Transmission of bovine coronavirus and serologic responses in feedlot calves under field conditions

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Objective—To compare shedding patterns and serologic responses to bovine coronavirus (BCV) in feedlot calves shipped from a single ranch in New Mexico (NM calves) versus calves assembled from local sale barns in Arkansas (AR calves) and to evaluate the role of BCV on disease and performance.

Animals—103 feedlot calves from New Mexico and 100 from Arkansas.

Procedures—Calves were studied from before shipping to 35 days after arrival at the feedlot. Nasal swab specimens, fecal samples, and serum samples were obtained before shipping, at arrival, and periodically thereafter. Bovine coronavirus antigen and antibodies were detected by use of an ELISA.

Results—NM calves had a high geometric mean titer for BCV antibody at arrival (GMT, 1,928); only 2% shed BCV in nasal secretions and 1% in feces. In contrast, AR calves had low antibody titers against BCV at arrival (GMT, 102) and 64% shed BCV in nasal secretions and 65% in feces. Detection of BCV in nasal secretions preceded detection in feces before shipping AR calves, but at arrival, 73% of AR calves were shedding BCV in nasal secretions and feces. Bovine coronavirus infection was significantly associated with respiratory tract disease and decreased growth performance in AR calves.

Conclusions and Clinical Relevance—Replication and shedding of BCV may start in the upper respiratory tract and spread to the gastrointestinal tract. Vaccination of calves against BCV before shipping to feedlots may provide protection against BCV infection and its effects with other pathogens in the induction of respiratory tract disease. (*Am J Vet Res* 2006;67:1412–1420)

Abbreviations

BCV	Bovine coronavirus
BRDC	Bovine respiratory disease complex
NM calves	Calves shipped from a single ranch in New Mexico
AR calves	Calves assembled from local sale barns in Arkansas
GMT	Geometric mean titer for BCV antibody

Bovine coronavirus is a cultivable, enveloped, single-stranded RNA virus in the *Coronavirus* genus within the Coronaviridae family and the Nidovirales order. It was first reported in diarrheic calves by Mebus et al.[1] Bovine coronavirus is a pneumoenteric virus that causes mild or severe respiratory infections and severe lower intestinal infections with enteritis.[2] This virus causes diarrhea in calves and has been associated with winter dysentery in cows.[3]

Infection by BCV appears to be widespread in cattle when they enter feedlots in the United States, with prevalence estimates of BCV shedding in feces or nasal secretions of calves arriving at a feedlot as high as 53% as detected by an ELISA, 96% as detected by reverse transcriptase-PCR, and 100% as detected by serologic testing.[4-8] Feedlot calves become naturally infected with BCV shortly after they are assembled at order-buyer barns; virus spread is probably enhanced by stressful conditions during transport and associated crowding.[4,9] The nasal route of transmission is speculated to occur in addition to the fecal-oral route.[2]

The role of BCV as a pathogen of the respiratory tract in calves[10-13] and feedlot cattle is not completely defined. Several investigators have shown that BCV may be implicated in BRDC,[6,8,9,14-18] with decreased growth performance in feedlot cattle[4,5,7]; others have found no correlation between BCV shedding and respiratory tract disease under field conditions[4,5]; some have failed to reproduce respiratory tract disease after experimental inoculation of calves with BCV[19-21]; and others have isolated this virus from healthy cattle[22] or have not detected BCV shedding in all feedlot calves with respiratory tract disease.[6,9,14,18]

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It has been difficult to fulfill Koch's postulates in regard to BCV involvement in respiratory problems such as BRDC because of limitations of these postulates to deal with host and environmental interactions and polymicrobial infections.[3] Nevertheless, a preponderance of evidence[7,8,9,11,14,17,18] now suggests that this virus may likely be implicated in BRDC by predisposing cattle to induction of respiratory tract disease and poor health performance. If this is the case, a clear understanding of BCV epidemiologic characteristics and its role in disease causation under field conditions is necessary for development of preventive measures such as vaccination prior to shipping to control this pathogen in feedlots calves.

Currently, the transmission of BCV under field conditions and its role in respiratory tract disease in herds with variable amounts of antibodies to this virus are not well defined. Studies on shedding patterns and the relationship between the enteric and respiratory forms of this virus under field conditions and in feedlots are still needed to understand the epidemiologic characteristics of this virus. The main objective of this study was to determine BCV shedding patterns and serologic responses to BCV under field conditions in feedlot calves and the possible role of BCV in disease and on performance.

Materials and Methods

Study population—Two groups of feedlot calves from the southwestern and south-central United States were shipped to a feedlot research station in New Mexico where they were commingled and distributed in pens for a feedlot study. One group of 103 calves came from the state of New Mexico, and a second group of 100 calves came from the state of Arkansas. Calves from New Mexico originated from a ranch located about 20 miles north of the town of Clayton; these calves were predominantly Red Angus with some Hereford crossbreds. Calves were tagged and vaccinated systematically with an experimental modified-live *Mannheimia haemolytica–Pasteurella multocida* vaccine[a] or a viral vaccine.[b] They received a booster of both vaccines prior to shipping. Dams were vaccinated with a signal vaccine[c] to protect against bovine viral diarrhea virus, infectious bovine rhinotracheitis virus, bovine parainfluenza-3, and bovine respiratory syncytial virus before calving.

The AR calves were assembled from 3 local sale barns and were already commingled for at least 3 to 4 days prior to shipping to the feedlot in New Mexico. These calves were predominantly beef crossbreds representing Charolais, Angus, Hereford, and Brahman. After purchase, they were tagged and vaccinated, by use of the same scheme as for NM calves. No BCV vaccine was given to calves in either group.

Both NM and AR calves were shipped approximately 32 km (NM calves) or 1,046 km (AR calves) to the experimental feedlot research station in New Mexico (Clayton Livestock Research Center) where this study was performed. At arrival, all calves were assigned to pens on the basis of bacterial vaccine status (vaccinated or nonvaccinated). Calves were then randomly allocated to pens to balance virus vaccination status and source (Arkansas or New Mexico) within each pen with AR and NM calves evenly dispersed. Calves received baled alfalfa hay and a premixed grain ration diet containing steam-flaked corn, ground hay, minerals, and molasses made on site twice daily. Calves were treated with tilmicosin[d] as the first antibiotic of choice in case of signs of respiratory infections. Diarrhea and other miscellaneous illnesses were treated on an individual basis according to clinical signs.

