

Evaluation of the effect of serum antibody abundance against bovine coronavirus on bovine coronavirus shedding and risk of respiratory tract disease in beef calves from birth through the first five weeks in a feedlot

Aspen M. Workman PhD

Larry A. Kuehn PhD

Tara G. McDanel PhD

Michael L. Clawson PhD

Carol G. Chitko-McKown PhD

John Dustin Loy DVM, PhD

Received August 23, 2016.

Accepted November 21, 2016.

From the US Animal Research Center, Agricultural Research Service, USDA, Spur 18D, Clay Center, NE 68933 (Workman, Kuehn, McDanel, Clawson, Chitko-McKown); and Nebraska Veterinary Diagnostic Center, School of Veterinary Medicine and Biomedical Sciences, University of Nebraska, Lincoln, NE 68506 (Loy).

Address correspondence to Dr. Workman (aspen.workman@ars.usda.gov).

OBJECTIVE

To evaluate the effect of serum antibody abundance against bovine coronavirus (BCV) on BCV shedding and risk of bovine respiratory disease (BRD) in beef calves from birth through the first 5 weeks in a feedlot.

ANIMALS

890 natural-service crossbred beef calves from 4 research herds.

PROCEDURES

Serial blood samples for measurement of serum anti-BCV antibody abundance by an ELISA and nasal swab specimens for detection of BCV and other viral and bacterial BRD pathogens by real-time PCR methods were collected from all calves or subsets of calves at predetermined times from birth through the first 5 weeks after feedlot entry. Test results were compared among herds, over time, and between calves that did and did not develop BRD. The associations of various herd and calf factors with test results were also evaluated.

RESULTS

At the calf level, serum anti-BCV antibody abundance was not associated with BCV shedding, but BCV shedding was positively associated with BRD incidence before and after weaning. The mean serum anti-BCV antibody abundance at weaning for a group of calves was inversely related with the subsequent incidence of BRD in that group; however, the serum anti-BCV antibody abundance at weaning for individual calves was not predictive of which calves would develop BRD after feedlot entry.

CONCLUSIONS AND CLINICAL RELEVANCE

Results indicated that serum anti-BCV antibody abundance as determined with ELISA were not associated with BCV shedding or risk of BRD in individual beef calves from birth through the first 5 weeks after feedlot entry. (*Am J Vet Res*;78:1065–1076)

Bovine respiratory disease is a serious health and economic problem for dairy and beef cattle industries worldwide.^{1,2} This disease complex is frequently characterized by a primary viral infection in combination with stress, which suppresses the host's immune system, damages the epithelium of the respiratory tract, and allows opportunistic bacteria such as

Mannheimia haemolytica, *Pasteurella multocida*, *Histophilus somni*, and *Mycoplasma bovis* to infect the lungs.^{3–5} The major viral pathogens associated with BRD include BVDV, BRSV, BHV1, and PI3.^{6,7} Alterations in innate defenses, leukocyte recruitment to the lungs, and production of proinflammatory cytokines culminate in the pathological lesions associated with BRD.³ Although intervention strategies such as vaccination and metaphylactic or therapeutic administration of antimicrobials are commonly used to prevent and treat respiratory tract disease in cattle, the incidence of BRD remains high.

Within the past decade, BCV has been identified as another important contributor to BRD.⁸ Bovine coronavirus is ubiquitous in cattle populations worldwide and was initially associated with outbreaks of enteric disease. It is now recognized to have an etiologic role in 3 distinct clinical syndromes (diarrhea in calves, hemorrhagic diarrhea in adult cattle

ABBREVIATIONS

| | |
|--------|------------------------------------|
| BCV | Bovine coronavirus |
| BHV1 | Bovine herpesvirus-1 |
| BRD | Bovine respiratory disease |
| BRSV | Bovine respiratory syncytial virus |
| BVDV | Bovine viral diarrhea virus |
| BVDV1 | Bovine viral diarrhea virus type 1 |
| BVDV2 | Bovine viral diarrhea virus type 2 |
| Ct | Cycle threshold |
| MLV | Modified-live virus |
| PI3 | Parainfluenza-3 virus |
| RT | Real-time |
| USMARC | US Meat Animal Research Center |

[winter dysentery], and respiratory tract disease in cattle of all ages).⁸ Both killed virus and MLV vaccines are available to control enteric disease in calves and can be administered to calves at birth or to pregnant cows to induce production of anti-BCV antibodies in colostrum. Although all BCV isolates are believed to belong to a single serotype,⁸⁻¹² results of a 2013 study¹³ suggest that there may be differences in seroreactivity between strains isolated from the respiratory tract and a commonly used enteric reference strain. However, there currently is no BCV vaccine licensed in the United States to aid in the prevention of BRD.⁸

Despite the fact that BCV has been associated with respiratory tract infections in cattle of all ages, its role in the pathogenesis of BRD in cattle at various production stages remains unclear. Most research on the association between BCV and BRD has involved feedlot cattle. Shedding of BCV in the absence of other important respiratory tract pathogens has been observed in cattle with naturally occurring BRD,^{14,15} and BCV has been isolated from pneumonic lungs alone or in combination with other respiratory tract pathogens.^{7,14-18} Results of multiple studies^{14,19-23} indicate that cattle with high anti-BCV antibody titers at feedlot entry are less likely to shed BCV and develop BRD than are cattle with low anti-BCV antibody titers at feedlot entry. However, it is not uncommon for apparently healthy feedlot cattle to shed BCV,^{14,21,23,24} which raises questions about the respective associations of anti-BCV antibody titer and BCV shedding with the risk of BRD in feedlot cattle.

Compared with the information known about feedlot cattle, much less is known about the relationship between BCV and BRD in preweaned beef calves. Dairy calves, even those with fairly high passively (colostrum) or actively (vaccination) acquired anti-BCV antibody titers, can shed BCV during the first week after birth, and recurrent shedding of BCV by apparently healthy dairy calves is common, which suggests that many BCV infections are not associated with disease.^{25,26} To our knowledge, a comprehensive study to evaluate BCV shedding and anti-BCV antibody titers in preweaned beef calves (a population for which the risk of BRD differs substantially among both individual animals and herds over time) has not been conducted. Thus, the literature is lacking with regard to a temporal evaluation of BCV shedding, humoral immunity to BCV, and the risk for development of BRD throughout the entire production cycle of beef cattle. The purpose of the study reported here was to evaluate the effect of serum anti-BCV antibody abundance on BCV shedding patterns and the incidence of BRD in beef calves from birth through the first 5 weeks in a feedlot.

Materials and Methods

Animals

Eight hundred ninety natural-service crossbred beef calves born during April and May 2014

were monitored from birth to the end of their fifth week in a feedlot. All study calves originated from 4 herds that were managed in separate locations at the USMARC. Each of those 4 herds contained additional calves that were not enrolled in the study. Herds 1 and 2 consisted of calves that were born as a result of artificial insemination or natural service, whereas herds 3 and 4 consisted of calves that were born as a result of natural service only. Only natural-service calves were enrolled in the study because calves that were born as a result of artificial insemination were enrolled in another study. All calves were managed in the same manner regardless of whether they were enrolled in the study reported here. The 4 herds did not have fence-line contact, so there was no direct pathogen transmission among herds. All experimental protocols were approved by the USMARC Institutional Animal Care and Use Committee (approval Nos. 5438-31000-082-04 and 3040-32000-031-07).

Vaccination schedule and calf processing

The cows (dams) in all 4 herds received an MLV vaccine-bacterin combination product^a that contained antigens of BHV1, BVDV1, BVDV2, PI3, BRSV, and bacterins for *Campylobacter fetus* and 5 serovars of *Leptospira* spp prior to breeding (approx June) annually. The dams were also vaccinated against enteric pathogens approximately 30 days before the beginning of calving season. That vaccine^b contained killed strains of bovine rotavirus and BCV, a K99 *Escherichia coli* bacterin, and *Clostridium perfringens* type C toxoid to enhance the production and secretion of antibodies against those pathogens into the colostrum and aid in the prevention of diarrhea in calves.

