Antibody titers against bovine coronavirus and shedding of the virus via the respiratory tract in feedlot cattle

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Objective—To describe patterns of seroconversion to bovine coronavirus (BCV) and shedding of BCV from the respiratory tract in feedlot cattle.

Animals—1,074 calves in feedlots in Ohio, Texas, and Nebraska.

Procedure—Nasal swab specimens were obtained at time of arrival (day 0) and at various times during the initial 28 days after arrival at feedlots. Specimens were tested for BCV, using an antigen-capture ELISA. Serum samples were obtained at time of arrival and again 28 days after arrival; sera were analyzed for antibodies to BCV, using an antibody-detection ELISA.

Results—Samples from 12 groups of cattle entering 7 feedlots during a 3-year period revealed that 78 of 1,074 (73%) cattle were shedding BCV (range, 0 to 35.9% within specific groups). At time of arrival, 508 of 814 (62.4%) cattle had low (<50) or undetectable BCV antibody titers. Seroconversion to BCV during the initial 28 days after arrival was detected in 473 of 814 (58%) cattle tested (range, 20.3 to 84.1% within specific groups). In cattle shedding BCV from the nasal passages, 49 of 68 (72.1%) seroconverted, and 472 of 746 (63.3%) cattle that were not shedding the virus seroconverted.

Conclusions and Clinical Relevance—Bovine coronavirus can be detected in populations of feedlot cattle in the form of viral shedding as well as seroconversion to the virus. Although only a few cattle were shedding the virus at the time of arrival at a feedlot, most of the cattle seroconverted to BCV by 28 days after arrival. (Am J Vet Res 2000;61:1057–1061)

Cattle are exposed to a multitude of infectious agents during their journey from ranches to auction markets to feedlots. Infection by viruses and bacteria is common among these cattle, although it does not always cause clinical disease. Often, however, it results in bovine respiratory disease complex (BRDC). The generally accepted theory for the cause of BRDC is that a combination of viruses and physical stresses overwhelm the defense mechanisms of an animal’s respiratory tract, allowing commensal bacteria of the nasal cavity, including Mannheimia haemolytica (formerly known as Pasteurella haemolytica) and P. multocida, to infect the lungs. Numerous viruses have been implicated in BRDC, including bovine respiratory syncytial virus, bovine herpesvirus-1, parainfluenza-3 virus, and bovine viral diarrhea virus. Most cattle arriving at feedlots are vaccinated against these viruses. Recently, investigators isolated bovine coronavirus (BCV) from cattle in feedlots. Cattle shedding this virus at the time of arrival are at increased risk for developing respiratory tract disease; however, it is unknown whether this is an incidental finding, because the virus also can be isolated from healthy cattle. Furthermore, it is not certain that BCV is a contributor to BRDC. Given the acknowledged roles of other viruses in BRDC, it is possible that BCV also may act synergistically with other infectious agents and stressors to contribute to pneumonia in cattle in feedlots. However, the prevalence of respiratory tract infections attributable to BCV in feedlot populations and the rate of seroconversion to it are unknown. To define the epidemiologic characteristics of BCV infections in cattle in feedlots, an observational study was performed to obtain data on BCV shedding patterns from the respiratory tract and rates of seroconversion to BCV in feedlot cattle.

Materials and Methods

Study population—During a 3-year period, a survey was conducted that included 1,074 cattle consisting of 12 groups of cattle entering 7 feedlots in Ohio, Texas, and Nebraska. All cattle were mixed-breed steers and heifers between 4 and 7 months old. Three of the feedlots were located in Ohio, including 1 in the southern part of the state (Lucasville), 1 in the central part of the state (heifers from Jackson, housed in Columbus), and 1 in the northeastern part of the state (Wooster). Cattle entering the Lucasville feedlot were purchased from a mixed-source livestock auction market in Kentucky and were transported approximately 96 km to a backgrounding unit operated by the Ohio Department of Rehabilitation and Correction. The cattle at Jackson were raised on a farm that was part of the Ohio Agricultural Research and Development Center (OARDC), with steers being fed at the OARDC feedlot in Wooster and heifers being fed at The Ohio State University feedlot in Columbus. In the fall of 1996, cattle at Wooster consisted of steers raised at 3 separate OARDC farms that then were commingled with cattle purchased at a mixed-source livestock auction in Virginia. In the fall of 1997, cattle were purchased at the auction market in Virginia and subsequently transported to the feedlot in Wooster. Cattle were obtained from various ranches located in western Texas and were commingled at a feedlot located near Amarillo, Tex. In the fall of 1998, 3 groups of mixed-source.
cattle entering feedlots in Nebraska (Hastings, Milford, Scottsbluff) were included in the study.

