Performance, survival, necropsy, and virological findings from calves persistently infected with the bovine viral diarrhea virus originating from a single Saskatchewan beef herd

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Abstract — Fifty-one calves from 652 cows and heifers that calved on a Saskatchewan ranch in 1992 were identified as persistently infected with bovine viral diarrhea virus (BVDV), based on virological and necropsy findings. Herd records suggested a further 20 calves that died between birth and weaning were probably also persistently infected. Subsequent to weaning, all surviving persistently infected calves were transferred to one pen in a 10 000 head commercial feedlot, to mimic normal management practice in western Canadian beef herds.

On average, when compared with healthy, BVDV-negative herdmates, persistently infected calves were "poor doers" and had poor survivability, with only 4 persistently infected calves surviving to 1 year of age. There was no difference ($P > 0.05$) in survival between male and female persistently infected calves.

The clinical, pathological, and virological findings from these persistently infected calves varied over time. The majority of persistently infected calves had gross pathological lesions at necropsy, consistent with mucosal disease. However, approximately 25% of the persistently infected calves had gross pneumonic lesions at necropsy, with no or only mild lesions of mucosal disease. A wide variety of other lesions were also noted in persistently infected calves at necropsy. Therefore, the possibility that BVDV-induced lesions can be misdiagnosed is very real.

The results of this study indicate that persistent infection with BVDV should always be considered in calves with chronic ill thrift, chronic enteritis, or respiratory disease.

Résumé — Performance, survie, nécropsie et observations virologiques de veaux infectés de façon persistante par le virus de la diarrhée virale des bovins provenant d'un unique troupeau de bovins de boucherie de la Saskatchewan. Selon des observations virologiques et nécrologiques, 51 veaux provenant de 652 vaches et génisses ayant vêlé dans un ranch de la Saskatchewan en 1992 ont été identifiés comme étant infectés de façon persistante, par le virus de la diarrhée virale des bovins (VDVB). Les dossiers du troupeau laissaient supposer que 20 autres veaux morts entre la naissance et le sevrage étaient probablement aussi infectés de façon persistante. À la suite du sevrage, tous les veaux survivants infectés de façon persistante ont été transférés dans un enclos situé dans un parc d'engraissement commercial de 10 000 têtes, pour reproduire la façon habituelle de gestion des troupeaux de bovins de boucherie de l'Ouest canadien. En général, lorsque comparés avec des individus sains du troupeau, exempt de VDVB, les veaux infectés de façon persistante avaient un mauvais rendement et un faible taux de survie, puisque seulement 4 veaux infectés de façon persistante ont survécu jusqu'à l'âge d'un an. Il n'y avait pas de différence ($P > 0,05$) dans les taux de survie entre mâles et femelles chez les veaux infectés de façon persistante.

Les observations cliniques, pathologiques et virologiques provenant de ces veaux infectés de façon persistante ont varié au cours du temps. La majorité des veaux infectés de façon persistante montrait des lésions pathologiques macroscopiques à la nécropsie, compatibles avec une maladie des muqueuses. Une grande variété d'autres lésions ont aussi été notées à la nécropsie des veaux infectés de façon persistante. Cependant, la possibilité que des lésions produites par le VDVB puissent être mal interprétées est bien réelle. Les résultats de cette étude indiquent que l'infection persistante par le VDVB devrait toujours être prise en considération chez les veaux présentant une déficience chronique d'efficacité alimentaire, une entérite chronique ou une maladie respiratoire.

(Traduit par docteur André Blouin)

Can Vet J 1997; 38: 29–37

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Funding for this project was provided by the Saskatchewan Agriculture Development Fund and the Saskatchewan Horned Cattle Trust Fund. Preliminary results of this study were published in the proceedings of the Annual Meeting of the Society for Theriogenology, Gainesville, Florida, 1993.

Introduction

The most important outcome of fetal infections with bovine viral diarrhea virus (BVDV) from an epidemiological point of view is the subsequent birth of calves that are persistently infected with BVDV (1,2). Persistently infected (PI) calves remain chronic shedders of BVDV for life and, as such, are a major source of transmission of BVDV within and among herds of cattle (1–4). Persistently infected calves appear to be the source of both cytopathic and noncytopathic biotypes of BVDV (3,4) and are at risk of developing terminal mucosal disease (3,5).

