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 (7.3%) 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.[1-4] Numerous viruses have been implicated in BRDC, including bovine respiratory syncytial virus, bovine herpesvirus-1, parainfluenza-3 virus, and bovine viral diarrhea virus.[1-4] Most cattle arriving at feedlots are vaccinated against these viruses.[5]

Recently, investigators isolated **bovine coronavirus (BCV)** from cattle in feedlots.[6-8] Cattle shedding this virus at the time of arrival are at increased risk for developing respiratory tract disease[7]; however, it is unknown whether this is an incidental finding, because the virus also can be isolated from healthy cattle.[8] 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

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Table 1—Results of testing for bovine coronavirus (BCV) in samples obtained from cattle in various feedlots in Ohio (Jackson, Lucasville, Wooster), Nebraska (Hastings, Milford, Scottsbluff), and Texas (Amarillo), using an antigen-capture ELISA

Location and time	No. of calves	Body weight at time of arrival (kg)	Days after arrival*					BCV positive	
			0	3	7	14	28	No.	%
Lucasville, Spring 1996									
Lot No.1	69	273	0	7	0	—	—	7	10.1
Lot No. 2	70	263	0	0	0	—	—	0	0
Lot No.3	105	267	8	5	0	—	—	13	12.4
Lucasville, Fall 1996									
Lot No.1	86	284	4	0	—	0	0	4	4.7
Lot No. 2	92	246	8	23†	—	1	1	33	35.9
Wooster, Fall 1996	100	251	1	—	0	0	0	1	1.0
Jackson, Fall 1996	44	229	0	—	0	0	0	0	0
Amarillo, Fall 1996	109	282	2	—	—	1	—	3	2.7
Wooster, Fall 1997	162	243	8	—	2	1	1	12	7.4
Scottsbluff, Fall 1998	87	NA	6	—	—	—	—	6	6.9
Hastings, Fall 1998	100	NA	3	—	—	—	—	3	3.0
Milford, Fall 1998	50	NA	1	—	—	—	—	1	2.0
Total	**1,074**	**NA**	**41**	**35**	**2**	**3**	**2**	**83**	**7.3**

*Day of arrival at feedlot = Day 0. †Includes 5 cattle that also had positive results when tested for BCV on day 0.
NA = Not available. — = Samples not obtained on that day.

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.[8] 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 (1,000 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[9] 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.[8] Plates were analyzed at 414 nm on a computer-linked ELISA reader,[a] and values for optical densities were saved as computer files. A spreadsheet program[b] 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[10] 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[c] 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.[d] Each titer 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,[11] 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-

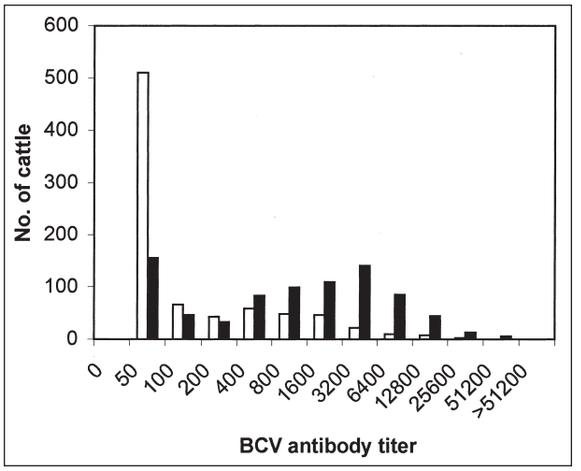

Figure 1—Frequency distribution of geometric mean antibody titer against bovine coronavirus (BCV) in 814 cattle at time of arrival (day 0; □) and 28 days after arrival (■) at various feedlots.

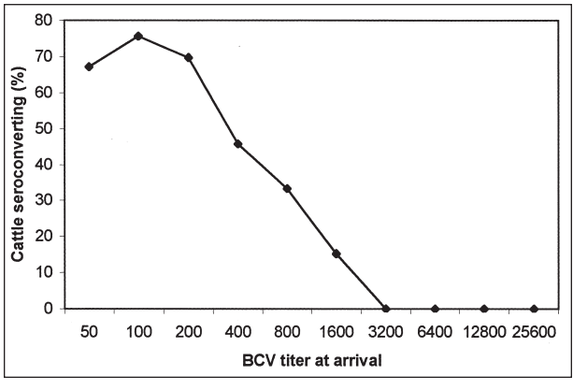

Figure 2—Percentage of cattle seroconverting to BCV, on the basis of initial antibody titer at time of arrival at a feedlot.

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.

