Bovine coronavirus (BCV) infections in transported commingled beef cattle and sole-source ranch calves

Robert W. Fulton, Douglas L. Step, Jackie Wahrmund, Lurinda J. Burge, Mark E. Payton, Billy J. Cook, Dirk Burken, Chris J. Richards, Anthony W. Confer

Abstract

This study investigated bovine coronavirus (BCV) in both beef calves direct from the ranch and commingled, mixed-source calves obtained from an auction market. The level of BCV-neutralizing antibodies found in the calves varied among ranches in 2 different studies in a retained-ownership program (ROP), from the ranch to the feedlot. Calves with low levels of BCV-neutralizing antibodies (16 or less) were more likely to be treated for bovine respiratory disease (BRD) than those with higher titers. In 3 studies of commingled, mixed-source calves, BCV was recovered from calves at entry to the feedlot and the infections were cleared by day 8. The BCV was identified in lung samples [bronchoalveolar lavage (BAL) collection] as well as in nasal swabs. Calves with low levels of BCV-neutralizing antibodies at entry were most likely to be shedding BCV. Bovine coronavirus was isolated from both healthy and sick calves, but not from sick calves after 4 d arrival at the feedlot. Bovine coronavirus (BCV) should be considered along with other bovine respiratory viruses in the diagnosis of etiologies in bovine respiratory disease, especially for animals that become sick shortly after arrival. If approved vaccines are developed, it would be best to carry out vaccination programs before calves are weaned, giving them sufficient time to gain active immunity before commingling with other cattle.

Résumé

L'objectif de la présente étude était d'enquêter sur le coronavirus bovin (BCV) chez les veaux d'embouche directement à la ferme et chez des veaux mis en groupe et provenant de sources variées obtenus à l'encan. Le titre d'anticorps neutralisant anti-BCV trouvé chez les veaux variait parmi les élevages dans 2 études différentes dans un programme de propriété retenue (ROP) de l'élevage au parc d'engraissement. Les veaux avec des titres d'anticorps neutralisants anti-BCV faibles, 16 ou moins, étaient plus susceptibles à être traités pour des maladies respiratoires bovines (BRD) que ceux avec des titres plus élevés. Dans 3 études sur des veaux provenant de sources variées, le BCV a été retrouvé chez les veaux à l'entrée en parc d'engraissement et l'infection était éliminée au jour 8. Le BCV a été identifié à partir d'échantillons pulmonaires [lavage broncho-alvéolaire (BAL)] ainsi que d'écouvillons nasaux. Les veaux avec des titres d'anticorps anti-BCV faibles à l'entrée étaient plus susceptibles d'excréter du BCV. Du BCV a été isolé à partir de veaux en santé et malades, mais pas à partir de veaux malades 4 jours après leur arrivée. Le BCV devrait être considéré au même titre que les autres virus respiratoires bovins comme agent étiologique lors du diagnostic des maladies respiratoires bovines, spécialement chez les animaux qui deviennent malades peu de temps après leur arrivée en parc d'engraissement. Si des vaccins approuvés sont développés, il serait approprié d'effectuer les programmes de vaccination avant que les veaux ne soient sevrés, ce qui leurs donnerait suffisamment de temps pour acquérir une immunité active avant d'être mélangé avec d'autres veaux.

(Traduit par Docteur Serge Messier)

Introduction

Bovine respiratory disease (BRD) has a major impact on the cattle industry, with economic losses occurring due to morbidity, mortality, treatment and prevention costs, loss of production, and reduced carcass value (1). Infectious agents associated with BRD include viruses [bovine herpesvirus-1 (BHV-1), bovine parainfluenza-3 (PI-3V), bovine viral diarrhea virus (BVDV) 1 and 2, bovine respiratory syncytial virus (BRSV), bovine adenoviruses (BAdV), bovine coronavirus (BCV)], and bacteria *(Mannheimia haemolytica, Pasteurella multocida, Histophilus somni,* and *Mycoplasma* spp.*)* (1,2). From the virus standpoint, BCV has received recent attention as BRD continues to be a problem in the industry, despite the presence and widespread use of modified live virus (MLV) and killed BHV-1, BVDV, PI-3V, and BRSV products. Clinicians and diagnosticians are often called upon to examine for agents other than the 4 viruses listed, bacteria, and *Mycoplasma* spp.

