antibody titer (Table 3). Geometric mean titer (GMT) for BCV antibody increased during the 21 days after arrival. All cattle that shed BRCV from the respiratory tract seroconverted, whereas 26 of 29 (90%) cattle in which BRCV antigen was not detected seroconverted. All cattle that shed BECV in feces seroconverted, whereas 24 of 27 (89%) cattle in which BECV antigen was not detected seroconverted. All cattle that shed BRCV or BECV from the respiratory tract or via the enteric route seroconverted, whereas 23 of 26 (89%) cattle in which BRCV and BECV antigens were not detected seroconverted.

The GMT for days 0 and 21 for BRCV-positive cattle was 26 and 22,405, respectively, whereas GMT for days 0 and 21 for BECV-positive cattle was 46 and 22,537, respectively. The GMT for days 0 and 21 for cattle positive for BRCV or BECV was 46 and 23,047, respectively. The GMT for days 0 and 21 for cattle negative for BRCV was 1,485 and 34,103, respectively,

<table>
<thead>
<tr>
<th>Type of sample</th>
<th>No. of calves</th>
<th>No. of samples</th>
<th>Days after arrival*</th>
<th>Samples positive for BCV</th>
<th>Cattle positive for BCV</th>
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<tr>
<td>Nasal swab specimen</td>
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<td>224</td>
<td>0</td>
<td>27/224 (12%)</td>
<td>26 (46%)</td>
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<tr>
<td>Feces</td>
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<td>223</td>
<td>0</td>
<td>48/223 (22%)</td>
<td>29 (52%)</td>
</tr>
</tbody>
</table>

Table 2—Comparison of shedding status for BRCV and BECV in 56 feedlot cattle

<table>
<thead>
<tr>
<th>BRCV shedding status</th>
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<th>Negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cattle</td>
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<td>29</td>
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<tr>
<td>Negative</td>
<td>1</td>
<td>26</td>
<td>27</td>
</tr>
<tr>
<td>Total</td>
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<td>56</td>
</tr>
<tr>
<td>Samples</td>
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<td>48</td>
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<tr>
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</tr>
<tr>
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<td>27</td>
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<td>53</td>
</tr>
<tr>
<td>Total</td>
<td>37</td>
<td>186</td>
<td>223</td>
</tr>
</tbody>
</table>

Table 3—Association between shedding status for BRCV or BECV and seroconversion rates to BCV in 56 feedlot cattle

<table>
<thead>
<tr>
<th>Seroconversion status*</th>
<th>Positive</th>
<th>Negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>BRCV</td>
<td>27</td>
<td>0</td>
<td>27</td>
</tr>
<tr>
<td>Negative</td>
<td>26</td>
<td>3</td>
<td>29</td>
</tr>
<tr>
<td>Total</td>
<td>53</td>
<td>3</td>
<td>56</td>
</tr>
<tr>
<td>BECV</td>
<td>29</td>
<td>0</td>
<td>29</td>
</tr>
<tr>
<td>Negative</td>
<td>24</td>
<td>3</td>
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</tr>
<tr>
<td>Total</td>
<td>53</td>
<td>3</td>
<td>56</td>
</tr>
<tr>
<td>BRCV and BECV</td>
<td>30</td>
<td>0</td>
<td>30</td>
</tr>
<tr>
<td>Positive</td>
<td>23</td>
<td>3</td>
<td>26</td>
</tr>
<tr>
<td>Negative</td>
<td>53</td>
<td>3</td>
<td>56</td>
</tr>
</tbody>
</table>

* Seroconversion classified as a ≥ 4-fold increase in BCV antibody titer.

values in parentheses are percentages.

*Day 0 = Day of arrival at feedlot. †Samples positive for bovine respiratory coronavirus (BRCV).

**Represents cattle that shed BCV at least once. 9One fecal sample was not collected at time of arrival. ||Samples positive for bovine enteric coronavirus (BECV).
whereas on days 0 and 21, GMT for cattle negative for BECV was 1,917 and 37,878, respectively. The GMT for days 0 and 21 for cattle negative for BRCV and BECV was 2,262 and 37,362, respectively.

Twenty-six of 56 (47%) cattle had low (≤ 100) BCV antibody titers on arrival (Fig 1). Cattle with BCV antibody titers ranging from < 25 to 100 were included in this classification, because 1:25 was the lowest serum dilution tested. The GMT for BCV on day 21 ranged from 3,200 to > 51,200. For cattle arriving with a GMT for BCV < 3,200, the percentage that seroconverted by day 21 was 100%, whereas for cattle arriving with titers of ≥ 6,400, none seroconverted by day 21 (Fig 2). Neither BRCV or BECV antigens were detected in cattle that had GMT for BCV of > 800 and > 1,600, respectively, in serum samples obtained on day of arrival.