Table 2—Number and percentage of each group of cattle that had an increase in titer to BCV and the geometric mean titer (GMT) to BCV at days 0 and 28 after arrival in various feedlots

	Seroconversion to BCV		GMT	
Location and time	**No.**	**%**	**Day 0**	**Day 28**
Lucasville, Spring 1996				
Lot No. 1	34	49.3	365.4	1,611
Lot No. 2	14	20.3	678	736
Lot No. 3	46	43.8	1,550.6	2,249
Lucasville, Fall 1996				
Lot No. 1	67	77.9	160.2	4,853
Lot No. 2	71	77.2	247	2,405
Wooster, Fall 1996	56	56	65.1	959
Jackson, Fall 1996	37	84.1	112.5	5,526
Amarillo, Fall 1996	62	56.4	218.1	2,188
Wooster, Fall 1997	86	53.1	173.4	1,700
Total	**473**	**58.0**	**404**	**2,242**

Table 3—Status for BCV antigen shedding and seroconversion to BCV in 814 cattle after their arrival at a feedlot

	Seroconversion		
Antigen shedding	**Positive**	**Negative**	**Total**
Positive	29	39	68
Negative	444	302	746
Total	**473**	**341**	**814**

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.[6,12,e,f] In 1996, BCV was isolated from 38 of 100 cattle arriving at feedlots in Kansas and Arizona that had signs of respiratory tract disease.[6] However, samples were not obtained from healthy cattle in that study. In a 1995 study of cattle arriving at 4 feedlots in Ohio,[e] nasal shedding of BCV was identified in 105 of 489 (21.5%) 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 a study in 1998,[f] BCV was isolated from 87 of 105 (83%) cattle involved in an outbreak of respiratory tract disease at a feedlot in Texas. Nine of the 10 cattle that died during that outbreak had BCV infections, and 69 of the surviving 95 (73%) cattle were infected with BCV but not with other viruses. In another study in 1998,[12] investigators found seroconversion to BCV, as defined by a > 4-fold increase in BCV antibody titer, in 61 to

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 between 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 before arrival at the feedlot, allowing many of them to mount an immune response prior to arrival at Lucasville. Although BCV was not found in the cattle at Jackson, 37 of 44 (84.1%) seroconverted to the virus, as measured by ELISA. These cattle were mixed with calves from other farms in the OARDC system, and those other calves may have been shedding the virus and serving as a source of exposure. Because samples were obtained only once per week from the cattle at Jackson, BCV may have been shed for only a few days during the interval between sample collections.

Although only a relatively few cattle were found to be actively shedding the virus in the populations from which samples were obtained, which could have been attributable to shedding of the virus on days when samples were not obtained or shedding during lairage and transport after exposure to the virus at auction barns, most cattle had been exposed to the virus and developed a measurable seroresponse by the end of the initial 28-day period in the feedlots.

Distributions of titers on days 0 and 28 were similar for most groups. Most cattle arrived with a relatively low titer to BCV, which then increased during the initial 28 days after arrival. As mentioned previously, cattle at Lucasville in spring 1996 tended to have higher BCV antibody titers at arrival than cattle in other groups, probably because of exposure to the virus at their farms of origin or, more likely, at the auction barn. Cattle with higher BCV antibody titers at arrival (Fig 2) appeared less likely to seroconvert, because they most likely had recent exposure and had seroconverted to the virus prior to arrival; thus, they would not produce the 4-fold increase in antibody titer needed for classification of seroconversion. This finding is in agreement with that of other investigators[12] who found that the change in BCV antibody titer during the initial month after arrival at feedlots in Canada was strongly negatively correlated with titer at time of arrival.

Analysis of the available data on BCV shedding and seroconversion from the survey reported here suggested that BCV followed a pattern typical of other respiratory tract pathogens in feedlots. A few cattle arriving at the feedlots were shedding the virus, most commonly during the first week after arrival. 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.[13] 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 after arrival at feedlots in Canada.[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.

[a]Titertek Multiscan plate reader, Flow Laboratories Inc, McLean, Va.
[b]Quattro Pro Windows, version 7.0, Borland International Inc, Scotts Valley, Calif.
[c]Emax Precision microplate reader, Molecular Devices, Sunnyvale, Calif.
[d]Microsoft Excel 97, Microsoft Corp, Seattle, Wash.
[e]Lathrop SL, Wittum TE, Morley PS, et al. Bovine coronavirus respiratory infections in feedlot cattle (abstr), in *Proceedings*. Conf Research Workers Anim Dis 1996;200.
[f]Storz J, Purdy CW, Lin XQ, et al. Market-stressed cattle of a shipping fever epizootic in a Texas feedlot have a high infection rate with respiratory bovine coronaviruses (abstr), in *Proceedings*. 31st Annu Convention Am Assoc Bovine Pract 1998;31:224.

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