Bovine coronavirus (BCV) has been identified in cattle pulled and treated for BRD and/or in healthy cattle in numerous studies in the United States and Canada and in European countries using viral isolations from nasal swabs and serology-detecting seroconversions indicating active infections (3,4,5–12). These cited studies have focused on virus isolations from the nasal cavity for the materials for virus isolation. Bovine coronavirus has also been identified in

Department of Veterinary Pathobiology (Fulton, Burge, Confer), Department of Veterinary Clinical Sciences (Step), Department of Animal Sciences (Wahrmund, Burken, Richards), and Department of Statistics (Payton), Oklahoma State University, Stillwater, Oklahoma 74078, USA; Agricultural Division, The Noble Foundation, Ardmore, Oklahoma 73401, USA (Cook).

Address all correspondence to Dr. Robert W. Fulton; telephone: 405-744-8170; fax: 405-744-5275; e-mail: robert.fulton@okstate.edu

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pneumonic lungs, often in combination with other viruses, bacteria, and/or *Mycoplasma* spp. (2,13,14). Experimental studies have identified BCV-infected cattle with epithelial lesions in the turbinates, trachea, and lungs as well as with interstitial pneumonia (15).

Previous studies have demonstrated that the presence or absence of various levels of BCV antibodies can be used to predict whether a calf would be treated in the feedlot (9,10). Several studies have indicated that cattle may be shedding BCV in the nasal secretions on arrival at the feedlot (d 0) or perhaps before delivery to the feedlot (6,12). It is therefore important to examine practices in the beef-breeding herd and the immune status of the calves for BCV before their entry into the auction-market system where they might be exposed to cattle that are shedding BCV.

The objectives of the present study were to: 1) compare BCV antibody levels in beef calves from different herds in samples collected post-weaning and before commingling with other herds; 2) correlate serum BCV antibodies in fresh calves (ranch-reared, non-commingled) collected before delivery to commercial feedlot with treatment for BRD after arrival at the feedlot; and 3) use virus isolation from nasal swabs and from lungs and serology to determine the dynamics of BCV infection in commingled, mixed-source calves transported to a research feedlot.

Materials and methods

Cattle studies

For the 2001 and 2002 studies (OSU-2001 and OSU-2002) conducted at Oklahoma State University (OSU), calves from southern Oklahoma and north-central Texas participated in the Noble Foundation (NF) Retained Ownership Program (ROP). The ROP was an educational program to enable ranchers to evaluate herd health and vaccination programs in their ranch-raised calves and to measure their performance in the feedlot. In the 2001 study, there were 159 spring-born calves from 18 different herds and in the 2002 study; there were 156 spring-born calves from 17 herds. The calves were raised on the respective ranches and had not been commingled with cattle from other ranches before delivery to feedlots. The calves had been on the premises for 45 d before shipment and were weaned, vaccinated with viral and bacterial vaccines, dehorned, and the males were castrated. The calves ranged in age from 8 to 10 mo in both studies. The calves were delivered to the Noble Foundation ranch in Marietta, Oklahoma, where they were identified and serums collected for serotesting. All calves tested negative for BVDV persistent infection (PI) status (virus isolation).

In the 2001 study, calves were then shipped by truck to a commercial feedlot near Guymon, Oklahoma. At the feedlot, the calves were administered a modified live viral (MLV) vaccine containing BHV-1, BVDV1a, PI-3V, and BRSV (BoviShield 4 MLV; Pfizer Animal Health, New York, New York, USA). The normal pull and treat regimen for that feedlot was followed, whereby a calf was pulled from the pen for respiratory disease when 1 or more of the following was present: depression, nasal discharge, lack of rumen fill, and lethargy. If the rectal temperature was 104°F or above, the animal was treated with an antimicrobial medication according to standard feedlot protocol. Calves were then shipped to a commercial feedlot near Oberlin, Kansas. Calves were negative for BVDV PI status by negative virus isolation. At the feedlot, the calves received a MLV vaccine containing BHV-1, BVDV1a, BVDV2a, PI-3V, and BRSV (Express 5 MLV; AgriLabs, Saint Joseph, Missouri, USA). The normal pull and treat regimen for that feedlot was followed. The calves were pulled as sick based on visual appearance in the pens and taken to the hospital pen. If the calf had a rectal temperature of 104°F or above, it was treated with an antimicrobial medication.

Three studies were also performed at Oklahoma State University (OSU) in 2009 (OSU-1, OSU-2, and OSU-3) using commingled, mixed-source, auction market calves delivered to the Willard Sparks Beef Research Center (WSBRC) feedlot at the Department of Animal Sciences, Oklahoma State University. All calves in the 2009 OSU studies tested negative for BVDV PI status by negative immunohistochemistry (IHC) BVDV tests using skin samples collected in formalin on d 0 of each study (16).

