Bovine viral diarrhea viral infections in feeder calves with respiratory disease: Interactions with *Pasteurella* spp., parainfluenza-3 virus, and bovine respiratory syncytial virus

Robert W. Fulton, C.W. Purdy, Anthony W. Confer, J.T. Saliki, Raymond W. Loan, Robert E. Briggs, Lurinda J. Burge

Abstract

The prevalence of bovine viral diarrhea virus (BVDV) infections was determined in a group of stocker calves suffering from acute respiratory disease. The calves were assembled after purchase from Tennessee auctions and transported to western Texas. Of the 120 calves, 105 (87.5%) were treated for respiratory disease. Sixteen calves died during the study (13.3%). The calves received a modified live virus BHV-1 vaccine on day 0 of the study. During the study, approximately 5 wk in duration, sera from the cattle, collected at weekly intervals, were tested for BVDV by cell culture. Sera were also tested for neutralizing antibodies to BVDV types 1 and 2, bovine herpesvirus-1 (BHV-1), parainfluenza-3 virus (PI-3V), and bovine respiratory syncytial virus (BRSV). The lungs from the 16 calves that died during the study were collected and examined by histopathology, and lung homogenates were inoculated onto cell cultures for virus isolation. There were no calves persistently infected with BVDV detected in the study, as no animals were viremic on day 0, nor were any animals viremic at the 2 subsequent serum collections. There were, however, 4 animals with BVDV type 1 noncytopathic (NCP) strains in the sera from subsequent collections. Viruses were isolated from 9 lungs: 7 with PI-3V, 1 with NCP BVDV type 1, and 1 with both BVHV-1 and BVDV. The predominant bacterial species isolated from these lungs was *Pasteurella haemolytica* serotype 1. There was serologic evidence of infection with BVDV types 1 and 2, PI-3V, and BRSV, as noted by seroconversion (≥ 4-fold rise in antibody titer) in day 0 to day 34 samples collected from the 104 survivors: 40/104 (38.5%) to BVDV type 1; 29/104 (27.9%) to BVDV type 2; 71/104 (68.3%) to PI-3V; and 81/104 (77.9%) to BRSV. In several cases, the BVDV type 2 antibody titers may have been due to crossreacting BVDV type 1 antibodies; however, in 7 calves the BVDV type 2 antibodies were higher, indicating BVDV type 2 infection. At the outset of the study, the 120 calves were at risk (susceptible to viral infections) on day 0 because they were seronegative to the viruses: 98/120 (81.7%), < 1:4 to BVDV type 1; 104/120 (86.7%) < 1:4 to BVDV type 2; 86/120 (71.7%) < 1:4 to PI-3V; 87/120 (72.5%) < 1:4 to BRSV; and 111/120 (92.5%) < 1:10 to BHV-1. The results of this study indicate that BVDV types 1 and 2 are involved in acute respiratory disease of calves with pneumonic pasteurellosis. The BVDV may be detected by virus isolation from sera and/or lung tissues and by serology. The BVDV infections occurred in conjunction with infections by other viruses associated with respiratory disease, namely, PI-3V and BRSV. These other viruses may occur singly or in combination with each other. Also, the study indicates that purchased calves may be highly susceptible, after weaning, to infections by BHV-1, BVDV types 1 and 2, PI-3V, and BRSV early in the marketing channel.

Résumé

La prévalence de l'infection par le virus de la diarrhée virale bovine (BVDV) fut déterminée chez un groupe de veaux affectés de maladie respiratoire aiguë. Les veaux furent regroupés après avoir été achetés dans des encans au Tennessee et transportés au Texas. Un total de 105 veaux (87,5 %) furent traités pour des problèmes respiratoires, et 16 animaux (13,3 %) sont morts au cours de l'étude. Les veaux reçurent un vaccin BHV-1 vivant modifié au jour 0 de l'étude. Au cours des 5 semaines suivantes, du sérum fut prélevé des animaux et la présence de BVDV vérifiée par culture cellulaire. Les sérums furent également éprouvés pour la présence d'anticorps neutralisants envers BVDV 1 et 2, l'herpesvirus bovin de type 1 (BHV-1), le virus parainfluenza-3 (PI-3) et le virus respiratoire syncytial bovin (BRSV). Les poumons des 16 veaux morts au cours de l'étude furent soumis à un examen histopathologique, et des préparations homogénéisées de poumon

Department of Infectious Diseases and Physiology (Fulton, Saliki, Burge), Department of Anatomy, Pathology and Pharmacology (Confer), Oklahoma Animal Disease Diagnostic Laboratory (Saliki), College of Veterinary Medicine, Oklahoma State University, Stillwater, Oklahoma 74078 USA; United States Department of Agriculture (USDA), Agriculture Research Service (ARS), Conservation and Production (Purdy), Bushland, Texas 79012 USA; Department of Veterinary Pathobiology, College of Veterinary Medicine, Texas A & M University, College Station, Texas 77843 USA (Loan); and USDA, ARS, National Animal Disease Center, P.O. Box 70, Ames, Iowa 50010 USA (Briggs).

