# The Prevalence of Bovine Viral Diarrhea Virus Infection in a Population of Feedlot Calves in Western Canada

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# ABSTRACT

The prevalence of bovine viral diarrhea virus (BVDV) infection was examined in a population of 5129 recently weaned steer calves entering a large feedlot in central Saskatchewan from September to December 1991.

Serum samples were collected within 24 h of arrival at the feedlot from every fifth calf processed and again 96 d postarrival. A microtiter virus isolation test was used to determine the prevalence of calves viremic with BVDV on entry to the feedlot. An enzyme-linked immunosorbent assay (ELISA) which detects antibody against glycoprotein 53 of the BVDV was used on paired sera to determine the seroconversion risk during the first 96 d in the feedlot. A virus neutralization (VN) test for BVDV was conducted on a sub-sample of paired sera to measure agreement in determination of seroconversion risk with the ELISA. A polymerase chain reaction (PCR) test which detects BVDV was used to determine if cattle were acutely viremic when treated for disease.

The estimated prevalence of persistently infected calves in this population was <0.1%. The seroconversion risk for BVDV was 27% (236/ 864) according to the ELISA and it varied from 0 to 63% among the 20 pens sampled. According to the VN test, the seroconversion risk for BVDV was 40% (132/327) and it varied from 0 to 100% among the 11 pens tested. The agreement between the ELISA and VN tests in seroconversion risk to BVDV was very poor (kappa =  $0.15 \pm 0.039$  SE). The prevalence of acute viremia in calves treated at the feedlot hospital was low at 4% (6/149).

Although the prevalence of persistent infection was low, serological tests suggested a high risk of seroconversion to BVDV. Unfortunately, the detailed epidemiology of acute BVDV infection in this feedlot could not be described because of the lack of agreement between the two serological tests. The two tests classified different individuals and groups of calves as having been infected with BVDV during the first 96 d in the feedlot. While the benefits of the ELISA for BVDV may seem considerable, the dynamics of the titer change and their interpretation subsequent to natural exposure to BVDV, need to be better defined before these tests can be used with confidence in future feedlot studies.

# RÉSUMÉ

Entre les mois de septembre et décembre 1991, la prévalence de l'infection par le virus de la diarrhée virale bovine (VDVB) a été évaluée dans une population de 5129 veaux récemment sevrés, à leur entrée dans un parc d'engraissement en Saskatchewan.

Des échantillons de sérum ont été prélevés à partir d'un veau sur cinq, au cours des premières 24 heures suivant l'arrivée, ainsi qu'au 96 jours suivant la date d'arrivée. Un test d'isolement viral sur microplaque a été utilisé dans le but de déterminer la prévalence de veaux virémiques au moment de leur entrée. Un test immunoenzymatique (ELISA) détectant les anticorps dirigés contre la glycoprotéine 53 du VDVB était utilisé sur des échantillons pairés de sérum afin de déterminer le risque de séroconversion durant les 96 premiers jours de présence dans le parc d'engraissement. Un test de séroneutralisation (SN) du VDVB a été réalisé sur un sous-échantillonnage des sérums pairés afin de mesurer l'agrément avec le risque de séroconversion détecté par l'ELISA. Un test d'amplification en chaîne par la polymérase détectant le VDVB a été utilisé afin de déterminer si les bovins étaient en virémie aigüe lorsque traité pour la maladie.

La prévalence des veaux infectés de façon chronique est évaluée à moins de 0.1 %. Le taux de séroconversion (ELISA) pour VDVB est de 27 % (236/864) avec une variation de 0 à 63 % dans les 20 enclos échantillonnés. Le test de SN indiquait une séroconversion pour le VDVB de 40 % (132/327) avec une variation de 0 à 100 % dans les 11 enclos échantillonnés. L'agrément entre les tests ELISA et SN pour la séroconversion pour le VDVB était très faible  $(kappa = 0.15 \pm 0.039 \text{ SE})$ . La prévalence de veaux virémiques en phase aiguë traités à l'hôpital du parc d'engraissement était faible (4 % ).