Mucosal disease occurs when cattle that are persistently infected with noncytopathic biotype BVDV become infected with a homologous cytopathic biotype BVDV (5). This homologous cytopathic biotype BVDV would appear to originate in most cases from the noncytopathic biotype BVDV, after subtle changes in its molecular makeup (4).

This increased understanding of the pathogenesis of BVDV infection has led to extensive studies of the epidemiology of BVDV infection in herds of cattle (1,2). The performance, survival, and necropsy findings in PI cattle have been reported to be extremely variable (3,6–9). The survivability of female PI cattle has also been reported to be greater than that of male PI cattle (10).

The advent of acute BVDV infections that produce gross pathology resembling mucosal disease makes it difficult to determine at necropsy if cattle are acutely or persistently infected with BVDV (11). Often, only the history of the animal or the antemortem isolation of BVDV allows the differentiation between acute and persistent infection. When cattle leave the ranch of origin, they are typically sorted into similar lines of cattle and sold via the auction market system. Any history is lost and a comparison with their herdmates can no longer be made.

The objectives of this study were to describe the performance, survival, necropsy, and virological findings in a cohort of PI calves born on a ranch in Saskatchewan. Persistently infected calves that survived past weaning were relocated to a commercial feedlot to mimic normal management conditions in western Canada. This study was unique in that a large number of known PI calves could be monitored over time.

Materials and methods

Study population

The herd studied was located in central Saskatchewan. The PI calves were from a cohort of 652 cows and heifers bred from April to June in 1991 and calved in the spring of 1992. The epidemiological factors that led to the birth of such a large number of PI calves from these cows and heifers are described in an accompanying paper (12).

History

The herd history is summarized as a time line in Figure 1. Due to a problem with excessive calf losses from birth to weaning over the 4 y prior to this study, all calf losses were investigated as part of a herd health program conducted by the Western College of Veterinary

1992	
Jan 15	First calf born
Jan 21	Abortions were noted in cows. Convalescent sera were collected from 5 cows. Aborted fetuses were not found.
April	Problems with ill-thrift, chronic respiratory disease and enteritis were noted in calves — 123 plasma samples were collected for vitamin E and selenium from every 5th calf branded and also from obviously unthrifty calves. 14 samples were subsequently tested for BVDV by virus isolation. These were calves that died prior to pregnancy testing or were subsequently found to be BVDV positive.
May 4	The last of 560 calves was born. 7 calves had been moved to another farm and were hand reared because they were mismothered.
May–July	Dams of unthrifty calves were identified and both cow and calf were placed with the cull cows and their calves.
July 17	170 yearling replacement heifers were pregnancy tested and blood samples were collected. None were BVDV positive.
July 24	45 cull cows, 40 calves (including a number of unthrifty calves), and 1 bull had blood samples taken for BVDV isolation. The calves were weaned and all the cows were sold.
Sept 7/8/11/16	Blood samples were collected from 395 cows and 95 calved heifers as they were pregnancy tested. Blood samples were also collected from 525 surviving calves of these cows (including 39 surviving calves bled on July 24) and 22 bulls.
Oct 15	521 calves were weaned off cows (including 7 hand reared calves). Blood samples were collected from 32 surviving calves that were BVDV-positive at pregnancy testing and they were sorted off. One calf was BVDV-negative and antibody positive when retested. Therefore, 31 persistently infected calves remained.
Oct 27	28 persistently infected and 2 normal calves were purchased and transferred to a 10 000 head capacity commercial feedlot. 2 persistently infected calves remained at the ranch.
Oct 28	Blood samples were collected from 7 hand reared calves.
1993	
March-April	Salvage slaughtered 4 remaining persistently infected calves, 3 from the feedlot and 1 from the ranch.

Figure 1. Time line summarizing the history of the bovine viral diarrhea virus (BVDV) problem on the ranch.

Medicine (WCVM). Previously, problems with owner compliance meant that few dead calves were necropsied. In 1992, any carcasses that were found in a fresh state and any calves that were euthanized were submitted for necropsy to the diagnostic laboratory at the WCVM.

