

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

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**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 BECV were positive for BRCV. In contrast, only 10/175 (6%) paired nasal swab specimens and fecal samples negative for BECV 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)

**B**ovine coronavirus (BCV) was first identified as a possible cause of diarrhea in calves in 1972<sup>1</sup> and is now recognized as a primary pathogen in diarrhea of neonatal calves<sup>2-6</sup> and epizootic diarrhea of adult cattle.<sup>3,7-11</sup> Bovine coronaviruses replicate in the differenti-

ated epithelium of the small and large intestines as well as the respiratory tract.<sup>12-16</sup>

Feedlot cattle are exposed to a multitude of infectious agents during transport from ranch to auction market to feedlot.<sup>17-19</sup> Infection by viruses and bacteria is common among these cattle, and although cattle do not always have signs of clinical disease, mild to severe respiratory tract disease associated with the bovine respiratory disease complex is common. Recently, investigators have detected and isolated BCV from nasal swab specimens obtained from feedlot cattle.<sup>20-23</sup> Cattle shedding BCV via the nasal passages at time of arrival in a feedlot have an increased risk for developing respiratory tract disease.<sup>24</sup> In 1 study,<sup>a</sup> high mortality associated with BCV infection was observed.

Although BCV infects epithelial cells in the intestinal and respiratory tracts of calves,<sup>12-16</sup> the prevalence of enteric infections attributable to BCV, the association between shedding of BCV via the respiratory tract and enteric routes, and the rate of seroconversion to BCV are unknown in feedlot cattle. To define epidemiologic characteristics of BCV-induced enteric and respiratory tract infections of feedlot cattle and the association of BCV with respiratory tract and enteric infections, we evaluated cattle entering a local feedlot in Ohio. Information collected on clinical signs, treatment rates for respiratory tract and enteric disease, and average daily weight gain was used to identify associations between these variables, shedding of bovine respiratory coronavirus (BRCV) and bovine enteric coronavirus (BECV), and seroconversion to BCV.

## Materials and Methods

**Animals**—Fifty-six crossbred steers between 6 and 7 months old were included in the study. They were in a group of 216 cattle purchased from a mixed-source livestock auction market in West Virginia and transported to the feedlot of the Ohio Agricultural Research and Development Center in Wooster, Ohio.

**Sample Collections**—Paired nasal swab specimens and fecal samples were collected from cattle at time of arrival (day 0) and 4, 14, and 21 days after arrival in the feedlot, using a technique described elsewhere.<sup>20</sup> Briefly, samples were obtained from 56 cattle in a newly arrived group. These 56 comprised cattle with apparent respiratory tract and enteric disease as well as those that appeared to be clinically normal. Using sterile cotton-tipped applicators, swab specimens were obtained from both nostrils of each calf; swabs then were placed in tubes containing 4 ml of maintenance medium.<sup>20</sup> Tubes were vortexed, swabs were removed, and samples were centrifuged (1,000 × g for 11 minutes). Supernatants were collected and frozen at -70 C for subsequent testing by use of an ELISA and virus isolation techniques.<sup>20</sup> Fecal samples

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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.<sup>7</sup>

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 florfenicol,<sup>b</sup> flunixin meglumine,<sup>c</sup> cephalosporin,<sup>d</sup> or tilmicosin<sup>e</sup> 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.<sup>21,25</sup> Three monoclonal antibodies (MAb; BC 22 F8.3 C for HE protein, BC 28 H1.2 C for N protein, and BC 29 G7.2 C for S protein) produced against the CD DB2 strain of BCV and hyperimmune antisera produced against the CD Mebus strain of BCV in guinea pigs were used for the antigen-capture ELISA. Briefly, 96-well microtiter plates were coated with a mixture of the 3 MAb developed against BCV structural proteins (HE, N, and S proteins) of the CD DB2 strain of BCV (antibody-positive coating) or BCV antibody-negative mouse ascitic fluids (antibody-negative coating). After incubating wells overnight at 4 C, 5% (wt:vol) nonfat, dry milk in PBS solution (PBSS; pH 7.4) was applied as a blocking reagent for 1 hour at 20 to 22 C. Fecal suspensions (1:25 dilution) and fluids from nasal swab specimens (2 nasal swabs in 4 ml of maintenance medium) were added to duplicate wells coated with the BCV-capture MAb or 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-ethylbenzothiazoline sulfonic acid with a final concentration of 0.03% hydrogen peroxide. Plates were read at a setting of 414 nm, using an ELISA reader,<sup>f</sup> and absorbances were saved as computer files. A computer spreadsheet program<sup>g</sup> was used to calculate ELISA values for the samples by subtracting the average absorbance of the paired BCV antibody negative-coated wells from the average absorbance of the paired BCV antibody positive-coated wells. Samples with an absorbance of  $\geq 0.03$  were considered positive for BCV antigen.