Address correspondence and reprint requests to Dr. Robert W. Fulton, telephone: 405-744-6738; fax: 405-744-8263; e-mail: rfulton@okstate.edu.

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inoculées sur culture cellulaire pour isolement viral. Aucun veau infecté de façon persistante par le BVDV ne fut détecté dans cette étude étant donné qu'aucun animal n'était virémique au jour 0 de même que lors des deux prélèvements subséquents. Toutefois, quatre animaux se sont révélés porteurs d'une souche non-cytopathogène de BVDV type 1 lors des prélèvements suivants. Des virus furent isolés à partir de 9 poumons : 7 avec du PI-3, 1 avec du BVDV type 1 non-cytopathogène, et 1 avec du BHV-1 et du BVDV. Le principal isolat bactérien retrouvé dans les poumons était Pasteurella haemolytica *type 1. Les analyses sérologiques des échantillons prélevés au jour 0 et au jour 34 ont démontré des évidences d'infection par BVDV types 1 et 2, PI-3 et le BRSV chez les 104 veaux survivants : 40/104 (38,5 %) pour le BVDV type 1; 29/104 (27,9 %) pour le BVDV type 2; 71/104 (68,3 %) pour le PI-3; et 81/104 (77,9 %) pour le BRSV. Dans plusieurs cas les titres d'anticorps envers le BVDV type 2 pourraient être attribuables à des réactions croisées avec des anticorps contre le BVDV de type 1. Toutefois, chez 7 veaux, les titres d'anticorps contre le BVDV type 2 étaient plus élevés, suggérant ainsi une infection par le type 2. Au début de cette étude, les 120 veaux étaient à risque (susceptible à une infection virale) dû au fait qu'ils étaient séro-négatifs envers les différents virus : 98/120 < 1 :4 au BVDV type 1; 104/120 (86,7 %) < 1 :4 au BVDV type 2; 86/120 (71,7 %) < 1 :4 au virus PI-3; 87/120 (72,5 %) > 1 :4 au BRSV; et 111/120 (92,5 %) < 1 :10 au BHV-1. Les résultats de cette étude démontrent que les virus BVDV de types 1 et 2 sont impliqués dans les problèmes respiratoires aigus chez les veaux en association avec la pasteurellose. La présence de BVDV peut être mise en évidence par isolement viral à partir du sérum et/ou du tissu pulmonaire ou par sérologie. Les infections par le BVDV surviennent en conjonction avec d'autres infections virales associées aux maladies respiratoires, tels les virus PI-3 et BRS. Ces autres virus peuvent être rencontrés lors d'infection unique ou mixte. De plus, cette étude démontre que des veaux achetés peuvent être très susceptibles après le sevrage à des infections par BHV-1, BVDV types 1 et 2, PI-3V et BRSV, tôt suite à leur introduction dans la chaîne de production.*

(Traduit par docteur Serge Messier)

Introduction

Bovine viral diarrhea virus (BVDV) is an important pathogen of the dairy and beef cattle populations and is associated with many clinical forms (1). The respiratory system is but one of the systems susceptible to BVDV infection (1,2). The role of BVDV in bovine respiratory disease has been the subject of a recent review (2). This virus is capable of primary infection of the bovine lung, as demonstrated by inoculation of calves with BVDV (3,4). Also, BVDV may act in combination with other agents, such as *Pasteurella haemolytica*, bovine herpesvirus-1 (BHV-1), and bovine respiratory syncytial virus (BRSV), as demonstrated in in vivo studies in calves (3,5,6).

There are numerous clinical epidemiological studies demonstrating that BVDV was associated with bovine respiratory disease (BRD). These studies have either been prospective studies, where the cattle were monitored from the cow herd, through the marketing channels, to the feedlot, and/or retrospective studies of BRD cases from the diagnostic laboratories with virus identification and serologic results. Based on serology, active infection with BVDV has been described in several studies with BRD disease (7–15). A study from a diagnostic laboratory in Texas involved with diagnosis of "shipping fever" in feedlot cattle reported BVDV was the most common (21%) viral isolate from pneumonic lungs and that there was seroconversion to both BVDV and *P. haemolytica* in treated cattle (16).