Records and data analysis

Calves were uniquely tagged at birth in the order born. The date of birth, identity of the dam, and birth weight were recorded. The number of calves that died within a 50-day period after birth was calculated from herd records. All calves that survived were weighed at weaning using electronic scales. An adjusted 205-day weaning weight was calculated to control for age at weaning. The birth weight was subtracted from the weaning weight, divided by the age of the calf at weaning, and multiplied by 205 d. Calves tagged at birth but missing at pregnancy testing and weaning were noted. Herd records were used to determine if these calves had died of BVDV-related disease. Many of the calves that subsequently died at pasture had previously had BVDV isolated from their blood or had been noted by the owners as being unthrifty or sick.

On October 27, 1992, surviving calves that were viremic when their dams were pregnancy tested in September 1992 were purchased and transferred to a single pen in a 10 000 head capacity commercial feedlot in an attempt to simulate what might normally happen to these calves under typical management conditions for western Canadian beef cattle. Logistic and legal constraints prevented us from selling these calves into the open auction system and following their survival and performance in the "real world."

Financial constraints prevented us from purchasing an age-matched group of normal calves from this herd as controls to commingle with the infected calves. However, 2 BVDV-negative, antibody-positive calves were in the pen. One was included by mistake because of a misread ear tag at weaning; the other was BVDV-positive and antibody-negative at pregnancy testing in September and later found to be BVDV-negative and antibody-positive when retested. Replacement heifers were selected and the remaining BVDV-negative calves were eventually sold at a local auction market. Two PI calves remained at the ranch and were commingled with the "tail end" of the BVDV-negative calves into feedlot pens adjacent to the replacement heifers.

Feedlot management

Within the feedlot, the PI calves were housed in an older section of the feedlot between 2 pens of fat yearling cattle, sharing a water bowl with 1 pen. Usual feedlot management practices, including the usual processing routine, were applied to the calves. They were monitored closely during the fall and winter of 1992–93, and calves that died or were euthanized were submitted for necropsy. Blood samples were collected every 2 wk for the next 3 mo. At this time, rectal temperatures, body weight, and any obvious clinical signs were recorded.

An apparent outbreak of bovine respiratory disease occurred 3 wk after the calves were moved to the feedlot. The case definition for bovine respiratory disease was depression, inappetence, signs attributable to the respiratory system, and a rectal temperature ≥40.3°C. Some of these calves also had diarrhea. All calves were mass medicated by individual parenteral injection with 20 mg/kg body weight (BW) of long-acting oxytetracycline (Liquamycin LA, rogar/STB, Pointe-Claire, Quebec) twice, 2 d apart. At subsequent blood samplings, calves that had a rectal temperature ≥40.3°C were treated with 10 mg/kg BW of tilmicosin (Micotil, Provel, Scarborough, Ontario). Calves were only treated with antimicrobials until early January. After this time, no antimicrobial therapy was provided, even if calves were found to be febrile. The 2 PI calves that remained on the ranch were also observed regularly during the same period. One of these calves subsequently died but was not necropsied. The 4 surviving PI calves were slaughtered in mid-April 1993 at a local abattoir. They were inspected at slaughter by government veterinarians and all passed ante and postmortem examinations. Tissue samples were collected for virological and histological examination.

Case definition for persistent infection

Calves were classified as PI if BVDV was isolated by virus isolation from blood or tissues on 2 successive occasions, at least 2 wk apart. Calves were also considered to be PI if BVDV was isolated from tissues collected at necropsy and there was evidence of chronic ill-thrift and pneumonia or mucosal disease. Calves that died on pasture but were not necropsied were classified as probably PI, if BVDV had been isolated from a blood sample collected prior to death.

Pathology

Complete necropsies were performed on calves by trained pathologists as soon as possible after death (usually within 24 h). In some cases, only respresentative tissues were submitted. Calves that were sent for salvage slaughter were inspected at slaughter and tissues were collected. Representative tissues were collected and fixed in phosphate-buffered formalin for routine examination. Representative samples of grossly affected organs were collected for aerobic and anaerobic bacterial culture and virus isolation. Based on gross pathological findings, the cause of death was classified as "mucosal disease" or "other." Mucosal disease was defined as extensive ulcerative lesions of the alimentary tract with/or without lymphoid atrophy (9). Other diseases were classified according to the organ system involved.

Virological techniques

All BVDV isolations on live calves more than 2-months old were from sera. Whole blood was collected in 10 mL vacuum tubes (Vacutainer serum separation tubes, Becton Dickinson, Missisauga, Ontario) and stored at 4°C for 24 h. Blood samples were centrifuged at 5000 × *g* for 10 min and approximately 3 mL of serum were separated and stored at −20°C until assayed. Stored plasma samples collected from calves in April 1992 and assayed for vitamin E and selenium were also tested.