The first study in 2009 (OSU-1) consisted of 148 calves from an auction market in Baton Rouge, Louisiana and 38 head from an auction market in El Reno, Oklahoma. The calves were processed on June 2 and June 6, respectively. At processing, the calves received a modified live virus (MLV) vaccine containing BHV-1, BVDV1a, BVDV2a, PI-3V, and BRSV (Vista 5 SQ MLV; Intervet, Millsboro, Delaware, USA). All the calves received a second dose of MLV vaccine containing BHV-1, BVDV1a, BVDV2a, PI-3V, and BRSV on June 16 (Express 5 MLV; AgriLabs). Twenty-two calves were selected as sentinels for the study and were dispersed among the pens for the 184 cattle (2 were removed from the study). Nasal swabs (Universal Viral Transport Medium; BD Diagnostic Systems, Sparks, Maryland, USA) were collected from the sentinel calves on day 0 and at intervals thereafter. Serums were collected on day 0 and day 56. Nasal swabs were collected from the calves pulled for clinical BRD treatment along with serums on the day of treatment and day 56 of the study.

In both OSU-1 and OSU-2, bronchoalveolar lavage (BAL) washing samples were collected from sentinel animals and the cattle pulled for treatment. The procedure was modified from a technique described in a previous study (17). Calves were restrained in the chute and a bronchoalveolar lavage tube (Bivona Medical Technology, Gary, Indiana, USA) was inserted into the ventral meatus of a nostril, passed on into the trachea, and directed past the tracheal bifurcation and advanced into a distal lung lobe. The 60 mL of normal saline was then delivered so that the material would enter the lungs. Approximately 50% to 75% (30 to 45 mL) of the solution was aspirated and stored for later viral isolation procedures. The BAL samples were collected in OSU-1 and OSU-2 at d 0 and at intervals thereafter and also from the calves that met the treatment criteria. The criteria for pulling cattle from the pens for treatment were based on visual signs of clinical BRD and assigned a subjective severity score of 1 to 4. Calves that met the treatment protocol guidelines were treated with antimicrobial medication.

The second study in 2009 (OSU-2) consisted of 164 calves entered into a protocol from 180 calves purchased from an Ohio auction market, shipped to the WSBRC facility at Oklahoma State University (OSU), and processed on September 13, 2009 (day 0). The calves received a MLV vaccine on day 0 and day 14. The vaccine

Table I. OSU-2001 calves with bovine coronavirus antibodies before entering feedlot

Herd number	Number of head	Range of antibodies	Geometric mean	Statistical differences[a]
1	10	256 to 2048	896.00	C,D
2	5	4 to 16	8.80	J
3	10	256 to 1024	512.00	D,E
4	10	1024 to 8192	4096.00	A
5	9	32 to 512	202.67	F
6	8	< 4 to 8	2.63	K
7	5	16 to 32	19.20	I,J
8	10	< 4 to 256	50.50	I
9	10	8 to 256	49.60	H,I
10	10	64 to 256	96.00	F,G
11	10	128 to 1024	537.60	D,E
12	10	128 to 2048	883.20	C,D
13	10	256 to 1024	512.00	D,E
14	10	128 to 1024	345.60	E
15	10	512 to 4096	1280.00	B,C
16	7	1024 to 8192	2925.71	A,B
17	10	128 to 2048	486.40	E
18	5	32 to 128	64.00	G,H
Total	159	< 4 to 8192	2.63 to 4096.00	

[a] Values with letter(s) indicate that there was no statistical difference in the geometric mean titer of a herd from other herds with the same letter ($P > 0.05$).

Table II. OSU-2002 calves with bovine coronavirus antibodies before entry to feedlot

Herd number	Number of head	Range of antibodies	Geometric mean	Statistical differences[a]
1	10	256 to 1024	640.00	A
2	10	8 to 512	106.40	C,D
3	10	< 4 to 8	3.60	G
4	4	128 to 4096	1824.00	A
5	10	< 4 to 32	10.90	F
6	10	4 to 64	37.20	D,E
7	10	4 to 512	99.20	D,E
8	19	64 to 2048	640.00	A
9	10	32 to 1024	326.40	B,C
10	10	8 to 64	33.60	D,E
11	6	4 to 64	20.67	E,F
12	10	16 to 4096	516.80	C
13	10	32 to 2048	547.20	A,B
14	10	512 to 1024	665.60	A
15	4	128 to 512	256.00	A,B,C
16	5	< 4 to 8	3.00	G
17	8	< 4 to 16	4.50	G
Total	156	< 4 to 4096	3.00 to 1824	

