Evaluation of diagnostic tests used for detection of bovine viral diarrhea virus and prevalence of subtypes 1a, 1b, and 2a in persistently infected cattle entering a feedlot

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RUMINANTS

Objective—To evaluate diagnostic tests used for detection of bovine viral diarrhea virus (BVDV) and determine the prevalence of BVDV subtypes 1a, 1b, and 2a in persistently infected (PI) cattle entering a feedlot.

Design—Prospective study.

Animals—21,743 calves.

Procedures—Samples were obtained from calves initially testing positive via antigen capture ELISA (ACE) performed on fresh skin (ear notch) specimens, and ACE was repeated. Additionally, immunohistochemistry (IHC) was performed on skin specimens fixed in neutral-buffered 10% formalin, and reverse transcriptase PCR (RT-PCR) assay and virus isolation were performed on serum samples. Virus was subtyped via sequencing of the 5' untranslated region of the viral genome.

Results—Initial ACE results were positive for BVDV in 88 calves. After subsequent testing, results of ACE, IHC, RT-PCR assay, and viral isolation were positive in 86 of 88 calves; results of all subsequent tests were negative in 2 calves. Those 2 calves had false-positive test results. On the basis of IHC results, 86 of 21,743 calves were PI with BVDV, resulting in a prevalence of 0.4%. Distribution of BVDV subtypes was BVDV1b (77.9%), BVDV1a (11.6%), and BVDV2a (10.5%).

Conclusions and Clinical Relevance—Rapid tests such as ACE permit identification and segregation of PI cattle pending results of further tests, thus reducing their contact with the rest of the feedlot population. Although vaccines with BVDV1a and 2a components are given to cattle entering feedlots, these vaccines may not provide adequate protection against BVDV1b. (*J Am Vet Med Assoc* 2006;228:578–584)

Bovine viral diarrhea viruses are a diverse group of viruses that cause infections in domestic ruminants worldwide.[1] Bovine viral diarrhea viruses can be classified by biotypes as cytopathic or noncytopathic on the basis of the presence or absence of observable cytopathic changes in infected cell cultures.[1] Additionally, BVDVs are diverse by their genotypic differences, which are also reflected by antigenic differences with the subtypes (BVDV1a, 1b, and 2a) found in the United States.[2-8] Bovine viral diarrhea virus infections cause clinically inapparent to severe disease involving 1 or more organ systems.[1] Historically, BVDV has been associated with gastrointestinal tract disease with high mortality rates. However, BVDV is presently associated with respiratory disease and fetal infections. The outcome of BVDV fetal infections in susceptible heifers and cows is dependent on the age of the fetus when exposed. Infection results in abortions, stillbirths, congenital malformations, and birth of PI calves.[1] Persistent infection in a calf develops when a susceptible heifer or cow is exposed to noncytopathic BVDV during pregnancy at approximately 42 to 125 days of gestation.[9] Persistently infected calves are born alive, are immunotolerant to the initial virus infecting the fetus, and shed the virus for life.[1] However, PI calves are able to respond with an active humoral immune response (antibodies) to heterologous BVDV, including naturally occurring strains or vaccine strains.[10] Thus, antibodies including maternally derived BVDV antibodies and those from vaccine-induced immunity may develop in PI calves. Persistently infected cattle are likely the most important reservoir or source of virus for susceptible cattle[1]; 70% to 100% of susceptible nonvaccinated calves become infected after exposure to PI calves.[11]

Bovine viral diarrhea virus and other viruses such as BHV-1, PI-3V, and BRSV contribute to BRD and bacterial pneumonia caused by *Mannheimia haemolytica, Pasteurella multocida, Histophilus somni,* and *Mycoplasma* spp.[12,13] The role of BVDV in BRD is 2-fold: as a primary

BVDV	Bovine viral diarrhea virus
PI	Persistently infected
BHV-1	Bovine herpesvirus type 1
PI-3V	Parainfluenza-3 virus
BRSV	Bovine respiratory syncytial virus
BRD	Bovine respiratory disease
IHC	Immunohistochemistry
ACE	Antigen capture ELISA
RT	Reverse transcription
MDBK	Madin Darby Bovine Kidney
OD	Optical density
UTR	Untranslated region