In 1992, serum and plasma samples were inoculated directly into cultures of BVDV-free fetal bovine tracheal cells, incubated for 5 d at 37°C, and then stained by an indirect fluorescent antibody technique to demonstrate

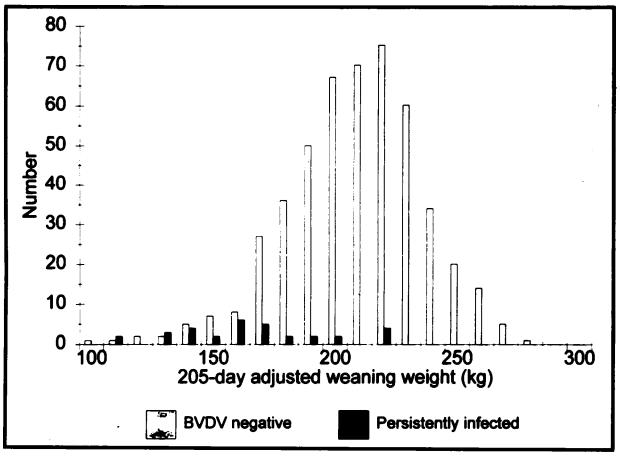

Figure 2. Frequency distribution of the 205-day adjusted weaning weights of calves persistently infected (PI) with bovine viral diarrhea virus (BVDV) and BVDV-negative calves in 1992.

the presence of BVDV (13). Serological screening for BVDV was undertaken using an enzyme-linked immunosorbent assay (14).

Three techniques were utilized to identify BVDV in tissues from necropsy specimens. Frozen sections were stained using a direct fluorescent antibody technique (13). When BVDV could not be demonstrated in frozen sections of tissue from cattle suspected of being PI with BVDV (based on previous isolation of BVDV from serum), formalin-fixed tissue samples were tested using an immunoperoxidase technique (15). For virus isolation, tissues were homogenized in phosphate-buffered saline, treated with penicillin/streptomycin, and centrifuged. The resulting supernatant was inoculated onto bovine tracheal cells. The presence of BVDV was confirmed using the direct fluorescent antibody technique.

A BVDV plaque assay was used for the separation of cytopathic biotype BVDV from noncytopathic BVDV. One hundred µL of serum from each sample collected over a 6-month period from 10 of the PI calves was serially diluted 1/100 and 1/10 000 and inoculated onto 2×10^5 bovine turbinate cells in 6-well plates. Virus was allowed to adsorb for 6–8 h; the medium was then replaced with 0.5% agarose, containing minimal essential medium and 10% fetal calf serum. Cells were incubated for 48 h and then further overlaid with 0.5% agarose containing neutral red. Plaques were usually visible 12 h after staining. The presence of cytopathic effect could then be determined visually.

Survival analysis

A survival curve was generated for PI calves by plotting the proportion that were still alive by 50 d intervals after birth. The log rank technique of survival analysis was used to determine if female PI calves had a greater survivability than male PI calves (17).

Results

In 1992, 560 calves were tagged. Bovine viral diarrhea virus was isolated from 2 of the 14 plasma samples collected for vitamin E and selenium assays from unthrifty calves at branding in April 1992. The 2 BVDV-positive calves died soon after testing. They were probably PI, because they were unthrifty. Bovine viral diarrhea virus was subsequently isolated from serum samples or tissue samples collected at necropsy from 7 more of these calves. Bovine viral diarrhea virus was isolated from 6 of the 40 calves sampled on July 24, 1992.

Figure 3. This photograph shows the extreme variation in weaning weight that can occur between healthy normal calves and persistently infected calves. The small calf is a persistently infected calf weaned in 1993 and is the same age as the other healthy heifers in the photograph.