The BVDV is a member of the genus *Pestivirus* of the family Flaviviridae (17). There are 2 biotypes, based on presence or absence of cytopathology in inoculated cell cultures [cytopathic (CP) or noncytopathic (NCP)] (1). There are 2 genotypes, based on RNA genomic differences detected by polymerase chain reaction (PCR), and 2 antigenic differences (type 1 and type 2) (18,19).

When susceptible cattle are exposed to BVDV postnatally they usually recover and shed virus temporarily, for a few days only. However, when pregnant susceptible heifers/cows are exposed to BVDV, the fetus is highly susceptible to infection and the outcome depends on the gestational stage of the fetus at the time the virus crosses the placenta (1,20). Fetuses infected with NCP BVDV strains prior to day 125 of gestation may become persistently infected. These calves are born immunotolerant to that strain and shed virus throughout their lifetime (20). These persistently infected cattle are believed to be the most important reservoirs of infection for susceptible cattle.

The purpose of this study was 4-fold: (1) to detect presence of persistently infected calves entering the livestock market system and determine their potential to expose other cattle; (2) to determine the presence of BVDV in BRD cases by viral isolation from samples from cattle and/or by seroconversion; (3) to reaffirm concurrent BVDV infections with other viruses associated with BRD; and 4) to examine if both BVDV types (1 and 2) can occur in cattle with BRD.

Material and methods

Cattle

The cattle were involved in a study to determine the factors associated with acute respiratory disease in young beef cattle (post-weaning), subsequent to entering the marketing system. The cattle, weighing from 315 to 495 lbs (143 to 225 kg), were purchased from 3 local auctions (3 sale barns) in eastern Tennessee on September 29 and 30, 1998, and arrived at a nearby order buyer's pens on September 30 and October 1, 1998. There, they received their initial treatments and vaccinations and were initially sampled. These samples represented the day 0 samples for this study. On day 0, the calves received a clostridial vaccine, (Ivovec Plus injection; 1% ivermectin w/v + 10% clorsulon w/v; Merck AgVet Division, Merck and Company, Rahway, New Jersey, USA) and a monovalent BHV-1 modified live virus (MLV) vaccine (Reliant; Rhone Merieux,

Table I. Serum antibody titers to BVDV types 1 and 2, PI-3V, and BRSV in 120 calves at initial collection on day 0

	Virus			
Dilution[a]	BVDV type 1	BVDV type 2	PI-3V	BRSV
0[b]	98 (81.7%)[c]	104 (86.7%)	86 (71.7%)	87 (72.5%)
4	5	3	12	21
8	4	1	9	8
16	4	3	7	3
32	3	4	1	1
64	0	2	4	0
128	1	2	0	0
256	0	0	1	0
512	2	0	0	0
1024	3	0	0	0
2048	0	1	0	0

[a] Reciprocal of highest dilution with complete neutralization of virus in both wells

[b] No neutralization at 1:4, the lowest dilution tested

[c] Number of animals

Inc., Athens, Georgia, USA). The calves were held at the order buyer's pens until shipment on October 4 via semi-trailer truck (approximately 1990 km), and arrived at a research feedyard at Bushland, Texas on October 5. The calves were sampled the next day, October 6 (day 6) and weekly thereafter (days 13, 20, 27 and 33). The study also involved treatments for metaphylaxis with an injectable *Pasteurella haemolytica* bacterin, and investigation of the environmental dust in the feedyard. These studies are subjects of other reports.

Serology

A virus neutralization test (VNT) in Madin-Darby bovine kidney (MDBK) cells in 96-well microtiter plates was used to quantitate viral neutralizing antibodies to BVDV types 1 and 2, PI-3V, and BRSV (21,22). The viruses used in the microtiter VNT were: CP BVDV type 1 (Singer strain), CP BVDV type 2 (125-C strain), PI-3V (SF-4 strain), and a BRSV vaccine strain (21,22). The 1:4 final dilution was the lowest dilution tested. A plaque reduction assay (PRA) was used to detect virus neutralizing antibodies to BHV-1 using the Cooper strain in MDBK cells with 24-well plates (21). The 1:10 final dilution was the lowest dilution tested. In this study, 0 or negative titers represented < 1:4 for BVDV types 1 and 2, PI-3V, BRSV, and < 1:10 for BHV-1. Titers expressed represent reciprocals of the endpoint titers (ie, a titer of 4 is actually 1:4).

