Bovine viral diarrhea virus (BVDV) 1b: predominant BVDV subtype in calves with respiratory disease

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Abstract

The prevalence of bovine viral diarrhea virus (BVDV) infections was determined in 2 groups of stocker calves with acute respiratory disease. Both studies used calves assembled after purchase from auction markets by an order buyer and transported to feedyards, where they were held for approximately 30 d. In 1 study, the calves were mixed with fresh ranch calves from a single ranch. During the studies, at day 0 and at weekly intervals, blood was collected for viral antibody testing and virus isolation from peripheral blood leukocytes (PBLs), and nasal swabs were taken for virus isolation. Samples from sick calves were also collected. Serum was tested for antibodies to bovine herpesvirus-1 (BHV-1), BVDV1a, 1b, and 2, parainfluenza 3 virus (PI3V), and bovine respiratory syncytial virus (BRSV). The lungs from the calves that died during the studies were examined histopathologically, and viral and bacterial isolation was performed on lung homogenates. BVDV was isolated from calves in both studies; the predominant biotype was noncytopathic (NCP). Differential polymerase chain reaction (PCR) and nucleic acid sequencing showed the predominant subtype to be BVDV1b in both studies. In 1999, NCP BVDV1b was detected in numerous samples over time from 1 persistently infected calf; the calf did not seroconvert to BVDV1a or BVDV2. In both studies, BVDV was isolated from the serum, PBLs, and nasal swabs of the calves, and in the 1999 study, it was isolated from lung tissue at necropsy. BVDV was demonstrated serologically and by virus isolation to be a contributing factor in respiratory disease. It was isolated more frequently from sick calves than healthy calves, by both pen and total number of calves. BVDV1a and BVDV2 seroconversions were related to sickness in selected pens and total number of calves. In the 1999 study, BVDV-infected calves were treated longer than noninfected calves (5.643 vs 4.639 d; $P = 0.0902$). There was a limited number of BVDV1a isolates and, with BVDV1b used in the virus neutralization test for antibodies in seroconverting calves' serum, BVDV1b titers were higher than BVDV1a titers. This study indicates that BVDV1 strains are involved in acute respiratory disease of calves with pneumonic *Mannheimia haemolytica* and *Pasteurella multocida* disease. The BVDV2 antibodies may be due to cross-reactions, as typing of the BVDV strains revealed BVDV1b or 1a but not BVDV2. The BVDV1b subtype has considerable implications, as, with 1 exception, all vaccines licensed in the United States contain BVDV1a, a strain with different antigenic properties. BVDV1b potentially could infect BVDV1a-vaccinated calves.

Résumé

La prévalence des infections par le virus de la diarrhée virale bovine (BVDV) fut déterminée dans deux groupes de veaux d'embouche souffrant de maladie respiratoire aiguë. Lors des deux études des veaux achetés à l'encan furent rassemblés par un acheteur et transportés dans des parcs d'engraissement où ils furent gardés pour approximativement 30 jours. Lors d'une des études les veaux furent mis avec des veaux provenant d'un élevage. Au cours des études, au jour 0 et à des intervalles de 1 semaine, des échantillons de sérum furent prélevés pour détecter la présence d'anticorps de même que des échantillons sanguins dans des tubes avec EDTA pour isolement viral à partir des leucocytes sanguins (PBL) ainsi que des écouvillons nasaux pour isolement viral. Des échantillons provenant des veaux malades furent également prélevés. On testa les échantillons de sérum pour la présence d'anticorps dirigés contre l'herpès virus bovin de type 1 (BHV-1), le virus BVDV1a, 1b et 2, le virus parainfluenza-3 (PI-3V) et le virus respiratoire syncitial bovin (BRSV). Les poumons des veaux qui moururent au cours des études