Collection of samples—For each group of calves, paired nasal swab specimens and fecal samples were obtained prior to shipping, at arrival at the feedlot research station, and periodically after arrival. Collection times were 22 days prior to shipping for NM calves (day–22) and 3 days prior to shipping for AR calves (day–3). After commingling and distribution of calves in both groups, fecal samples and nasal swab specimens were collected on day 0 (arrival) and on days 3, 7, 14, and 21 after arrival. Serum samples were obtained on each collection day except for day 3, with an additional collection of blood to obtain serum on day 35 after arrival. The difference in the collection day for samples prior to shipping for both groups (day–22 and day–3 for NM and AR calves, respectively) was the result of differences in management practices between NM and AR calves. Nasal swab specimens were collected from both nostrils by use of sterile cotton-tipped applicators and placed into plastic tubes containing 3 mL of sterile minimum essential medium.[e] Blood was collected by jugular venipuncture, and about 2 mL of serum/calf was obtained and placed in plastic vials. Fecal samples were collected by rectal stimulation directly into 60-mL sterile plastic containers. All samples were frozen at 20°C, shipped to the Food Animal Health Research Program at The Ohio State University, placed in aliquots, and stored at 70°C until tested for BCV. Tubes with nasal swab specimens were vortexed and swab specimens removed, and the fluid was stored in microcentrifugation Eppendorf tubes[f] at 70°C. Ten percent fecal suspensions were prepared in PBS solution (pH, 7.4) and centrifuged (3,000 × *g* for 20 minutes), and the supernatants were aspirated and stored at 70°C. After arrival at our laboratory, sera were thawed, heat inactivated at 56°C for 30 minutes, and stored at 70°C.

Weight gains and respiratory tract disease—Calves were weighed at each sample collection time at the feedlot and were observed daily at morning feedings for signs of respiratory tract disease and other illnesses. Calves with clinical signs of depression, anorexia, nasal discharge, ocular discharge, or labored breathing were restrained in a chute to obtain rectal temperatures. Only calves with suspected undifferentiated respiratory tract disease that had a rectal temperature of ≥ 40°C were treated. Affected calves were first treated with tilmicosin.[d] For the purpose of analysis in our study, treated calves were considered to have respiratory tract disease.

ELISA for detection of BCV antigen—An indirect double-sandwich antigen-capture ELISA was used to detect BCV antigen in feces[23] and as modified by Hasoksuz et al[24] for detection of BCV antigen in nasal swab specimens. On the basis of results of a study done by Cho et al,[19] the sensitivity of the ELISA used in our study for BCV antigen detection in fecal suspensions from calves was 24% higher than for BCV antigen detection in nasal swab specimens.

Briefly, 96-well plates[g] were coated overnight at 4°C by use of a pool of 3 monoclonal antibodies directed against the S, N, and HE structural proteins of BCV[23,25] or unrelated ascites fluids, then blocked for 2 hours at room temperature (approx 23°C) with 5% nonfat dried milk.[h] Specimens were applied for 1 hour at room temperature, and a secondary antibody (guinea pig hyperimmune serum against Mebus strain of BCV) was then applied for 1 hour at room temperature. Rabbit polyclonal anti–guinea pig immunoglobulin conjugated with horseradish peroxidase[i] was added, and the plates were incubated for 1 hour at room temperature. Reactions were developed by use of 2,2-azino-bis-3-ethyl-benzthiazoline-6-sulfonic acid applied for 10 minutes and then stopped with 5% SDS. The absorbance value of each well was read

with a computer-linked ELISA plate reader,[j] and the readings were saved as computer files and opened on a spreadsheet program.[k] Absorbance values for each sample were calculated by subtracting the mean absorbance of paired negative-coated wells from the mean absorbance of paired positive-coated wells. Samples with a resulting absorbance greater than the cutoff value were considered positive for BCV. The cutoff value was calculated as the mean value for absorbance of paired negative-coated wells plus 3 times the SD.

ELISA for detection of antibodies to BCV—An indirect antibody ELISA developed by Smith et al[26] for detection of antibodies to enteric BCV was adapted to detect IgG antibodies to BCV in serum samples of feedlot cattle as modified by Lathrop et al.[6] Briefly, 96-well plates[g] were coated with a mixture of the same 3 monoclonal antibodies as used in the antigen capture ELISA. After blocking as described for the antigen ELISA, clarified human rectal tumor-18 cell-culture supernatants from human rectal tumor-18 cells infected with the Mebus strain of BCV were added to each well in duplicate as positive rows; clarified human rectal tumor-18 cell-culture mock-infected supernatants were used for negative duplicate rows and incubated for 2 hours at room temperature. Serial 2-fold (range, 1:25 to > 1:51200) dilutions in PBS solution (pH, 7.4) of serum samples were applied, and then plates were incubated for 1 hour at room temperature. Goat anti-bovine IgG (heavy and light chains) conjugated to horseradish peroxidase[l] was added, and the plates were incubated for 1 hour at room temperature. The same chromogen described for the antigen ELISA (2,2-azino-bis-3-ethyl-benzthiazoline-6-sulfonic acid) was applied to each well for 10 minutes. The color development was stopped by use of 5% SDS, and the plates were read with a computer-linked ELISA plate reader.[j] Absorbencies were saved as computer files, transferred to spreadsheet programs as before, and used to calculate absorbance values for the samples by subtracting the mean absorbance of wells coated with mock-infected cell-culture supernatants from the mean absorbance of BCV-infected cell-culture supernatant-coated wells at each dilution for each sample. Titers were determined as the serum dilution at which the absorbance values were higher than the cutoff point value. The cutoff point value was calculated as described for the antigen capture ELISA.

Statistical analysis—The prevalence of BCV shedding in feces and nasal secretions on each collection day was calculated to identify and describe BCV shedding patterns. The effect of BCV shedding on weight gain was assessed by use of a multivariable ANOVA. Comparison of GMT was conducted by use of the Mann-Whitney *U* test. Associations between GMT and respiratory tract disease and between GMT and BCV shedding were determined by the Kruskal-Wallis test for each group (AR and NM calves) independently (ie, within analysis according to cattle source). Odds ratios for the association between BCV shedding and seroconversion, between BCV shedding and source of calves, and between BCV shedding and respiratory tract disease were determined by use of a multivariable logistic regression model with generalized estimating equations. Pen assignment and source where appropriate were included in multivariable models to account for potential confounding. Data were analyzed by use of a commercial computer software program.[m]

Some results are expressed on the basis of 102 and 100 NM calves instead of the original 103 NM calves or on the basis of 97 and 99 AR calves instead of the original 100 AR calves because some samples were unsuitable for diagnostic testing or a sample (feces, nasal swab, or blood) from an individual calf was not collected on a specific sample collection day as a result of logistic problems during the collection process. Values of $P < 0.05$ were considered significant.