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invader and as an immunosuppressor adversely affecting host defenses.[1] Involvement of BVDV strains in BRD in stocker and feeder production has been detected in calves after weaning that were shipped to a feedlot and observed for 35 days.[12,13] In those studies, BVDV was isolated more frequently from sick calves with BRD than healthy calves. Additionally, BVDV1a and 2a seroconversions were associated with illness.[13] Likewise, calves with BVDV were treated longer (days duration) than noninfected calves.[13] In those studies, BVDV strains were involved with acute BRD caused by *M haemolytica* and *P multocida*.[12,13] In addition, high levels of immunity against BVDV at entry to the feedlot were associated with increased protection and economic benefits.[14] Calves from herds with low BRD morbidity rates had higher concentrations of antibodies against BVDV1a than herds with higher morbidity rates, and the cost of treatment in calves with low concentrations of antibodies against BVDV1a and 2a increased.[14] Calves treated twice or more had lower concentrations of antibodies against BVDV1a than those treated once or not at all. Thus, a goal for cattle entering a feedlot would be adequate or enhanced immunity against BVDV.

Results of 1 study[15] indicate that the prevalence of cattle PI with BVDV entering a feedlot was 0.3%. Cattle PI with BVDV are important sources of virus and shed large quantities of virus, thus exposing other cattle in direct or close contact, including penmates or those in adjacent pens. Risk of initial treatment for BRD was 43% in cattle exposed to a PI calf.[15] In that study, 0.3% of cattle entering a feedlot were PI, 2.6% of chronically ill cattle were PI, and 2.5% of cattle that died were PI. The authors of that study reported that 15.9% of initial cases of BRD were attributed to exposure to PI calves.[15] Although the prevalence of PI cattle entering a feedlot is low, considerable disease may develop in exposed cattle. Thus, control of feedlot diseases appears to be aided by removal of PI calves, therefore minimizing risks attributable to those calves.

Control of BVDV involves enhancement of immunity against BVDV by vaccination and biosecurity by use of identification and removal of PI cattle with maintenance of BVDV-negative status in the animal population. Diagnostic testing becomes important in the detection of and surveillance for PI cattle. Several procedures are available by diagnostic laboratories, including viral isolation to detect infectious virus, PCR assays for BVDV genomic material, antigen detection by fluorescent antibody testing, IHC, and ELISA.[16] Skin (ear notch) specimens fixed in neutral-buffered 10% formalin are becoming the samples of choice for detection of PI cattle. These specimens are easily collected and remain stable. The virus is detected by performing IHC on skin specimens fixed in neutral-buffered 10% formalin[15,17,18] or by ACE performed on fresh skin (ear notch) specimens.[16] Ideally, a rapid test for detection of PI cattle may be beneficial so that PI cattle can be removed as quickly as possible from the feedlot, thus minimizing continued exposure to BVDV.

Bovine viral diarrhea viruses are, in reality, a diverse group of viruses based on biotypes and genotype and antigen differences. Erroneously, some have equated virulence with cytopathic strains, which is not correct. Cytopathic strains are often used in vaccines.[19] The BVDV genotypes (subtypes) present in North America are primarily BVDV1a, 1b, and 2a.[6-8] However, a BVDV2b strain was isolated in the United States from an animal that died from BRD pneumonia.[20] In the United States, most vaccines against BVDV contain BVDV1a; however, a growing number of vaccines contain BVDV2a.[19] Control of BVDV in cattle entering feedlots relies on vaccines given during processing before entering a feedlot.

The purposes of the study reported here were to compare various diagnostic tests used for detection of BVDV and determine the prevalence of BVDV subtypes 1a, 2a, and 2b in PI calves entering a feedlot. Diagnostic tests compared in our study included ACE performed on fresh skin (ear notch) specimens, IHC performed on skin (ear notch) specimens fixed in neutral-buffered 10% formalin, and an RT-PCR assay.