[a] Values with letter(s) indicate that there was no statistical difference in the geometric mean titer of a herd from other herds with the same letter ($P > 0.05$).

contained BHV-1, BVDV1a, BVDV2a, PI-3V, and BRSV (Express 5 MLV; AgriLabs). Twenty calves were selected as sentinel calves and dispersed among the pens with the other cattle in the shipment of 164 calves. Nasal swabs, serums, and BAL samples were collected from the sentinel calves on day 0 and at intervals thereafter. In addition, nasal swabs, serums, and BAL samples were collected from the sick calves pulled for treatment when they were treated for BRD. Serums were collected from the sentinels and sick calves on day 56 of the study.

The third study in 2009 (OSU-3) consisted of 363 calves purchased from auction markets in Louisiana, Mississippi, and Oklahoma, shipped to Oklahoma State University (OSU), and processed on October 2, 2009 (day 0). At processing, the calves received a MLV vaccine containing BHV-1, BVDV1a, BVDV2a, PI-3V, and BRSV (Pyramid 5 MLV, Fort Dodge Animal Health, Fort Dodge, Iowa, USA). Twenty calves were selected at processing to serve as sentinels and dispersed among the pens with the other calves. At day 0, nasal swabs and serums were collected. Due to the protocol for the study, BAL samples were not collected at day 0 and there were no further nasal swabs collected from the sentinels or sick cattle. Serums were collected for the convalescent sample later in the study on day 177 or day 195. Calves were identified for treatment and their temperatures were recorded. The protocol for this study called for treatment based on the animal's subjective clinical attitude score and a rectal temperature of 104.5°F or above.

These studies were approved by the Oklahoma State University Institutional Animal Care and Use Committee (#VM0818 and VM#0819).

Serotesting

A microtitration virus neutralization test (VNT) in 96-well plates was used to quantitate antibodies to BCV using duplicate rows for the serum dilutions (18–23). The cell cultures used in the serotest were HRT (human rectal tumor) cells (18G; American Type Culture, Manassas, Virginia, USA). The challenge virus used in the VNT was a cytopathic BCV (USDA APHIS NVSL, Ames, Iowa, USA). Serial 2-fold dilutions of serum were made and the challenge virus was diluted to contain 100 $TCID_{50}$. Plates were incubated for 5 d in the 37°C incubator. The endpoint was the final serum/virus dilution that completely inhibited the viral cytopathic effects in both wells. Thus, 1:4 (1:2 dilution of serum to virus) was the lowest dilution tested. Positive and negative controls were used in each test run. The titers were expressed as the reciprocal of the endpoint dilution. Geometric mean titers (GMTs) were performed from the endpoint titers for each group. Animals with less than the detectable VNT antibodies (< 1:4) were assigned the whole number of 1 when the GMTs were determined. The data were analyzed by 1-way analysis of variance (ANOVA) or as a completely randomized design. When significant in the ANOVA, the effect of treatment was further analyzed with pair-wise *t*-tests using alpha = 0.05 (SAS Institute, Cary, North Carolina, USA).

Virus isolation

Filtered nasal swab samples and BAL samples were inoculated into freshly seeded HRT cells in 25-cm flasks containing 6 mL of cell culture medium, minimal essential medium (MEM) containing antibiotics, and 2% BVDV-free bovine fetal serum. Cultures were

Table III. OSU-1 sentinel calves with bovine coronavirus infections in commingled, mixed source calves after arrival at feedlot