Calves at approximately 20 to 70 days of age (June [same time as dams received prebreeding vaccine]) received an initial vaccination with an MLV vaccine-bacterin combination product^c that contained antigens of BHV1, BVDV1, BVDV2, PI3, BRSV, and *M haemolytica* and a multivalent bacterin-toxoid product^d intended to protect animals against disease caused by *Clostridium chauvoei*, *Clostridium septicum*, *Clostridium novyi*, *Clostridium sordellii*, *C perfringens* types C and D, and *Moraxella bovis*. Calves at 111 to 161 days of age (approx 21 days prior to weaning [ie, preconditioning]) received a booster of the respiratory vaccine^c and another multivalent clostridial vaccine^e intended to protect animals against disease caused by *C chauvoei*, *C septicum*, *Clostridium haemolyticum*, *C novyi*, *C sordellii*, and *C perfringens* types C and D. Calves at approximately 130 to 190 days of age (weaning and feedlot entry) received another respiratory vaccine^f that contained antigens of BHV1, BVDV1, BVDV2, PI3, and BRSV.

Calves were weaned between September 24 and October 3, 2014, and moved from their herds of origin to a feedlot where they were processed and commingled in 14 pens, each of which had capacity for approximately 70 calves. Calves were allocated to pens such that each pen contained the same pro-

portion of calves from each of the 4 herds of origin. That method of allocation was chosen to maximize exposure among calves from all 4 herds and simulate the commingling of calves that occurs at commercial feedlots.

Over the next 5 weeks, calves with suspected BRD were removed from each pen for sample collection and treated in accordance with the standard operating procedure established for cattle maintained at the USMARC. Diagnosis of BRD was made on the basis of the presence of clinical signs such as lethargy, anorexia, abnormal breathing, nasal and ocular discharge, and pyrexia. Calves were treated at the discretion of feedlot staff and the attending veterinarian, all of whom remained unaware of (were blinded to) the herd of origin for each calf at the time of BRD diagnosis. All adverse health events and treatments were recorded for each study calf so that associations between BRD and other comorbidities could be evaluated.

Sample collection procedures

A blood sample (approx 9 mL) for plasma acquisition was collected between 18 and 48 hours after birth from a small subset ($n = 34$) of calves for which both birth and the first nursing episode were observed. A blood sample (approx 9 mL at initial vaccination and 25 mL at preconditioning and weaning) for serum acquisition and nasal swab specimens were collected from all 890 calves at the time of each vaccination (initial vaccination, preconditioning, and weaning). Those 3 sample acquisition times were selected to eliminate additional stress associated with excessive handling because calves were already being handled at those times for routine processing and vaccination.

Additional blood samples (approx 9 mL) and nasal swab specimens were obtained from each calf removed from a pen because of suspected BRD (cases; $n = 67$) as well as apparently healthy calves (controls; 109). Two controls were selected for sample collection from each pen on a weekly basis during the first 4 weeks after feedlot entry. Because of a low number of cases in the fifth week, controls were selected only from the pens with cases. Controls were selected by use of a convenience method, rather than a randomized sampling method, to prevent or minimize stress associated with sorting needed to remove specific calves. Notches were made in the ear tags of calves treated for BRD as well as control calves to prevent those calves from being selected as controls at future sampling dates.

Blood samples were obtained by jugular venipuncture with an 18-gauge needle at 18 to 48 hours after birth and initial vaccination and with a 16-gauge needle at all sample acquisition times thereafter. Blood samples were centrifuged at 1,650 $\times g$ at 4°C for 25 minutes. Then, the plasma or serum from each sample was harvested and stored at -80°C until analysis.

Nasal swab specimens were obtained by passing 2 sterile 6-inch-long cotton-tipped swabs into each nostril to swab the nasopharyngeal region. For each calf during each sample acquisition time, 1 nasal swab specimen from each nostril was obtained and placed into a cryovial that contained 1 mL of transport medium (buffered peptone water with 12% glycerol). Vials were snap-frozen in liquid nitrogen and stored at -80°C until analysis.

Detection of viral pathogens by use of RT-PCR assay

Real-time PCR methods were used to detect respiratory viruses (BHV1, BVDV, BCV, and BRSV) in nasal swab medium that was used for transport and storage of nasal swab specimens. Viral shedding was determined for 60 calves that were randomly selected by means of a random number generator from each of the 4 herds at each of the 3 routine sample acquisition times (initial vaccination, preconditioning, and weaning). That sample size was selected because it was calculated to have a 95% probability for detection of at least 1 infected animal if the true prevalence of viral shedding in the herd at the sample acquisition time was at least 5%.²⁷ Viral shedding was also determined for cases and controls on the basis of disease status and sample collection date. Briefly, pools consisting of nasal swab medium from 5 calves were created on the basis of herd of origin at each routine sample acquisition time as well as on the basis of disease status (case or control) and sample collection date.

Each pool contained 100 μL of nasal swab medium from each of the 5 designated calves. From each pooled sample, RNA was extracted by use of a phenol and guanidine isothiocyanate reagent,⁸ and DNA was extracted with a silica membrane-based nucleic acid purification kit.^h Then, an RT-PCR assay was performed to detect the major viral pathogens associated with BRD (BHV1, BRSV, BVDV, and BCV). Primers and probes used for the assay were the same as those described in other studies.²⁸⁻³¹ For each sample analyzed, cyclic amplification was conducted on a 25- μL reaction solution containing 2.5 μL each of extracted RNA and DNA, 4.5mM magnesium chloride, 400 μM of deoxyribonucleotide triphosphate, 0.4 μM concentration of each primer, 0.2 μM concentration of each probe, and 1 μL of an enzyme mixⁱ with reverse transcriptase and a hot-start *Taq* polymerase. Cycling conditions included reverse transcription at 50°C for 30 minutes, inactivation of the reverse transcriptase enzyme and activation of the *Taq* polymerase at 95°C for 15 minutes, and 40 cycles of 94°C for 30 seconds, 55°C for 60 seconds, and 72°C for 60 seconds. Positive, negative, no-template, and extraction control samples were included in each assay. Samples with a Ct value < 40 were considered to have positive results for the virus of interest. For pooled samples that yielded positive results for a particular virus, the RT-PCR assay was repeated on individual nasal swab

medium from each of the 5 calves that contributed to the pooled sample to identify the calf or calves that were shedding the virus of interest.

Detection of bacterial pathogens by RT-PCR assay

Samples of nasal swab medium collected from cases (calves with BRD) and controls during the first 5 weeks after feedlot entry were sent to the University of Nebraska-Lincoln Veterinary Diagnostic Center for detection of *M baemolytica*, *P multocida*, *H somni*, and *M bovis* by use of multiplex RT-PCR methods that have been validated for nasal swab specimens and lung tissue matrices obtained from cattle.^j This RT-PCR assay was performed on individual samples (ie, samples were not pooled).