Bien que la prévalence d'infection persistente était faible, les résultats des tests sérologiques suggèrent un fort risque de séroconversion au VDVB. Le manque de corrélation entre les deux tests sérologiques empêche toute possibilité de description épidémiologique d'une infection aigüe par VDBV dans ce parc d'engraissement. Les deux test classaient différemment

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des individus et des groupes de veaux comme ayant été infectés par le VDVB durant les premiers 96 jours en parc d'engraissement. Bien que les bénéfices du test ELISA pour le VDVB paraissent considérables, les dynamiques du titre change et leur interprétation suivant une exposition naturelle au VDVB doivent être mieux définies avant que ces tests puissent être utilisés avec confiance dans des études ultérieures. (*Traduit par Dr Serge Messier*)

## INTRODUCTION

Knowledge of the pathogenesis of disease caused by the bovine viral diarrhea virus (BVDV) has increased dramatically over the last 10 y (1). While the importance of acute infection with BVDV in feedlot calves is debatable (2-16), it is becoming increasingly evident that calves persistently infected (PI) with BVDV are important in the epidemiology of the disease (1). Persistently infected calves tend to have poor growth rates, low survivability and typically die from chronic ill-thrift, secondary bacterial and viral infections or mucosal disease (MD) (17-20). The importance of PI calves was not considered in previous feedlot studies (4-7). The prevalence of PI calves born in western Canadian beef herds has never been determined, but previous studies in other populations of cattle have estimated that approximately 1% of calves are PI (1.21-22).

Epidemiological studies of BVDV infection rely on serological tests to predict the prevalence of acute subclinical infection (23). Previously, these studies have relied on virus neutralization (VN) tests to classify cattle as infected with BVDV(4-7). However, because of antigenic variation among isolates of BVDV, VN tests lack sensitivity (24,25) and cattle may be misclassified as infected or uninfected. The cost and labor requirements associated with VN tests are also considerable. Enzyme-linked immunosorbent assays have been developed as an alternative to VN tests and can detect antibodies to various antigens of the BVDV (26,27). An ELISA is particularly attractive

TABLE I. Summary of samples collected and tested for bovine viral diarrhea virus and antibody

	п	<i>n</i> tested with microtiter			
Pen	sampled	virus isolation test	paired sera	Total in pen	
1	49	49	<b>O</b> <sup>a</sup>	253	
2	54	54	<b>O</b> <sup>a</sup>	275	
3 4	21	21	Oª	108	
4	37	37	36	168	
5	58	58	57	284	
6	53	53	49	253	
7	55	55	49	258	
8	21	21	20	100 167 267	
9	35	34	33		
10	54	54	51		
11	51	44	43	253	
12	55	48	44	276	
13	60	60	56	297 283 247	
14	57	56	54		
15	50	50	50		
16	61	61	58	301	
17	57	57	56	282	
18	43	43	39	211	
19	57	57	56	281	
20	57	57	57	283	
21	12	12	12	51	
22	36	36	32	176	
23	12	12 12		55	
Total	1045	1029 <sup>b</sup>	864 <sup>b</sup>	5129	

<sup>a</sup>Calves in the first three pens were only sampled at reimplanting approximately 96 days after arrival. All other calves were sampled at processing, within 24 h of arrival at the feedlot <sup>b</sup>Some vials were broken and therefore not tested

because of the ease with which the procedure can be automated. In this study, an ELISA was developed to detect antibody to the glycoprotein 53 (gp53) antigen because antibody to gp53 effectively neutralizes BVDV infectivity (28,29). By detecting antibodies to the gp53 antigen, which is common among BVDV isolates (29), this ELISA should be less strain specific than the VN test.

The objectives of this study were: 1) to determine the prevalence of persistent infection with BVDV in calves entering a western Canadian feedlot, 2) to evaluate a new ELISA to detect antibody to gp53 of the BVDV, 3) to determine the prevalence of acute BVDV infection in calves during the first 96 d in the feedlot and 4) to determine the prevalence of BVDV viremia in calves that were treated for disease.