Materials and Methods

Cattle and sample collection—After weaning, calves weighing approximately 227 kg (499 lb) were processed at a southwest Kansas feedlot prior to placement in a starter yard for approximately 60 days. Calves arrived after purchase by order buyers in several southern and southeastern states. A total of 21,743 cattle were processed from July 1, 2004, to December 21, 2004. Processing at arrival included administration of a modified-live virus vaccine containing BHV-1, PI-3V, and BRSV strains and killed BVDV1a and 2a strains.[a] Clostridial and *M haemolytica–P multocida* immunogens,[b] an anthelmentic, and a metaphylactic antimicrobial were also administered during processing. Ten days after processing, calves received a second modified-live virus vaccine containing BHV-1, BVDV1a, BVDV2a, PI-3V, and BRSV.[c] Fresh skin (ear notch) specimens were collected and placed in phosphate-buffered saline solution during initial processing and tested for BVDV antigen via ACE.[d] The ACE was performed at the veterinary clinic of the attending veterinarian (BH). Cattle testing positive via ACE were removed from the feedlot within 48 hours for retesting. Fresh skin specimens were obtained and placed in phosphate-buffered saline solution for a second ACE and neutral-buffered 10% formalin for IHC. Serum was obtained for virus isolation and RT-PCR assay. All tests were performed at the Center for Veterinary Health Sciences, Oklahoma State University. Additional samples and nasal swab[e] specimens were obtained from certain calves determined to be PI via ACE for virus isolation and titration.

Virus isolation and subtyping—To isolate BVDV, serum samples were inoculated onto MDBK cell monolayers in 24-well plates, as previously described.[13,21] Monolayers were observed and biotypes (cytopathic or noncytopathic) determined on the basis of the presence or absence of cytopathic effects. For subtyping, serum samples (100 µL) were inoculated into freshly seeded MDBK cells in a flask containing 6 mL of medium. Cultures were incubated for 6 days, subjected to a freeze-thaw cycle, and clarified by centrifugation. Supernatants were stored at –70°C until subtyping.

The BVDV isolates were typed as BVDV1a, 1b, 2a, or 2b on the basis of phylogenetic comparison of sequences of the 5′-UTR of the viral genome, as previously described.[6] Reference BVDV1a, 1b, 2a, and 2b strains were included in the analysis.

Antigen-capture ELISA—Detection of BVDV antigen in skin (ear notch) specimens was performed by use of a commercially available kit[e] following the manufacturer's recommendations. Standardized OD results were calculated by use of the following equation: standardized OD = (raw OD of sample – raw OD of negative control)/(raw OD of positive control – raw OD

of negative control). Samples with standardized OD values < 0.20 were considered negative, and those with OD values > 0.39 were considered positive. Samples with OD values from 0.2 to 0.39 were retested with detector reagents with or without antibody. Standardized OD values for those samples that were retested were calculated by use of the following equation: standardized OD = (raw OD of sample with antibody – raw OD of sample without antibody)/(raw OD of positive control – raw OD of negative control). For samples that were retested, standardized OD values < 0.20 were considered negative and those with values ≥ 0.20 were considered positive. For quality control for each run to be acceptable, the raw OD values for negative and positive controls in the kit[d] must have been < 0.5 and > 0.8, respectively.

IHC for BVDV—A skin (ear notch) specimen was collected from each calf with positive ACE results and immediately placed in a tube with neutral-buffered 10% formalin and stored at 22°C until shipment to the laboratory. Samples were stored at 22°C at the laboratory until submission for IHC (approx 48 hours after initial collection). Immunohistochemistry was performed on skin specimens as described[22] by use of primary anti-BVDV monoclonal antibody 3.12 Fl.[f] Positive tissue controls included those fixed skin specimens from calves confirmed to be PI by multiple viral isolations performed at least 3 weeks apart and positive IHC results. Positive IHC results were characterized by red distinct granular intracytoplasmic staining in the epithelium of the stratum spinosum and stratum basale of the epidermis and follicular infundibulum in > 1 location. Calves from which skin specimens were considered positive via IHC were classified as PI.