Results

BCV shedding in feces and nasal secretions—A low percentage of NM calves, which had a high GMT (1,928) on arrival at the feedlot, shed BCV in nasal secretions (2/102; 2%) and feces (1/103; 1%) on day 3; these calves did not subsequently shed BCV during the remainder of the study (**Figure 1**). In contrast, a high percentage of AR calves, which had a low GMT (102) on arrival at the feedlot, shed BCV in nasal secretions (day 0, 60/100; 60%) and feces (day 0, 61/99; 62%) during the entire period (**Figure 2**). Shedding of BCV in nasal secretions and feces differed significantly on day 0 (arrival) between AR and NM calves. On the basis of the odds ratios, AR calves were 56 times as likely to shed BCV as NM calves during the study period.

Serologic responses—Most NM calves had a high GMT (2,541) before arrival (day–22). Seroconversion, as determined by a > 4-fold increase in antibody titers against BCV, was observed in only 7 of 102 (6.9%) NM calves between day–22 (before arrival) and day 0 (arrival) and mainly in NM calves that had a low GMT (ie, ≤ 400) prior to shipping, indicating that they were exposed at the farm of origin. By day 35 after arrival, some NM calves seroconverted to BCV (41/100; 41%); of these, 2 (2/41; 4.9%) calves maintained a progressive 5- to 8-fold increase in antibody titers against BCV from day–22. Although the remaining NM calves that seroconverted by day 35 (39/41; 95%) had a substantial GMT (1,014) on day 0, they apparently became reexposed after arrival. The high antibody titer on day 0 in these NM calves suggests previous exposure to the virus at the farm of origin.

The AR calves had a low GMT (102) at arrival. Between days 0 and 35, most (89/99; 89.9%) AR calves seroconverted, having a high GMT (5,955) at the end of the study period (day 35). Thus, these AR calves became infected at the auction house, during transportation, or on arrival at the feedlot. Ten (10/99; 10.1%) AR calves with a high GMT (2,111) before arrival and on day 0 did not seroconvert within the 35-day test period.

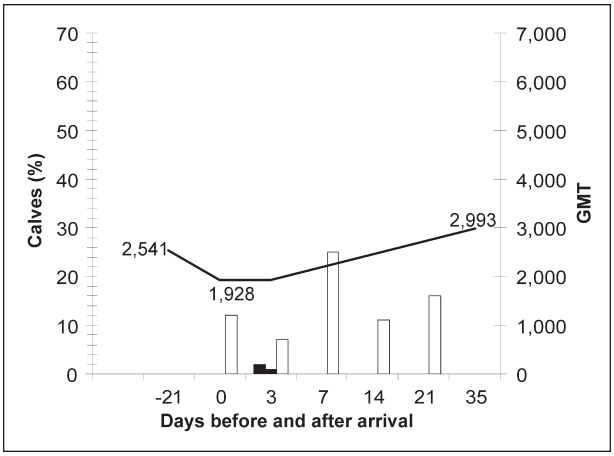

Figure 1—Percentage of NM calves that shed BCV in nasal secretions (black bar), shed BCV in feces (gray bar), or had signs of respiratory tract disease (white bar)* as well as GMT of NM calves (solid line) versus test day before and after arrival (day 0) at feedlot. *Calves considered as having respiratory tract disease between days 0 and 2, 3 and 6, 7 and 13, 14 and 21, and 21 and 29 are represented on days 0, 3, 7, 14, and 21, respectively.

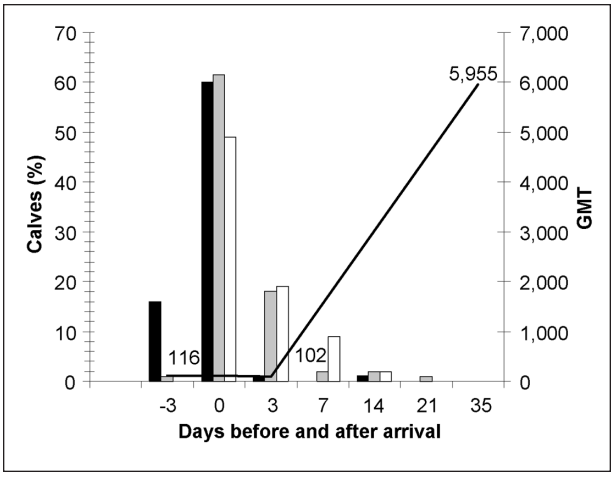

Figure 2—Percentage of AR calves that shed BCV in nasal secretions, shed BCV in feces, or had signs of respiratory tract disease as well as GMT of AR calves versus test day before and after arrival (day 0) at feedlot. *See* Figure 1 for key.

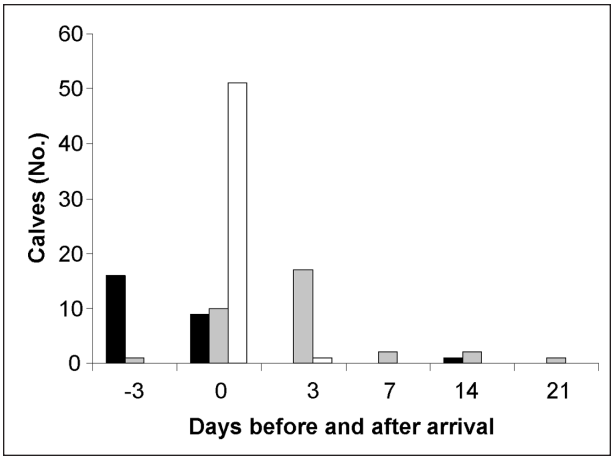

Figure 3—Number of AR calves that shed BCV in nasal secretions (black bar), feces (gray bar), or both (white bar) versus test day before and after arrival (day 0) at feedlot.