**ELISA for BCV antibody**—An antibody-detection ELISA validated for BRCV by Lathrop et al<sup>21</sup> and for BECV by Smith et al<sup>26</sup> was used to detect IgG antibodies to BCV in serum sam-

ples from feedlot cattle. Briefly, 96-well ELISA plates were coated with a mixture of the same 3 MAb used for the antigen-capture ELISA and incubated overnight at 4 C. Plates were washed 5 times between each step with PBST. Next, clarified semipurified human rectal tumor (HRT)-18 cell-culture supernatants containing BCV were added to each well and incubated for 1 hour at 25 C. After washing, serial 2-fold dilutions (range, 1:400 to 1:6,400) of serum samples diluted in PBST were applied to a row of wells. Samples from cattle with out-of-range titers were retested at 2-fold serial dilutions from 1:50 to 1:51,200. Plates with sample dilutions were incubated for 1 hour at 25 C and washed. Diluted rabbit anti-bovine IgG horseradish-peroxidase-conjugated antibody<sup>h</sup> in PBST was added to each well; wells were incubated for 1 hour at 25 C and then washed. The same chromogen substrate that was used in the antigen-capture ELISA was applied to each well. The color reaction was stopped after 20 minutes by adding 50  $\mu$ l of 5% sodium dodecyl sulfate per well. Plates were read at a setting of 414 nm, using an ELISA reader,<sup>f</sup> and absorbances were saved as computer files. A computer spreadsheet program<sup>g</sup> 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 serum 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.<sup>20</sup> Briefly, cells were washed with cell culture medium (Eagle minimal essential medium [EMEM] containing 1% antibiotics [penicillin, dihydrostreptomycin, and cycostatin] and 1% NaHCO<sub>3</sub>) and inoculated in duplicate wells along with selected ELISA-positive filtered (0.45- $\mu$ m) 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  $\mu$ g/ml) was added. Cultures were incubated for 3 to 4 days at 37 C in a 5% CO<sub>2</sub> atmosphere. Cultures were examined daily for evidence of cytopathic effects, and BCV was confirmed by use of immunofluorescence tests, as described elsewhere.<sup>16</sup> 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  $\chi^2$  test was used to assess the relationship between shedding of BECV and BRCV. In addition, the  $\kappa$  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 56 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

Table 1—Results for samples obtained from cattle at a feedlot in Ohio that were tested for bovine coronavirus (BCV) by use of an antigen-capture ELISA

| Type of sample      | No. of calves | No. of samples | Days after arrival* |         |        |    | Samples positive for BCV | Cattle positive for BCV |
|---------------------|---------------|----------------|---------------------|---------|--------|----|--------------------------|-------------------------|
|                     |               |                | 0                   | 4       | 14     | 21 |                          |                         |
| Nasal swab specimen | 56            | 224            | 8 (14)              | 22 (39) | 7 (13) | 0  | 37/224 (17)†             | 26 (46)†‡               |
| Feces               | 56            | 223§           | 17 (31)             | 25 (45) | 6 (11) | 0  | 48/223 (22)              | 29 (52)                 |

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. §One fecal sample was not collected at time of arrival. ||Samples positive for bovine enteric coronavirus (BECV).

virus decreased to 13%, and this value decreased to 0% by day 21. Shedding rate for BECV at time of arrival and day 4 was 31 and 45%, respectively. For samples obtained on day 14, the percentage of fecal samples that yielded positive results for coronavirus decreased to 11%, and this value decreased to 0% by day 21.

Both BECV and BRCV were recovered at least once during the 3-week period from 25 of 56 (45%) calves in the study (Table 2). Twenty-nine cattle had positive results for BECV, and 25 (86%) of them also shed BRCV. In contrast, 27 cattle had negative results for BECV, and only 1 (4%) of them shed BRCV. There was a 91% agreement for shedding of BECV and BRCV in the same cattle. Value of the  $\kappa$  coefficient (0.82) indicated a high degree of agreement beyond chance between shedding of BRCV and BECV in the same animal. However, the McNemar test did not detect the large difference, probably because of our relatively small sample size.