RT-PCR assay—A nested multiplex RT-PCR assay was used to detect BVDV in serum.[23] The typing detected BVDV1 and 2 but did not separate the major types into subtypes. Reference strains included in each RNA extraction and RT-PCR assay included BVDV1a (Singer) and BVDV2a (125 C).

Viral titration of BVDV—A microtiter viral titration assay in 96-well plates was used to quantitate viral infectivity in serum and nasal swab specimens.[15] The $TCID_{50}$ per 0.025 mL of sample was calculated by the Spearman-Karber method.[15] Ten-fold dilutions and 4 wells/dilution were used for each sample.

To calculate the viral quantity per 1.0 mL of serum, a dilution factor of 40 was used (log_{10}, 1.6). For nasal swab specimens, it was determined that each swab would absorb 100 µL (0.1 mL). After arrival at the laboratory, each nasal swab specimen was placed in 2 mL of cell culture medium (nasal swab specimen dilution). A 0.025-mL volume of a 1:10 dilution of nasal swab specimen dilution was used as the inoculum. Thus, to determine the viral quantity/1.0 mL of nasal swab specimen dilution, a dilution factor of 40 × 2 × 10 (800) was used (log_{10}, 2.9).

Statistical analysis—Differences among viral isolations of each BVDV subtype were compared by use of a 2-tailed Fisher exact test by use of a computer software program.[g] Values of $P < 0.05$ were considered significant.

Results

A total of 21,743 calves were tested during processing performed at entry to the feedlot. Calves were purchased from order buyers in auction markets of 10 southern and southeastern states; the prevalence of PI cattle per state ranged from 0.13% to 2.0% (**Table 1**). Eighteen order buyers supplied cattle (**Table 2**). Results of initial ACEs performed on fresh skin specimens were positive in 88 calves. Those calves were moved to a quarantine pen. Within approximately 48 hours of the initial collection and ACE, a second set of samples was collected and sent to the diagnostic laboratory. Of the original 88 calves with positive ACE results, 86 had positive ACE results on the second set of samples. In all 86 calves, results of IHC performed on skin specimens fixed in neutral-buffered 10% formalin, RT-PCR assays performed on serum, and isolation of infectious virus by cell culture inoculation were also positive. Two calves with negative ACE results also had negative IHC, RT-PCR assay, and cell culture isolation results. In those 2 calves, initial OD values for ACE were 0.51 and 0.45, both of which were > 0.39 and considered positive. The OD values for the second samples from those calves were 0.04 and 0.01, which were < 0.20 and therefore considered negative. On the basis of negative ACE, IHC, and RT-PCR assay results and because virus was not isolated from the second samples, these calves had false-positive test results because of unknown reasons. Additional fresh skin specimens were collected 21 and 27 days after the initial ACE was performed, and results for both tests were negative. The initial detection of 88 calves with positive ACE results followed by subsequent identification of 86 calves in which results of various diagnostic tests were positive indicated that the predictive value for the initial ACE was 97.7%.

The prevalence of cattle PI with BVDV entering a feedlot as determined by IHC was 0.40%

Table 1—Prevalence of cattle PI with BVDV by order buyer location.

State of buyer	No. of cattle	No. of PI cattle	BVDV subtypes			Prevalence of PI cattle (%)	Total No. of pens	No. (%) of pens with PI cattle	Percentage of pens with PI cattle
			BVDV1a	BVDV1b	BVDV2a				
Arkansas	403	8	0	7	1	2.0	4	3 (75)	75.0
North Carolina	850	4	0	4	0	0.47	11	4 (36.4)	36.4
Florida	1,930	3	0	3	0	0.16	18	3 (16.7)	16.7
Kentucky	416	1	0	1	0	0.24	4	1 (25)	25.0
Missouri	1,323	2	0	2	0	0.15	15	2 (13.3)	13.3
Mississippi	756	1	1	0	0	0.13	8	1 (12.5)	12.5
Oklahoma	8,184	42	5	33	4	0.51	88	36 (40.9)	40.9
Tennessee	1,227	7	2	3	2	0.57	14	7 (50)	50.0
Texas	5,691	15	2	12	1	0.26	67	15 (22.4)	22.4
Virginia	963	3	0	2	1	0.31	11	2 (18.2)	18.2
Total	**21,743**	**86**	**10**	**67**	**9**	**0.40**	**240**	**74 (30.8)**	**30.8**

Table 2—Prevalence of cattle PI with BVDV by individual buyer.