The GMT at arrival was significantly ($P < 0.001$) different between AR calves (GMT, 102) and NM calves (GMT, 1,928). Also, the total seroconversion rate from days 0 to 35 was significantly ($P < 0.001$) different between AR calves (89/99; 89.9%) and NM calves (41/100; 41%). The 130 AR and NM calves in total that seroconverted between days 0 (arrival) and 35 had a GMT of 151 at arrival; in contrast, 69 AR and NM calves in total that did not seroconvert had a GMT of 1,860 at arrival. When individual calves were analyzed, some calves (n = 5) that seroconverted had a moderate GMT (3,200) on day 0, which suggests that even with a high GMT, BCV reinfections are possible, which might be influenced by the exposure dose or route or host susceptibility factors. When AR calves were analyzed independently from NM calves, a significant ($P < 0.001$) difference was observed in the GMT (54.7 vs 1,877.7) at arrival between AR calves that seroconverted versus AR calves that did not seroconvert by day 35, respectively. Similarly, when NM calves were analyzed independently from AR calves, a significant ($P < 0.001$) difference was observed in the GMT (1,042.8 vs 1,668.6) at arrival between NM calves that seroconverted versus NM calves that did not seroconvert by day 35, respectively. Accordingly, calves that seroconverted to BCV in this feedlot were more likely to have low antibody titers against BCV at arrival, regardless of their source of origin.

Viral shedding and serologic responses—In general, calves shedding BCV, either in feces or nasal secretions, had a significantly ($P < 0.001$) lower GMT on day 0 than calves that were not shedding BCV. On the basis of odd ratios, calves that seroconverted were 7 times as likely to shed BCV as calves that did not seroconvert. A significant ($P < 0.001$) association between BCV shedding, either in feces or nasal secretions, and seroconversion was found, which indicates that BCV shedding, as detected by the ELISA, was a good indicator of infection in our study. When within-group analyses were performed, AR calves shedding BCV, either in feces or nasal secretions, had a significantly ($P < 0.001$) lower GMT on day 0 than calves that were not shedding BCV. However, because few NM calves shed BCV throughout the study (ie, in nasal secretions [2/102; 2%] or in feces [1/103; 1%]), no association could be detected.

BCV shedding patterns—Shedding of BCV in nasal secretions of AR calves preceded BCV shedding in feces. When AR calves that shed BCV were analyzed independently (**Figures 3 and 4**), 17 calves shed BCV before arrival (day–3), of which 16 shed BCV exclusively in nasal secretions and 1 shed BCV only in feces (this calf shed BCV in feces only one other time on day 0). Among the 16 calves that shed BCV in nasal secretions on day–3, 10 shed BCV in both feces and nasal secretions on day 0 (arrival); of these 10 calves, 8 did not shed BCV on subsequent sample collection days, 1 shed BCV only in feces on day 3, and 1 shed BCV only in feces on days 14 and 21. Of the remaining 6 calves that shed BCV in nasal secretions on day 3, 2 did not shed BCV on any subsequent sample collection day, 1 shed BCV only in feces on days 0 and 3, 1 shed BCV only in feces on day 0, and 2 shed BCV only in nasal secretions on day 0.

Of the 70 AR calves that shed BCV on day 0, 51 (72.9%) shed BCV through both routes, 9 (12.9%) shed BCV only in nasal secretions, and 10 (14.3%) shed BCV only in feces (Figures 3 and 4). At arrival, a significant ($P < 0.001$) association was found between BCV shedding in nasal secretions and feces in a single calf. On day 3, only 18 calves were shedding BCV, of which 17 shed BCV only in feces and 1 shed BCV in feces and nasal secretions. The calf on day 3 that shed through both routes had previously shed BCV only in nasal secretions on day 0 and subsequently shed BCV only in feces on day 7. Of the 17 calves that shed BCV in feces only on day 3, 1 had shed BCV only in nasal secretions on day 0, 13 had shed BCV by both routes on day 0, 1 had shed BCV only in nasal secretions on day–3 and only in feces on day 0, and 2 had shed BCV only in feces without having any previous or subsequent shedding during the selected sample collection days. Among 74 AR calves that shed BCV at some time

during the study (in nasal secretions, feces, or both), 14 had BCV shedding in nasal secretions that preceded BCV shedding in feces. Interestingly, none of these 74 AR calves had BCV shedding in feces preceding BCV shedding in nasal secretions at any of the sample collection days.

Respiratory tract disease—Both NM (74/103; 72%) and AR (79/100; 79%) calves were similarly affected with respiratory tract disease throughout the study period. However, the distribution of disease differed between NM and AR calves (Figures 1 and 2). Forty-nine percent (49/100) of AR calves had respiratory tract disease at arrival. The percentage of AR calves with respiratory tract disease decreased to 19% (19/100) on day 3, 9% (9/100) on day 7, and 2% (2/100) on day 14. No AR calves had signs of respiratory tract disease after day 14. In contrast, respiratory tract disease in NM calves peaked on day 7 after arrival. On day 0, 12% of NM calves had respiratory tract disease, peaking at 25% on day 7 and decreasing thereafter to 11%, 16%, and 1% on days 14, 21, and 29, respectively.

BCV shedding and antibody titers against BCV versus respiratory tract disease—A significant ($P < 0.001$) association between BCV shedding in nasal secretions and respiratory tract disease during the first 3 days after arrival was observed. Calves shedding BCV in nasal secretions were 1.5 times as likely ($P < 0.001$) to have respiratory tract disease during this period as calves that were not shedding. Viral shedding was not associated with respiratory tract disease on any other sample collection day. When NM and AR calves were analyzed independently, the association between BCV shedding in nasal secretions and respiratory tract disease was observed only for AR calves ($P < 0.001$) during the first 3 days after arrival. Few (n = 2) NM calves were shedding BCV in nasal secretions during the first 3 days after arrival.

Similarly, when the association between antibody titers against BCV and respiratory tract disease was studied, AR calves with respiratory tract disease during the first 3 days after arrival also had a significantly ($P < 0.001$) lower GMT than AR calves that did not have respiratory tract disease. However, when NM calves were analyzed independently, no significant ($P = 0.7$) difference was found in antibody titers against BCV at arrival between calves with respiratory tract disease and calves that did not have respiratory tract disease. A significant ($P = 0.001$) association between seroconversion on day 35 and treatment for respiratory tract disease at arrival was found when AR calves were analyzed independently. However, when the NM calves were analyzed independently, no association between seroconversion on day 35 and treatment for respiratory tract disease was found.