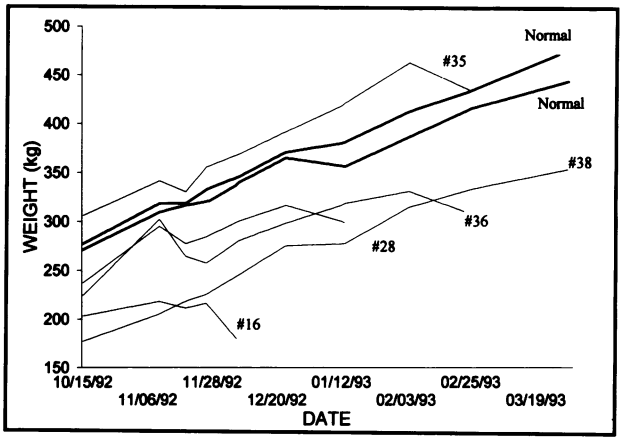

Figure 4. Postweaning growth of 5 persistently infected calves and 2 BVDV-negative calves commingled with them in a single feedlot pen in the commercial feedlot.

Bovine viral diarrhea virus was isolated from 40 of the 525 calves sampled when the cow herd was pregnancy tested in September 1992. Four of these calves had tested positive in July (2 had died), so were confirmed PI. Thirty-one of the 40 calves that were BVDV-positive at pregnancy testing were confirmed PI at weaning. Eight BVDV-positive calves died at pasture and 1 calf that was BVDV-positive, antibody-negative in September was subsequently found to be BVDV-negative and antibody-positive when retested in October. This was the only acute infection with BVDV recorded in this study.

In total, 40 calves died prior to weaning, 10 were definitely PI, and a further 10 had BVDV isolated from blood samples collected prior to death, so were probably PI. Most of these calves died when the cow herd was grazing pasture during the summer. It is very likely that the remaining 20 calves that were unaccounted for and died at pasture were also PI. No BVDV-positive cows, heifers, or bulls were identified. Therefore, the prevalence of PI calves born in this herd in 1992 was between 9.1% and 12.7% (51 and 71/560).

Table 1. Clinical signs in persistently infected (PI) calves at the feedlot in 1992–93

Date	BVDV[a] status	Number	Temp≥ 40.3°C	Nasal ulcers	Diarrhea	Lame	Average wt (kg)
10/15/92	+	31[b]	n/a	n/a	n/a	n/a	227
	−	2[c]	n/a	n/a	n/a	n/a	274
11/10/92	+	27	n/a	3	0	0	255
	−	2	n/a	0	0	0	314
11/19/92	+	26	26	0	0	0	257
	−	2	2	0	0	0	317
11/22/92	+	25	13	0	0	0	n/a
	−	2	0	0	0	0	n/a
11/26/92	+	25	8	3	4	0	266
	−	2	2	0	2	0	326
07/12/92	+	24	21	3	2	2	272
	−	2	1	0	0	0	342
12/23/92	+	22	10	2	0	0	297
	−	2	0	0	0	0	367
1/13/93	+	16	11	0	0	1	313
	−	2	2	0	0	0	367
2/04/93	+	11	9	6	3	0	334
	−	2	1	0	0	0	400
2/25/93	+	7	2	4	0	0	354
	−	2	1	0	0	0	423
3/30/93	+	3	0	0	0	0	339
	−	2	0	0	0	0	457

[a]Bovine viral diarrhea virus
[b]Two of these PI calves remained at the ranch
[c]One calf was BVDV-positive at this time but was subsequently found to be negative

Most of the PI calves were unthrifty at weaning. The average adjusted weaning weight of the PI calves was 160 kg compared with 203 kg for the BVDV-negative calves. Only 4 PI calves had 205 day adjusted weaning weights above the average of the BVDV negative calves (Figure 2). An extreme example of ill-thrift in a PI calf born in 1993 is shown in Figure 3. Several BVDV-negative calves had very low adjusted weaning weights. These calves appeared very stunted, almost dwarfed, and were resampled several times for BVDV to ensure that they were not PI. Subsequent to weaning, PI calves continued to grow until they developed signs of mucosal disease, when most suffered dramatic weight loss prior to death (Figure 4). None of the weaned PI calves developed detectable antibody to BVDV after being moved to the feedlot. However, the 2 BVDV-negative calves in the feedlot pen had very high levels of antibody to BVDV.

Ten days after arrival at the feedlot, 1 calf died from fibrinous bronchopneumonia and the other calves in the pen began to show clinical signs of bovine respiratory disease. Calves were all injected with long-acting oxytetracycline, and their average feed intake and individual body weights increased dramatically. The clinical findings, BVDV status, rectal temperature, and average body weights of the calves placed into the feedlot over time are listed in Table 1. The weights of 5 PI calves and 2 normal calves in the feedlot pen are shown over time Figure 4.