Buyer	State	Total No. of cattle purchased	No. of PI cattle	Prevalence (%)	BVDV Subtypes			No. of pens	No. (%) of pens with PI cattle
					BVDV1a	BVDV1b	BVDV2b		
1	Oklahoma	7,480	38	0.51	5	30	3	80	32 (40)
2	Missouri	1,323	2	0.15	0	2	0	15	2 (13.3)
3	Virginia	277	2	0.72	0	1	1	3	1 (33.3)
4	North Carolina	850	4	0.47	0	4	0	11	4 (36.4)
5	Florida	1,604	2	0.12	0	2	0	15	2 (13.3)
6	Texas	3,689	12	0.33	1	11	0	45	12 (26.7)
7	Kentucky	314	0	0.0	0	0	0	3	0 (0)
8	Virginia	686	1	0.15	0	1	0	8	1 (12.5)
9	Tennessee	1,227	7	0.57	2	3	2	14	7 (50)
10	Mississippi	756	1	0.13	1	0	0	8	1 (12.5)
11	Kentucky	102	1	0.98	0	1	0	1	1 (100)
12	Florida	326	1	0.31	0	1	0	3	1 (33.3)
13	Oklahoma	221	1	0.45	0	1	0	2	1 (50)
14	Arkansas	403	8	2.0	0	7	1	4	3 (75)
15	Texas	1,575	1	0.06	0	1	0	17	1 (5.9)
16	Oklahoma	100	1	1.0	0	1	0	1	1 (100)
17	Texas	427	2	0.47	1	0	1	5	2 (40)
18	Oklahoma	383	2	0.52	0	1	1	5	2 (40)
	Total	**21,743**	**86**	**0.4**	**10**	**67**	**9**	**240**	**74 (30.8)**

(86/21,743 cattle). Normal sorting procedures would have resulted in 74 of 240 (30.8%) pens with at least 1 PI calf. Some pens would have had numerous PI calves.

Of the 86 PI calves in which RT-PCR assay results were positive, 77 were infected with BVDV1 strains and 9 were infected with BVDV2 strains. The RT-PCR assay did not segregate BVDV1 and BVDV2 into subtypes. Calves with positive RT-PCR results also had positive IHC, viral isolation, and ACE results.

As expected, BVDV isolates from the 86 PI calves were noncytopathic. On the basis of sequencing of a 5'-UTR, there were 67 BVDV1b (77.9%), 10 BVDV1a (11.6%), and 9 BVDV2a (10.5%) strains. The most common ($P < 0.05$) BVDV subtype isolated in PI calves was BVDV1b. There was no significant difference in the number of isolates that were BVDV1a and 2a. There was 100% agreement among results of RT-PCR assays and subtyping of cell culture isolates for the 2 major subtypes, BVDV1 and BVDV2.

The 86 PI calves were held in quarantine pens or subsequently remained in the starter yard. Within approximately 60 days after processing and arrival, 22 of the 86 PI calves had died; 14 had mucosal disease, 6 had respiratory tract disease, 1 had bloat, and the diagnosis in 1 calf was not known. Twenty-five of the original 86 calves that were tested 1 to 4 months after initial sample collection for IHC testing had positive IHC results on the second test.

The 86 PI cattle were from order buyers in 10 southern and southeastern states (Tables 1 and 2). Cattle were purchased at auction markets, sorted, and shipped to a feedlot in Kansas. State of origin information for breeding herds was not available. The industry practice is for calves from each shipment (from the shipping truck) to be placed in pens with approximately 60 to 100 calves/pen. The 21,743 calves were placed in 240 pens. Cattle from each buyer's individual shipment could then be placed in separate pens and the number of pens with PI cattle identified. The percentage of pens with PI cattle was expected. There would have been 74 of 240 (30.8%) pens with PI calves based on each shipment being placed in 1 pen (74 shipments with 1 or more PI calves). The percentage of PI calves by order buyer state ranged from 0.13% to 2.0%; the expected percentage of pens with PI cattle for order buyers in all 10 states ranged from 12.5% to 75.0%.