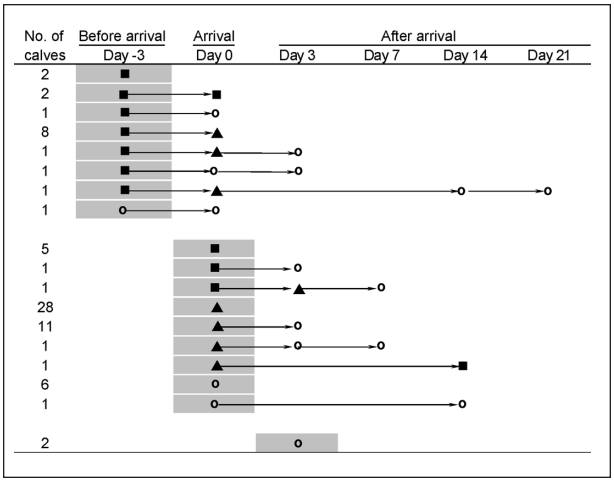

Figure 4—Bovine coronavirus transmission pattern in AR calves shedding nasally (closed square), rectally (open circle), or both nasally and rectally (closed triangle) in the AR group. The blank spaces represent no shedding detected for the corresponding

BCV shedding and weight gain—After adjusting for pen distribution, source, and treatment for respiratory tract disease, it was found that NM and AR calves shedding BCV gained, on average, 8 kg less ($P = 0.025$) than calves that were not shedding BCV. When AR and NM calves were analyzed separately, AR calves had a significant association between BCV shedding and low weight gain, whereby AR calves that shed BCV in nasal secretions, feces, or both gained 8.8 kg less than calves not shedding BCV ($P = 0.04$). A similar finding was observed in NM calves where calves that were shedding BCV also had lower weight gains (14.7 kg), but this difference was not significant ($P = 0.12$).

Discussion

To our knowledge, BCV transmission dynamics under field conditions and their role in respiratory tract disease in herds with variable amounts of antibodies to this virus are not well defined. Further studies of BCV shedding patterns and the association between enteric and respiratory forms of this virus under field conditions and in feedlots are required to understand the epidemiologic characteristics of this virus. The main objective of our study was to determine shedding patterns and serologic responses to BCV under field conditions in feedlot calves and the possible role of this virus on disease and growth performance.

Most of the NM calves had a high GMT (1,928) against BCV at arrival to the feedlot. On the basis of the age (6 to 7 months) of NM calves during which maternal antibodies to BCV will have declined and the fact that these calves were not vaccinated against BCV, the high antibody titers against BCV in this group presumably correspond to previous infection at the farm of origin. Only a low number of NM calves shed BCV in nasal secretions and feces. In contrast, AR calves had a low GMT (102) at arrival to the feedlot and had high BCV shedding rates (in nasal secretions and feces) throughout our study as well as high seroconversion rates. Shedding of BCV between the 2 groups differed significantly, and AR calves shed BCV at a higher rate than NM calves. It is important to note that AR calves not only differed from NM calves in the lower GMT against BCV at arrival but also that AR calves had a different exposure histories and stressors (such as commingling in a sale barn and longer transport to the feedyard) than NM calves.

Results of our study indicate that under field conditions, a serum GMT of > 1,860 was associated with low seroconversion rates, and calves with a serum GMT of 1,928 had a low incidence of BCV shedding. Taken together, these findings indicate that calves with a high GMT are less likely to become infected with BCV. In 1 study,[27] a serum IgG1 GMT of > 2,048 in gnotobiotic calves protected them against reinfection and BCV shedding in nasal secretions after challenge exposure to BCV. In another field study,[4] a serum GMT of > 2,262 was associated with calves that had negative results for BCV infection. In a similar study, Hasoksuz et al[5] found that calves that had a serum GMT of > 1,600 did not shed BCV in feces or nasal secretions. The protective serum GMT reported in these studies is similar to the serum GMT associated with a decreased likelihood of BCV infection in our report.

Therefore, if antibody titers against BCV at arrival are high, a decreased likelihood of BCV infection will be found, as was observed in the large number of NM calves that had low BCV shedding and low seroconversion after arrival and commingling at the feedlot. A similar outcome was observed in some AR calves with a high GMT. However, it is also possible that the low BCV shedding and BCV infection rates (documented by seroconversion) in the NM calves was not because of a high GMT at arrival but because they faced less stressors than AR calves. Nonetheless, the NM calves were directly exposed to the numerous AR calves shedding BCV after commingling at the feedlot, after which only a few NM calves had detectable BCV shedding and seroconversion.

Our observation that calves with a high GMT at arrival were protected from BCV infection, shedding, or both has also been observed in experimental challenge studies,[19,27] and our finding is in agreement with results of previous field studies, whereby cattle arriving at feedlots with high antibody titers against BCV appeared less likely to seroconvert to BCV than cattle without detectable BCV titers at arrival.[4,6,7] In addition, results[4,5,9,18] of other reports indicate that calves with a low GMT were as likely to shed and seroconvert to BCV after transport and arrival at feedlots. Therefore, if an association between antibody titers against BCV and protection exists, as ours and previous results suggest, we hypothesize that vaccination of calves against BCV, at least 3 weeks prior to shipping to feedlots to induce a serum IgG GMT of ≥ 1,860, could provide protection from BCV infection and its direct or combined effects with other pathogens of BRDC. Further supporting this suggestion are the results from a recent study,[16] which indicate that intranasal vaccination of feedlot calves with an enteric BCV modified-live vaccine prior to arrival at the farm appeared to reduce the risk for treatment for respiratory tract disease.

Shedding of BCV in nasal secretions of AR calves preceded BCV shedding in feces, with peak BCV shedding in nasal secretions at 3 days before arrival and in feces at 3 days after arrival. Results of 1 study[21] indicate that the route of inoculation of BCV influences the sequence of initial detection of coronavirus antigen from feces or nasal swab specimens. In that study,[21] when BCV was inoculated via the intranasal route, virus was detected initially by immunofluorescence of nasal epithelial cells in the upper respiratory tract of most calves and later by immune electron microscopy of fecal filtrates. In contrast, if calves were orally inoculated, the BCV shedding was first detected in feces and later in the nasal swab specimens of most calves. Findings in our study indicate that the fecal-oral and nasal transmission for this virus may be important. Other investigators have also suggested that nasal and fecal-oral transmission of BCV may be important under field conditions.[28,29]

A possible explanation for the fact that we detected virus initially in the respiratory tract and later in feces is that under field conditions, the replication and shedding of BCV by feedlot cattle was initiated through the respiratory route (BCV shedding in nasal secretions) and then spread to the gastrointestinal tract with subsequent BCV shedding in feces. This pattern of shedding has not been reported previously for BCV under field conditions in feedlot cattle but has been reported in longitudinal studies[28,29] of BCV shedding by dairy cattle and supports the hypothesis of this virus being a pneumoenteric agent as previously proposed.[21] Previous feedlot studies[4,5] of BCV have not clearly shown this pattern of shedding as was observed in our study. A possible reason is the experimental design used in these studies, where samples were collected from calves only after their arrival to the feedlot and not before arrival, which likely missed the initial replication period of the virus.