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furent prélevés et soumis à un examen histopathologique et l'isolement viral et bactérien effectués à partir de poumons homogénéisés. Le BVDV fut isolé des veaux lors des deux études et le biotype non-cytopathogène (NCP) était prédominant. Le typage des isolats de BVDV par réaction d'amplification en chaîne par la polymérase (PCR) différentielle et séquençage des acides nucléiques démontra que dans les deux études le type prédominant était le type BVDV1b. En 1999, on détecta dans le temps, à partir de plusieurs échantillons provenant d'un veau infecté de façon persistante (PI), du BVDV1b NCP, ainsi que le fait que ce veau ne démontra pas de séroconversion contre BVDV1a ou BVDV2. Dans les deux études, du BVDV fut isolé à partir d'échantillons de sérum, des PBL et des écouvillons nasaux des veaux, et dans l'étude de 1999, à partir de tissu pulmonaire prélevé lors des nécropsies. Selon les résultats de l'isolement viral et des analyses en sérologie il fut démontré que le BVDV était un facteur contribuant aux problèmes respiratoires chez les veaux. Le BVDV était isolé plus fréquemment des veaux malades que des veaux en santé, autant en considérant les parcs séparément que le nombre total d'animaux. La séroconversion envers BVDV1a et BVDV2 était reliée à la maladie dans des parcs sélectionnés et sur le total des veaux. Au cour de l'étude réalisée en 1999 les veaux infectés par BVDV furent traités plus longtemps que les veaux non-infectés (5,643 vs 4,639 j, P = 0,0902). L'isolat de BVDV prédominant dans ces études était le type BVDV1b alors que seulement un nombre limité d'isolats de BVDV1a a été retrouvé. En utilisant le BVDV1b dans l'épreuve de neutralisation virale, on nota une prédominance de titres plus élevée avec BVDV1b que BVDV1a. Les résultats de l'étude indiquent que les isolats de BVDV1 sont impliqués dans les infections respiratoires aiguës chez les veaux atteints de pneumonie causées par Mannheimia haemolytica *et* Pasteurella multocida. *Les anticorps envers BVDV2 pourraient être dus à des réactions croisées étant donné que le typage des isolats de BVDV ne révéla que la présence de BVDV1b ou 1a, mais non celle de BVDV2. Le BVDV fut détecté par isolement viral à partir du sérum, des PBL, des écouvillons nasaux et du poumon prélevé lors de la nécropsie. Plusieurs implications sont associées avec le sous-type BVDV1b sauf une exception, tous les vaccins homologués par le USDA contiennent le BVDV1a, une souche possédant des propriétés antigéniques différentes. Le BVDV1b pourrait donc potentiellement infecté des veaux vaccinés avec BVDV1a.*

(Traduit par Dr Serge Messier)

Introduction

Bovine viral diarrhea virus (BVDV) causes infection and disease in cattle, with involvement of 1 or more organ systems (1,2). The conditions range from inapparent infection in postnatal calves to severe, fatal systemic diseases, such as mucosal disease (1). BVDV has been isolated from several clinical forms of disease and from necropsy samples, including from cattle with signs and, or, lesions of bovine respiratory disease (BRD) (2).

BVDV is classified by biotype and genotype (1,3,4). Biotypes, cytopathic (CP) and noncytopathic (NCP), are based on the presence or absence of visible cytopathic effects (CPE) in infected cell cultures. BVDV genotypes (1 and 2) are detected by polymerase chain reaction (PCR) and antigenic differences (3,4). The type 1 genotype has been further subdivided into types 1a and 1b on the basis of PCR and nucleic acid sequencing (5,6). A recent study indicated that BVDV could be clustered into BVDV1a and BVDV1b and also into 11 phylogenetic groups (7).

BVDV has been associated with clinical signs and lesions of BRD (8–28). The involvement of BVDV in BRD has been demonstrated by (1) experimental infections, (2) isolation of virus and, or, identification of BVDV antigen in lesions and, or, other respiratory tract samples from calves with respiratory signs or lesions, and (3) demonstration of active infection through seroconversions in groups of cattle with BRD.

BVDV genotypes have been associated with particular disease forms, PCR being used to differentiate the genotypes. In 1 study, in which clinical conditions were described by veterinarians submitting samples, BVDV NCP biotypes were isolated more frequently than BVDV CP biotypes and BVDV1 genotypes more frequently than BVDV2 genotypes from cattle with respiratory disease (2). Also, BVDV1 genotypes were isolated more frequently than BVDV2 genotypes from necropsy samples from calves with fibrinous pneumonia (2).

Knowledge of the BVDV1 subtypes specific for BRD is limited. However, both of the BVDV strains isolated from Venezuelan dairy calves with BRD were of the 1b subgroup (26). Besides BVDV1a and 1b subtypes, 2 additional clusters have been identified: 1 cluster, 1d, was predominantly associated with field cases of respiratory disease in the southern region of Africa (29). Subsequently, calves experimentally challenged with a BVDV1d subtype developed primary respiratory disease (27).

In this study, postweaning calves were held for approximately 5 wk and observed for natural cases of acute respiratory disease. The purpose was 4-fold: (1) to detect the presence of persistent BVDV infection in calves and determine its role in BVDV transmission; (2) to determine the susceptibility of seronegative calves to BVDV infection; (3) to determine the presence of BVDV in BRD cases by virus isolation and, or, seroconversion; and (4) to determine the BVDV1 subtype involved in BRD cases.

Materials and methods

Cattle

The cattle were involved in 2 studies to determine factors associated with acute respiratory disease in young (postweaning) beef cattle. In both the 1999 study and the 2000 study, cattle were purchased from local auctions in eastern Tennessee and transported to a nearby order buyer's (OB) pens. There they received their initial treatments and vaccinations, and samples were collected, including nasal swabs for virus isolation, EDTA blood for isolation of virus

from peripheral blood leukocytes (PBLs), and clotted blood samples for viral serologic testing (20). On day 0 of both studies, the calves received anthelmintic, monovalent BHV-1 modified-live virus (MLV) vaccine, and clostridial vaccine, as previously described (20). The calves were then shipped via semi-trailer truck to research feedyards: in Clayton, New Mexico, for the 1999 study and in Bushland, Texas, for the 2000 study. Samples were collected as described above at weekly intervals until the final day of each study: in the 1999 study, day 33; and in the 2000 study, day 32. The isolation and transmission of *Mannheimia haemolytica* and *Pasteurella multocida*, primary causes of bacterial pneumonias in BRD, are the subjects of other investigations.