Shedding status for BRCV and BECV in the samples obtained at arrival and on days 4, 14, and 21 from the 56 cattle revealed concurrent shedding of BRCV and BECV in 27/223 (12%) nasal swab specimens and fecal samples collected at the same time from the same animal (Table 2). Twenty-seven of 48 (56%) paired samples that had positive results for BECV also had positive results for BRCV. In contrast, only 10 of 175 (6%) paired samples with negative results for BECV had positive results for BRCV. Analysis revealed 86% agreement between shedding of BECV and BRCV in concurrent samples. Although the McNemar test again did not detect this difference, the value of the  $\kappa$  coefficient indicated good agreement beyond chance between shedding of BECV and BRCV at time of arrival ( $\kappa = 0.49$ ) and on day 4 ( $\kappa = 0.67$ ). The  $\kappa$  value for agreement between shedding of BECV and BRCV for the entire period was 0.81.

Analysis of results of the ANOVA revealed a significant ( $P = 0.033$ ) difference in weight gain between calves that shed BECV at any time and those that did not shed the virus. Calves that had negative results for 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  $\geq 4$ -fold

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

| BECV shedding status | BRCV shedding status |            | Total      |
|----------------------|----------------------|------------|------------|
|                      | Positive             | Negative   |            |
| <b>Cattle</b>        |                      |            |            |
| Positive             | 25                   | 4          | 29         |
| Negative             | 1                    | 26         | 27         |
| <b>Total</b>         | <b>26</b>            | <b>30</b>  | <b>56</b>  |
| <b>Samples</b>       |                      |            |            |
| Positive             | 27                   | 21         | 48         |
| Negative             | 10                   | 165        | 175        |
| <b>Total</b>         | <b>37</b>            | <b>186</b> | <b>223</b> |

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

| Shedding status      | Seroconversion status* |          | Total     |
|----------------------|------------------------|----------|-----------|
|                      | Positive               | Negative |           |
| <b>BRCV</b>          |                        |          |           |
| Positive             | 27                     | 0        | 27        |
| Negative             | 26                     | 3        | 29        |
| <b>Total</b>         | <b>53</b>              | <b>3</b> | <b>56</b> |
| <b>BECV</b>          |                        |          |           |
| Positive             | 29                     | 0        | 29        |
| Negative             | 24                     | 3        | 27        |
| <b>Total</b>         | <b>53</b>              | <b>3</b> | <b>56</b> |
| <b>BRCV and BECV</b> |                        |          |           |
| Positive             | 30                     | 0        | 30        |
| Negative             | 23                     | 3        | 26        |
| <b>Total</b>         | <b>53</b>              | <b>3</b> | <b>56</b> |

\*Seroconversion classified as a  $\geq 4$ -fold increase in BCV antibody titer.

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,

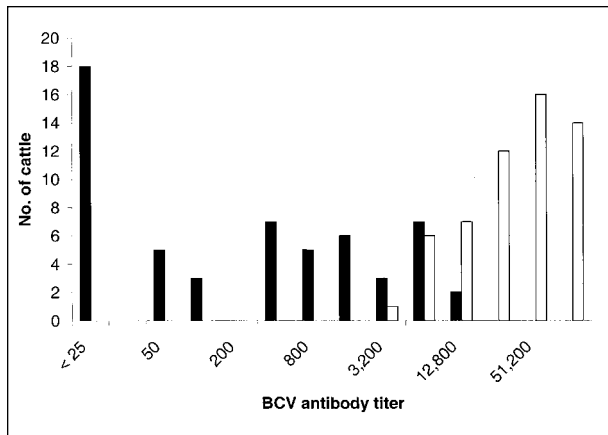


Figure 1—Frequency distribution of geometric mean titers (GMT) for bovine coronavirus (BCV) antibody in samples obtained from 56 cattle at the time of arrival (day 0; black bar) and day 21 after arrival at a feedlot (white bar).