Virus isolation

The viral isolations were performed at the Oklahoma Animal Disease Diagnostic Laboratory (OADDL), Oklahoma State University. A monolayer enzyme-linked immunosorbent assay (M-ELISA) was used to detect BVDV in sera (23). This procedure, as described, was modified from established indirect ELISA procedures. Reagents and cells included: bovine turbinate (BT) cells in 96-well plates; monoclonal antibodies (MAbs), 15c5 and 20.10.6, reactive to BVDV; peroxidase-conjugated sheep anti-mouse immunoglobulin G; and a 3,3′,5,5′-tetramethyl benzidine-hydrogen peroxide chromogen-substrate mixture. A sample was considered negative if there was no positive color reaction after one passage.

To isolate viruses from lungs, tissue homogenates were inoculated onto BT monolayers in 24-well plates as described (23). After 5 to 7 d, a second passage was made by subculturing trypsinized cells, and for another 5 to 7 d, samples were observed for viral cytopathology. At the end of the second passage, the trypsinized cells were stained using an indirect fluorescent antibody (FA) procedure with MAbs 15c5 and 20.10.6. Cytopathic agents other than BVDV (BRSV, PI-3V, and BHV-1) were tested for by direct FA tests by the OADDL.

Genotyping of BVDV

The BVDV isolates from the sera and lung tissues were typed by using a 2-step reverse transcription nested PCR assay, as described previously (24–26).

Bacterial isolation and identification

Bacterial isolation from lung tissues and serotyping of the isolates were performed as described previously (27,28).

Pathology

Lung tissues collected at necropsy were fixed in 10% buffered formalin, and processed for histopathology. Sections were stained with hematoxylin and eosin, and, in a blinded study, were examined by one of the authors (AWC) and morphologic diagnosis was rendered.

Results

Serology at arrival

The initial sera collected on day 0 and tested for BHV-1, BVDV types 1 and 2, PI-3V, and BRSV antibodies indicated numerous seronegative calves. There were 111/120 (92.5%) calves negative for

Table II. Pulmonary lesions, bacterial isolates, and viral isolates in 16 calves dying during a study with respiratory signs

Animal number	Day of death	Pneumonia	Bacterial isolates	Viral isolates
36	27	Subacute, severe, fibrinopurulent bronchiolitis and bronchopneumonia	*Pasteurella multocida*	PI-3V
72	7	Acute, severe, fibrinopurulent alveolitis/pneumonia Acute bronchiolitis, suppurative Alveolar hemorrhage, severe	*Pasteurella haemolytica* A1 *P. multocida*	PI-3V
85	5	Acute, fibrinopurulent alveolitis/pneumonia Pulmonary emphysema and acute minimal bronchiolitis with mild alveolar hemorrhage	*P. haemolytica* A1	PI-3V
100	36	Fibrinopurulent bronchopneumonia Acute interstitial pneumonia Giant cells present	*P. multocida*	PI-3V
104	13	Acute fibrinopurulent alveolitis/pneumonia with extensive hemorrhage and necrosis	*P. haemolytica* A1	PI-3V
110	6	Acute, severe, fibrinous, pleuropneumonia with multifocal necrosis Acute, severe, bronchiolitis with epithelial necrosis and regeneration	*P. haemolytica* A1 *P. multocida*	PI-3V
15	31	Acute to subacute suppurative bronchopneumonia	Unknown isolate; possibly *P. haemolytica* A2 or A6	PI-3V
102	11	Peracute fibrinopurulent bronchopneumonia with extensive lobular necrosis and thrombosis of vessels	*P. haemolytica* A1	BVDV type 1
115	14	Acute severe fibrinopurulent pneumonia with extensive hemorrhage and necrosis	*P. haemolytica* A1	BVDV[a] BHV-1
10	6	Moderate fibrinous pleuropneumonia (alveolitis)	*P. haemolytica* A1	Negative
11	6	Moderate fibrinous pleuropneumonia with moderate necrotizing bronchiolitis	*P. haemolytica* A2	Negative
14	6	Moderate to severe fibrinous pleuropneumonia with segmental bronchiolitis and fibrinoid arterial necrosis	*P. haemolytica* A1 *P. multocida*	Negative
71	7	Acute fibrinopurulent alveolitis/pneumonia	*P. haemolytica* A1 *P. multocida*	Negative
91	6	Fibrinopurulent alveolitis/pneumonia, acute	*P. haemolytica* A1	Negative
106	7	Severe acute fibrinous pneumonia with alveolitis	*P. haemolytica* A1	Negative
114	7	Severe acute fibrinohemorrhagic pneumonia with minimal necrosis	*P. haemolytica* A1	Negative

[a] BVDV not typed due to interference of BHV-1

BHV-1, 98/120 (81.7%) were negative for BVDV type 1, 104/120 (86.7%) were negative for BVDV type 2, 86/120 (71.7%) were negative for PI-3V, and 87/120 (72.5%) were negative for BRSV (Table I).