Detection of anti-BCV antibodies

For each of the 240 calves evaluated for BCV shedding at initial vaccination and weaning, serum anti-BCV antibody abundance was measured by use of a commercially available indirect antibody ELISA^k in accordance with the manufacturer's instructions. Serum anti-BCV antibody abundance was also measured in samples collected at initial vaccination and weaning for the 67 cases and 109 controls evaluated during the first 5 weeks after feedlot entry and in samples collected from birth through weaning for a small subset of calves from herds 3 ($n = 21$) and 2 (13) that did and did not, respectively, have evidence of BCV infection in preweaned calves. Briefly, test plates were assessed at a wavelength of 450 nm with a computer-linked ELISA plate reader.^l Optical density of the tested sample was divided by the optical density of the positive control sample provided in the kit to calculate the anti-BCV antibody percentage positivity for the tested sample. Samples with a percentage positivity < 10 were considered negative for anti-BCV antibodies (seronegative), whereas those with a percentage positivity ≥ 10 were considered positive for anti-BCV antibodies (seropositive). A percentage positivity > 100 could be obtained if the concentration of anti-BCV antibodies in the tested sample was greater than that in the positive control sample provided in the kit. A dilution series for the positive control sample was used to determine the assay limit of linearity because the linear range for measurement of anti-BCV antibodies was not provided by the manufacturer. Results indicated that the percentage positivity of the positive control sample had a good linear range from 10 to 100. When measured values were compared with expected values, the linearity equation was $Y = 1.657X - 0.08916$, with $R^2 = 0.999$. All test samples with results greater than that linear range were diluted and retested. The adjusted percentage positivity was calculated as the dilution factor of the sample multiplied by the percentage positivity, and that value was accepted as the relative anti-BCV antibody abundance.

Statistical analysis

Individual calves were the experimental unit for all analyses. Two-way contingency tables^m were created, and χ^2 tests were used to assess the respective associations between the development of BRD during the first 5 weeks after feedlot entry (case or control status) and BCV shedding and the presence of specific bacterial pathogens in the nasopharynx. The respective associations between serum anti-BCV antibody ELISA results and herd of origin or case status were evaluated by use of a 1-way ANOVA followed by t tests for pairwise comparisons only if the overall F test was significant. Separate linear regression models were developed for the dependent variables of weaning age and weaning weight. Both models included fixed effects for herd of origin, case status, and the interaction between herd of origin and case status. Logistic regression analysis was used to determine the effect of herd on case status. All ANOVA and regression analyses were performed with a commercially available statistical software program.ⁿ Values of $P < 0.05$ were considered significant for all analyses.

Results

Calves

The number of calves enrolled in the study from each herd, median dam parity within each herd, and mean \pm SD weaning age and weight for the study calves were summarized (**Table 1**). Although cows in all 4 herds were exposed to bulls during the same 60-day period, the time during that period at which the study calves were conceived differed among herds. Thus, the mean weaning age and weaning weight of study calves differed among herds. Of the 890 calves enrolled in the study, 4 were excluded prior to weaning because of injury or death, although none of the conditions were known to be related to infectious disease.

Respiratory virus shedding and BRD incidence prior to feedlot entry

Shedding of each of 4 respiratory viruses (BHV1, BVDV, BRSV, and BCV) in 60 randomly selected study calves from each herd was determined at initial vaccination (20 to 70 days old), preconditioning (111 to 161 days old), and weaning (130 to 190 days old). Bovine viral diarrhea virus was not detected in any calf at any sample acquisition time prior to feedlot entry. Bovine herpesvirus-1 was detected in 2 calves of herd 4 at weaning; BRSV was also detected in 1 of those calves. The number of calves from which BCV was detected in nasal swab specimens was summarized (**Table 2**). Bovine coronavirus was detected in 14 (23%) calves of herd 3 at initial vaccination and 8 (13%) and 9 (15%) calves of herds 1 and 3, respectively, at weaning. The overall prevalence rate of BCV shedding was 6% (14/240) at initial vaccination, 0% at preconditioning, and 7% (17/240) at weaning, and the overall prevalence rate of BCV shedding prior to feedlot entry was

Table 1—Descriptive characteristics for 890 natural-service crossbred beef calves born in 4 USMARC herds during April and May 2014 that were monitored from birth to the end of their fifth week in a feedlot to evaluate the effect of serum anti-BCV antibody abundance on BCV shedding and the incidence of BRD.

| Variable | Herd | | | |
|--|------------------------|-----------------------|-----------------------|-------------------------|
| | 1 | 2 | 3 | 4 |
| Total No. of calves | 534 | 624 | 241 | 116 |
| No. of calves enrolled in study | 138 | 413 | 226 | 113 |
| Dam parity | 2.5 (1–4) | 5.2 (2–9) | 7.3 (4–11) | 6.0 (4–8) |
| Weaning age (d) | 146 ± 12 ^{ab} | 148 ± 18 ^a | 162 ± 17 ^c | 158 ± 15 ^{b,c} |
| Weaning weight (kg) | 203 ± 23 ^a | 211 ± 31 ^a | 236 ± 33 ^b | 236 ± 31 ^b |
| No. (%) of study calves that developed BRD prior to feedlot entry | 2 (1.5) | 0 (0) | 25 (11) | 0 (0) |
| No. (%) of study calves that developed BRD during the first 5 wk after feedlot entry | 3 (2) | 51 (12) | 12 (5) | 4 (4) |

Values represent the mean ± SD or median (range) unless otherwise specified. A diagnosis of BRD was made on the basis of the presence of lethargy, anorexia, abnormal breathing, nasal and ocular discharge, and pyrexia.

^{a-c}Within a row, values with different superscript letters differ significantly ($P < 0.05$).

Table 2—Number (percentage) of calves from which BCV was detected in nasal swab specimens at initial vaccination (20 to 70 days old), preconditioning (111 to 161 days old), and weaning (130 to 190 days old).

| Sample acquisition time | Herd | | | | Total |
|-------------------------|--------------|--------------|----------------|--------------|---------------|
| | 1 | 2 | 3 | 4 | |
| Initial vaccination | 0 (0) | 0 (0) | 14 (23) | 0 (0) | 14 (6) |
| Preconditioning | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| Weaning | 8 (13) | 0 (0) | 9 (15) | 0 (0) | 17 (7) |
| Total | 8 (4) | 0 (0) | 23 (13) | 0 (0) | 31 (4) |

For each herd at each specified sample acquisition time, nasal swab specimens from 60 arbitrarily selected calves were assayed by use of RT-PCR assay for determination of BCV shedding.

4% (8/180) for herd 1, 0% for herd 2, 13% (23/180) for herd 3, and 0% for herd 4. The incidence of BRD (defined as the presence of lethargy, anorexia, abnormal breathing, nasal and ocular discharge, and pyrexia) in calves prior to feedlot entry differed among the 4 herds and ranged from 0% (herds 2 and 4) to 11% (herd 3). The incidence rate of BRD in calves prior to feedlot entry for herd 3 (25/226 [11%]) was significantly greater than that for herd 1 (2/138 [1.5%]). None of the 27 calves that developed BRD prior to feedlot entry had comorbidities such as diarrhea, and only 3 calves were treated for diarrhea prior to feedlot entry.

Respiratory virus shedding and BRD incidence during the first 5 weeks after feedlot entry

Of the calves enrolled in the study, 70 (7.9%) developed BRD during the first 5 weeks after feedlot entry (cases). The incidence rate of BRD in calves during the first 5 weeks after feedlot entry differed among the 4 herds of origin. The incidence rate of BRD for herd 2 (51/413 [12%]) was significantly greater than that for herds 1 (3/138 [2%]), 3 (12/226 [5%]), and 4 (4/113 [4%]).

Of the 70 cases, 39 (55.7%) were steers and 31 (44.3%) were heifers. The cases were distributed fairly equally among the 14 pens, with at least 1 BRD case

identified in 11 pens during the first week after feedlot entry (**Supplementary Table S1**, available at avmajournals.avma.org/doi/suppl/10.2460/ajvr.78.9.1065). Although controls were selected on the basis of convenience, neither the mean weaning age nor mean weaning weight differed significantly between cases and controls regardless of whether herd of origin was or was not controlled (data not shown), which suggested that the case and control populations were well matched.

Sixty-seven of the 70 BRD case samples were available for analysis. Bovine viral diarrhea virus was detected in 1 case, and sequence analysis of the 5' untranslated region of the isolated virus revealed that it was a BVDV1a strain that was 98% homologous to the NADL strain used in the administered MLV vaccine (data not shown). Bovine herpesvirus-1 and BRSV were not detected in any calf during the first 5 weeks after feedlot entry.