Lung samples were collected at necropsy from each calf dying during the studies. These samples were tested for viruses and bacteria at the Oklahoma Animal Disease Diagnostic Laboratory (OADDL), Oklahoma State University, Stillwater, Oklahoma. Samples were also collected for histopathological study.

Serologic tests

A virus neutralization test (VNT) in Madin–Darby bovine kidney (MDBK) cells in 96-well microtiter plates was used to quantitate virus-neutralizing antibodies to BVDV types 1a, 1b, and 2, PI3V, and BRSV. The viruses used in this test were CP BVDV type 1a (Singer strain), CP BVDV1b (TGAC 8HB), CP BVDV type 2 (125-C strain), PI3V (SF-4 strain), and a BRSV vaccine strain (20,30,31). The 1:4 final dilution was the lowest dilution tested. A plaque reduction assay in MDBK cells in 24-well plates was used to detect virus-neutralizing antibodies to the Cooper strain of BHV-1 (20,30,31). 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, PI3V, and BRSV, and $<$ 1:10 for BHV-1. Titers expressed represent reciprocals of the endpoint titers.

Microbiologic studies

The virus isolations were performed at the OADDL. A monolayer enzyme-linked immunosorbent assay was used to detect BVDV in serum (20,32). To isolate viruses, nasal swabs, PBLs, and lung samples were inoculated onto BT monolayers in 24-well plates, as previously described (20,32). Cytopathic agents other than BVDV (BRSV, PI3V, and BHV-1) were tested for by direct fluorescent antibody tests. The BVDV isolates were typed with the use of differential PCR and sequencing of the 5′ untranslated region, as previously described (5,6). Bacterial isolation from lung tissues was also performed by the OADDL.

Pathological studies

Lung samples collected at necropsy were fixed in 10% buffered formalin and processed for histopathological study. Sections were stained with hematoxylin and eosin, and, in a blinded study, were examined by 1 of the authors (A.W.C.), who rendered a morphologic diagnosis.

Subtypes in vaccines

Vaccine manufacturers in the United States were contacted to determine the name(s) of the BVDV strain(s) in both killed and MLV vaccines licensed and marketed in the United States. The genotype and biotype for each vaccine strain was provided by the vaccine manufacturer. In addition, product descriptions were available to the public in a reference text containing bovine-vaccine product information (33); these descriptions are presented in Table I.

Table I. Strains and types of bovine viral diarrhea virus (BVDV) in modified-live virus (MLV) and killed vaccines (33)

Vaccine type and trade name	Name of strain	Genotype/biotype
MLV		
Express 5	Singer	1a CP
	296	2 CP
BoviShield 4	NADL	1a CP
Pyramid 4	Singer	1a CP
Reliant 4	NADL	1a CP
Frontier 4 Plus	C24V	1a CP
	296	2 CP
Titanium 5	C24V	1a CP
	296	2 CP
Jencine 4	WRL	1 NCP
Herd Vac 3	Singer	1a CP
Killed		
Elite4	Singer	1a CP
Horizon 4 Plus	C24V	1a CP
	125C	2 CP
Master Guard 5	C24V	1a CP
	125C	2 CP
Respishield 4	Singer	1a CP
Triangle 4 + type II	Singer	1a CP
	5912	2 CP
CattleMaster 4	5960	1a CP
	6309	1 NCP
ViraShield 5	KY22	1a CP
	TN 131	2 NCP
Surround 4	Singer	1a CP
	NY	1b NCP

CP — cytopathic; NCP — noncytopathic

Statistical analysis

The percentages of isolations from sick vs healthy calves and of seroconversions were compared with Fisher's exact 2-sided test (34). The effect of BVDV infection on the duration of treatment was analyzed with a 2-population t-test (34). All tests were performed at the 0.05 level of significance.