Collection of samples—At the time of arrival at a feedlot (day 0) and on various days after arrival, nasal swab specimens were obtained from cattle, using a technique described by Hasoksuz et al. Briefly, samples were collected from all cattle in an incoming group, including those with signs of respiratory tract disease and those that appeared to be healthy. Using 6-inch sterile cotton-tipped applicators, samples were collected from both nostrils, and the swab specimens were placed in tubes containing 4 ml of viral transport medium. Tubes were centrifuged at 2,000 X g for 11 minutes, swabs were removed, and supernatants were collected and frozen at –70 C.

Serum samples were obtained at time of arrival and on day 28 to enable us to test for seroconversion to BCV. Samples (10 to 15 ml) of blood were obtained via jugular venipuncture. Blood samples were centrifuged at 2,000 X g for 20 minutes; serum then was removed, allocated into duplicate aliquots, and frozen at –20 C.

Enzyme-linked immunosorbent assay for BCV antigen—An indirect, double-antibody sandwich antigen-capture ELISA developed by Smith et al to detect BCV in fecal samples obtained from adult dairy cattle was adapted to detect BCV in supernatant fluids of nasal swab specimens, using the procedure described by Hasoksuz et al. Plates were analyzed at 414 nm on a computer-linked ELISA reader, and values for optical densities were saved as computer files. A spreadsheet program was used to calculate the ELISA values for each sample by subtracting the mean value for absorbance of paired negative-coated wells from the mean value for absorbance of paired positive-coated wells. Samples with a resulting absorbance > 0.1 were considered positive for BCV.

Enzyme-linked immunosorbent assay for antibodies to respiratory tract BCV—An antibody-detection ELISA developed by Smith et al for detection of enteric BCV was adapted to detect antibodies to BCV in serum samples from the feedlot cattle. Alterations from the aforementioned protocol included use of tissue-culture supernatants of a BCV strain (isolated from the respiratory tract of a calf in a feedlot in Ohio and grown in human rectal tumor [HRT]-18 cells) as antigen and use of an affinity-purified goat anti-bovine IgG antibody conjugated to horseradish peroxidase for antibody detection. Absorbance from a row of wells coated with mock-infected cell-culture supernatant, rather than BCV antigen, was subtracted from the absorbance of the BCV-coated wells at each dilution for each sample, using a spreadsheet program. Each tier was determined to be the serum dilution at which the positive-coated well had an absorbance value of 0.1 or more greater than that of the negative well.

Virus neutralization assay—A subset of 47 paired serum samples (5% of the samples obtained for each group) was tested, using a virus neutralization (VN) assay, for comparison with ELISA results. A plaque-reduction VN test was performed, using the bovine enteric coronavirus (BECV) Mebus strain and HRT-18 cells. Antibody titers determined by VN testing were expressed as the reciprocal of the highest dilution of serum that resulted in an 80% reduction in plaque formation, compared with that of a virus-control sample. The correlation coefficient for titers determined by use of ELISA and VN assay was 0.56.

Results

Schedules for collection of samples varied among feedlots, depending on their handling procedures and concurrent studies being conducted (Table 1). The number of cattle shedding BCV was relatively low, with only 83 positive samples from 1,074 cattle. Five of the positive samples were from cattle that previously shed the virus before entering the feedlots; thus, the overall shedding rate was 7.3% (78 of 1,074). Shedding rate for cattle at time of arrival was 3.8% (41 of 1,074), and it was 8.3% (35 of 422) for cattle on day 3. By day 7, the percentage of nasal swab specimens that had positive results when tested for coronavirus decreased to 0.36% (2 of 550), and it remained low for samples obtained on days 14 (3 of 593 [0.5%]) and 28 (2 of 484 [0.4%]).

During the initial 28 days after arrival, 473 of 814 (58%) cattle tested seroconverted to BCV, as deter-
mined by results of the ELISA (Table 2). Geometric mean BCV titers appeared to increase during the first 28 days after arrival for several groups of cattle, including the cattle at Lucasville in the fall of 1996 and the cattle at Wooster, Jackson, and Amarillo in the fall of 1996. Cattle in Lot No. 2 at Lucasville in the spring of 1996 appeared to have only a slight increase in geometric mean BCV titers during the first 28 days after arrival. Less than half (29 of 68 [42.6%]) of the cattle shedding BCV from the respiratory tract seroconverted, whereas 444 of 736 (60.3%) cattle in which BCV antigen was not detected seroconverted (Table 3).

Geometric mean titers for days 0 and 28 for the BCV-positive cattle were 84 and 1,413, respectively, whereas those for BCV-negative cattle were 434 and 2,318, respectively.

The majority of cattle (508 of 814 [62.4%]) had low (< 50) BCV antibody titers at the time of arrival (Fig 1). This classification included cattle with BCV antibody titers ranging from 0 to 50, because 1:50 was the lowest serum dilution tested. Geometric mean BCV titers on day 28 ranged from < 50 to > 51,000. Similar trends were detected for specific groups (data not shown), with most cattle having low BCV antibody titers at arrival but having higher titers after the initial 28 days in the feedlots.