#### **MATERIALS AND METHODS**

#### THE STUDY POPULATION

This study was conducted at a 10 000 head capacity commercial feedlot in central Saskatchewan

during the fall and winter of 1991-1992. A total of 5129 recently weaned steer calves, approximately 7 to 10 mo of age and weighing between 250 and 350 kg, were purchased from auction markets across western Canada. Within 24 h of arrival at the feedlot calves were processed. Processing included unique identification with an eartag, branding, implantation with zeranol (Ralgro, I.M.C., Terre Haute, Indiana), treatment with ivermectin (Ivomec pour on, MSD Agvet, Kirkland, Quebec), injection with 2 mL of vitamins A and D (Poten AD, Rogar/STB, Pointe Claire-Dorval, Quebec), and immunization with a multivalent clostridial bacterin (Tasvax 8, Coopers Agropharm Inc., Ajax, Ontario), a Pasteurella haemolytica vaccine (Presponse, Langford Inc., Guelph, Ontario or Pneumo-Star, Biowest Inc., Saskatoon, Saskatchewan), and a modified-live infectious bovine rhinotracheitis virus and parainfluenza-3 virus vaccine (Bovishield 2, Smith Kline Beecham Animal Health, Missisauga, Ontario). A rectal temperature was measured on all calves and those with a fever ( $\geq 40.3^{\circ}$ C) were

treated with tilmicosin (Micotil, Provel, Division Eli Lilly Canada Inc., Scarborough, Ontario) and all others were treated prophylactically with long-acting oxytetracycline (Liquamycin LA, Pfizer, Montreal, Ouebec). Calves were housed in pens containing from 50 to 300 head (Table I). A pen contained calves originating from 1 to 9 different auction market sources and calves from each auction market came from many different farms. Each pen was managed as a homogeneous unit (a "lot") until slaughter, approximately 180-200 d after arrival.

Blood samples were collected at processing in a systematic random manner from every 5th calf processed (n = 1045). Calves that were sampled on arrival were sampled again at reimplanting 90-100 (median 96) days postarrival. The number of calves sampled for virological (n = 1029) and serological (n = 864) testing for BVDV from each pen is summarized in Table I. Whole blood was collected in 10 mL vacuum tubes (Vacutainer, Becton Dickinson, Mississauga, Ontario) and stored at 4°C for 24 h. Blood samples were centrifuged at  $5000 \times g$  for 10 min and approximately 3 mL of serum were separated and stored at  $-70^{\circ}$ C until assayed.

#### PERSISTENT INFECTION WITH BVDV

Calves were classified as PI if BVDV was isolated from blood or tissue samples collected more than 2 wk apart. If BVDV was isolated from blood samples prior to death or from tissues obtained at necropsy and the calf died with lesions consistent with MD (30) it was considered to be PI.

#### PREVALENCE OF PERSISTENT INFECTION WITH BVDV

Serum samples that were collected at processing were sent to the diagnostic laboratory, New York State College of Veterinary Medicine. A microtiter virus isolation test was used to determine the prevalence of PI calves. Calves that were positive for BVDV at processing were sampled again at reimplanting. Each serum sample was added in duplicate to bovine testis cells in 96 well microtiter plates. After incubation for 4 d at 37°C, the cells were fixed with acetone. The presence of BVDV-infected cells was determined by an indirect immunocytochemical detection method using monoclonal antibodies to BVDV, biotinylated rabbit anti-mouse IgG, Strepavidin, and 3amino-9-ethylcarbazole as chromogen. A red cytoplasmic coloration of the cells, observed by light microscopy, indicated the presence of BVDV in the sample.

Tissues were collected at necropsy from calves with obvious lesions of MD or with enteritis. Virus isolation for BVDV was undertaken on these tissues to confirm whether the animals were PI. Tissue samples were homogenized in phosphate buffered saline, treated with penicillin/streptomycin solution, centrifuged, and the supernatant inoculated into fetal bovine trachea cell cultures. Cell cultures were incubated at 37°C for 5 d and then stained by an indirect fluorescent antibody technique to demonstrate the presence of BVDV. Non-BVDV infected fetal bovine trachea cell cultures were concurrently stained using the indirect fluorescent antibody technique and served as controls.

#### ACUTE BVDV INFECTION

An ELISA and a VN test were used to detect antibodies to BVDV. A significant increase in antibody titer (seroconversion) during the first 96 d in the feedlot was then used as evidence of acute infection with BVDV during that period.