Results

Serologic findings at arrival

The blood collected on day 0 in both the 1999 and 2000 studies was tested for BHV-1, BVDV1a, BVDV2, PI3V, and BRSV antibodies. Of the OB calves in the 1999 and 2000 studies, respectively, 92.6% and 95.0% were seronegative for BHV-1, 76.9% and 65.8% for BVDV1a, 82.6% and 80.0% for BVDV2, 55.4% and 45.0% for PI3V, and 86.0% and 87.5% for BRSV. Of the ranch calves in the 1999 study, 70.2%

Table II. Pulmonary lesions of and bacteria and viruses isolated from calves dying during the 1999 study[a]

Animal no.	Day of death[b]	Type of pneumonia	Bacteria isolated	Viruses isolated
108T	29	Subacute, severe fibrinopurulent bronchopneumonia with severe bronchiolar necrosis	*Mannheimia haemolytica*, *Pasteurella multocida*, *Archanobacterium pyogenes*	BVDV CP1b
57T	28	Acute to peracute severe fibrinohemorrhagic pneumonia	*M. haemolytica*, *A. pyogenes*	None
17T	15	Acute, severe fibrinopurulent pleuropneumonia	*M. haemolytica*	None
87T	5	Acute, severe fibrinopurulent pleuropneumonia	*M. haemolytica*, *P. multocida*	None

[a] Necropsy tissues were not available from order-buyer (OB) calf 63T and ranch calf 40NM

[b] Day after start of study

Table III. Pulmonary lesions of and bacteria and viruses isolated from calves dying during the 2000 study

Animal no.	Day of death	Type of pneumonia	Bacteria isolated	Viruses isolated
69	11	Severe fibrinopurulent pleuropneumonia	*M. haemolytica*	None
48	11	Severe fibrinopurulent pleuropneumonia	*M. haemolytica*	None
36	12	Fibrinopurulent pleuropneumonia with extensive hemorrhage and necrosis	*M. haemolytica*, *P. multocida*	None
99	12	Fibrinopurulent pleuropneumonia with necrotizing suppurative bronchiolitis	*M. haemolytica*	None
111	16	Severe fibrinopurulent pleuropneumonia	None	None
72	28	Suppurative bronchopneumonia with abscess formation	*P. multocida*, *A. pyogenes*	PI3V

were seronegative for BHV-1, 71.4% for BVDV1a, 81.0% for BVDV2, 30.0% for PI3V, and 41.7% for BRSV. The titers of antibody to BVDV1a, BVDV2, PI3V, and BRSV could be measured for evidence of active infection (4-fold or greater rise) or maternal antibody decay (a drop of 1 dilution or more). BHV-1 maternal antibody determinations could not be made, as all the calves in each study had received the MLV BHV-1 vaccine. Based on the above-defined evidence of maternal antibody decay, there were animals in both studies with maternal antibodies at entry. It was subsequently learned that the cow herd from which the ranch calves were obtained for the 1999 study had been receiving annual vaccinations with viral vaccine containing BHV-1, BVDV1, PI3V, and BRSV (CattleMaster 4, Pfizer Animal Health, Exton, Pennsylvania, USA).

Respiratory disease morbidity and mortality

During the 1999 study, the morbidity rate was 124/205 (60.5%) among the calves, which included the 118 surviving calves treated after clinical signs of BRD were observed and the calves that died with BRD lesions (6). The morbidity rate was 81/121 (66.9%) for the OB calves and 43/84 (51.2%) for the ranch calves. Of the 6 calves (2.9%) that died during the 1999 study, 5/121 (4.1%) were OB calves and 1/84 (1.2%) ranch calves. In the 2000 study, the morbidity rate was 70/120 (58.3%) for the OB calves, and 6/120 (5%) died during the study. Respiratory disease signs included fever [temperature $\geq$ 104°F (40°C)], nasal and ocular discharge, coughing, and labored breathing. Treatment included antimicrobial therapy. The duration of treatment ranged from 1 to 7 d in the 1999 study and 3 to 13 d in the 2000 study.

Calves that died: lung lesions and isolates

Necropsy tissue was not available from 1 OB calf and 1 ranch calf. The lesions in the lungs of the other 10 calves that died during the studies were consistent with those of pneumonia caused by *M. haemolytica* and, or, *P. multocida* — primarily fibrinopurulent pneumonia in 9 cases and bronchopneumonia in 1 case (Tables II and III). *M. haemolytica* and, or, *P. multocida* was isolated from the lungs in both studies. Viruses were isolated from the lung homogenates in each study: 1 lung in the 1999 study was positive for CP BVDV (subtype 1b), and 1 in the 2000 study was positive for PI3V.

Virus isolates from survivors

In 1999, there was only 1 calf positive for BVDV on day 0 (Table IV). This calf, 72T, an OB calf, proved to be persistently infected, as samples were positive through day 33; in addition, BHV-1 was isolated on day 26 and PI3V on day 19. Of the remaining 9 BVDV-positive calves, all but 2 had NCP BVDV; 1 isolate from a nasal swab from calf 72T was CP, as was an isolate from necropsy lung tissue. Six of the 10 pens (nos. 1 to 4, 9, and 10) had calves from which BVDV was isolated. The only subtype was 1b. Of the live calves from which BVDV was isolated, 8 were sick, and 1 was healthy. The probability of BVDV being isolated from sick calves more frequently than healthy calves approached significance ($P = 0.0887$) with Fisher's exact 2-sided test.