Respiratory disease morbidity and mortality

During this study, 105 (87.5%) of the 120 calves were treated for disease after clinical signs were observed. Respiratory disease signs included nasal and ocular discharges, coughing, labored breathing, and elevated body temperatures. Diarrhea was observed occasionally. Treatments included a regimen of oral neomycin sulfate, injectable oxytetracycline, or injectable tilmicosin. The number of treatments required for the sick calves ranged from 1 to 7 treatments. There were 16 animals (13.3%) that died during the study. The first death occurred during the experiment on day 5, and the last death occurred on day 36, 3 d after the end of the experiment (Table II). The calf that died on day 36 had been treated 6 times during the study and was considered part of the mortality group. There were 4 calves that died that had not been treated. These occurred early in the study, and the animals were either found dead or were found with severe signs and moribund.

Lung lesions with bacteriology and virology testing

The lesions in the lungs of the 16 calves that died were consistent with those involved in pneumonic pasteurellosis, primarily fibrinopurulent pneumonia (Table II). *Pasteurella haemolytica* serotype A1 was isolated from 12 of 16 cases; *P. haemolytica* A2 from 1 case; and an unknown isolate, possibly *P. haemolytica* A2 or A6, from 1 case. In 8 of the lungs tested, *Pasteurella haemolytica* A1 was the only species isolated; *P. multocida* was isolated from 6 lungs, 4 of which were concurrently infected with *P. haemolytica* A1.

Viruses were isolated from the homogenates prepared from each of 9 lungs. Seven were positive for PI-3V, 1 was positive for NCP BVDV type 1, and 1 was positive for both BHV-1 and BVDV. The genotype of the BVDV isolated in combination with BHV-1 could not be determined by PCR, possibly due to interference by BHV-1. Lung samples, nasal swabs, and sera were sent to the

Table III. Seroconversion (4-fold or greater) to BVDV types 1 and 2, PI-3V, and BRSV from day 0 to day 33 in 104 surviving calves

	Dilutions indicating seroconversion[a]		
BVDV type 1	BVDV type 2	PI-3V	BRSV
0[b] → 8 (3)[c]	0 → 8 (5)	0 → 8 (6)	0 → 8 (11)
0 → 16 (4)	0 → 16 (8)	0 → 16 (15)	0 → 16 (18)
0 → 32 (10)	0 → 32 (5)	0 → 32 (19)	0 → 32 (20)
0 → 64 (8)	0 → 64 (4)	0 → 64 (16)	0 → 64 (12)
0 → 128 (2)	0 → 128 (1)	0 → 128 (2)	0 → 128 (1)
0 → 256 (5)	0 → 1024 (2)	0 → 256 (2)	0 → 512 (1)
0 → 512 (2)	0 → 2048 (1)	0 → 512 (1)	4 → 16 (4)
0 → 1024 (1)	0 → 4096 (1)	4 → 16 (3)	4 → 32 (3)
0 → 2048 (1)	16 → 512 (1)	4 → 64 (1)	4 → 64 (4)
0 → 4096 (1)	16 → 1024 (1)	8 → 64 (1)	4 → 1024 (1)
4 → 16 (1)		8 → 256 (1)	8 → 32 (1)
4 → 32 (1)		16 → 64 (1)	8 → 1024 (2)
8 → 8192 (1)		16 → 128 (2)	16 → 64 (1)
		32 → 512 (1)	16 → 128 (1)
			16 → 256 (1)
Total 40/104 (38.5%)	29/104 (27.9%)	71/104 (68.3%)	81/104 (77.9%)

[a] Reciprocal of highest dilution with complete neutralization of virus in both wells
[b] No neutralization at 1:4, the lowest dilution tested
[c] Number of animals

laboratory of Dr. J. Storz, Louisiana State University, for viral isolation and serology of bovine coronavirus (29).

Viral isolation from sera

None of 120 calves were positive for BVDV by viral isolation at day 0. Non-cytopathic BVDV type 1 was isolated from 4 calves later in the study, 3 calves (#60, #115 and #100) on day 13, and one calf (#17) on day 34. All 4 BVDV-viremic calves were treated during the study for BRD. Interestingly, calf #100 died on day 36, 23 d later, but only PI-3V was isolated from the lung. Calf #115, which was BVDV-positive on day 13, died 1 d later, and the lungs were positive for both BHV-1 and BVDV. Attempts were not made to differentiate the BHV-1 from the MLV vaccinal BHV-1 strain.