Signs of respiratory tract disease characterized by coughing and nasal discharge were observed in 21 of 56 (38%) cattle arriving at the feedlot. By day 4, the number of cattle that had signs of respiratory tract disease increased markedly (48; 86%) and remained high on days 14 (50; 90%) and 21 (47; 84%). Proportion of cattle with signs of respiratory tract disease was not different for calves that shed BRCV versus calves that did not shed BRCV (Table 4). Diarrhea was observed in cattle on the day of arrival (5 calves; 9%) and days 4 (22; 39%), 14 (16; 29%), and 21 (18; 32%) after arrival. Proportion of cattle with diarrhea was not different between calves that had positive results for BECV and calves that had negative results for BECV. We did not detect an association between virus shedding from the respiratory tract or in feces, clinical signs, and treatment. However, many of the cattle continued to have signs of respiratory tract disease > 21 days after arrival in the feedlot (the last day of our study).

Of the 10 concurrent ELISA-positive fecal samples and nasal swab specimens selected randomly from ELISA-positive samples, 8 BECV and 8 BRCV strains were isolated and serially passaged in HRT-18 cells. After 3 to 5 initial blind passages, cytopathic effects characterized by enlarged, rounded, detached, dark cells usually were observed approximately 72 hours after inoculation. Using fluorescein isothiocyanate-fluorescein isothiocyanate-conjugated bovine anti-Mebus BCV serum, immunofluorescence was observed after 2 to 5 passages following infection of HRT-18 cells with the 16 BCV isolates.

**Discussion**

Although BCV infection of the respiratory tract of feedlot cattle after transportation has been described, to our knowledge, none of those investigations have provided detailed observations of an association between BCV infections of the respiratory tract and enteric system in feedlot cattle. In the study reported here, 46 and 52% of feedlot cattle shed BCV from the respiratory tract and in feces, respectively. Analysis of the κ value revealed that during the peak of BECV and BRCV shedding (days 0 and 4), an association was detected between these 2 events, indicating a strong relationship between shedding of BCV via the respiratory tract and enteric route. This result suggests that cattle become infected with BCV during, shortly before, or shortly after transport to a feedlot and shed BCV from the respiratory tract or in feces. This finding also supports results of our previous study, which documented BCV shedding from the respiratory tract and in feces in experimentally infected young calves. The low number of cattle or samples involved in the current study could explain why the McNemar test did not detect an association between shedding of BCV via the respiratory tract and enteric route. However, the κ value obtained for cattle (0.82) and for paired nasal swab specimens and fecal samples (0.81) indicated a high agreement between BECV and BRCV shedding in this study.

In the study reported here, BRCV and BECV were
isolated from samples and specimens obtained from the respiratory tract and enteric system of the same feedlot cattle at the same time. This result was consistent with observations in experimentally inoculated calves to support the fact that BCV replicates in the respiratory and enteric tracts. Strains of BRCV frequently were detected by ELISA and isolated from nasal swab specimens of feedlot cattle with respiratory tract disease after transport. Although BCV can replicate in the enteric and respiratory tracts, it is still unclear whether BRCV and BECV are distinctive in biological, antigenic, and genetic properties. There are reports that BCV strains isolated from the respiratory tract had biological and antigenic properties that differed from BCV strains isolated from the enteric tract, whereas other investigators could not detect differences between BCV isolated from the enteric and respiratory tracts. Recently, Fukutomi et al found that BRCV and BECV isolated from the respiratory and enteric tracts, respectively, of a cow with winter dysentery belonged to different antigenic groups, as determined on the basis of their reactivity against anticapsid glycoprotein MAb. However, in another report, other investigators found a specific BRCV strain that was genetically similar to BECV, with 98.7% nucleotide similarity of the spike gene sequence between the BRCV and BECV isolates. However, these latter investigators compared only 1 BRCV and 2 BECV strains. Therefore, additional studies of the biological, antigenic, and genetic properties of BCV isolated from the enteric and respiratory tracts of the same feedlot cattle are needed. To date, all enteric and respiratory tract strains of BCV that have been examined by use of virus neutralization tests belong to a single BCV serotype, although subtypes exist. In addition, although the antigen-capture ELISA used in the study reported here could detect 20 BECV and > 30 BRCV strains, including BCV subtypes (data not shown), we cannot rule out the possibility that this method might not detect BRCV and BECV strains with dramatically differing antigenic properties including distinct group antigens that do not react with the BCV anti-N MAB used.