In 2000, no calves were positive for BVDV on day 0, and none were persistently infected during the study. There were 35 BVDV isolates, from 32 calves; all isolates were NCP (Table V). All 6 pens had cattle

Table IV. BVDV isolations from calves in the 1999 study

Pen no.	Animal no.	Origin	Collection day	Sample origin	Health status	BVDV subtype[a]
1	56T	OB	5	PBLs	H	—[b]
2	65T	OB	8	PBLs	S	—
			10	Nasal		1b
3	45T	OB	12	Nasal	S	1b
	72T	OB	0	Serum[c]	S	—
			0	Nasal		—
			2	Nasal		1b
			5	Nasal		1b CP
			6	Nasal		1b
			33	Nasal		1b
			6	PBLs		—
			12	PBLs		—
			33	PBLs		—
	84T	OB	12	Nasal	S	—
4	89T	OB	6	PBLs	S	—
9	2T	OB	4	PBLs	S	1b
	108T	OB	29	Lung (necropsy)	Died on day 29	1b CP
10	56NM	Ranch	11	PBLs	S	—
	68T	OB	7	PBLs	S	1b

PBLs — peripheral blood lymphocytes; H — healthy; S — sick

[a] All isolates were NCP, except where noted as CP

[b] BVDV isolated, but not subtyped

[c] BVDV was also isolated from serum on days 5, 12, 19, and 26

from which BVDV was isolated, the numbers ranging from 9 calves in pens 1 and 8 calves in pen 3 to 2 calves in pen 4. BVDV was isolated from 24 sick calves and 8 healthy calves; the probability that BVDV was isolated more frequently from sick calves than from healthy calves was significant ($P = 0.0323$). Calves 116 and 118 had multiple isolations of BVDV. Of the 33 isolates available for subtyping, 27 were 1b and 6 were 1a. Three calves (nos. 34, 118, and 112) had subtype 1a alone, whereas calf 116 was BVDV1a-positive on days 10 and 11, yet BVDV1b-positive on day 25.

PI3V was isolated from nasal swabs of both healthy and sick calves, in all pens, in both studies. BHV-1 was isolated from nasal swabs of 15 OB calves in the 1999 study (1 in each of pens 1 and 4, 2 in pen 2, 3 in pen 3, and 4 in each of pens 8 and 9) but from no calves in the 2000 study. Generally, BHV-1 was isolated from calves only once; 2 calves were positive on days 19 and 26, and 1 calf was positive on days 5, 11, and 19. BHV-1 was isolated from 4 healthy and 11 sick calves. Eight calves had both PI3V and BHV-1 isolations, at the same or different collections. BVDV was isolated from either the nasal swabs or the PBLs of 2 BHV-1-positive calves.

Seroconversions to BVDV types 1a and 2, PI3V, and BRSV in the surviving calves

In 1999, 199 of the 205 calves survived the study duration; in 2000, 114 of the 120 calves survived. Seroconversion, or active infection with BVDV1a, BVDV2, PI3V, or BRSV, was defined by a 4-fold or greater rise in the antibody titer from day 0 to the final day of blood collection. The attempt to determine pen effect on viral infection and disease is illustrated in Tables VI to IX. For BVDV1a and BVDV2, the origin of the calf (OB or ranch) and the disease state (sick or healthy) is shown for each of pens. Owing to the low numbers by origin in the 1999 study, the 2 origins were pooled for comparisons by pen of the total sick to healthy ratios.

There were significantly more BVDV1a seroconversions in sick (47.5%) than in healthy (28.4%) calves ($P = 0.008$ by Fisher's exact 2-sided test) in the 1999 study. There was no significant difference by pen; however, for some pens, the difference approached significance: pen 1, $P = 0.1571$; pen 2, $P = 0.1273$; and pen 10, $P = 0.0867$. Pen 10 had the highest seropositivity rate among the pens for BVDV1a, 16/19 (84.2%), and all 10 sick calves in this pen seroconverted. Pen 3 contained the persistently infected calf, 72T, and had a BVDV1a seropositivity rate of 68.4% (13/19); of the seroconverting calves, 9 were sick and 4 healthy, which indicated transmission to both sick and healthy calves. It appears that the major source of the BVDV in the study was the OB calves, as only 2 of the 44 ranch calves in the 2 pens containing only ranch calves seroconverted to BVDV. Of the 116 OB calves, 61 (52.6%) seroconverted to BVDV1a, and of the 83 ranch calves, 18 (21.7%) did so (considering only surviving calves). However, when, as in pen 10, ranch calves were mixed with OB calves that had a high rate of seroconversion to BVDV1a among the sick calves (6/6), then most (8/9) of the ranch calves became infected with BVDV1a. For BVDV2 in that study, the P value was 0.0960 for the relation of BVDV infection to sickness; seroconversion occurred in 29.7% of the sick and 18.5% of the healthy calves.