A possible explanation for this pattern of shedding, as previously proposed,[21,30] is that after initial replication in the nasal passages, BCV spreads to the gastrointestinal tract through the swallowing of large quantities of virus. It is likely that this initial respiratory amplification of BCV allows larger amounts of this enveloped and unstable but infectious virus to transit to the gastrointestinal tract after swallowing. This pattern of replication and spread may be unique for BCV among other animal enteric and respiratory viruses. Understanding the biological characteristics and transmission of a pathogen is crucial when designing preventive measures.

On day 0, of the total AR calves shedding BCV, 73% (51/70) were concurrently shedding BCV in nasal secretions and feces. On this day, a significant ($P < 0.001$) association between BCV shedding in nasal secretions and feces was detected, indicating a strong relationship between these 2 events. Similar findings have been shown, where a 91% agreement between BCV shedding through the respiratory tract and the gastrointestinal tract in the same animal was found.[4] In a field study[20] of an outbreak of diarrhea in calves, a significant association between the simultaneous detection of coronavirus in feces and nasal swab specimens in calves with diarrhea was also found. During the last 2 decades, there have been questions in regard to the relationship between BCV isolated from the enteric and respiratory tracts and whether respiratory and enteric BCV strains are the same virus or are distinct in biological, antigenic, and genetic properties. The pattern of shedding of BCV observed in our study and the fact that a high proportion of calves (73%)

shed the virus in nasal secretions and feces at arrival (day 0), with a high degree of association between nasal and BCV shedding in feces, suggest that enteric and respiratory BCV may be the same virus detected at different stages of its infectious life cycle in the host. Several previous findings[4,13,20-22,25,27,31-33] also support this hypothesis, whereby no major differences between respiratory and enteric BCV isolates were found at the antigenic, biological, or sequence level (point mutations), which indicates that most isolates may be the same or similar. However, because BCV, like other RNA viruses, represents a quasispecies,[34,35] it is possible that some viruses within the quasispecies are more suited for replication in the respiratory tract, whereas others replicate more efficiently in the intestine. A similar suggestion was proposed for the minor genetic differences observed in respiratory versus enteric isolates of the porcine respiratory coronavirus.[36]

Bovine respiratory tract disease in feedlot calves is more prevalent during the first weeks after arrival at feedlots.[37,38] This overlaps with a high prevalence of respiratory viral infections that include viruses such as infectious bovine rhinotracheitis virus, bovine respiratory syncytial virus, bovine viral diarrhea virus, bovine parainfluenza-3, and BCV and bacteria such as *M haemolytica* and *P multocida* during the first weeks after arrival to feedlots.[4,6-8,14,18,38-41] Because of this overlap and the fact that BCV has been isolated from a high proportion of calves with respiratory tract disease in some studies,[8,18] it was proposed that BCV could play a role as a causative agent in BRDC.[18] During the last 10 years, there have been a growing number of reports that collectively provided epidemiologic evidence to suggest that BCV may be part of this complex of entities that result in respiratory tract disease in feedlot cattle.[4-9,14-18]

In our study, calves shedding BCV appeared to be at greater risk of receiving treatments against respiratory tract disease than calves that were not shedding during the first 3 days after arrival (odds ratio, 1.5). However, when a within-group analysis was performed to determine this association, it was observed that such risk was not the same for both groups. In AR calves, we detected an association between BCV shedding in nasal secretions and respiratory tract disease during the first 3 days after arrival, but in the NM calves, no association could be established because the low number of calves that were shedding BCV did not allow statistical analysis among such variables.

Other researchers have shown a similar association between BCV shedding in nasal secretions and treatments for respiratory tract disease[16] as was observed in AR calves. In that study,[16] intranasal vaccination of calves against BCV on entry to a feedlot was protective against BRDC and BCV shedding in nasal secretions increased the risk of treatment for respiratory tract disease. Additionally, in a study by Lathrop et al,[14] calves shedding this virus were 1.6 times as likely to have respiratory tract disease and 2.2 times as likely to have pulmonary lesions at slaughter as calves that did not shed BCV. Similarly, in a study by Hasoksuz et al,[5] calves shedding BCV in nasal secretions were 2.7 times as likely to have respiratory tract disease as calves that were not shedding; However, in this latter study[5] and also in another,[4] the use of a smaller number of calves resulted in failure to detect an association between clinical signs of respiratory tract disease and BCV shedding or seroconversion.[4,5]

For AR calves with respiratory tract disease on day 0, a significantly lower GMT was found, compared with AR calves that did not have respiratory tract disease. This finding is in agreement with the results of several other investigations of young calves and feedlot cattle, whereby BCV-seropositive cattle or those with higher antibody titers against BCV at arrival had higher protection rates against respiratory tract disease.[7,9,10,15,16,39] However, this protective effect against respiratory tract disease was not observed for NM calves. Because both groups of calves had approximately the same percentage of calves with respiratory tract disease during the entire study period, it can be inferred that BCV is not the only contributing factor in the induction of respiratory tract disease. Therefore, the increase in respiratory tract disease among the NM calves after arrival suggests that other viral, viral-bacterial, or bacterial causes also played a role, as documented in previous studies.[37,39,42-48]

During the first 35 days after arrival, BCV infection was significantly associated with decreased growth performance in the feedlot calves examined in our study, results that are consistent with those of previous researchers[4,5] that suggest that BCV may have an impact on the health status of a herd. Similarly, high antibody titers against BCV were associated with increased weight gains in other studies.[7,15] In our study, after adjusting for pen distribution, source, and BCV shedding, respiratory tract disease was also significantly associated with decreased growth performance. Calves treated for respiratory tract disease gained 6 kg less, on average, than calves that were not treated. This is consistent with previous research findings that indicate that respiratory tract disease in feedlot cattle has an effect on weight gain.[7,39,44] However, in other studies, no association was found between BCV antibody titers against BCV at arrival and improved weight gains[39] or between BCV shedding and decreased growth,[14] and no effect of respiratory tract disease on weight gain was found.[4]

The correlation between the detection of an agent and a disease process is not definitive proof of a causal role. Results of our study, in addition to those of several other reports,[4,14,16,17,18] indicate that BCV infection might be a risk factor for poor health and low performance in feedlot calves. However, from our data, it is not possible to discern whether the viral infection was the direct cause of poor health and low performance or whether other underlying variables were the true cause and BCV shedding was a secondary effect.