The ELISA for BVDV was designed to detect antibodies to the glycoprotein 53 (gp53) of BVDV. A recombinant vaccine virus containing a truncated gp53 gene of BVDV was used to infect BSC-1 cells. Secreted gp53 was purified from the cell culture medium by immunoaffinity chromatography using BVDV monoclonal antibodies linked to Affi-Gel 10 (31). An indirect ELISA for titrating BVDV antibodies in microtiter plates was undertaken using a robotic Biomek ELISA system. Ninety-six well Immunolon 2 plates (Dynatech Laboratories Inc., Alexandria, Virginia) were coated with 100 µL purified gp53 diluted in 0.05 M sodium bicarbonate buffer, pH 9.6 (17 ng/well) overnight at 4°C. After 3 washes with distilled water, 100  $\mu$ L of serum serially diluted with diluent (phosphate buffered saline containing 0.05% Tween 20) were

added to each well. The plates were incubated for 2 h at room temperature and washed. Then 100 µL of goat antibovine alkaline phosphatase conjugate, diluted 1:5000 in diluent were added to each well. After incubation at room temperature for 2 h, plates were washed and 1 mg/mL p-nitrophenyl phosphate and a 1% solution of diethylamine containing 0.5 mM MgCl, at pH 9.8 were added to the wells. After incubation for 2 h at room temperature, the enzymatic reaction was stopped by adding 30 µL 0.3M EDTA at pH 8.0 to each well. Absorbance was measured at 405 nm.

There were 864 paired sera available for testing with the ELISA test. A total of 905 sera were transferred to plates for storage, but some plates were not tested due to breakages. A convenience sample of 327 paired sera from 11 pens were tested using the VN test for BVDV antibody (28). Samples were from cattle that entered the feedlot after the 16th October 1991. The Singer isolate of BVDV was the reference strain.

#### DETERMINATION OF "SEROCONVERSION"

A four-fold increase in VN titer during the first 96 d in the feedlot was regarded as a significant increase in BVDV titer and therefore as seroconversion. Because the ELISA was a new serological test and had only been evaluated under laboratory conditions using a limited number of samples from cattle experimentally infected with specific isolates of BVDV, we had to determine how well the test worked on field isolates. Cattle in this feedlot may have been infected with many antigenically different isolates of BVDV. Therefore, our aim was to develop a serological test that was not strain specific.

Ideally, both tests should have been performed on a large number of paired sera from naive cattle experimentally infected with isolates of BVDV that varied vastly in their antigenicity. However, we did not have sufficient resources to create a "gold standard." Therefore, we compared the results of this new ELISA test with VN test results (26,27).

Our aim in this case was to determine if the new ELISA would accurately classify cattle as having been TABLE II. Agreement between the virus neutralization (VN) and enzyme-linked immunosorbent assay (ELISA) tests for prediction of seroconversion to bovine viral diarrhea virus during first 96 d in the feedlot

		V	N			
Е		YES	NO		Observed agreement	61.2%
L	YES	46	41	87	Expected agreement	54.4%
I	NO	84	151	235	% actual agreement beyond chance	6.8%
S		130	192	322	% potential agreement beyond chance	45.6%
Α					Карра	0.15

infected with BVDV during the first 96 d in the feedlot. Therefore, the use of correlations (26,27) was not appropriate. Instead, we determined the kappa statistic for agreement between the VN and ELISA test in classifying cattle as infected with BVDV.

To measure agreement in classification we had to determine a "seroconversion" cutpoint for the ELISA. Several arbitrary cutpoints were tried. The cutpoint which gave the best kappa for agreement with the subsample of calves tested with the VN test was then chosen.

A kappa statistic of 0 indicates no agreement and a kappa of 1 indicates total agreement; a kappa of 0.4–0.6 would indicate moderate agreement between the 2 tests (32). For a fourfold increase in ELISA titer, the kappa was 0.13. For an eight-fold increase in ELISA titer, the kappa was 0.15 (Table II). For a sixteen-fold increase in ELISA titer the kappa was 0.09. Therefore, an eight-fold increase in ELISA titer was chosen as seroconversion.