In the 2000 study, BVDV1a seroconversion, based on the total from the 6 pens, was positively related to sickness ($P = 0.0517$), with 32.8% of the sick and 16.0% of the healthy calves seroconverting. Pen 1 had

Table V. BVDV isolations from calves in the 2000 study

Pen no.	Animal no.	Collection day	Sample origin	Health status	BVDV subtype[a]
1	14	18	PBLs	S	1b
	26	5	PBLs	S	1b
	34	18	Nasal	S	1a
	61	11	PBLs	S	1b
	67	4	PBLs	H	1b
	90	21	PBLs	H	1b
	111	3	PBLs	Died on day 15	1b
	114	4	Serum	S	—[b]
	116	10	PBLs	S	1a
		11	PBLs		1a
		25	PBLs		1b
2	9	11	PBLs	S	—
	66	4	PBLs	H	1b
	96	18	PBLs	S	1b
	99	3	PBLs	Died on day 12	1b
	104	11	PBLs	H	1b
	118	11	PBLs	H	1a
		25	PBLs		1a
3	44	11	PBLs	S	1b
	76	25	PBLs	H	1b
	88	25	PBLs	S	1b
	105	25	PBLs	S	1b
	107	25	PBLs	S	1b
	113	25	PBLs	S	1b
	117	11	PBLs	H	1b
	119	11	PBLs	S	1b
4	91	25	PBLs	H	1b
	94	25	PBLs	S	1b
5	18	18	PBLs	S	1b
	57	4	PBLs	S	1b
	87	25	PBLs	S	1b
	112	11	PBLs	S	1a
6	16	18	PBLs	S	1b
	39	4	PBLs	S	1b
	75	25	PBLs	S	1b

[a] All were NCP

[b] BVDV isolated, but not subtyped

a P value of 0.0377 (7/12 sick calves and 0/6 healthy calves seroconverted), and pen 5 had a P value of 0.1032 (7/11 sick calves and 0/18 healthy calves seroconverted), which demonstrated that seroconversions occurred at a greater rate in the sick calves than in the healthy calves. Similarly for BVDV2, among the total group of survivors, seroconversion was positively related to sickness ($P = 0.0387$), with 29.7% of the sick and 12.0% of the healthy calves seroconverting. Pens 1 and 5 had the same P values in demonstrating a relationship between seroconversion to BVDV1a and sickness.

In 1999, 64.3% of the calves seroconverted to BRSV and 37.7% to PI3V. There was no relationship between seroconversion and sickness for either virus, for either the total group of survivors or by pen. In 2000, 86.8% of the calves seroconverted to BRSV and 65.8% to PI3V. Again, there was no relationship between seroconversion and health status for either virus, for either the total group or by pen.

Effect of BVDV infection on duration of treatment

For this evaluation, a calf was classified as having BVDV infection if either the virus was isolated from PBLs or nasal swabs or there was seroconversion (a 4-fold or greater rise in titer of antibody to either BVDV1a or BVDV2). In the 1999 study, duration of treatment was 5.643 d for BVDV-infected calves and 4.639 d for calves without BVDV. The probability that BVDV caused the greater duration of treatment approached significance ($P = 0.0902$). In the 2000 study, the duration of treatment was 2.15 d for BVDV-infected calves and 1.90 d for calves without BVDV; the probability that BVDV caused the greater duration of treatment did not approach significance ($P = 0.3013$).

BVDV infection in calves that died

During the 1999 study, 6 calves died with BRD, and 4 were necropsied. Calf 108T died on day 29; BVDV1b CP was isolated from

Table VI. BVDV1a seroconversions by pen in the 1999 study

Pen no.	No. (and %) of seroconversions						Total surviving	No. that died
	OB		Ranch		Total			
	Sick	Healthy	Sick	Healthy	Sick	Healthy		
1	8/14	1/6	—	—	8/14	1/6	9/20	0
2	5/8	1/2	5/7	0/3	10/15	1/5	11/20	0
3	9/14	4/5	—	—	9/14	4/5	13/19	1
4	1/5	1/5	1/8	1/2	2/13	2/7	4/20	0
5	—	—	1/6	1/16	1/6	1/16	2/22	0
6	4/7	1/3	0/4	1/6	4/11	2/9	6/20	0
7	—	—	0/13	0/9	0/13	0/9	0/22	0
8	6/13	2/5	—	—	6/13	2/5	8/18	2
9	6/9	4/10	—	—	6/9	4/10	10/19	2
10	6/6	2/4	4/4	4/5	10/10	6/9	16/19	1
Total	45/76 (59.2)	16/40 (40.0)	11/42 (26.2)	7/41 (17.1)	56/118 (47.5)	23/81 (28.4)	79/199 (39.7)	6