Despite an absence of respiratory signs, a number of PI calves remained febrile and began to develop signs of severe mucosal disease around the end of November. These included laminitis, ulceration of the nasal and interdigital skin, and a persistent, often bloody, diarrhea (Table 1).

Calf #35 grew better than the 2 "normal" calves in the pen, only to die 3 d after first appearing sick in March 1993 from peracute mucosal disease (Table 2, Figure 4). In general, PI cattle grew quite well after weaning, only to lose weight suddenly with the onset of mucosal disease.

The necropsy findings are summarized in Table 2. It is important to note that every single calf that was necropsied in this herd in 1992 either had evidence of mucosal disease or had BVDV isolated either from tissues collected at necropsy or blood samples collected prior to death. Several cows died, but infection with BVDV was ruled out as the cause. In total, 36 calves were necropsied and the viscera from 4 PI yearlings that were salvage slaughtered were examined.

Two of the PI calves that died from mucosal disease (#3, #4) had a pronounced hemorrhagic diathesis. Another calf (#11) died of severe anemia and had obvious chronic bone marrow depletion. In total, 12 of the 36 calves had hemorrhages evident in the carcass. Severe lymphoid depletion was evident in 29, making it almost a universal finding, even in the carcasses of the 4 healthy PI yearlings that were slaughtered.

Localised dysplasia of the subepiphyseal primary spongiosa or "growth arrest lines" were evident in the long bones of 15 calves. In many cases, chronic bone marrow dysplasia was also evident in conjunction with the growth arrest lines.

Thirteen calves had gross pneumonic lesions. One calf (#9) had a severe laryngotracheitis and secondary bronchopneumonia. Whether this was due to BVDV

Table 2. Distribution of lesions in necropsied calves that died of bovine viral diarrhea virus (BVDV)-related disease during 1992 and 1993. Virological findings from these calves are also summarized for comparison

Calf ID	Date of Death	Age (d)	Ulceration of gut	Ulceration of skin	Respiratory lesions	Growth arrest lines	Lymphoid depletion	Hemorrhages	BVDV tissues	BVDV blood	Gross diagnosis
1	4/21/92	58	O,E,P	–	—	–	+	–	n/a	0	MD
2	6/26/92	120	O,E,A	–	L	–	+	+	Yes	1	MD
3	6/30/92	97	A	–	L	+	+	+	Yes	1	BRD/HD
4	7/10/92	93	O,A,C	–	L	+	+	+	Yes	1	MD/HD
5	7/22/92	151	O,E,A	+	L	+	–	–	Yes	0	MD
6	7/27/92	119	A	–	L	–	+	–	Yes	1	BRD
7	8/17/92	158	O,E,A	–	—	–	+	+	Yes	1	MD
8	9/8/92	144	O	–	L	–	+	–	Yes	2	BRD
9	10/1/92	239	O,E,A,I	–	U,L	–	+	–	Yes	1	MD/BRD
10	10/16/92	248	O,E,R	+	—	–	+	–	n/a	2	MD
11	10/26/92	197	E	–	—	+	–	–	Yes	2	Anemia
12	11/8/92	269	I	–	—	–	–	–	Yes[a]	2	MD
13	11/18/92	270	—	–	L	+	+	–	Yes	3	BRD
14	11/22/92	295	—	–	L	+	–	–	Yes	4	BRD
15	12/7/92	292	O,P	–	—	–	+	+	Yes	7	MD
16	12/7/92	262	I,P	+	—	+	+	+	Yes	9	MD
17	12/16/92	275	—	–	L	–	–	+	n/a	7	BRD
18	12/25/92	276	O,R,A,I,P	–	—	+	+	+	Yes	9	MD
19	1/1/93	308	O,A	+	L	–	–	–	n/a	8	BRD
20	1/8/93	339	O,A,P	–	—	–	+	+	Yes	8	MD
21	1/8/93	312	P	–	—	–	+	–	n/a	8	MD
22	1/13/93	339	A,P	–	—	–	+	+	Yes	8	MD
23	1/13/93	345	P	–	—	–	+	–	Yes	8	MD
24	1/30/93	334	O,E,Om,A,P	–	—	–	+	+	Yes	9	MD
25	1/30/93	351	O,E,Om,A,P	–	—	+	+	–	Yes	9	MD
26	2/2/93	316	O,E,A,P	+	L	–	+	–	No[a]	11	MD
27	2/4/93	365	A,C	–	—	+	–	–	No[a]	9	MD
28	2/4/93	358	O,E,A	+	L	–	+	–	Yes	9	MD/BRD
29	2/4/93	361	O,E	+	—	+	–	–	Yes	9	MD
30	2/4/93	342	O,E,A	–	—	+	–	–	No	9	MD
31	2/11/93	339	O,I	+	—	+	–	–	Yes[a]	9	MD
32	2/19/93	382	A,I,P	–	—	–	+	–	Yes	9	MD
33	3/3/93	359	—	–	—	–	–	–	No	10	MD
34	3/12/93	397	O,E,R,A,C	–	—	–	+	–	n/a	10	MD
35	3/16/93	401	E,A,P,C	+	—	–	+	+	Yes	10	MD
36	3/16/93	368	O,E,C	–	—	+	+	–	Yes	10	MD
37	3/24/93	349	—	–	—	–	+	–	Yes	3	SLAUGHTER
38	4/14/93	439	—	–	—	–	+	–	Yes	11	SLAUGHTER
39	4/14/93	401	—	–	—	–	+	–	Yes	11	SLAUGHTER
40	4/14/93	400	—	–	—	–	+	–	Yes	11	SLAUGHTER