Blood samples were collected using convenience sampling procedures from calves treated for BRD and other diseases on arrival or when treated at the feedlot hospital. The objective of this testing was to determine if BVDV infection was detectable in calves when they were treated for disease. The case definition of BRD morbidity was depression, inappetance, clinical signs attributable to the respiratory system and no other body system, and a rectal temperature  $\geq 40.3^{\circ}$ C at processing or >40.0°C if treated at the feedlot hospital after processing. Calves treated at the feedlot hospital for conditions other than BRD were sampled for comparison. Blood samples were collected into EDTA blood tubes (Vacutainer, Becton Dickinson, Missisauga, Ontario) and stored overnight at 4°C. Samples were centrifuged at 5000  $\times$  g and the buffy coat removed and stored at  $-70^{\circ}$ C. A total

of 149 calves were sampled. Out of 922 calves treated for BRD, 117 (13%) were sampled and of 423 treated for other conditions, 32 (8%) were sampled. These samples were tested at the Department of Microbiology, Western College of Veterinary Medicine, using the Polymerase Chain Reaction (PCR) test for BVDV (33). This cohort of cattle was then followed until slaughter.

### NECROPSY TECHNIQUE

All cattle that died in the feedlot were necropsied within 24 h of death by the attending feedlot veterinarians. A gross diagnosis was made and when necessary, specimens were submitted to the diagnostic pathology laboratory at the WCVM to determine the cause of death. Mortality was subdivided into death from BRD, apparent Haemophilus somnus infection (hemophilosis), MD, and other causes. Mortality associated with BRD included death from fibrinous pneumonia or bronchopneumonia. Apparent hemophilosis mortality included death from myocarditis, pericarditis, endocarditis, pleuritis, polyarthritis, thrombotic meningoencephalitis and septicaemia (34).

#### STATISTICAL ANALYSIS

All processing, treatment, and necropsy information were recorded at the feedlot using a computerized recording system (Master-Med, Hi-Plains System Inc., Amarillo, Texas). Data were transferred to a database management system (Paradox, Borland International, Scotts Valley, California), and subsequently to a database in SAS (SAS Institute Inc., Cary, North Carolina).

#### RESULTS

The prevalence of viremia with BVDV at first sampling was 0.097% (1/1029) using the microtiter virus

isolation test. However, we could not determine if the calf that tested positive was PI because it was from one of the first three pens to enter the feedlot. These calves were not sampled at processing and were only sampled at reimplanting. Therefore, they were subsequently lost to followup. Therefore, based on the results of the microtiter virus isolation test in the sampled population, the prevalence of PI calves in the feedlot was between 0 and 0.097%. However, 3 calves (3/5129, 0.06%) died from MD in the total (unsampled) population. Bovine viral diarrhea virus was isolated from all 3 calves. Therefore, the apparent prevalence of PI calves in this population was <0.1%.

The kappa statistic for agreement in classification of seroconversion between the VN test and ELISA was  $0.15 \pm 0.039$  (SE) (Table II). This represents very poor agreement between the 2 tests in the classification of cattle acutely infected with BVDV during the first 96 d in the feedlot. The two tests classified different calves and different pens of calves as being infected or not infected with BVDV during the first 96 d of the feeding period.

The overall seroconversion risk to BVDV was 27% (236/864) according to the ELISA and it varied from 0 to 63% among the 20 pens sampled (Table III). This variation was statistically significant ( $\chi^2 = 108.69$ , P <0.00001). According to the VN test, the overall seroconversion risk to BVDV was 40% (132/327) and it varied from 0–100% among the 11 pens tested (Table III). This variation was significantly different among pens ( $\chi^2 = 71$ , P < 0.00001).

In total, 6 (4%) of the 149 treated calves tested with the PCR test were positive for BVDV. Thirty-nine calves were treated for BRD on arrival at the feedlot and none of these calves were positive for BVDV. Five calves (4.27%) from the 78 treated for BRD other than on arrival were positive for BVDV. One calf (3.13%) from the 32 treated for conditions other than BRD was positive for BVDV and it subsequently died of MD. Seven of the 149 treated calves that were sampled subsequently died.