Table VII. BVDV2 seroconversions by pen in the 1999 study

Pen no.	No. (and %) of seroconversions						Total surviving	No. that died
	OB		Ranch		Total			
	Sick	Healthy	Sick	Healthy	Sick	Healthy		
1	2/14	1/6	—	—	2/14	1/6	3/20	0
2	6/8	1/2	1/7	0/3	7/15	1/5	8/20	0
3	7/14	3/5	—	—	7/14	3/5	10/19	1
4	1/5	1/5	1/8	1/2	2/13	2/7	4/20	0
5	—	—	1/6	1/16	1/6	1/16	2/22	0
6	2/7	1/3	0/4	1/6	2/11	2/9	4/20	0
7	—	—	0/13	0/9	0/13	0/9	0/22	0
8	5/13	1/5	—	—	5/13	1/5	6/18	2
9	5/9	1/10	—	—	5/9	1/10	6/19	2
10	2/6	1/4	2/4	2/5	4/10	3/9	7/19	1
Total	30/76 (39.5)	10/40 (25.0)	5/42 (11.9)	5/41 (12.2)	35/118 (29.7)	15/81 (18.5)	50/199 (25.1)	6

the lung tissues. In the 2000 study, 6 calves died with BRD, and 4 were necropsied. Calf 99 died on day 12 of the study; BVDV1b had been isolated from PBLs on day 4. Calf 111 died on day 16; BVDV1b had been isolated from PBLs on day 4.

Comparison of BVDV1a and BVDV1b titers

The BVDV1a (Singer) and BVDV2 (125-C) viruses were used initially in the VNT to detect BVDV antibodies. This serology was done prior to subtyping, which showed that BVDV1b subtypes predominated in both studies. In both 1999 and 2000, the serum from calves seroconverting to BVDV1a was then tested for BVDV1b antibodies, in both acute and convalescent phases. This test was performed to determine if the BVDV1b antibody titers were higher than the BVDV1a titers using the Singer strain.

There were 75 serum samples available for BVDV1b serologic testing in the 1999 study. Of the 58 from OB calves, 34 had higher BVDV1b than BVDV1a titers (by 1 dilution or more), 14 had the same titer, and 10 had lower BVDV1b than BVDV1a titers (by 1 dilution or more). Of the 17 samples from ranch calves, 7 had higher and 10 had lower BVDV1b than BVDV1a titers. The only ranch-calf BVDV isolate was not available for typing. In the 2000 study, there were 29 serum samples available for BVDV1b serologic testing: 12 had higher BVDV1b than BVDV1a titers, 7 had the same titer, and 10 had lower BVDV1b than BVDV1a titers.

BVDV subtypes in vaccines

At the time of the studies, the vaccines in the United States primarily contained BVDV1a CP strains, although some killed and MLV vaccines contained BVDV2 CP strains (Table I). In all but 2 vaccines, the biotypes were CP; 1 killed and 1 MLV vaccine contained NCP strains. One MLV vaccine contained an NCP strain, WRL, referred to as BVDV1; however, the subtype was not identified. One killed vaccine contained strain 6309, a BVDV1 NCP strain whose subtype was not specified. Only 1 vaccine contained BVDV1b.

Discussion

BVDV was identified in cases of BRD in 2 separate studies of calves of mixed source (OB procured) that were commingled, transported to an experiment feedyard, and observed for 4 to 5 wk. In 1 study, fresh ranch calves were mixed with the OB calves. These 2 studies, in 1999 and 2000, are continuing studies of BRD and follow, in general, our study from 1998 (20). In contrast to the 1998 study, in which blood was collected only at days 0 and 33 for virus

Table VIII. BVDV1a seroconversions by pen in the 2000 study

	No. (and %) of seroconversions			No. that
Pen no.	Sick	Healthy	Total	died
1	7/12	0/6	7/18	2
2	3/5	5/14	8/19	1
3	3/14	1/6	4/20	0
4	0/10	1/9	1/19	1
5	4/11	0/8	4/19	1
6	4/12	1/7	5/19	1
Total	21/64 (32.8)	8/50 (16.0)	29/114 (25.4)	6

Table IX. BVDV2 seroconversions by pen in the 2000 study

	No. (and %) of seroconversions			No. that
Pen no.	Sick	Healthy	Total	died
1	7/12	0/6	7/18	2
2	2/5	4/14	6/19	1
3	3/14	0/6	3/20	0
4	0/10	0/9	0/19	1
5	4/11	0/8	4/10	1
6	3/12	2/7	5/19	1
Total	19/64 (29.7)	6/50 (12.0)	25/114 (21.9)	6

isolation and serologic testing, additional collections were made in the 1999 and 2000 studies: nasal swabs and additional blood samples were collected for virus isolation from PBLs on a weekly basis and from sick calves. The addition of nasal swabs was designed to detect viruses in upper respiratory tract secretions that might not be present in serum or PBLs. PBLs were used to detect systemic viruses and, especially, those causing acute BVDV infection. Weekly collection permitted virus detection over time. Samples of lung tissue from calves dying during the studies were assayed for viruses and bacteria, and viral serologic testing was performed, with an additional BVDV subtype, BVDV1b. The plan was to identify additional viruses in calves held over this period. The number of viruses, both BVDV and others, in both the 1999 and 2000 studies was increased, as expected. Also, viruses were isolated from both healthy and sick calves.