In conclusion, calves arriving at the feedlot with low antibody titers against BCV were significantly more susceptible to BCV infection than calves with high antibody titers against BCV. Under field conditions, a GMT of ≥ 1,860 was adequate to decrease the likelihood of BCV infection in most of the calves in the herd. Therefore, vaccinating calves with an effective BCV vaccine at least 3 weeks prior to shipping to feed-

lots to induce a similar GMT might provide protection against BCV infection and its direct or combined effects with other pathogens on health performance. Results of our study also indicate that under field conditions, BCV is first detectable in the nasal secretions and later in fecal samples; a possible explanation for this is that replication of this virus may be initiated via the upper respiratory tract, through the nasal passages, and then spread to the gastrointestinal tract. In addition, a significant association between BCV infection, decreased growth performance, and respiratory tract disease was observed in our study, at least short term. This finding further supports the idea that BCV may have a negative effect on the health status of feedlot calves, which, together with previous reports[4,14,16-18] of low growth performance and respiratory tract disease associated with BCV in calves and feedlot cattle, may warrant vaccination in this age group.

a. USDA, National Animal Disease Center, Ames, Iowa.
b. Titanium-5 (MLV IBR/PI-3/BVD1/BVD2/BRSV), Diamond Animal Health Inc, Des Moines, Iowa.
c. Cattle Master 4 (IBR, PI-3, BVD1, BVD2, BRSV), Pfizer Animal Health, Exton, Pa.
d. Micotil, Elanco Animal Health, Indianapolis, Ind.
e. Minimum essential medium, Gibco Invitrogen Corp, Grand Island, NY.
f. Eppendorf, Brinkmann Instruments Inc, Westbury, NY.
g. Nunc-immuno maxisorp surface, Nunc Brand Products, Nalge Nunc International, Rochester, NY.
h. Nonfat dry milk, Nestlé, Solon, Ohio.
i. Rabbit anti–guinea pig Ig peroxidase, Boehringer Mannheim Corp, Indianapolis, Ind.
j. Titertek multiskan MCC/340, Labsystems & Row Lab, Helsinki, Finland.
k. Microsoft Excel 2000, Microsoft Corp, Seattle, Wash.
l. Goat anti-bovine IgG, Kirkegaard & Perry Lab, Gaithersburg, Md.
m. SAS 9.1 TS level 1M2 XP_PRO platform, SAS Institute Inc, Cary, NC.

References

1. Mebus CA, Stair EL, Rhodes MB, et al. Neonatal calf diarrhea: propagation, attenuation, and characteristics of a coronavirus-like agent. *Am J Vet Res* 1973;34:145–150.

2. Saif LJ. Bovine coronavirus infection. In: Coetzer JAW, Tustin RC, eds. Infectious diseases of livestock. Vol 2. 2nd ed. Oxford, England: Oxford University Press, 2004;795–802.

3. Saif LJ. A review of evidence implicating bovine coronavirus in the etiology of winter dysentery in cows: an enigma resolved? *Cornell Vet* 1990;80:303–311.

4. Cho KO, Hoet AE, Loerch SC, et al. Evaluation of concurrent shedding of bovine coronavirus via the respiratory tract and enteric route in feedlot cattle. *Am J Vet Res* 2001;62:1436–1441.

5. Hasoksuz M, Hoet AE, Loerch SC, et al. Detection of respiratory and enteric shedding of bovine coronaviruses in cattle in an Ohio feedlot. *J Vet Diagn Invest* 2002;14:308–313.

6. Lathrop SL, Wittum TE, Loerch SC, et al. Antibody titers against bovine coronavirus and shedding of the virus via the respiratory tract in feedlot cattle. *Am J Vet Res* 2000;61:1057–1061.

7. Martin SW, Nagy E, Shewen PE, et al. The association of titers to bovine coronavirus with treatment for bovine respiratory disease and weight gain in feedlot calves. *Can J Vet Res* 1998;62:257–261.

8. Storz J, Stine L, Liem A, et al. Coronavirus isolation from nasal swab samples in cattle with signs of respiratory tract disease after shipping. *J Am Vet Med Assoc* 1996;208:1452–1455.

9. Lin XQ, Oe KL, Storz J, et al. Antibody responses to respiratory coronavirus infections of cattle during shipping fever pathogenesis. *Arch Virol* 2000;145:2335–2349.

10. Ganaba R, Belanger D, Dea S, et al. 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* 1995;59:26–33.

11. McNulty MS, Bryson DG, Allan GM, et al. Coronavirus infection of the bovine respiratory tract. *Vet Microbiol* 1984;9:425–434.

12. Mostl K, Burki F. Ursachliche Beteiligung boviner Coronaviren an respiratorischen Krankheitsausbruchen bei Kalbern und pathogenetisch-immunologische Uberlegungen hierzu. *Dtsch Tierarztl Wochenschr* 1988;95:19–22.

13. Thomas LH, Gourlay RN, Stott EJ, et al. A search for new microorganisms in calf pneumonia by the inoculation of gnotobiotic calves. *Res Vet Sci* 1982;33:170–182.

14. Lathrop SL, Wittum TE, Brock KV, et al. Association between infection of the respiratory tract attributable to bovine coronavirus and health and growth performance of cattle in feedlots. *Am J Vet Res* 2000;61:1062–1066.

15. Martin SW, Nagy E, Armstrong D, et al. The associations of viral and mycoplasmal antibody titers with respiratory disease and weight gain in feedlot calves. *Can Vet J* 1999;40:560–567.

16. Plummer PJ, Rohrbach BW, Daugherty RA, et al. Effect of intranasal vaccination against bovine enteric coronavirus on the occurrence of respiratory tract disease in a commercial backgrounding feedlot. *J Am Vet Med Assoc* 2004;225:726–731.