O — Oropharynx, E — Esophagus, R — Rumen/reticulum, Om — Omasum, A — Abomasum, I — Ileum, P — Peyer's patches, C — Colon/rectum
U — Upper respiratory tract (larynx and trachea), L — Lower respiratory tract (Lower airways and lungs)
BVDV isolations from blood was the number of times bovine viral diarrhea virus was isolated from blood prior to death
MD Mucosal disease
BRD Bovine respiratory disease
n/a Not attempted
[a]Immunohistochemistry was attempted on tissues from these cattle

or a secondary viral infection was not determined. Six calves would have been diagnosed grossly as having died from bovine respiratory disease if BVDV had not been isolated from tissues. A further 2 calves had respiratory tract lesions and mucosal ulceration but could easily have been misclassified as respiratory disease on gross necropsy under field conditions, if the upper alimentary tract had not been fully examined. The remaining 5 calves had very obvious mucosal disease, as well as pneumonia.

Ulceration of the skin of the nose, conjunctival membranes, and interdigital skin occurred mostly in the older calves that died in the feedlot of acute mucosal disease. Other incidental findings in calves that died from mucosal disease were nephritis in calves #3 and #6, retinal hypoplasia in calves #8 and #16, and necrosis of the adrenal gland in calves #10 and #16. Lesions in muscle tissue due to sarcocystis were present in 9 calves. Hepatic necrosis and abscessation was noted in 12 calves. This hepatic necrosis and abscessation was possibly secondary to chronic rumenitis caused by BVDV, because it was evident in preweaned calves, as well as in the weaned calves on grain-based feedlot rations.

Bovine viral diarrhea virus could not be isolated from tissues obtained at necropsy from 6 calves, although it was evident in 2 using immunohistochemistry (Table 2). The reason for this inability to isolate virus was not determined.

The proportion of the 10 calves that had cytopathic BVDV present in their sera is shown in Table 3. It would appear that the increase in the proportion of calves with cytopathic BVDV in their sera coincided with the increase in mortalities from mucosal disease. However, cytopathic BVDV was present in the serum of

Table 3. Results of plaque assay for cytopathic bovine viral diarrhea virus (September 1992 through March 1993)

Calf ID	Sept 8	Oct 15	Nov 10	Nov 19	Nov 22	Nov 26	Dec 7	Dec 23	Jan 13	Feb 25	Mar 30	Necropsy diagnosis
9	neg	—	—	—	—	—	—	—	—	—	—	MD/BRD
14	neg	neg	neg	neg	—	—	—	—	—	—	—	BRD
20	neg	neg	**pos**	**pos**	**pos**	**pos**	**pos**	**pos**	—	—	—	MD
22	neg	neg	neg	**pos**	**pos**	**N/D**	**pos**	**pos**	—	—	—	MD
27	neg	neg	neg	neg	neg	neg	neg	neg	neg	—	—	MD
29	**pos**	neg	**pos**	**pos**	**pos**	**pos**	**pos**	**pos**	**pos**	—	—	MD
32	**pos**	neg	neg	neg	neg	neg	neg	**pos**	**pos**	—	—	MD
34	neg	neg	**pos**	**pos**	**pos**	**pos**	**pos**	**pos**	**pos**	neg	—	MD
35	neg	**pos**	**pos**	**pos**	**pos**	neg	**pos**	neg	**pos**	**pos**	—	MD
38	neg	neg	neg	neg	neg	**pos**	**pos**	**pos**	**pos**	**pos**	**pos**	Slaughtered