Twelve (1.15%) of the 1045 calves sampled on arrival at the feedlot died. Five (0.47%) deaths were from apparent hemophilosis, 2 (0.19%)were from BRD, and 5 (0.47%) were from other causes. There were no deaths from MD in this group of calves. In the total population of 5129 calves, 75 (1.46%) died, 41 (0.80%)from apparent hemophilosis, 13 (0.25%) from BRD, 3 (0.06%) from MD, and 18 (0.35%) from other causes (bloat, atypical interstitial pneumonia, injuries, diffuse cellulitis and undifferentiated nervous disease).

#### DISCUSSION

The most significant finding of this study was the very low prevalence of PI calves in this feedlot. The estimated prevalence of PI calves of <0.1% is unusually low compared to the estimated 1% reported in other studies (16,21,22). This feedlot population consisted of calves that were well grown for their age (7 to 10 mo of age and 250-350 kg in body weight). Persistently infected calves have been shown to suffer from poor performance and survivability (17-20). Therefore, it is possible that very few PI calves were purchased by this feedlot operator. Whether the prevalence would have been different in other feedlots with different buying specifications, particularly feedlots buying calves that are poorly grown for their age, is something future studies of BVDV in feedlots should address.

Another possible reason for the low prevalence of persistent infection is the poor sensitivity of the microtiter virus isolation test. Also, this test might not detect antigenically different isolates of BVDV. However, our own unpublished observations would suggest that the sensitivity of the microtiter virus isolation test for the detection of PI calves is equal to the traditional virus isolation technique for BVDV.

It is possible that there were PI calves in the feedlot that were not in the sampled population. Some of these may have died from diseases other than MD, or survived until slaughter. However, based on our estimated prevalence of PI calves, this number should have been very low. Tissues for BVDV isolation were only collected from calves with enteritis or those suspected of dying from MD. Tissues should have been collected TABLE III. Prevalence of BVDV infection by pen including the number of calves that tested positive to BVDV using the microtiter virus isolation test (MVI); geometric mean titers (GMT) to BVDV determined by an enzyme-linked immunosorbent assay (ELISA) detecting antibody to gp53 of the BVDV at processing (day 0) and d 96; seroconversion risk determined by the ELISA and a virus neutralization (VN) test; the prevalence of acute BVDV infection determined in sick cattle using the polymerase chain reaction (PCR) test and the total number sampled for PCR testing

		GMT	GMT	ELISA	VN	PCR	n tested
Pen	MVI	Day 0	Day 96	(%)	(%)	positive	with PCR
1	1	***	12 765	***	***	0	4
2	0	***	239	***	***	0	8
3	0	***	13 493	***	***	0	1
4	0	5557	11 268	17	***	0	1
5	0	7231	12 766	12	***	1	15
6	0	4330	20 453	35	***	0	18
7	0	7538	24 492	22	***	0	5
8	0	7585	23 494	15	***	0	2
9	0	1097	8540	39	***	1	2
10	0	695	8659	43	***	1	28
11	0	84	2702	63	***	1	20
12	0	174	7332	55	65	0	16
13	0	2288	6841	34	46	1	6
14	0	1128	7231	43	24	1	3
15	0	4837	8780	12	13	0	2
16	0	5043	14 869	9	36	0	15
17	0	1010	7750	30	35	0	3
18	0	5634	9410	21	44	***	0
19	0	6747	27 746	29	100	***	0
20	0	6841	14 869	23	77	***	0
21	0	3468	17 080	17	0	***	0
22	0	222 227	14 067	0	29	***	0
23	0	1783	12 766	25	***	***	0
Total	1	2628	14 462	27	40	6	149

from all dead calves to determine if BVDV could be isolated from calves that died of causes other than MD, particularly respiratory disease and apparent hemophilosis. However, had we been able to isolate BVDV from the tissues of these calves, we would not have been able to differentiate if this infection was an acute infection or persistent infection without previous isolation of BVDV. Based on previous estimates of the prevalence of PI calves in the bovine population (16,21-22), we had hoped to have a significant number of known PI calves in the sampled population which could have been monitored for survival and performance relative to healthy calves.