Eighteen order buyers supplied cattle in our study (Table 2). The prevalence of PI cattle purchased by each buyer ranged from 0.0% to 2.0%. The percentage of pens with PI cattle for each buyer ranged from 0% to 100%. These numbers may be misleading as buyer 16 supplied only 100 cattle (1 pen), buyer 11 supplied 102 cattle (1 pen), and buyer 7 supplied 314 cattle (3 pens). Eliminating those 3 buyers with limited numbers of cattle, the percentages of pens with PI cattle in the next closest herds based on numbers of calves (from low to high) were 5.9% (buyer 15) and 75.0% (buyer 13). Because several shipments had numerous calves that were PI with BVDV, only 74 rather than 86 pens had PI cattle.

Concentrations of infectious virus in serum samples of PI calves were determined for available calves (**Table 3**). Serum samples from 12 calves were assayed for infectious virus by cell culture inoculation with the titers adjusted to 1.0 mL. Viral titers per milliliter among the 12 calves ranged from 4×10^2 $TCID_{50}$/1.0 mL to 7×10^4 $TCID_{50}$/1.0 mL of serum. Paired serum samples from 5 calves assayed for virus had < 1 log_{10} difference in viral titers.

Nasal swab specimens and serum samples collected simultaneously from 3 PI calves were assayed for infectious virus (**Table 4**). Results of IHC performed on skin specimens obtained at the same time and fixed in neutral-buffered 10% formalin were positive for BVDV. Viral titers of nasal swab specimens in the 3 calves ranged from 1.4×10^4 $TCID_{50}$ to 4.5×10^4 $TCID_{50}$. Viral titers in serum were similar to viral titers in nasal swab specimens, with < 0.5 log_{10} difference for each calf.

Table 3—Results of serum viral titers in calves PI with BVDV entering a feedlot.

Calf	BVDV subtype	Date of sample	Viral titer per milliliter ($TCID_{50}$)
621	2a	7/20/04	1.3×10^4
15011	1b	7/20/04	4×10^4
		1/04/05	2.2×10^4
1381	1a	8/04/04	4×10^3
6704	1a	8/12/04	4×10^2
6499	1b	9/01/04	7×10^3
		1/04/05	1.3×10^4
17213	1a	9/30/04	4×10^4
9180	1a	10/19/04	1.3×10^4
3910	1a	10/27/04	7×10^3
		1/04/05	4×10^4
4276	1a	11/10/04	7×10^4
17503	1b	10/07/04	2×10^4
		1/04/05	1.3×10^4
4345	2a	11/10/04	2×10^3
6351	1b	8/04/04	4×10^4
		1/04/05	4×10^4

In each calf, results of IHC performed on skin (ear notch) specimens fixed in neutral-buffered 10% formalin were positive.

Table 4—Results of serum and nasal swab specimen viral titers in calves PI with BVDV entering a feedlot.

Calf	BVDV subtype	Date sample obtained	Sample	Viral titer per milliliter ($TCID_{50}$)
4276	1b	4/22/05	Serum	4.0×10^4
			Nasal swab specimen	1.4×10^4
4061	1b	4/22/05	Serum	2.2×10^4
			Nasal swab specimen	4.5×10^3
13984	1b	4/22/05	Serum	2.2×10^4
			Nasal swab specimen	4.5×10^4

In each calf, results of IHC performed on skin (ear notch) specimens fixed in neutral-buffered 10% formalin were positive.