Sera were available for several of these calves on day 13 and were tested for antibodies to BVDV type 1 and 2. Calf #17, which was BVDV type 1-positive on day 34, had a BVDV type 1 titer of 128 on day 13 and 512 on day 34. This calf thus had both virus and antibody at the same time. Calf #60, which was BVDV type 1-positive on day 13, was seronegative to BVDV type 1 on day 13 but seroconverted to BVDV type 1 (titer of 64) by the day 34 collection. Likewise, calf #100 was BVDV type 1-positive on day 13, and was BVDV type 1 seronegative on that day but seroconverted to BVDV type 1 (titer of 64) by day 33. Calf #115 was BVDV type 1-positive on day 13 and serum for this calf was negative for BVDV type 1 antibodies on day 13. Calf #115 died the next day (day 14). The positive BVDV sera collected on days 13 and 34 for calves #17, #60 and #100 were, in each case, higher to BVDV type 1 than to BVDV type 2. Therefore, the serologic response to BVDV type 1 was consistent with the virus detected in the calf. All the other calves that remained alive during the study and had blood samples taken at the weekly collections were negative for BVDV in the serum.

Seroconversion to BVDV types 1 and 2, PI-3V, and BRSV in surviving calves

There were 104 calves that survived this respiratory disease outbreak subsequent to shipment. The presence of BVDV types 1 and 2, PI-3V, and BRSV in this outbreak was also determined by active infection, defined by 4-fold rise or greater in the antibody titers from the day 0 to day 34 serum samples (Table III). By definition, seroconversion occurred when the titer at day 0 (<1:4, with 1:4 the lowest dilution tested) rose to ≥ 8 at later sampling. Samples with titers of 0 (<1:4) at day 0 that had become 4 by day 34 were not considered to have seroconverted. Any sample with a titer of ≥ 4 on day 0 that had developed 4-fold or greater at day 34 was evidence of seroconversion. The seroconversion rates were: 40/104 (38.5%) to BVDV type 1; 29/104 (27.9%) to BVDV type 2; 71/104 (68.3%) to PI-3V; and 81/104 (77.9%) to BRSV, respectively (Table III). Because the BVDV types 1 and 2 have crossreacting antigens, the type 2 BVDV titers may have been a result of BVDV type 1 antibodies. However, there were 5 calves that seroconverted to BVDV type 2 that had higher antibody titers to type 2 BVDV than to BVDV type 1. The following BVDV titers were recorded for these 5 calves at the day 34 sampling: calf #19, BVDV1, 8 and BVDV2, 1024; calf #54, BVDV1, 16 and BVDV2, 4096; calf #68, BVDV1, 16 and BVDV2, 512; calf #76, BVDV1, 64 and BVDV2, 128; and calf #98, BVDV 1, 32 and BVDV2, 2048. There was, therefore, serologic evidence of both BVDV types in this group of calves.

Combinations of viral infections detected by serology

Various combinations including single viral seroconversions or seroconversions to multiple viruses are summarized in Table IV. Of

Table IV. Combinations of seroconversion to single or multiple viruses in the 104 surviving calves: treated vs untreated calves

	Number of Calves	
Positive for single or combination	Treated (93)	Untreated (11)
BVDV1, BVDV2, PI-3V, BRSV	11	—[a]
BVDV1, BVDV2, PI-3V	5	1
BVDV1, BVDV2, BRSV	6	3
BVDV1, PI-3V, BRSV	8	—
BVDV1, BVDV2	1	—
BVDV1, BRSV	4	—
BVDV1	1	—
BVDV2	1	—
BVDV2, PI-3V	1	—
PI-3V, BRSV	31	6
PI-3V	8	—
BRSV	11	1
Negative to all	5	
Total	93	11

[a] No calves with this seroconversion

the 104 calves that survived, 93 (89.4%) had been treated for respiratory disease. There were 5 calves with BRD that did not seroconvert to any of the 4 viruses (BVDV types 1 and 2, PI-3V, or BRSV). There were, however, 11 calves that seroconverted to all 4 viruses. The virus combination found most often, for both the treated and untreated groups, was PI-3V + BRSV, in 31 and 6 calves, respectively. The next most frequently seen combination in the treated group was BRSV alone (11), followed by PI-3V alone (8), and BVDV type 1 + PI-3V + BRSV (8).

In the 11 untreated cattle, there was evidence of seroconversion for all of the viruses, either singly or in combination.