Bovine coronavirus RNA was detected in nasal swab specimens from 65 of 67 (97%) cases and 64 of 109 (59%) controls (**Table 3**) and was detected in all cases and controls sampled during the first 2 weeks after feedlot entry. However, for calves that had positive results for BCV ($Ct < 40$) by use of the RT-PCR assay, the mean Ct value was significantly ($P < 0.001$) lower for cases than for controls during the first week after feedlot entry (**Figure 1**), which suggested that cases were

Table 3—Number of BRD case and control calves from which BCV was detected in nasal swab specimens during each of the first 5 weeks after feedlot entry.

| Time after feedlot entry (wk) | BRD status | Herd | | | | | | | | | |
|-------------------------------|------------|----------------------|-------------|----------------------|-------------|----------------------|-------------|----------------------|-------------|----------------------|-------------|
| | | 1 | | 2 | | 3 | | 4 | | Total | |
| | | No. positive for BCV | No. sampled | No. positive for BCV | No. sampled | No. positive for BCV | No. sampled | No. positive for BCV | No. sampled | No. positive for BCV | No. sampled |
| 1 | Case | 2 | 2 | 19 | 19 | 2 | 2 | 1 | 1 | 24 | 24 |
| | Control | 1 | 1 | 13 | 13 | 10 | 10 | 2 | 2 | 26 | 26 |
| 2 | Case | 0 | 0 | 13 | 13 | 2 | 2 | 2 | 2 | 17 | 17 |
| | Control | 4 | 4 | 8 | 8 | 8 | 8 | 6 | 6 | 26 | 26 |
| 3 | Case | 0 | 0 | 7 | 7 | 3 | 3 | 0 | 0 | 10 | 10 |
| | Control | 0 | 4 | 4 | 6 | 0 | 11 | 0 | 5 | 4 | 26 |
| 4 | Case | 0 | 1 | 8 | 8 | 2 | 3 | 0 | 0 | 10 | 12 |
| | Control | 0 | 5 | 6 | 12 | 0 | 5 | 2 | 4 | 8 | 26 |
| 5 | Case | 0 | 0 | 2 | 2 | 1 | 1 | 1 | 1 | 4 | 4 |
| | Control | 0 | 0 | 0 | 3 | 0 | 1 | 0 | 1 | 0 | 5 |
| Total | Case | 2 | 3 | 49 | 49 | 10 | 11 | 4 | 4 | 65 | 67 |
| | Control | 5 | 14 | 31 | 42 | 18 | 35 | 10 | 18 | 64 | 109 |

Bovine coronavirus was detected by means of RT-PCR assay methods, and samples with a Ct value < 40 were considered to have positive results. See Table 1 for remainder of key.

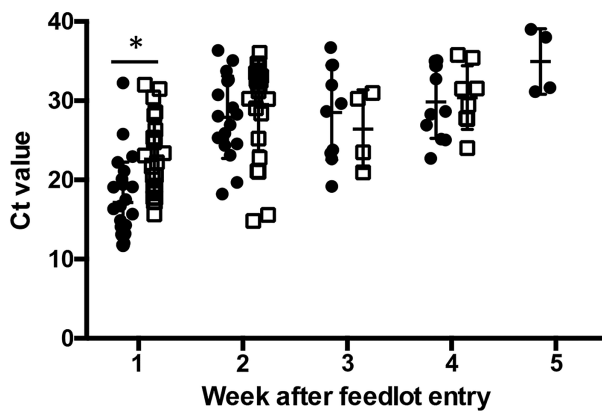


Figure 1—Scatterplots of RT-PCR assay results for calves in which BCV was detected in nasal swab specimens and that did (cases; circles) or did not (controls; squares) develop BRD during each of the first 5 weeks after feedlot entry. At feedlot entry, 886 natural-service crossbred beef calves from 4 herds of origin were commingled and allocated among 14 pens such that each pen contained the same proportion of calves from each herd. Cases were defined as calves with clinical signs of BRD, including lethargy, anorexia, abnormal breathing, nasal and ocular discharge, and pyrexia. Controls were apparently healthy calves, and 2 controls were selected on the basis of convenience from each pen weekly for sample collection. Cases and controls were excluded from future sampling; thus, each case and control is represented only once in the figure. Results were reported as Ct values. The Ct value is inversely associated with the amount of BCV RNA detected; therefore, the lower the Ct value, the greater the amount of BCV RNA in the sample. Samples with a Ct value < 40 were considered to have positive results for BCV. For each scatterplot, the horizontal line represents the mean and the vertical line delimits the SD. *Within a given week, the mean Ct value differs significantly ($P < 0.05$) between cases and controls.

shedding a greater amount of BCV in their nasal secretions than controls during that week. During weeks 3, 4, and 5 after feedlot entry, BCV was not detected in any control that originated from herds 1 and 3 but was detected in 10 of 21 controls that originated from herd 2 and 2 of 10 controls that originated from herd 4. Thus, the majority (10/12) of controls that had posi-

tive results for BCV during that period originated from herd 2. In contrast, BCV was detected in 24 of 26 (92%) cases during weeks 3, 4, and 5, and those BCV-positive cases originated from all herds, except herd 1. During the first 5 weeks after feedlot entry, cases were 23 times (OR, 23; 95% confidence interval, 5 to 142; $P < 0.001$) as likely to shed BCV as were controls. However, given that BCV was detected in all 41 cases and 52 controls evaluated during the first 2 weeks after feedlot entry, weeks 3 through 5 after feedlot entry were analyzed separate from weeks 1 and 2 after feedlot entry. During weeks 3 through 5 after feedlot entry, cases were 45 times (OR, 45; 95% confidence interval, 8 to 322; $P < 0.001$) as likely to shed BCV as were controls.

Bacterial shedding and BRD incidence during first 5 weeks after feedlot entry

The numbers of cases and controls from which *M haemolytica*, *P multocida*, *H somni*, and *M bovis* were detected in nasal swab specimens during each of the first 5 weeks after feedlot entry were summarized (Table 4). During week 4 after feedlot entry, the proportion of cases (11/12 [92%]) that had positive results for *M bovis* was significantly ($P = 0.002$) greater than the proportion of controls (8/26 [31%]) that had positive results for *M bovis*. However, the proportion of test-positive cases did not differ significantly from the proportion of test-positive controls for any bacterial species evaluated during any other week. Additionally, for test-positive calves, the mean Ct value for cases did not differ significantly from the mean Ct value for controls for any bacterial species or week after feedlot entry (Figure 2), which indicated that, for each bacterial species evaluated, the relative abundance of bacteria (bacterial load) in the nasopharynx did not differ between cases and controls.

Effect of serum anti-BCV antibody abundance on BCV shedding

Relative serum anti-BCV antibody abundance for the calves from each herd that were evaluated

Table 4—Number of case and control calves for which *Mannheimia haemolytica*, *Pasteurella multocida*, *Histophilus somni*, and *Mycoplasma bovis* were detected in nasal swab specimens during the first 5 weeks after feedlot entry.

| Time after feedlot entry (wk) | BRD status | No. sampled | No. positive for <i>M haemolytica</i> | No. positive for <i>P multocida</i> | No. positive for <i>H somni</i> | No. positive for <i>M bovis</i> |
|-------------------------------|------------|-------------|---------------------------------------|-------------------------------------|---------------------------------|---------------------------------|
| 1 | Case | 24 | 8 | 8 | 13 | 0 |
| | Control | 26 | 7 | 9 | 9 | 0 |
| 2 | Case | 17 | 5 | 6 | 6 | 2 |
| | Control | 26 | 12 | 10 | 9 | 5 |
| 3 | Case | 10 | 6 | 6 | 5 | 5 |
| | Control | 26 | 12 | 17 | 8 | 4 |
| 4 | Case | 12 | 7 | 10 | 7 | 11* |
| | Control | 26 | 10 | 20 | 7 | 8 |
| 5 | Case | 4 | 4 | 4 | 2 | 2 |
| | Control | 5 | 2 | 3 | 2 | 1 |
| Total | Case | 67 | 30 (45) | 34 (51) | 32 (48) | 20 (30) |
| | Control | 109 | 43 (39) | 59 (54) | 35 (32) | 18 (17) |

All bacterial species were detected by means of RT-PCR assay methods, and samples with a Ct value < 40 were considered to have positive results for the bacterial species of interest. Numbers within parentheses represent the percentage of the total cases or controls.