Discussion

Results of the study reported here indicated that an antigen detection system for BVDV, in this case, the initial ACE performed on fresh skin specimens in phosphate-buffered saline solution from all entering cattle, identified a high percentage (97.7%) of cattle as PI with BVDV when subsequent samples were collected, and additional tests were performed. The initial ACE identified all PI calves confirmed by various diagnostic tests performed on subsequent samples. Only 2 of 88 calves were considered to have false-positive ACE results, whereas the remaining 86 (97.7%) calves were determined to be PI on the basis of positive results obtained by use of ACE, IHC, RT-PCR assay, and virus isolation. The ACE has several advantages over the other diagnostic tests. Antigen capture ELISA can be performed within hours of skin specimen collection, permitting the feedlot veterinarian and manager to make decisions on biosecurity and control. Calves with positive ACE results can be isolated while additional samples are being collected and submitted for further testing. Immunohistochemistry requires use of hazardous material (ie, neutral-buffered 10% formalin), shipment to a diagnostic laboratory, and processing with visual diagnosis by a pathologist. Antigen capture ELISA gives results for individual cattle, whereas serum samples used for RT-PCR assays are pooled. Although RT-PCR assay is useful for detection of PI cattle if results are negative, further testing is required for all individual samples in the pool if results are positive. Therefore, additional time would be required to identify PI cattle.

Results of the study reported here indicated that the prevalence of PI cattle entering a feedlot was 0.4% and are in agreement with results of other studies.[15,24,25] Lonergan et al[15] reported that 0.3% of cattle entering a feedlot were PI with BVDV as determined by IHC performed on skin (ear notch) specimens. Larson et al[24] reported that 3 of 938 (0.32%) calves in 2 stocker operations were PI with BVDV as determined by IHC performed on skin (ear notch) specimens. Wittum et al[25] performed a multistate study of beef herds represented by 18,931 cattle from which serum samples were assayed for infectious virus by microtiter viral isolation. In that study, 33 calves (0.17%) were confirmed to be PI on the basis of 2 sequential positive test results.

Our study as well as 2 other recently published studies[15,24] in PI cattle used results of 1 IHC test for BVDV performed on skin (ear notch) specimens fixed in neutral-buffered 10% formalin as the criterion used to classify cattle as PI. However, results of another study[18] indicate that cattle acutely or transiently infected with BVDV could test positive via IHC performed on fixed skin (ear notch) specimens and via ACE performed on fresh skin specimens. In that study, results for IHC, ACE, or both performed monthly for a minimum of 3 months were positive in 8 calves, whereas results of viral isolation and PCR assay performed

monthly were negative; these calves were considered as acutely or transiently infected. In our study, we did not have continual access to calves for repeated testing. Criteria for IHC or virulence of isolates used in the study by Cornish et al[18] may have been different from those used in our study. By use of the same IHC diagnostic test and criterion for positive IHC results and PI status, personnel at the Oklahoma Animal Disease Diagnostic Laboratory have consistently (100%) identified PI calves (n = 60) for which samples were available at 6-month to 1-year intervals.

Results of our study also yielded information on the prevalence of BVDV genotypes and subgenotypes in PI cattle and further support the high prevalence of BVDV1b (79%), compared with BVDV1a (11.6%) and BVDV2a (10.5%), in PI cattle entering a feedlot in the United States detected in previous studies. Results of a study[26] of dairy operations in the United States by use of bulk milk samples and samples from infected dairy cattle indicate that the prevalence of BVDV1b, 1a, and 2a were 49.1%, 11.3%, and 39.33%, respectively, from 53 isolates. Results of a survey[27] of BVDV isolates from diagnostic laboratory accessions (n = 131 cases) indicate that 45.8% were BVDV1b, 28.2% were BVDV1a, and 26.0% were BVDV2a. The prevalence of each genotype is important because it relates to vaccine use for control and diagnostic testing. The diagnostic tests used in the study reported here detected all 3 subtypes found in the United States; BVDV1a, 1b, and 2a were detected via IHC and ACE performed on skin (ear notch) specimens, and both major types (BVDV1 and 2) were detected in serum samples via RT-PCR assay. However, vaccines used in North America contain predominantly BVDV1a, although a growing number of vaccines now contain BVDV2a.[12] Reportedly, there is only 1 vaccine manufactured with BVDV1b, although it may be sold to and marketed by multiple companies.[12] Vaccines with BVDV1a and 2a should be evaluated for protection against BVDV1b, or BVDV1b vaccines should be developed for protection against this common BVDV subtype.