Response to BHV-1 vaccination

Because the 120 calves had received an MLV BHV-1 vaccine on day 0, the seroconversion rates were not included in the seroconversion studies indicated in Tables III and IV. There were no unvaccinated controls in this study. Thus, it is not absolute that the seroconversions were due only to vaccination. With the exception of one lung found to be positive for BHV-1 (in combination with BVDV), there was no other evidence of BHV-1 infection in this study. No gross lesions caused by BHV-1 were observed at necropsy, nor were microscopic lesions, such as intranuclear inclusions, observed by histopathology.

The humoral immune response to BHV-1 was examined in 41 seronegative (< 1:10) calves on day 0 and on day 33. Several of the 41 calves were tested on day 13 as well. All 41 calves had titers ranging from 20 to 384. Nine of the 120 calves were seropositive to BHV-1 (titers of 14 to 53) on day 0. This may have been due either to positive transfer of maternal antibodies or to active infections. One calf with BHV-1 antibody titer of 40 on day 0, died 6 d later. Of the 8 seropositive calves, 6 had increased (≥ 2-fold) numbers of antibodies. In fact, one calf with titer of 18 on day 0 developed the highest titer in the study (640). There were 2 seropositive calves on day 0 that demonstrated a continued decline in BHV-1 antibodies after vaccination. Interestingly, one of these 2 calves (#41) had titers to BVDV types 1 and 2 of 32 and 16, respectively, at day 0, and these titers declined to 4 and 0 by day 33. In that calf, these antibody levels and their decline are suggestive of passively derived titers from colostrum. Thus, it is possible that in a limited number of calves, maternally derived BHV-1 antibodies may have blocked active immune response to BHV-1 after vaccination.

Discussion

There were no persistently infected (PI) calves detected in this study. It was believed, initially, that PI animals were the source of BVDV infection in this study. The number of PI animals reflected by prevalence is often low, around 1%, with the number of PI animals in the study centered on a few herds (30,31). For example, one study reported 1.7% PI animals out of 66 herds, with the PI animals from 2 of 6 herds yielding BVDV on the initial collection (30). Twenty dairy herds in Michigan were examined, and 7/5481 (0.13%) animals were PI, and they were found in only 3 herds (31). A Danish survey of 19 dairy herds found 1.4% of the animals to be PI, and they were found in 10/19 herds (32). Yet, in some cases, there may be a herd with a higher prevalence of PI calves. For example, 8 PI calves were detected in a beef herd that had 143 calves, and their dams were not PI (33).

The results of this study were similar to a Canadian study. Calves were purchased from auction markets in Canada and transported to a central Saskatchewan feedlot (34). The calves were processed, with sera collected at day 0, upon arrival, and at reimplanting (90–100 d postarrival), and the sera were tested for BVDV. One calf was viremic upon arrival, but the calf was not available for retesting. However, another criterion was used to detect PI animals: mucosal disease diagnosis in calves. Three calves died of mucosal disease. The mucosal disease form is believed to occur when PI calves become infected with a related cytopathic strain (1).

The source of the BVDV in this study is not likely to be a PI calf from among the 120 purchased calves. It is possible, however, that a PI calf was the initial source, or, possibly, multiple PI animals or acutely infected calves that shed the virus only for a short time. Persistently infected cattle are considered the most important source of BVDV, as they shed high titers of virus in several secretions/excretions throughout their lifetime (1,35). The calves in this study may have been exposed at the farm of origin or to PI animals in the auction markets. Experimental studies have demonstrated how effectively PI animals transmit infection to susceptible cattle (36,37). In one study, 22/35 (62.9%) susceptible heifers became seropositive after exposure to a PI cow and her PI calf in a 25 m × 12 m pen for approximately 24 h (36). In another study, 5 sentinel calves seroconverted to BVDV after exposure to air from a stable that housed 2 PI calves (37). Potentially, calves acutely infected postnatally will shed the virus, albeit for a short interval, then cease shedding while seroconverting. Animals may be viremic up to 15 d after infection (38,39). Virus was shed in nasal secretions of specific-pathogen-free calves exposed intranasally to a noncytopathic strain from 1 to 9 d after exposure and virus was found in peripheral leukocytes from day 4 to day 8 (40).

The role of BVDV in this acute respiratory disease outbreak, including pneumonic pasteurellosis, is substantiated by: (1) isolation of BVDV type 1 from serum of affected cattle; (2) isolation of BVDV type 1 from pneumonic lungs; (3) active infection detected by seroconversion to both BVDV type 1 and 2 in paired sera; and (4) demonstrating the presence of BVDV in concert with other viruses associated with pneumonic pasteurellosis (PI-3V and BRSV). The results of this study are consistent with a prior study that used diagnostic laboratory isolates from cattle with various clinical/necropsy diagnoses. In the current study, the predominant BVDV respiratory disease isolate was NCP type 1, similar to the other study (25). Most likely, the infections caused by BVDV types 1 and 2 were natural infections, as there were no BVDV vaccines used in these calves.