17. Storz J, Lin X, Purdy CW, et al. Coronavirus and Pasteurella infections in bovine shipping fever pneumonia and Evans' criteria for causation. *J Clin Microbiol* 2000;38:3291–3298.

18. Storz J, Purdy CW, Lin X, et al. 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* 2000;216:1599–1604.

19. Cho KO, Hasoksuz M, Nielsen PR, et al. 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* 2001;146:2401–2419.

20. Reynolds DJ, Debney TG, Hall GA, et al. Studies on the relationship between coronaviruses from the intestinal and respiratory tracts of calves. *Arch Virol* 1985;85:71–83.

21. Saif LJ, Redman DR, Moorhead PD, et al. Experimentally induced coronavirus infections in calves: viral replication in the respiratory and intestinal tracts. *Am J Vet Res* 1986;47:1426–1432.

22. Tsunemitsu H, Yonemichi H, Hirai T, et al. Isolation of bovine coronavirus from feces and nasal swabs of calves with diarrhea. *J Vet Med Sci* 1991;53:433–437.

23. Smith DR, Tsunemitsu H, Heckert RA, et al. Evaluation of two antigen-capture ELISAs using polyclonal or monoclonal antibodies for the detection of bovine coronavirus. *J Vet Diagn Invest* 1996;8:99–105.

24. Hasoksuz M, Lathrop SL, Gadfield KL, et al. Isolation of bovine respiratory coronaviruses from feedlot cattle and comparison of their biological and antigenic properties with bovine enteric coronaviruses. *Am J Vet Res* 1999;60:1227–1233.

25. Hasoksuz M, Lathrop S, Al-dubaib MA, et al. Antigenic variation among bovine enteric coronaviruses (BECV) and bovine respiratory coronaviruses (BRCV) detected using monoclonal antibodies. *Arch Virol* 1999;144:2441–2447.

26. Smith DR, Nielsen PR, Gadfield KL, et al. Further validation of antibody-capture and antigen-capture enzyme-linked immunosorbent assays for determining exposure of cattle to bovine coronavirus. *Am J Vet Res* 1998;59:956–960.

27. El-Kanawati ZR, Tsunemitsu H, Smith DR, et al. Infection and cross-protection studies of winter dysentery and calf diarrhea bovine coronavirus strains in colostrum-deprived and gnotobiotic calves. *Am J Vet Res* 1996;57:48–53.

28. Heckert RA, Saif LJ, Hoblet KH, et al. A longitudinal study of bovine coronavirus enteric and respiratory infections in dairy calves in two herds in Ohio. *Vet Microbiol* 1990;22:187–201.

29. Heckert RA, Saif LJ, Myers GW, et al. Epidemiologic factors and isotype-specific antibody responses in serum and mucosal secretions of dairy calves with bovine coronavirus respiratory tract and enteric tract infections. *Am J Vet Res* 1991;52:845–851.

30. Saif LJ, Smith KL. Enteric viral infections of calves and passive immunity. *J Dairy Sci* 1985;68:206–228.

31. Hasoksuz M, Sreevatsan S, Cho KO, et al. Molecular analy-

sis of the S1 subunit of the spike glycoprotein of respiratory and enteric bovine coronavirus isolates. *Virus Res* 2002;84:101–109.

32. Reynolds DJ. Coronavirus replication in the intestinal and respiratory tracts during infection of calves. *Ann Rech Vet* 1983;14:445–446.

33. Zhang X, Herbst W, Kousoulas KG, et al. Comparison of the S genes and the biological properties of respiratory and enteropathogenic bovine coronaviruses. *Arch Virol* 1994;134:421–426.

34. Condit RC. *Fields virology*. 4th ed. Philadelphia: Lippincott Williams & Wilkins, 2001.

35. Domingo E, Baranowski E, Ruiz-Jarabo CM, et al. Quasispecies structure and persistence of RNA viruses. *Emerg Infect Dis* 1998;4:521–527.

36. Costantini V, Lewis P, Alsop J, et al. Respiratory and enteric shedding of porcine respiratory coronavirus (PRCV) in sentinel weaned pigs and sequence of the partial S gene of the PRCV isolates. *Arch Virol* 2003;149:957–974.

37. Hoerlein AB. *Shipping fever*: Santa Barbara, Calif: American Veterinary Publications Inc, 1980.

38. Jensen R, Pierson RE, Braddy PM, et al. Shipping fever pneumonia in yearling feedlot cattle. *J Am Vet Med Assoc* 1976;169:500–506.

39. O'Connor A, Martin SW, Nagy E, et al. The relationship between the occurrence of undifferentiated bovine respiratory disease and titer changes to bovine coronavirus and bovine viral diarrhea virus in 3 Ontario feedlots. *Can J Vet Res* 2001;65:137–142.

40. Yates WD, Kingscote BF, Bradley JA, et al. The relationship of serology and nasal microbiology to pulmonary lesions in feedlot cattle. *Can J Comp Med* 1983;47:375–378.

41. Hoerlein AB, Mansfield ME, Abinanti FR, et al. Studies of shipping fever of cattle. I. Para-influenza 3 virus antibodies in feeder calves. *J Am Vet Med Assoc* 1959;135:153–160.

42. Jakab GJ. Viral-bacterial interactions in pulmonary infection. *Adv Vet Sci Comp Med* 1982;26:155–171.

43. Potgieter LN, McCracken MD, Hopkins FM, et al. Experimental production of bovine respiratory tract disease with bovine viral diarrhea virus. *Am J Vet Res* 1984;45:1582–1585.

44. Yates WD. A review of infectious bovine rhinotracheitis, shipping fever pneumonia and viral-bacterial synergism in respiratory disease of cattle. *Can J Comp Med* 1982;46:225–263.

45. Jericho KW, Magwood SE, Stockdale PH. Prevention of experimental bovine pneumonic pasteurellosis by exposure to IBR virus (lett). *Can Vet J* 1976;17:194–195.

46. Lopez A, Thomson RG, Savan M. The pulmonary clearance of *Pasteurella hemolytica* in calves infected with bovine parainfluenza-3 virus. *Can J Comp Med* 1976;40:385–391.

47. Jericho KW, Langford EV. Pneumonia in calves produced with aerosols of bovine herpesvirus 1 and *Pasteurella haemolytica*. *Can J Comp Med* 1978;42:269–277.

48. Hamdy AH, Trapp AL, Gale C, et al. Experimental transmission of shipping fever in calves. *Am J Vet Res* 1963;24:287–294.