*Within a week, the proportion of cases that had positive results for the given bacterial species differs significantly ($P < 0.05$) from the corresponding proportion for controls.

See Table 1 for remainder of key.

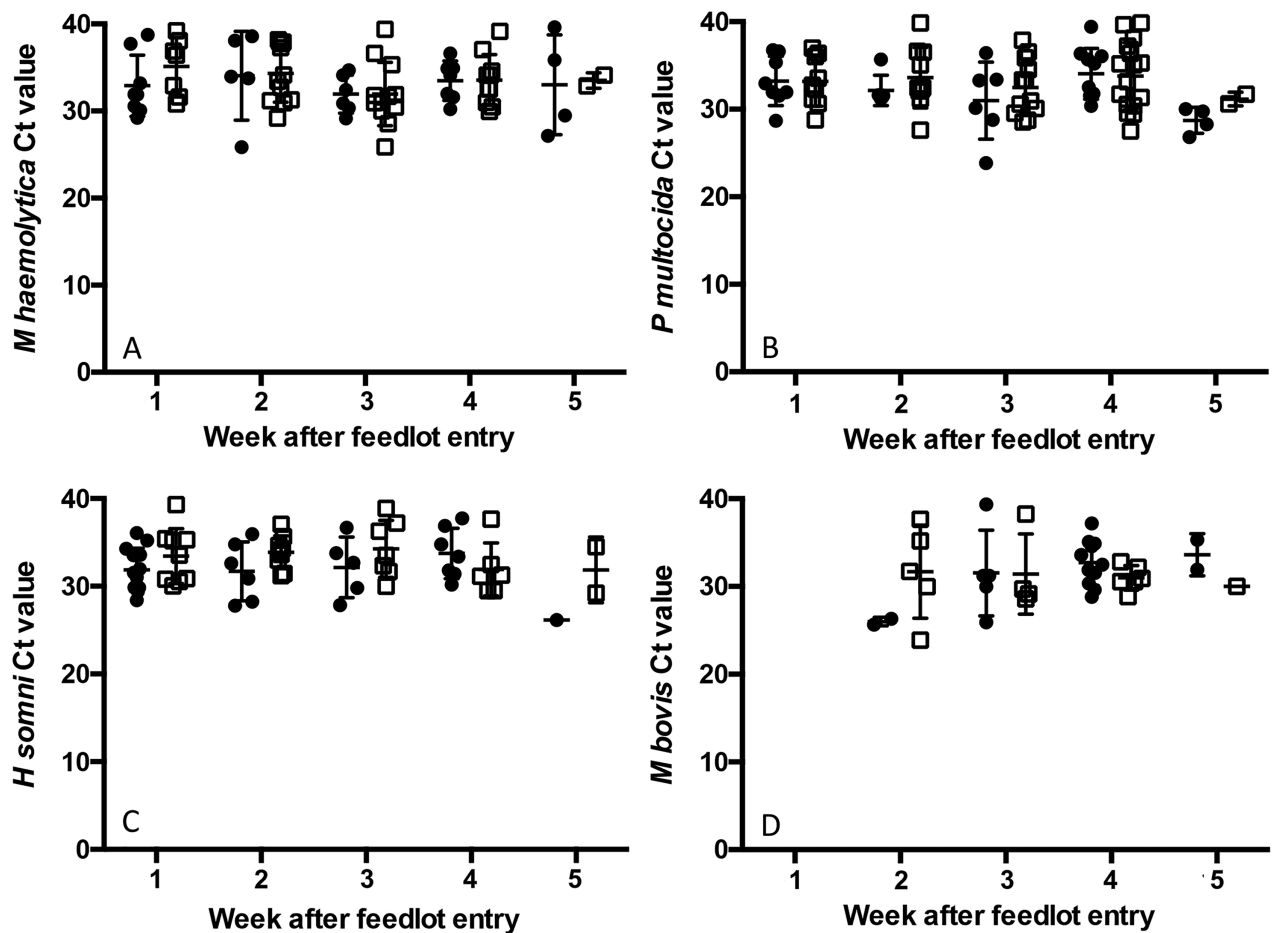


Figure 2—Scatterplots of RT-PCR assay results for the cases (circles) and controls (squares) of Figure 1 in which *Mannheimia haemolytica* (A), *Pasteurella multocida* (B), *Histophilus somni* (C), and *Mycoplasma bovis* (D) were detected in nasal swab specimens during each of the first 5 weeks after feedlot entry. For all bacterial species, samples with a Ct value < 40 were considered to have positive results. See Figure 1 for remainder of key.

for BCV shedding at initial vaccination and weaning were plotted (**Figure 3**). At initial vaccination, BCV shedding was detected in 14 of 60 (23%) calves from

herd 3 but was not detected in any calves evaluated from the other 3 herds. The calves evaluated from herd 3 had the highest mean relative anti-BCV anti-

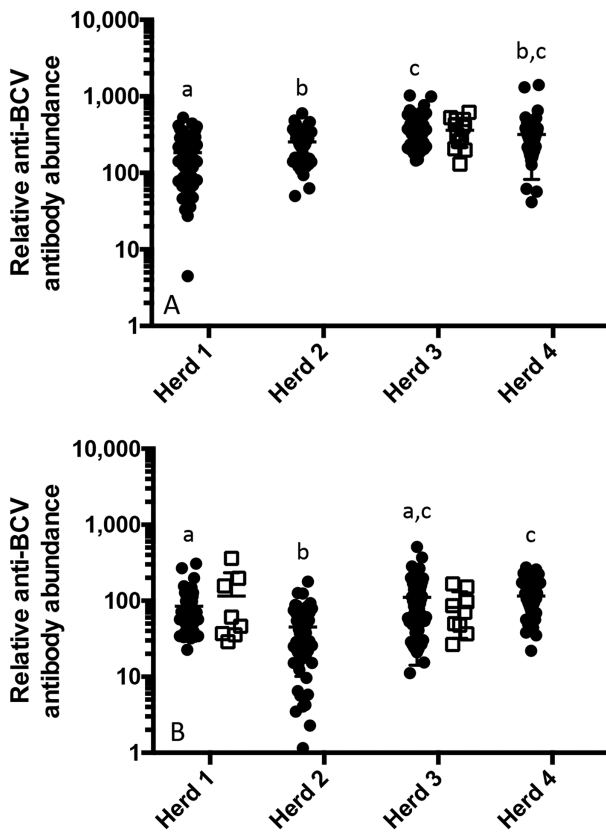


Figure 3—Scatterplots of the relative serum anti-BCV antibody abundance for calves that were (squares) or were not (circles) shedding BCV at initial vaccination (20 to 70 days old; A) and weaning (130 to 190 days old; B). Relative anti-BCV antibody abundance and BCV shedding were determined for 60 randomly selected calves from each herd at both initial vaccination and weaning. ^{a-c}Mean relative anti-BCV antibody abundance for herds with different letters differs significantly ($P < 0.05$). See Figure 1 for remainder of key.

body abundance (382), which was significantly greater than that for herds 1 (184) and 2 (254) but did not differ from that for herd 4 (317). The mean anti-BCV antibody abundance at initial vaccination for herd 1 was significantly less than that for the other 3 herds, and that for herd 2 was significantly less than that for herd 3. Within herd 3, the mean anti-BCV antibody abundance did not differ significantly ($P = 0.65$) between calves that were and were not shedding BCV at initial vaccination.