		Virus isolation		Virus isolation		Virus isolation		Serology	
Calf number	Treated	Day 0 NS	Day 0 BAL	Day 8 NS	Day 8 BAL	Day 14 NS	Day 14 BAL	Acute	Convalescent
499	Neg	Neg	Neg	Neg	Neg	Neg	Neg	128	128
513	Neg	Pos	Pos	Neg	Neg	Neg	Neg	4	256
517	Neg	Neg	Neg	Neg	Neg	Neg	Neg	32	256
531	Neg	Neg	Neg	Neg	Neg	Neg	Neg	32	512
538	Neg	Pos	Pos	Neg	Neg	Neg	Neg	< 4	128
542	Pos	Pos	Pos	Neg	Neg	Neg	Neg	8	256
544[a]	Neg	Neg	Neg	Neg	Neg	Neg	Neg	128	NS
545	Neg	Pos	Pos	Neg	Neg	Neg	Neg	4	1024
546	Neg	Neg	Neg	Neg	Neg	Neg	Neg	256	1024
548	Neg	Neg	Neg	Neg	Neg	Neg	Neg	64	512
552	Neg	Pos	Pos	Neg	Neg	Neg	Neg	< 4	256
557	Neg	Neg	Neg	Neg	Neg	Neg	Neg	256	512
559	Neg	Neg	Neg	Neg	Neg	Neg	Neg	512	512
562[a]	Pos	Neg	Neg	Neg	Neg	Neg	Neg	64	NS
563	Neg	Pos	Pos	Neg	Neg	Neg	Neg	< 4	256
576	Neg	Pos	Pos	Neg	Neg	Neg	Neg	< 4	256
580	Neg	Neg	Neg	Neg	Neg	Neg	Neg	32	512
587	Neg	Neg	Neg	Neg	Neg	Neg	Neg	256	32
592	Neg	Pos	Pos	Neg	Neg	Neg	Neg	< 4	64
609	Neg	Pos	Pos	Neg	Neg	Neg	Neg	< 4	256
512	Neg	Neg	Neg	Neg	Neg	Neg	Neg	256	128
526	Neg	Neg	Neg	Neg	Neg	Neg	Neg	64	512

NS — No sample collected.

[a] Calf removed from study.

incubated for 6 d and observed daily for viral cytopathic effect (CPE). At the end of the incubation, the cultures were subjected to a freeze-thaw cycle, clarified by centrifugation, and stored frozen (23). Regardless of viral CPE being observed, all samples were tested for BCV using a reverse transcriptase gel-based polymerase chain reaction (PCR) assay (24). Samples from the infected flasks were considered positive for BCV virus if the PCR results were positive, regardless of whether viral CPE was observed or not.

Selected BCV from the infected cell cultures were further examined by neutralization using a BCV monoclonal antibody, lot WR99316 BC28 H1.2C against N protein (3). Equal volumes of the diluted monoclonal antibody and undiluted virus were mixed and incubated for 1 h at 37°C. A negative control serum negative for BCV antibodies was also used. After the incubation, the mixtures were then assayed in 96-well plates using HRT cells and the cultures were returned to the incubator for 6 d and observed daily for CPE (18,19). The infectivity in the BCV positive serum plus virus and the negative BCV serum plus virus were titrated for $TCID_{50}$ (18,19).

Statistical analysis

All statistical analyses were done using PC SAS Version 9 (SAS Institute). Means, standard errors, and ranges were calculated and reported. Analysis of variance procedures were used to ascertain overall differences in herds (treatments) and if significant, pair-wise *t*-tests were calculated to further investigate relevant herd effects. A significant level of 0.05 was used for all comparisons.

Results

Bovine coronavirus (BCV) serology in beef calves from ranches

In the OSU study carried out in 2001, BCV-neutralizing antibody titers in the calves for the respective herd in the group ranged from < 4 to 8192 (Table I). The lowest to highest geometric mean titers were 2.63 to 4096. There were significant differences in the BCV antibody titers among the 18 herds, ($P < 0.05$) (Table I). Ninety out of 159 calves (56.6%) from these herds were treated in the feedlot. In the OSU study carried out in 2002, the BCV-neutralizing antibody titers in the calves in the group ranged from < 4 to 4096 and the geometric mean titers among the 2002 herds ranged from 3.00 to 1824 (Table II). There were significant differences in the BCV antibody titers among the 17 herds, ($P < 0.05$) (Table II). Twenty-seven out of 156 calves (17.3%) in the feedlot were treated for bovine respiratory disease (BRD).

The BCV antibody titers for the treated and non-treated calves in each study were compared to determine a relationship between antibody levels at entry to the feedlot and when treated for BRD. In both the 2001 and 2002 OSU studies, calves with a BCV-neutralizing antibody titer of 16 or below were more likely to be treated for BRD than those calves with a titer of 32 and above. Thus for the 2001 study, $P = 0.0207$, for the 2002 study, $P = 0.0007$, and for both studies combined, $P = 0.0018$.