BVDV infection occurred in both studies and in numerous instances appeared to contribute to BRD. This was demonstrated by both virus isolation from affected animals and seroconversion in calves with BRD signs. Subtyping of the BVDV isolates was important, in that the predominant subtype in these studies was BVDV1b. The expectation that BVDV1 would be more common than BVDV2 was based on our prior study (2), in which we found that BVDV1 and NCP strains were more common in cattle with a history of BRD than were CP and BVDV2 strains (2). Also, BVDV1 was isolated more frequently than BVDV2 from necropsied cattle with fibrinous pneumonia. The results in this study are similar to the Venezuelan study, in which BVDV1b was isolated from dairy calves with respiratory disease (26).

There are implications for BVDV vaccines to control these infections. BVDV has been isolated from cattle receiving BVDV1a vaccines (2,35). The current BVDV vaccines, both MLV and killed, contain BVDV1a, with 1 exception: a killed BVDV containing the NCP BVDV New York strain, of subtype 1b (reference 33, plus individual product descriptions in the text, and correspondence from the company listing the vaccine components). There are antigenic differences between BVDV1a and BVDV1b, as demonstrated by a recent study (36). Calves were either vaccinated with a BVDV1a MLV vaccine or inoculated intranasally with either an NCP BVDV1b strain or an NCP BVDV2 strain. Postexposure serum samples were tested for antibodies in the VNT with the CP BVDV1a NADL strain, the CP BVDV1b TGAC strain, or the CP BVDV2 125-C strain. Cross-reactive and type-specific antibodies to all 3 viruses were found in the individual animals. Usually the titers were higher to the same genotype (subtype) as the challenge or vaccine strain. Knowledge of the ability of the BVDV1a vaccines to protect against BVDV1b strains is limited. It is known, however, that BVDV1a vaccines, both killed and MLV, induce a wide range of antibodies to several BVDV1a strains (37). The presence of BVDV1 strains in calves subsequent to BVDV1a vaccination may represent field strains for which there was little protection afforded by the BVDV1a vaccine strain. Both experimental and field studies should be performed to demonstrate the efficacy of BVDV1a vaccines against BVDV1b strains. Also, it is likely that BVDV1b strains may be incorporated into the BVDV1a vaccines, as have the BVDV2 strains (33).

Our studies have also demonstrated concurrent infections with BHV-1, PI3V, and BRSV. The PI3V and BRSV seroconversions were expected, as these viruses appear to circulate in commingled cattle, as had been observed in our prior study (20). From seroconversions in the 1999 and 2000 studies in both healthy and sick calves, we could not demonstrate a significant relationship between the presence of either virus and BRD status. The calves had not received any PI3V, BRSV, or BVDV vaccines at entry to the study; thus, it is most likely these infections represent field infections. However, the detection of PI3V in nasal secretions, late in the holding period in both studies, supports widespread infection. Perhaps, if the cattle had been held 2 to 3 wk longer, there would have been time for an increase in antibody titers, indicating active infection.

The isolation of BHV-1 from calves in the 1999 study was also a repeat of the finding in our prior study (20). The calves in all 3 studies had received an MLV monovalent BHV-1 vaccine at day 0. In the 1999 study, several calves were shedding BHV-1 nasally. Since all calves had received the BHV-1 vaccine, there were no unvaccinated calves to serve as sentinels for field BHV-1 strains. It is likely that the BHV-1 isolates represented natural infections brought by the OB calves during commingling and transportation, because BHV-1 was not isolated from any of the ranch calves. BHV-1 was also found in outbreaks of BHV-1 disease in calves that had been vaccinated with an MLV BHV-1 vaccine after arrival at a feedlot (38). It is not unexpected to find multiple viruses in both sick and healthy calves involved in BRD, as BHV-1, BVDV, PI3V, BRSV, bovine adenoviruses, and bovine coronaviruses are listed as etiologic agents for BRD (39).

In summary, BVDV1b was the predominant BVDV subtype identified in 2 studies of acute BRD in commingled calves that had received no BVDV vaccines. In 1 study, a calf persistently infected with BVDV1b likely exposed other calves during the commingling and transportation. The transmission of virus from calves with acute BVDV infection cannot be ruled out in either study, particularly

since the 2000 study had no persistently infected calves. The implications for effective vaccination against BVDV1b are obvious: (1) there must be demonstration of the efficacy of current BVDV1a vaccines against BVDV1b; (2) new BVDV1b components to add to current BVDV1a vaccines should be developed; or (3) current BVDV1b vaccine should be used. From a diagnostic standpoint, 2 issues should be addressed: (1) the epidemiologic features of the BVDV subtypes should be determined from isolates from clinical cases by differential PCR and sequencing; and (2) diagnostic laboratories should consider incorporating serologic testing for BVDV1b in addition to the tests for BVDV1a and BVDV2 that most laboratories currently use to detect active infection by seroconversion.

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