At weaning, BCV shedding was detected in 8 of 60 (13%) calves from herd 1 and 9 of 60 (15%) calves from herd 3. Mean relative anti-BCV antibody abundance at weaning was greatest for the calves of herd 4 (115) but did not differ significantly from that for the calves of herd 3 (106). Mean relative anti-BCV antibody abundance at weaning was lowest for the calves of herd 2 (45) and differed significantly from that for the calves of the other 3 herds. Herd 2 also had the highest proportion of calves that were seronegative for antibodies against BCV. Mean relative anti-BCV antibody abundance at weaning for the calves of herd 1

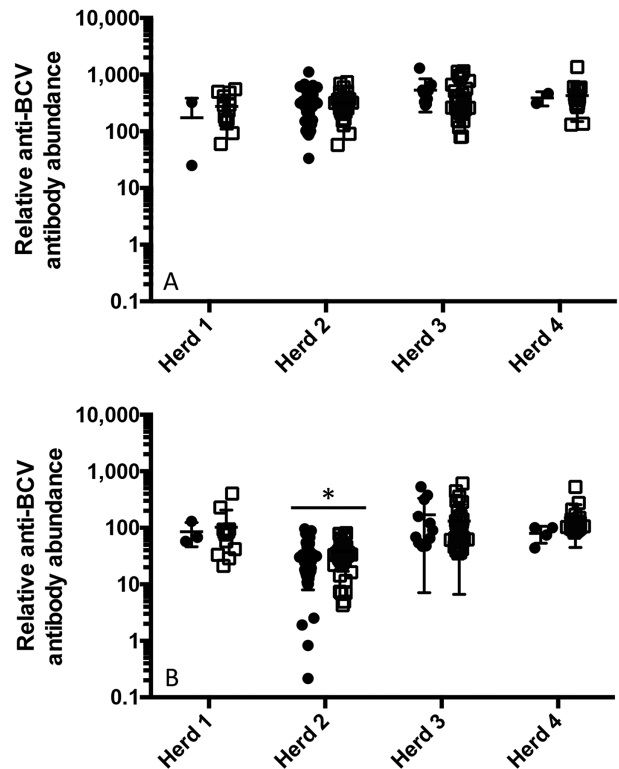


Figure 4—Scatterplots of the relative serum anti-BCV antibody abundance at initial vaccination (A) and weaning (B) for the cases (circles) and controls (squares) of Figure 1. *Mean relative anti-BCV antibody abundance for herd 2 differed significantly ($P < 0.001$) from that for each of the other 3 herds. See Figure 1 for remainder of key.

(89) was significantly greater than that for the calves of herd 4 but did not differ significantly from that for the calves of herd 3. At weaning, mean anti-BCV antibody abundance did not differ significantly between calves that were and were not shedding BCV within herds 1 ($P = 0.22$) and 3 ($P = 0.37$).

Effect of serum anti-BCV antibody abundance on BRD incidence during the first 5 weeks after feedlot entry

Within each herd, the mean relative anti-BCV antibody abundance at initial vaccination and weaning did not differ significantly between cases and controls. Mean relative anti-BCV antibody abundance for all calves (cases and controls) did not differ significantly among herds at initial vaccination but was significantly ($P < 0.001$) lower for the calves of herd 2, compared with that for the calves from each of the other 3 herds, at weaning (Figure 4). Within herds, mean relative anti-BCV antibody abundance at weaning was inversely associated with the proportion of cases. The incidence of BRD during the first 5 weeks after feedlot entry was greatest for calves that originated from herd 2 (51/413 [12%]), which also had the lowest mean relative anti-BCV antibody abundance. Herd 2 was also the only herd that had calves seronegative for anti-BCV antibodies at weaning (ie, feed-

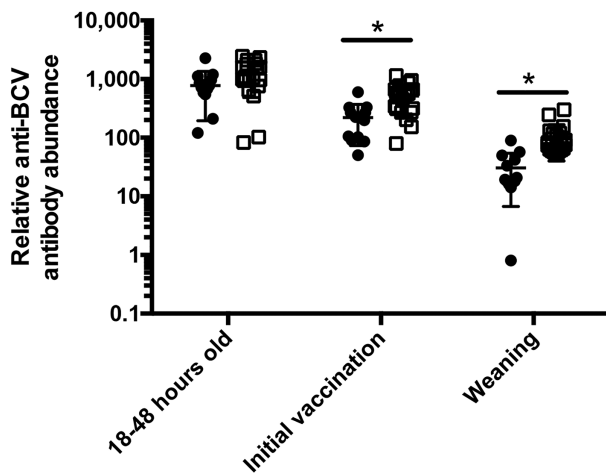


Figure 5—Scatterplots of the relative serum anti-BCV antibody abundance for a subset of calves from herds 2 (circles; $n = 13$) and 3 (squares; 21) that were evaluated at 18 to 48 hours old, initial vaccination, and weaning. Prior to feedlot entry, BCV shedding was not detected in any of the calves evaluated from herd 2, but it was detected in 23 of 180 calves evaluated in herd 3. The subset of calves from herd 3 represented in this figure included 8 calves from which BCV was detected in nasal swab specimens prior to feedlot entry. *Within a sample acquisition time, mean relative anti-BCV antibody abundance differs significantly ($P \leq 0.001$) between the 2 herds. See Figure 1 for remainder of key.

lot entry); 13 (8 cases and 5 controls) of 104 (12.5%) calves evaluated from herd 2 were seronegative for anti-BCV antibodies at weaning. When those 13 calves were removed from the analysis, mean anti-BCV antibody abundance for herd 2 was still significantly ($P < 0.001$) lower than that for each of the other 3 herds (data not shown).

Relative anti-BCV antibody abundance over time in herds with and without evidence of BCV shedding prior to feedlot entry

Relative serum anti-BCV antibody abundance was plotted for a subset of calves from herds 2 ($n = 13$) and 3 (21) that were evaluated at all 3 sample acquisition times prior to feedlot entry (18 to 48 hours old, initial vaccination, and weaning; **Figure 5**). Prior to feedlot entry, BCV was not detected in nasal swab specimens from any of the calves evaluated from herd 2 but was detected in nasal swab specimens from 23 of 180 calves evaluated from herd 3. The subset of calves evaluated from herd 3 included 8 calves from which BCV was detected in nasal swab specimens prior to feedlot entry. Relative serum anti-BCV antibody abundance for samples collected from calves at 18 to 48 hours old likely reflected passively acquired (maternally derived) anti-BCV antibodies, and the mean relative anti-BCV antibody abundance did not differ significantly between herds 2 and 3 at that sample acquisition time. However, the mean relative anti-BCV antibody abundance for herd 2 was significantly

lower than that for herd 3 at both initial vaccination ($P = 0.001$) and weaning ($P < 0.001$).

Discussion

Although there is mounting evidence that BCV is involved in the etiology of BRD, information regarding the respective temporal relationships between humoral immunity against BCV and BCV shedding and the risk for the development of BRD in beef calves at various times before and after feedlot entry is lacking. Results of the present study indicated that serum anti-BCV antibody abundance was not associated with BCV shedding; however, BCV shedding was positively associated with the incidence of BRD. Also, the mean serum anti-BCV antibody abundance for a group of calves was inversely related to the subsequent incidence of BRD in that group. However, serum anti-BCV antibody abundance at weaning for individual calves within a group was not predictive of which calves would subsequently require treatment for BRD.