Table IV. OSU-2 sentinel calves — Bovine coronavirus infections in commingled, mixed source calves after arrival at feedlot

		Virus isolation		Virus isolation		Serology	
Calf number	Treated	Day 0 NS	Day 0 BAL	Day 8 NS	Day 8 BAL	Acute	Convalescent
665	Pos	Pos	Neg	Neg	Neg	16	256
667	Neg	Pos	Neg	Neg	Neg	8	128
678	Neg	Pos	Neg	Neg	Neg	4	512
692	Neg	Neg	Neg	Neg	Neg	256	1024
724	Neg	Neg	Neg	Neg	Neg	64	256
739	Neg	Pos	Neg	Neg	Neg	4	256
746	Neg	Pos	Pos	Neg	Neg	< 4	1024
747	Neg	Pos	Pos	Neg	Neg	4	1024
749	Neg	Neg	Neg	Neg	Neg	32	512
764	Neg	Pos	Neg	Neg	Neg	128	1024
773	Neg	Neg	Neg	Neg	Neg	32	2048
776	Neg	Pos	Neg	Neg	Neg	8	512
778	Neg	Pos	Pos	Neg	Neg	8	32
801	Pos	Pos	Pos	Neg	Neg	4	128
802	Pos	Pos	Pos	Neg	Neg	8	256
813	Pos	Pos	Pos	Neg	Neg	128	4096
820	Neg	Neg	Neg	Neg	Neg	256	256
821	Neg	Pos	Neg	Neg	Neg	32	256
833	Pos	Pos	Pos	Neg	Neg	8	256
834	Neg	Pos	Pos	Neg	Neg	4	512

Bovine coronavirus (BCV) infections in commingled mixed source auction market calves after arrival at feedlot

The sentinel calves (10,15,24) in the 3 OSU studies carried out in 2009 were studied by virus isolation and serology, detecting active infection by a 4-fold or higher rise in BCV-neutralizing antibody titers in acute to convalescent samples (Tables III to V). Of the 22 calves used as sentinel calves in OSU-1, 9 out of 22 (40.9%) were BCV virus positive in both the nasal swabs and the BAL samples on the day of processing, day 0 (Table I). Calves shedding the virus on day 0 cleared the virus by day 8 as nasal swab and BAL samples were all negative at collection day 8. Convalescent serum was not collected from 2 of the calves as 1 calf died with BRD (#562) and another calf (#544) was removed from the study due to lameness. Fifteen of the remaining 20 sentinel calves (75%) seroconverted. Calves that were shedding BCV at day 0 had BCV antibody levels of 8, 4, or < 4 on day 0, whereas calves with BCV antibody titers of 32 or higher at d 0 did not shed virus during the study, although they often seroconverted. Six sentinel animals remained healthy and seroconverted to BCV.

In the OSU-1 study, 41 out of 184 calves (22.3%) were pulled for BRD treatment in the first 14 d after arrival, 13 in the first 4 d, and 28 from day 5 to day 14. The only calves with BCV isolations were 6 out of 13 in the first 4 d (Table VI) and all 6 were BCV virus positive in both nasal swabs and BAL samples. In those 6 calves, BCV antibody titers were 8 in 1 animal or < 4 in 5 animals. Of the remaining sick calves that were negative for BCV in the first 4 d, only 1 seroconverted. The 5 calves that were not seroconverting had BCV titers of 64 or higher at d 0.

In the OSU-2 study, 15 out of 20 (75%) of the sentinel calves were shedding virus in the nasal swabs on d 0 and 8 of those (8 out of 20, 40%) were shedding virus for BCV in the BAL samples (Table IV). When they were negative for BCV in both the nasal swabs and BAL samples, calves cleared the BCV infection by d 8. Nineteen of the 20 sentinel calves (95%) seroconverted to BCV. All but 3 calves (#764, #813, and #821) had BCV antibody titers of 16 or less at day 0, whereas calves #764 and #813 had titers of 128, and calf #821 had a titer of 32. All 3 of those calves, however, seroconverted to BCV. Five calves that were virus positive at day 0 were later pulled for BRD treatment.

In the OSU-2 study, 34 out of 164 calves (20.7%) were pulled for treatment in the first 14 d after arrival (Table VI). Four out of 14 calves pulled in the first 4 d were positive for BCV in the nasal swabs and 3 of those 4 positive calves were also positive for BCV in the BAL samples. Eleven of the 14 calves pulled for BRD treatment in the first 4 days seroconverted to BCV and the remaining calves had BCV titers of > 2048 when they were pulled for treatment. There were 20 calves pulled for BRD treatment from day 5 to day 14 and all were negative for BCV in both the nasal swabs and BAL samples. Of those 20 calves, only 5 seroconverted. These 5 calves that seroconverted had titers at day of pull of 32 to 128 and still seroconverted. The titers for the remaining calves that did not seroconvert ranged from 128 to $\geq 32\,768$ on the day of treatment.