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 ( $\leq 100$ ) 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  $< 3,200$  to  $> 51,200$ , the percentage that seroconverted by day 21 was 100%, whereas for cattle arriving with titers of  $\geq 6,400$ , 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-

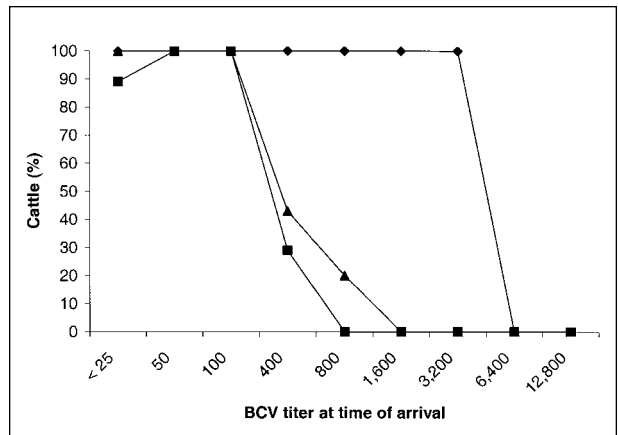


Figure 2—Percentage of cattle that shed bovine respiratory coronavirus (square) or bovine enteric coronavirus (triangle) and that seroconverted to BCV (diamond), on the basis of GMT for BCV at time of arrival in a feedlot.

Table 4—Association between shedding of BRCV or BECV and clinical signs in 56 feedlot cattle

| Shedding status* | BRCV       |           |            | BECV      |            |            |
|------------------|------------|-----------|------------|-----------|------------|------------|
|                  | Positive   | Negative  | Total      | Positive  | Negative   | Total      |
| Positive         | 29         | 8         | 37         | 11        | 37         | 48         |
| Negative         | 137        | 50        | 187        | 40        | 135        | 175        |
| <b>Total</b>     | <b>166</b> | <b>58</b> | <b>224</b> | <b>51</b> | <b>172</b> | <b>223</b> |

\*Shedding status of BRCV for respiratory tract disease and BECV for diarrhea.

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,<sup>20-24,a</sup> 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  $\kappa$  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,<sup>16</sup> 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  $\kappa$  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.<sup>12-16</sup> Strains of BRCV frequently were detected by ELISA and isolated from nasal swab specimens of feedlot cattle with respiratory tract disease after transport.<sup>20-24,a</sup> 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<sup>20,23</sup> 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<sup>15,27</sup> could not detect differences between BCV isolated from the enteric and respiratory tracts. Recently, Fukutomi et al<sup>28</sup> 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 antispikes glycoprotein MAb. However, in another report,<sup>29</sup> 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.<sup>11,20</sup> 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 95% 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<sup>21,30</sup> 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<sup>21</sup> 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.<sup>21</sup> 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.<sup>21</sup> 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<sup>21,30</sup> 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 BECV and cattle that did not shed the virus revealed a significant ( $P = 0.033$ ) difference. The estimated difference between these groups was 8.17 kg. However, there was not an apparent effect of BRCV shedding or seroconversion on average daily weight gain (data not shown). These findings are consistent with those of another study<sup>24</sup> 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 BRCV is uncertain. In a recent study,<sup>24</sup> 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<sup>24</sup> indicated detection of BRCV 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.<sup>31,32</sup> Some of the viruses are believed to act as predisposing agents for bacterial pathogens, such as *Mannheimia* sp.<sup>33</sup> 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.<sup>33</sup>

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 coronavirus and 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 cattle<sup>34</sup> or immunosuppressed adult cattle exposed to winter dysentery or strains of BCV that cause diarrhea in calves.<sup>35</sup>

<sup>a</sup>Storz J, Purdy CW, Lin ZQ, et al. Market-stressed cattle of a shipping fever epizootic in a Texas feedlot have a high infection rate with respiratory bovine coronaviruses, in *Proceedings. 31th Annu Conv Am Assoc Bovine Pract* 1998;31:224.

<sup>b</sup>Nuflor, Schering-Plough Animal Health Corp, Union, NJ.

<sup>c</sup>Banamine, Schering-Plough Animal Health Corp, Union, NJ.

<sup>d</sup>Excenel, Pharmacia and Upjohn Co, Kalamazoo, Mich.

<sup>e</sup>Micotil, Elanco Animal Health, Eli Lilly Co, Indianapolis, Ind.

<sup>f</sup>Titertek Multiscan plate reader, Flow Laboratories Inc, McLean, Va.

<sup>g</sup>Quattro Pro Windows version 7.0, Borland International Inc, Scotts Valley, Calif.

<sup>h</sup>Anti-Bovine IgG (Rabbit) Affin, HRP Conj, ICN Biomedical, Costa Mesa, Calif.

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