The prevalence of BVDV subgenotypes by state of order buyer origin was determined for each state. Because procurement of cattle for this feedlot relies on auction market cattle, the herd of origin (breeding herd) by state was not always readily available. When a national animal identification program becomes operational, each animal's place of birth and subsequent movement will be accurately determined. Strain BVDV1b was detected in cattle from order buyers in 9 of 10 states. Mississippi, which was negative for BVDV1b or 2a, only had 1 PI calf. This calf was infected with a BVDV1a strain. Yet, order buyers in states to the west (Arkansas), south (Florida), and north (Tennessee) of Mississippi all had cattle infected with BVDV1b. Strain BVDV1b was detected in cattle from order buyers in states from Florida to Virginia and west to Texas and Oklahoma.

The effect of a PI calf in a feedlot has been characterized in another study.[15] Exposure to a PI calf was defined as housing in the same pen with or a pen adjacent to a PI calf, which resulted in exposed cattle having a 43% greater risk for respiratory tract disease, compared with cattle that were not exposed to a PI calf.[15] Exposed cattle also had greater risk for treatment of respiratory tract disease and received more treatments than cattle that were not exposed.[15] In that study, 15.9% of initial treatments in cattle were attributable to exposure to a PI calf.[15] The ability of a PI calf to transmit infection to penmates is considerable. Results of 1 study[11] indicate that susceptible nonvaccinated calves in feedlot pens exposed to PI calves become infected as defined by seroconversion, viral isolation, or both in 70% to 100% of the calves in multiple pens. The potential for 1 PI calf to transmit infection and cause disease in a feedlot is extraordinary. In our study, cattle in 74 of 240 (30.8%) pens would have had direct exposure to a PI calf. Inclusions of pens adjacent to a pen containing a PI calf would have greatly increased this percentage.

Viral load in serum, vaginal mucus, feces per gram, and urine of PI calves has been determined.[28,29] In 1 study,[28] serum viral titers in 7 calves ranged from 5×10^3 $TCID_{50}$/1.0 mL to 5×10^5 $TCID_{50}$/1.0 mL. In the study reported here, serum titers of PI calves were similar and ranged from 2×10^3 $TCID_{50}$/1.0 mL to 7×10^4 $TCID_{50}$/1.0 mL. Results of another study[27] indicate that the median cell culture infectious dose in vaginal mucus, feces per gram, and urine from 1 calf was 1×10^4/mL and the median cell culture infectious dose in serum was 1×10^6. Results of the study reported here indicated that viral concentrations per milliliter of serum and nasal swab specimens are similar. Thus, nasal mucus contains a considerable concentration of virus and is a likely source of virus to susceptible calves. Results of a study[30] in which IHC was performed on biopsy and necropsy specimens of various regions of the respiratory tract from PI calves indicate that BVDV antigen is present in the nasal mucosa. In that study, BVDV was detected in mixed tubuloalveolar glands, including serous secretory cells and ductular epithelium. Thus, BVDV in nasal secretions is the result of locally produced infectious virus in the nasal mucosa. In addition, the virus could potentially come from leukocytes and serum (local production or migrating cells). Finding the virus in nasal swab specimens underscores the importance of removing PI cattle to prevent exposure by direct or close contact of PI cattle with susceptible cattle.

a. Prism 4, Fort Dodge Animal Health, Fort Dodge, Iowa.
b. Pulmo-Guard PhM-1, Boehringer Ingelheim Vet Medica Inc, St Joseph, Mo.
c. Express 5, Boehringer Ingelheim Vet Medica Inc, St Joseph, Mo.
d. Bovine virus diarrhea antigen test kit, Syracuse Bioanalytical Inc, East Syracuse, NY.
e. Viral culturette, Becton-Dickinson & Co, Franklin Lakes, NJ.
f. Saliki JT, Oklahoma Animal Disease Diagnostic Laboratory, Stillwater, Okla.
g. PC SAS, version 8.2, SAS Institute, Cary, NC.

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