Serology has often been reported to be a useful tool to examine single and/or multiple infections, especially for respiratory disease in cattle. Seroconversion has been used as a measure of active infection, and, when present, has been ascribed to diseases present in the population tested. Infections by BVDV for single or multiple herds, as detected by seroconversions, have been reported in several publications. The prevalence rates for the BVDV infections varied from 10% to 89.7% (7–11,16). In most of these studies, the BVDV type was specified by the virus neutralization test (VNT) used to measure antibodies. The Singer or the NADL (NADC) CP type 1 strains are the more common isolates used in the United States and Canada. Because there are cross reactions between BVDV type 1 and type 2, these prior studies may have had BVDV type 2 antibodies in addition to type 1 antibodies (19,22). Only in recent years have diagnostic laboratories and/or researchers used available type 2 strains in the VNT in conjunction with the more traditional type 1. The 1993-1995 BVDV outbreak in Canada focused on the increased role of BVDV type 2 strains in cattle diseases (41).

Identification of additional BVDV-infected animals beyond the 4 of 120 already found to be positive might have been possible had peripheral blood leukocytes been tested. One study showed that, in acutely infected animals, there is greater recovery of BVDV from the peripheral leukocytes than from the serum of infected animals (42). The serum antibodies to BVDV may inhibit recovery of BVDV from the serum. Potentially, PCR assays may detect virus in the presence of antibodies.

Examples of the studies demonstrating BVDV seroconversion are: 1) 10% seroconversion in a single university herd with postweaning calves suffering from BRD, and the NADL strain was used in VNT (7); 2) 24% seroconversion (average from 5 separate groups) in Ontario feedlot calves, NADL strain was used in VNT (8); 3) 57% of Quebec calves with BRD (group 1 of 5 herds), and NADL strain was used in VNT (9); 4) 42% seroconversion in 1983–87 Ontario study from 15 different groups of feedlot calves (10); 5) 51% of one group of feedlot calves in Ontario with BRD, BVDV strain not specified for VNT (11); 6) 89.7% seroconversion in treated cattle (170 calves) with BRD in a research study in Texas, with only 11.2% of calves that did not require treatment seroconverting, and NADC strain was used in VNT (16); and (7) 40% of feedlot calves in a Saskatchewan feedlot with range of 0% to 100% seroconversion in 11 pens, and Singer strain was used in VNT (34).

In the current study, day 0 and day 34 samples from surviving calves indicated that 38.5% seroconverted to BVDV type 1, and 27.9% seroconverted to BVDV type 2. These seroconversions are most likely due to natural infections, and demonstrate the likelihood of BVDV infection when the initial samples from 120 calves were 81.7% and 86.7% seronegative to BVDV types 1 and 2, respectively. With some calves having higher BVDV type 2 antibodies than BVDV type 1, there is additional support for BVDV infections in the group of cattle.

The findings in this study demonstrated that there were often concurrent infections by BVDV and by PI-3V and BRSV. The PI-3V and BRSV viruses are causes of respiratory disease when acting alone. In several of the reports cited above, PI-3V and BRSV seroconversion was seen in affected cattle. The role of BVDV in these mixed infections, especially with respect to bovine pneumonic pasteurellosis, is important (43). It is considered to be immunosuppressive in cattle, causing diminished blood leukocyte and lung macrophage function. Thus, it is not surprising that BRSV, BHV-1, and *P. haemolytica* infections in cattle may be more severe when BVDV is present.

There are other agents such as bovine coronaviruses, bovine adenoviruses, rhinoviruses, and BHV (other than BHV-1) that may cause respiratory infections or disease in cattle, or that are detected by seroconversions in some cattle populations. No doubt, when only 1 virus or bacterium is investigated in epidemiological studies, emphasis may shift, potentially, to that one agent. In our study, we tested for several viruses by inoculation of cell cultures with lung homogenates of calves dead of BRD. We did not look for more than 5 viruses by serology. Coronavirus isolation from cattle with early morbidity and mortality during the receiving stage of this current study has been reported (29). Coronaviruses were especially prevalent in nasal swabs during the time of commingling at the order buyer's pens and arrival at the feedyard.

In summary, we identified 4 viruses, BVDV types 1 and 2, PI-3V, and BRSV, in an outbreak of respiratory disease in unvaccinated calves. Potential control measures might include appropriate vaccination, as currently licensed vaccines against all 4 viruses are available.

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