In the OSU-3 study, 17 out of 20 sentinel calves (85%) were positive for BCV in the nasal swabs at d 0 (Table V). No BAL samples were collected and no other subsequent collections were made from the sentinels or sick calves in the 363-head shipment. Two calves, both of which were BCV positive at day 0, died with BRD

Table V. OSU-3 sentinel calves — Bovine coronavirus infections in commingled, mixed source calves after arrival at feedlot

Calf number	Virus positive day 0	Date treated	Rectal temperature	Serology	
				Acute	Convalescent
3162	Pos	D3	105.3°F	32	512
3163	Pos	D2	105.9°F	16	512
3164	Pos	D22	106.2°F	8	2048
3165	Pos	D4	105.8°F	32	1024
3166[a]	Pos	D5	105.4°F	16	Died
3167	Pos	D1	107.4°F	8	4096
3168	Pos	D8	105.3°F	32	512
3169	Pos	D1	106.1°F	16	512
3170	Pos	D2	105.3°F	16	1024
3171[a]	Pos	D2	106.9°F	16	Died
3172	Pos	Neg	NT	32	2048
3173	Neg	Neg	NT	128	512
3174	Pos	D4	105.7°F	256	512
3175	Pos	D6	106.1°F	16	1024
3176	Pos	D5	104.6°F	16	512
3177	Neg	Neg	NT	64	1024
3178	Pos	Neg	NT	64	512
3179	Neg	Neg	NT	64	512
3180	Pos	Neg	NT	16	256
3181	Pos	D4	105.1°F	16	512

NT — not taken.

[a] Died with BRD.

(#3166 and #3171). Of the remaining 18 sentinel calves in the study, 17 out of 18 (94.4%) seroconverted to BCV (Table V). The 3 calves that were negative for BCV on d 0 also seroconverted. One calf that was positive for BCV at d 0 did not seroconvert (#3174) (acute titer, 256 and convalescent, 512). Fourteen sentinel calves were pulled for BRD treatment and all 14 were shedding BCV in nasal swabs at d 0. Of the remaining 6 calves not treated for BRD in the study, 3 were positive for BCV at d 0 and 3 were negative. All 6 calves not treated in the study seroconverted to BCV.

Neutralization results for bovine coronavirus (BCV)

Twelve BCV isolates (6 from sentinel calves and 6 from sick calves) from OSU-1 were used to study the ability of a BCV monoclonal antibody to neutralize the virus. The monoclonal antibody, WR 99316, reduced the infectivity of the 12 isolates by 4.0 to 5.25 $\log_{10}$. The reduction by at least 4 $\log_{10}$ or greater using 400 units of the monoclonal antibody indicates that these isolates had antigenic characterization for BCV in addition to the genomics for BCV detected by the polymerase chain reaction (PCR) assay.

Discussion

Bovine coronavirus has previously been investigated using nasal and serum samples from feedlot cattle undergoing treatment for BRD and these calves were from mixed sources, often reported as auction-market-derived calves (3,6–8,10–12,24). In 1 study of ranch-raised calves and commingled, auction-market-derived calves delivered to a feedlot, the ranch-raised calves had high antibody titers at arrival and probably represented calves that had been exposed to BCV at the ranch before delivery (12).

The current study, which focused on multiple aspects of BCV infections, differed substantially from previous reported studies. Firstly, the immune status for BCV on calves representing individual ranches was studied using serums collected from the calves delivered directly from the ranch and that had not been exposed to cattle from other ranches. Secondly, in contrast to other studies, in this study a virus neutralization test (VNT) was used on the ranch calves to derive the mean titers. Thirdly, the levels of BCV virus neutralization test (VNT) antibodies were used to make a correlation as to whether various levels of antibodies could be used to predict whether a calf would be treated for BRD at the feedlot and observed after arrival. Fourthly, we detected BCV in the respiratory tract of cattle using both nasal swabs and BAL samples.