Serologic analysis also indicated that 93% of the feedlot cattle seroconverted to BCV during the 21-day period after arrival at the feedlot. This agrees with the results of other studies that documented seroconversion to BCV in 61 to 100% of 604 calves in Canadian feedlots and 20 to 84% of 1,074 calves in feedlots in the United States. In addition, only 57% of cattle that developed a measurable seroresponse to BCV in the study reported here were actively shedding BCV from the respiratory tract or in feces on the days of sample collection. This could have been attributable to transient shedding of the virus on days when samples were not collected or to shedding during holding and transport following exposure to the virus at auction barns. This result is similar to that of another study in which investigators reported that the majority of cattle that did not shed virus from their respiratory tract seroconverted to BCV.

The distribution of GMT for BCV on days 0 and 21 in the cattle of our report is similar to values reported in another study. Most cattle arrived with a relatively low titer to BCV, which then increased during the 21-day period. Maximal antibody titers at the time of arrival for cattle that seroconverted was 3,200. Although these cattle seroconverted, BCV shedding was not detected during the 21 days in the feedlot. This may be attributable to prior exposure to the virus at the farms of origin or, more likely, at the auction barn. Cattle with a GMT for BCV > 6,400 at time of arrival did not seroconvert, because they probably had recent exposure and seroconversion to the virus prior to arrival and, thus, did not have a 4-fold increase in antibody titer needed to be classified in the seroconversion category. This finding is in agreement with that of other investigators who found that the change in BCV antibody titer during the initial 21 days in a feedlot was strongly negatively correlated with the titer at the time of arrival. Detection rates for shedding of BRCV and BECV were decreased in cattle that had GMT for BCV > 400 (Fig 2), suggesting that cattle that had a GMT > 400 were protected and did not shed BCV. This result provides information important for use in creating vaccination strategies against BCV in feedlot cattle.

In the study reported here, shedding of BECV by feedlot cattle was associated with reduced weight gain. Comparing weight gains between cattle that shed BCV and cattle that did not shed the virus revealed a significant difference (P = 0.033) difference. The estimated difference between these groups was 8.17 kg. However, there was not an apparent effect of BCV shedding or seroconversion on average daily weight gain (data not shown). These findings are consistent with those of another study in which investigators also found that shedding of BCV via the respiratory tract and seroconversion were not related to weight gain. There also was not an apparent effect of treatment and respiratory tract and enteric disease on average daily weight gain.

Although 39% of nasal swab specimens obtained on day 4 were positive when tested for BRCV antigen by use of an ELISA (Table 1), there was not a significant correlation between shedding of BRCV and development of respiratory tract disease during the 21-day period. The degree of clinically apparent disease of the respiratory tract attributable to BCV is uncertain. In a recent study, cattle that were shedding the virus and that had seroconverted to it during the first month after arrival at a feedlot were at increased risk for developing respiratory tract disease, compared with cattle that did not shed the virus or seroconvert. In the study reported here, it is noteworthy that almost all of the cattle that shed BRCV or seroconverted to BCV had signs of respiratory tract disease by day 21. Our findings in a prior study indicated detection of BCV during the first month after arrival at a feedlot was a marker for respiratory tract disease of sufficient severity to cause lesions that were still evident at time of slaughter. Indeed, feedlot cattle are susceptible to respiratory tract infection and disease induced by multiple pathogens, such as bovine respiratory syncytial virus, bovine viral diarrhea virus, bovine herpesvirus 1, and Mannheimia haemolytica, and it is known that concurrent infections exacerbate clinical signs of respiratory tract disease. Some of the viruses are believed to act as predisposing agents for bacterial pathogens, such as Mannheimia sp. Given the acknowledged roles of other viruses in the bovine respiratory
disease complex, it is possible that BCV also may act synergistically with other infectious agents and stressors to contribute to pneumonia in feedlot cattle.31

A number of pathogens cause enteric disease in cattle. In the study reported here, only 22% of the fecal samples from cattle with diarrhea were positive for BCV antigen when tested by use of an ELISA. When tested by use of immune electron microscopy, group A rotavirus, Bredavirus, and coronavirus were detected in the fecal samples (data not shown). Some of these fecal samples had mixed infections of Bredavirus and coronavirus or rotavirus. From these results, the diarrhea observed could have been attributable to infections with coronavirus, other enteric pathogens, or a combination of coronavirus and other pathogens. On the other hand, 22% of the fecal samples from cattle that did not have diarrhea were positive for BCV antigen when tested by use of an ELISA. It is possible that these cattle had diarrhea during shipping or at the auction barn and were still shedding BCV. Another possibility is that chronic or stress-induced shedding of BCV in the feces was observed in adult healthy cattle32 or immuno-suppressed adult cattle exposed to winter dysentery or strains of BCV that cause diarrhea in calves.35

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