Percentage of cattle that seroconverted among cattle arriving with relatively low BCV antibody titers (< 200) ranged from 67.3 to 75.8% (Fig 2). The percentage of cattle seroconverting decreased to < 50% for cattle with titers of ≥ 400 at arrival, and it decreased to 0 for cattle with titers of > 3,200 at arrival.

**Discussion**

The first step in determining the potential role of BCV in BRDC of feedlot cattle is to define epidemiologic characteristics of the virus in feedlot populations, including shedding patterns and exposure to the virus, as measured by seroconversion. In previous studies, investigators provided evidence that BCV could be isolated from cattle in feedlots, but a relatively small number of cattle were included in those studies. In 1996, BCV was isolated from 38 of 100 cattle arriving at feedlots in Kansas and Arizona that had signs of respiratory tract disease. In that study, calves shedding BCV at arrival were 2.5 to 3.2 times more likely to need treatment for respiratory tract disease than calves that were not shedding the virus at arrival. Furthermore, seroconversion to BCV, as defined by a > 4-fold increase in BCV antibody titer, was detected in 72 of 185 (38.9%) of the calves tested. In that study, calves shedding BCV at arrival were 2.5 to 3.2 times more likely to need treatment for respiratory tract disease than calves that were not shedding the virus at arrival. Furthermore, seroconversion to BCV, as defined by a > 4-fold increase in BCV antibody titer, was detected in 72 of 185 (38.9%) of the calves tested. In that study, calves shedding BCV at arrival were 2.5 to 3.2 times more likely to need treatment for respiratory tract disease than calves that were not shedding the virus at arrival.
100% of 604 calves at various feedlots in Canada, providing further evidence of BCV in feedlot cattle.

Analysis of our results indicated that BCV could be detected in cattle in feedlots in Ohio, Texas, and Nebraska at an overall rate of 7.3% of all cattle from which samples were obtained. Rates of shedding of BCV from the distal portion of the nasal cavity varied among years, locations, and days after arrival at feedlots, which would be expected for any pathogens at feedlots. We did not detect BCV in 2 of the groups (ie, cattle in Lot No. 2 at Lucasville in the spring of 1996 and cattle at Jackson in the fall of 1996; Table 2). Other groups also had low rates of BCV shedding, including calves at Milford and Amarillo. Two possibilities appear likely: the virus was not common in those groups of cattle, or the cattle shed the virus during sample-collection dates. After arrival, the second highest number of BCV-positive samples was found in samples obtained on day 3. To reduce stress on cattle after their arrival at a feedlot and assignment to home pens, most feedlots do not process cattle prior to day 7 after arrival. It is likely that more BCV-positive samples would have been found if more cattle had been available for sample collection on day 3, rather than just the cattle at Lucasville.

The majority of cattle (473 of 814 [58%]) seroconverted to BCV during the initial 28 days after arrival at a feedlot, with values for specific groups ranging from 20.3 to 77.9%. The higher mean BCV antibody titers at arrival apparently reduced the percentage of cattle at Lucasville in spring 1996 that seroconverted, with rates of 20.3 to 49.3%, compared with 53.1 to 84.1% in other groups. These cattle were purchased at a livestock auction and likely were exposed to the virus and the cattle at Jackson, 37 of 44 (84.1%) seroconverted, with rates of 53.1 to 84.1% in other groups. These cattle were purchased at a livestock auction and likely were exposed to the virus and the cattle at Jackson, 37 of 44 (84.1%) seroconverted to BCV during the initial 28 days after arrival, most cattle were exposed to the virus and developed a detectable antibody response. Cattle arriving with relatively high BCV antibody titers appeared less likely to seroconvert than cattle without detectable BCV titers at arrival. Similar patterns have been found with other respiratory tract pathogens in feedlot cattle. Extensive commingling of cattle from various origins at feedlots increases the risk for development of fatal fibrinous pneumonia by exposing the calves to pathogens to which they have not been previously exposed.14 Evidence of seroconversion to numerous pathogens in feedlot cattle during the initial month at the feedlots has been documented.14,15 It was found that >50% of the cattle seroconverted to M hemolytica, parainfluenza-3 virus, and bovine respiratory syncytial virus during the initial month at the feedlot.14 Seroconversion to the organism responsible for infectious bovine rhinotracheitis was rare (<5%), but 40% of the calves seroconverted to bovine viral diarrhea virus during the initial month at the feedlot.14 In that study, it also was reported that titers for these organisms in calves at the time of arrival were negatively correlated with subsequent seroconversion.14

It is apparent that BCV can be isolated from feedlot cattle in a number of geographic locations, and that most cattle develop an antibody response to BCV during their initial 28 days at a feedlot. However, further analysis is needed to determine the extent of damage caused by BCV and to more clearly define the contribution of BCV to BRDC. Identification of BCV in feedlot populations is incriminating but does not by itself prove that it causes respiratory tract disease.
References