In the 2001 and 2002 OSU studies, calves from 35 ranches were studied to measure the level of BCV antibodies in the post-weaned calves delivered to the feedlot. There were significant differences in the mean antibody levels represented in each herd and a wide range of mean antibody titers from almost non-detectable to substantial (4096). This indicates that there was considerable difference in the exposure to BCV among those herds. These calves had not received any BCV vaccines before delivery and none of the herds used BCV vaccinations in the pregnant cow to stimulate transfer of BCV antibodies via colostrum to the calf. In those calves 8 mo of age or older, the BCV-neutralizing antibodies detected in the calves could perhaps have represented those derived from maternal immunity. In a prior study measuring the maternal-derived antibodies in calves including BHV-1, BVDV1a, BVDV1b, BVDV2a, BRSV, and PI-3V, the mean age

Table VI. BCV virus isolation and serology for commingled, mixed source calves after arrival at feedlot and treatment for BRD

			Virus isolation			
	Calf	Day	Nasal		Serology	
Study	number	collected	swab	BAL	Acute	Convalescent
OSU-1	524	1	Neg	Neg	256	256
	575	2	Pos	Pos	8	128
	603	2	Pos	Pos	< 4	256
	611	2	Neg	Neg	1024	256
	486	3	Neg	Neg	128	128
	521	3	Pos	Pos	< 4	256
	529	3	Neg	Neg	128	NS
	554	3	Pos	Pos	< 4	256
	591	3	Pos	Pos	< 4	256
	482	4	Neg	Neg	64	512
	488	4	Neg	NS	128	128
	600	4	Pos	Pos	< 4	64
OSU-2	688	1	Neg	Neg	32	256
	740	1	Neg	Neg	16	512
	769	1	Pos	Pos	4	512
	787	1	Pos	Neg	16	512
	797	1	Pos	Pos	16	256
	676	2	Neg	Neg	64	1024
	681	2	Neg	Neg	64	512
	801	2	Neg	Neg	64	128
	802	2	Pos	Pos	32	256
	815	2	Neg	Neg	32	256
	718	4	Neg	Neg	> 2048	512
	762	4	Neg	Neg	64	512
	775	4	Neg	Neg	> 2048	1024
	829	4	Neg	Neg	> 2048	1024

BCV — bovine coronavirus; BRD — bovine respiratory disease; NS — no sample.

at which the calves from nonvaccinated cows became seronegative was: BHV-1, 122.9 d; BVDV1a, 192.2 d; BVDV1b, 179.1 d; BVDV2a, 157.8 d; PI-3V, 190.6 d; and BRSV, 186.7 d (23). With the calves in this study ranging from 8 to 10 mo in age, a few calves might have had maternal antibodies remaining or those antibodies may have been those remaining after decline. The half-life of the maternal antibodies to the respective virus listed above ranged from 21.2 to 35.9 d (23). A future project would be to investigate the maternal transfer of BCV antibodies to the neonate and the half-life of those antibodies.

A significant finding in this study was that the levels of the BCV-neutralizing antibodies in ranch-raised calves upon entry to the feedlot were predictive of whether a calf might be treated for clinical BRD in the feedlot after arrival. Calves with very low levels of BCV-neutralizing antibodies (≤ 16) were more likely to be treated than calves with higher levels. In future studies on BRD under feedlot conditions, serology might be used to determine the exposure and immune status for BCV.

The current study also identified and confirmed that calves commingled from mixed sources, from auction-market sources, and from wide geographic regions across the midwestern and south-central US states probably have BCV-active infections upon delivery to the feedlot and are shedding the virus. Similar to those in other studies, the calves in this study cleared the infections by day 8 after arrival. Also similar to other studies, the virus was found in the nasal swabs. In this study, BCV was also recovered in lung samples [bronchoalveolar lavage (BAL)], which were collected along with the nasal swabs. While BCV is not unlike other viruses that are shed in the nasal swabs during active infections, the finding of the BCV in the lung-derived samples suggests that BVC probably plays a role in lung lesions such as pneumonias.

Bovine coronavirus (BCV) appears to be an early type of infection among the commingled calves. Calves in 2 different groups in this study identified BCV infections (nasal swab and BAL virus isolations) from sick calves in the first 4 d after arrival, but not from calves from 5 to 14 d after arrival. Another aspect of this study was that BCV was recovered from some healthy calves as well. In addition, active infections for BCV appear quite common as noted by the large number of seroconversions in both sick and healthy animals. It is common to find seroconversions to several bovine viruses among cattle under feedlot conditions as noted for BVDV, PI-3V, and BRSV (20,21).

The finding of varied levels of BCV immunity among beef-breeding herds is an important aspect of this study. This study

also demonstrated again that calves entering the marketing channel where they may mix with calves from different sources might become infected during the process of sale and delivery to the feedlot or during backgrounding operations. It would appear from the results of this study that BCV immunoprophylaxis (delivery of vaccines) would be best for the calves before weaning, giving them sufficient time to gain active immunity before commingling with other cattle.

Further studies are needed to experimentally document the ability of BCV to cause lung lesions. Such studies would be especially important to demonstrate a challenge system to measure the efficacy of BCV vaccines.

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