For the calves of the present study, the rate of BCV shedding prior to weaning differed among the 4 herds of origin and over time; however, the BCV shedding pattern was not associated with serum anti-BCV antibody abundance. Dams of the calves were vaccinated with a vaccine containing an enteric BCV strain 30 days before the start of calving season to facilitate the passive transfer of anti-BCV antibodies via colostrum and aid in the prevention of diarrhea in calves. We hypothesized that BCV-induced disease outbreaks would occur in preweaned calves when a certain proportion of that population lost protective immunity owing to the natural decline of maternally derived anti-BCV antibodies. However, despite significant differences in the prevalence of BCV shedding among herds at the initial vaccination (20 to 70 days old) and weaning (130 to 190 days old) time points, no association was observed between mean anti-BCV antibody abundance and prevalence of respiratory virus shedding. Furthermore, within-herd analyses indicated that the mean serum anti-BCV antibody abundance did not differ significantly between calves that were and were not shedding BCV. These results suggested that the serum anti-BCV antibody abundance as determined by use of a currently available ELISA did not accurately predict BCV shedding in individual beef calves prior to weaning and were consistent with findings of studies^{25,26} involving preweaned dairy calves in which subclinical and recurrent BCV infections, albeit common, are not correlated with serum anti-BCV antibody titers. Differences in the rate of BCV shedding by preweaned calves among the 4 herds of origin were likely a function of the extent of exposure those calves had to the virus.

Contrary to serum anti-BCV antibody abundance, the prevalence of BCV shedding by preweaned calves in a herd appeared to be positively correlated with the incidence of BRD in those calves. The BRD incidence rate in preweaned calves was greatest for herds

1 (2/138 [1.4%]) and 3 (25/226 [11%]), the 2 herds in which calves shedding BCV were detected. Nasal swab specimens for pathogen detection were not collected at the time of BRD diagnosis from preweaned calves; therefore, it is unknown whether BCV was involved in the pathogenesis of clinical BRD in those calves. Herd-level risk factors associated with BRD in preweaned beef calves include large herd size, incidence rate of diarrhea in calves, duration of calving season, and introduction of cattle from outside sources.³²⁻³⁴ None of those factors were associated with the incidence of BRD in preweaned calves for the USMARC herds involved in the present study, although the introduction of outside cattle could not be assessed because those herds are closed populations. In another study,³⁵ the incidence of BRD in preweaned calves was negatively associated with dam parity (ie, calves from young cows were more likely to develop BRD than calves from older cows), which the investigators attributed to improvements in the quality and quantity of colostrum and milk, and thereby the passive transfer of maternally derived antibodies to calves, as cows age. Interestingly, the herd with the highest incidence of BRD in preweaned calves in the present study (herd 3) had the highest median dam parity. Additionally, mean serum anti-BCV antibody abundance for calves at 18 to 48 hours old did not differ significantly between the herds that did (herds 1 and 3) and did not have (herds 2 and 4) BCV or BRD detected in preweaned calves, which further suggested that passive transfer of maternally derived anti-BCV antibodies was not associated with BCV shedding or the risk for development of BRD. In fact, in the study reported here, BCV shedding was the only factor evaluated that was significantly associated with BRD incidence in calves both before and after weaning.

In the present study, the prevalence of calves shedding BCV was fairly low prior to weaning, but it was quite high during the first 5 weeks after feedlot entry. In fact, BCV was detected in the nasal swab specimens collected from all calves that developed BRD (cases) and apparently healthy calves (controls) evaluated during the first 2 weeks after feedlot entry. This indicated that BCV infection was associated with both subclinical and clinical disease. However, the amount of BCV RNA detected in the nasal swab specimens of cases was significantly greater than that detected in the nasal swab specimens of controls, which suggested that disease severity was positively associated with viral load. Results of other studies^{14,19,21,36-38} indicate that the rate of seroconversion to BCV can be as high as 100% in beef calves during the first few weeks after feedlot entry, which suggests that BCV transmission among calves is common at feedlot entry. In the present study, BCV shedding remained a significant factor in the development of BRD in calves during weeks 3 through 5 after feedlot entry. Furthermore, during weeks 3 through 5 after feedlot entry, *M bovis* was detected in nasal swab specimens from a significantly greater proportion of cases (18/27 [67%]) than controls (13/57

[23%]), which suggested that the presence of both BCV and *M bovis* in the nasopharynx in conjunction with other stressors (eg, weaning and changing pens) may have contributed to the development of BRD in calves during that period.

The role of BCV in the development of BRD in calves was further supported by the fact that, in the present study, the mean serum anti-BCV antibody abundance for a group of calves at weaning was inversely associated with the incidence of BRD for those calves during the first 5 weeks after feedlot entry. Mean serum anti-BCV antibody abundance at weaning for the calves of herd 2 was significantly lower than that for the calves of the other 3 herds (Figure 4), and calves of herd 2 had the highest incidence of BRD during the first 5 weeks after feedlot entry. As previously mentioned, mean serum anti-BCV antibody abundance for calves at 18 to 48 hours old did not differ among the 4 herds; therefore, the differences observed at weaning were most likely the result of differences in BCV exposure rather than differences in passive transfer of maternally derived antibodies. The findings of the present study were consistent with results of other studies^{14,19-23} that have indicated BCV infection before weaning is protective against development of BRD after weaning and that calves with high serum anti-BCV antibody abundance at feedlot entry are less likely to develop BRD than calves with low serum anti-BCV antibody abundance. However, in the study reported here, contrary to other studies,^{14,21} the serum anti-BCV antibody titer at weaning for individual calves was not predictive of which calves would be treated for BRD after feedlot entry.

The reasons serum anti-BCV antibody abundance was not associated with BCV shedding or BRD incidence in preweaned and weaned beef calves in the present study are unknown. The ELISA used to measure anti-BCV antibodies in the present study detected total reactive rather than neutralizing antibodies. It is also possible that anti-BCV antibodies generated in response to a natural infection might have had a weak protective effect against viral replication, which would protect against the development of clinical signs of disease but not against virus shedding. That would be consistent with results of other studies, which indicate that subclinical BCV infections^{14,21,23-26} and recurrent or intermittent^{25,26} shedding of BCV are common. It also may suggest a lack of long-term protective immunity after natural infection.⁸ The role of cell-mediated immunity in the mediation of viral shedding in calves with BRD is poorly characterized. It is possible that cell-mediated immune responses may correlate with BCV shedding and the development of BRD better than serum anti-BCV antibody abundance. Finally, BCV shedding and the serum anti-BCV antibody abundance were evaluated in the calves of this study fairly infrequently from birth through the first 5 weeks after feedlot entry. Thus, we based our results on a series of snapshots

in time. Calves that were not identified as BCV shedders may have shed BCV between sample acquisition times, which might have masked the effect of serum anti-BCV antibody abundance on BCV shedding. Future studies should be designed to distinguish between these possibilities and determine the best immune correlates of protection for BCV infection.

In the present study, detection of BCV in nasal swab specimens obtained from preweaned beef calves was associated with the incidence of BRD in those calves before and after weaning. Also, the mean anti-BCV antibody abundance at weaning for a group of calves was inversely associated with the incidence of BRD for those calves after feedlot entry, which suggested that calves infected with BCV prior to weaning were protected against BRD after weaning and feedlot entry. However, the serum anti-BCV antibody abundance at weaning for individual calves was not predictive of which calves would be treated for BRD after feedlot entry. Although BCV shedding (which was presumably indicative of infection) was not associated with death in the present study, BCV infection can have adverse effects on calf health and well-being as well as feedlot productivity owing to a decrease in weight gain^{8,20} and increase in veterinary care costs for affected calves. Thus, generation of immunity against BCV in calves prior to feedlot entry should decrease the incidence of BRD and associated adverse animal health issues and production losses.²⁰ Further studies are necessary to elucidate the role of BCV in the pathogenesis of BRD in beef cattle at various stages of the production cycle. Such information is necessary to guide BCV vaccine development and control strategies.