This is the first published study where an ELISA and VN test for BVDV have been evaluated by kappa. There are many possible reasons for the lack of agreement between the VN test and ELISA in the classification of infected individuals. The ELISA may lack accuracy (23). It may have been very sensitive and detected residual maternal antibody, with an apparent reduction in the increase in antibody titer during the

first 96 d in the feedlot. However, there is no published data to support this theory. A previous study has suggested that ELISA titers rise at a much higher rate than VN titers in seronegative cattle after challenge with BVDV (26). If a significant number of calves were infected with BVDV immediately prior to entering the feedlot, they may have had antibody titers which were detectable with the ELISA test and not with the VN test. The rate of decay of ELISA titers may also be somewhat different from VN titers (26). Therefore, the dynamics of the titer change with our ELISA test should be more thoroughly evaluated prior to use in further studies. Others have examined the dynamics of the titer change and shown promising results for ELISA tests when compared to a VN test (26). However, the increase in BVDV titers used as the baseline was due to vaccination with a known isolate of BVDV and not natural exposure. These authors pointed out that the correlation between the VN and ELISA tests was greatly reduced when sera from field submissions were tested. Cattle in a feedlot situation

may be exposed to many antigenically different isolates of BVDV.

Future evaluation of an ELISA for BVDV would be much easier if a bank of standard sera (23) were available from cattle experimentally challenged with a wide variety of antigenically different BVDV isolates. If these cattle were sampled frequently after exposure, the dynamics of the titer change could also be assessed.

If we had sampled the calves in this study more frequently, for example at 30-d intervals, very different results may have been obtained. However, this was not possible without constructing a separate handling facility, strictly for resampling calves. In order to cope with the demands of processing incoming calves and treating sick calves, both processing and hospital handling facilities in this feedlot were fully utilized during the early stages of this study.

Despite the low prevalence of PI calves in this population, the risk of acute infection with BVDV appeared to be high based on the increase in antibody titers during the feeding period (Table III). Infections with BVDV most likely occurred when naive calves came in contact with PI calves on the farm of origin or during the transport and selling process, establishing acute infections. The lack of PI calves in the feedlot population would suggest that once calves entered the feedlot environment, BVDV spread from acutely infected calves to naive calves, so that by the end of the feeding period, most calves had been exposed to BVDV. However, the calves in the 2nd pen appeared to have been protected from **BVDV** infection during the feeding period because they had a low geometric mean titer to BVDV after 96 d in the feedlot. Presumably, few or no calves in this pen were infected prior to or during their time in the feedlot.

We could not determine with confidence which individual calves were acutely infected with BVDV during the first 96 d of the feeding period because of the poor agreement between the VN and ELISA tests. Therefore, it was not possible to do more detailed studies of the seroepidemiology of BVDV infection in this feedlot, particularly with regard to its association with BRD.

The prevalence of acute viremia with BVDV in treated animals was surprisingly low, despite the apparent high risk of infection with BVDV based on the serological tests. Unfortunately, only a small proportion of sick cattle were sampled and because of delays in testing, positive calves were lost to followup. However, one of the viremic calves was obviously PI because it died from MD. The remaining 5 calves that were positive for BVDV did not die. It is possible that the PCR test is too specific and antigenically different BVDV isolates were missed. However, this is unlikely. Because the duration of acute viremia in calves experimentally infected with BVDV has been shown to range from 1-15 d (25). most infected calves should have still been viremic at the time of treatment. if infection with BVDV was associated with disease.

In conclusion, although the prevalence of persistent infection with BVDV in this feedlot was very low, the apparent risk of acute infections with BVDV based on the serological tests appeared to be high. Bovine viral diarrhea virus would appear to have spread from acutely infected calves to naive commingled calves in this feedlot. Since there were problems with agreement between the serological tests used, we could not confidently describe the epidemiology of acute BVDV infection in the feedlot. While the benefits of an ELISA test for BVDV may seem considerable, the dynamics of the titer change in naive calves subsequent to natural exposure to BVDV need to be better defined before they can be used with confidence in future feedlot studies.

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