Acknowledgments

Supported by the Agricultural Research Service (CRIS 3040-32000-031-00D).

Dr. Loy has served as a consultant for and has a substantial financial interest in Harrisvaccines. In accordance with the University of Nebraska-Lincoln's Conflict of Interest policy, the university's Conflict of Interest in Research Committee has determined that this must be disclosed.

Presented as a poster at the 35th Annual Meeting of the American Society for Virology, Blacksburg, Va, June 2016.

The use of product and company names is necessary to accurately report the methods and results; however, the USDA neither guarantees nor warrants the standard of the products. The use of names by the USDA implies no approval of the product to the exclusion of others that may also be suitable.

The authors thank Brad Sharp, Sam Nejezchleb, Tammy Sorensen, Stacy Bierman, and Gennie Schuller for technical assistance.

Footnotes

- a. Bovi-Shield Gold FP 5 VL 5, Zoetis, Florham Park, NJ.
- b. Scourguard 4KC, Zoetis, Florham Park, NJ.
- c. Bovi-Shield Gold One Shot, Zoetis, Florham Park, NJ.
- d. Vision 7 with Spur, Merck, Kenilworth, NJ.
- e. Vision 8, Merck, Kenilworth, NJ.
- f. Bovi-Shield Gold 5, Zoetis, Florham Park, NJ.
- g. TRIZOL LS, Life Technologies, Grand Island, NY.
- h. QIAamp DNA mini kit, Qiagen Inc, Valencia, Calif.
- i. OneStep RT-PCR kit, Qiagen Inc, Valencia, Calif.
- j. Pike L, Loy JD. Comparative assessment of multiplex PCR assays to culture and susceptibility testing methods for the

- detection of bovine bacterial respiratory pathogens and macrolide antimicrobial resistance determinants (abstr), in *Proceedings*. 58th Annu Meet Am Assoc Vet Lab Diagn 2015;41.
- k. BCV antibody ELISA, Boehringer Ingelheim Svanova, Uppsala, Sweden.
- l. Spectramax, Molecular Devices, Sunnyvale, Calif.
- m. JavaStat. Medical Research Consulting, Madison, Wis. Available at: medrescon.tripod.com/ctab2x2.html. Accessed Mar 6, 2017.
- n. SAS, version 16, SAS Institute Inc, Cary, NC.

References

1. Panciera RJ, Confer AW. Pathogenesis and pathology of bovine pneumonia. *Vet Clin North Am Food Anim Pract* 2010;26:191-214.
2. Griffin D. Bovine pasteurellosis and other bacterial infections of the respiratory tract. *Vet Clin North Am Food Anim Pract* 2010;26:57-71.
3. Griffin D, Chengappa MM, Kuszak J, et al. Bacterial pathogens of the disease complex. *Vet Clin North Am Food Anim Pract* 2010;26:381-394.
4. Caswell JL, Bateman KG, Cai HY, et al. *Mycoplasma bovis* in respiratory disease of feedlot cattle. *Vet Clin North Am Food Anim Pract* 2010;26:365-379.
5. Taylor JD, Fulton RW, Lehenbauer TW, et al. The epidemiology of bovine respiratory disease: what is the evidence for predisposing factors? *Can Vet J* 2010;51:1095-1102.
6. Fulton RW. Bovine respiratory disease research (1983-2009). *Anim Health Res Rev* 2009;10:131-139.
7. Fulton RW, Blood KS, Panciera RJ, et al. Lung pathology and infectious agents in fatal feedlot pneumonias and relationship with mortality, disease onset, and treatments. *J Vet Diagn Invest* 2009;21:464-477.
8. Saif LJ. Bovine respiratory coronavirus. *Vet Clin North Am Food Anim Pract* 2010;26:349-364.
9. Kanno T, Ishihara R, Hatama S, et al. Antigenic variation among recent Japanese isolates of bovine coronaviruses belonging to phylogenetically distinct genetic groups. *Arch Virol* 2013;158:1047-1053.
10. Tsunemitsu H, el-Kanawati ZR, Smith DR, et al. Isolation of coronaviruses antigenically indistinguishable from bovine coronavirus from wild ruminants with diarrhea. *J Clin Microbiol* 1995;33:3264-3269.
11. 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.
12. 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.
13. Fulton RW, Ridpath JF, Burge LJ. Bovine coronaviruses from the respiratory tract: antigenic and genetic diversity. *Vaccine* 2013;31:886-892.
14. Fulton RW, Step DL, Wahrmund J, et al. Bovine coronavirus (BCV) infections in transported commingled beef cattle and sole-source ranch calves. *Can J Vet Res* 2011;75:191-199.
15. Storz J, Purdy CW, Lin X, et al. Isolation of respiratory bovine coronavirus, other cytotidal viruses, and *Pasteurella* spp from cattle involved in two natural outbreaks of shipping fever. *J Am Vet Med Assoc* 2000;216:1599-1604.
16. Gagea MI, Bateman KG, van Dreumel T, et al. Diseases and pathogens associated with mortality in Ontario beef feedlots. *J Vet Diagn Invest* 2006;18:18-28.
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. Decaro N, Campolo M, Desario C, et al. Respiratory disease associated with bovine coronavirus infection in cattle herds in Southern Italy. *J Vet Diagn Invest* 2008;20:28-32.
19. 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.

20. Thomas CJ, Hoet AE, Sreevatsan S, et al. Transmission of bovine coronavirus and serologic responses in feedlot calves under field conditions. *Am J Vet Res* 2006;67:1412-1420.
21. 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.
22. Lin XQ, O'Reilly KL, Storz J, et al. Antibody responses to respiratory coronavirus infections of cattle during shipping fever pathogenesis. *Arch Virol* 2000;145:2335-2349.
23. 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.
24. 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.
25. Heckert RA, Saif IJ, 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.
26. Heckert RA, Saif IJ, 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.
27. Humphry RW, Cameron A, Gunn GJ. A practical approach to calculate sample size for herd prevalence surveys. *Prev Vet Med* 2004;65:173-188.
28. Boxus M, Letellier C, Kerkhofs P. Real Time RT-PCR for the detection and quantitation of bovine respiratory syncytial virus. *J Virol Methods* 2005;125:125-130.
29. Decaro N, Elia G, Campolo M, et al. Detection of bovine coronavirus using a TaqMan-based real-time RT-PCR assay. *J Virol Methods* 2008;151:167-171.
30. Mahlum CE, Haugerud S, Shivers JL, et al. Detection of diarrhoea virus by TaqMan reverse transcription polymerase chain reaction. *J Vet Diagn Invest* 2002;14:120-125.
31. Wang J, O'Keefe J, Orr D, et al. Validation of a real-time PCR assay for the detection of bovine herpesvirus 1 in bovine semen. *J Virol Methods* 2007;144:103-108.
32. Dutil L, Fecteau G, Bouchard E, et al. A questionnaire on the health, management, and performance of cow-calf herds in Québec. *Can Vet J* 1999;40:649-656.
33. Hanzlicek GA, Renter DR, White BJ, et al. Management practices associated with the rate of respiratory tract disease among preweaned beef calves in cow-calf operations in the United States. *J Am Vet Med Assoc* 2013;242:1271-1278.
34. Woolums AR, Berghaus RD, Smith DR, et al. Producer survey of herd-level risk factors for nursing beef calf respiratory disease. *J Am Vet Med Assoc* 2013;243:538-547.
35. Muggli-Cockett NE, Cundiff LV, Gregory KE. Genetic analysis of bovine respiratory disease in beef calves during the first year of life. *J Anim Sci* 1992;70:2013-2019.
36. 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.
37. 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.
38. 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.