— Animal had died and was not tested
MD Mucosal disease was gross postmortem diagnosis
BRD Gross lesions present in the respiratory tract at necropsy
N/D Not done

some cattle for months prior to death from mucosal disease. It was not present in the sera of 2 calves that died of pneumonia, even though 1 calf had mild mucosal ulceration. Cytopathic BVDV was not evident in the serum of calf #27 and could not be isolated from the tissues obtained from this animal at necropsy.

By the end of February 1993, 5 of the original 30 cattle remained in the feedlot pen. Two of these were the original normal cattle, the other 3 were PI. One of the PI cattle (#40) had shown evidence of mucosal disease since the beginning of February. These signs were mainly nasal and oral erosions; however, the calf was eating and was not considered to be suffering. These nasal and oral erosions subsequently healed, and the animal gained weight and was slaughtered.

The 3 surviving PI cattle in the feedlot were at least 15 cm shorter than the 2 normal cattle in the pen. Their body weights were also much less (Table 1). At no time did healthy, fat cattle in adjacent feedlot pens show evidence of BVDV infection or disease of any form. These cattle had not been vaccinated for BVDV on arrival at the feedlot.

There was no significant difference ($T = 0.29$; $P > 0.05$) in the survivability of male versus female PI calves.

Discussion

Under management conditions typical of western Canadian beef herds, the survivability of PI calves was poor compared with that of healthy BVDV-negative herdmates. Only 4 PI calves survived past 12 mo of age. Unfortunately, we were not able to observe these surviving cattle for a longer period.

The findings suggest that the observed prevalence of PI calves in this population would have varied significantly, depending on the age of the cattle when they were sampled. If yearling cattle had been sampled, the prevalence of PI cattle in this herd would have been grossly underestimated at around 0.8% (4/489). Therefore, the actual prevalence of PI calves born in a population of beef cattle could be grossly underestimated if you only determined the prevalence of PI cattle in the adult cattle population. Contrary to observations that female PI cattle live longer than male PI cattle (10), this study found that the survivability of male and female PI cattle was the same.

On average, when compared with BVDV-negative herd mates, PI calves were "poor doers." However, a small number of PI calves at the top end of the "normal distribution" performed quite well, having above average weaning weights. These calves grew quite well but later developed acute mucosal disease. Had the PI calves from this herd been sold, they would have been sorted by weight prior to sale. Producers and feedlot operators who selectively purchased the lightweight weaned calves from this herd would have acquired significantly more PI calves than producers who purchased the well-grown calves.

A number of BVDV-negative calves had low weaning weights, with some appearing dwarfed. While the exact cause of this ill-thrift was not determined, it is possible that these calves had been infected in utero with BVDV later in pregnancy, clearing the virus from their systems (5), but sustaining permanent bone damage.

Our findings support those of previous studies (3) indicating that the majority of PI calves develop mucosal disease. However, a wide variety of lesions were noted, in addition to the classical lesions of mucosal disease. The possibility that BVDV-induced lesions can be misdiagnosed is therefore very real. Most calves (32/36) had gross lesions of the alimentary tract consistent with mucosal disease, and the severity of this mucosal disease appeared to increase as the calves aged and moved into the feedlot. In general, calves that died before 250 d of age had a chronic, wasting form of mucosal disease, with evidence of severe emaciation, large oral ulcers, chronic abomasal ulceration, diffuse lymphoid depletion, and secondary bacterial pneumonia at necropsy. "Growth arrest lines" were common in the long bones. Older cattle confined in the feedlot developed a more acute form of mucosal disease, with more ulceration of the skin and more diffuse ulceration of the digestive tract and Peyer's patches. Death or euthanasia usually occurred within 1 wk of the onset of clinical signs. Unfortunately, we were not able to determine if changes in the BVDV infecting these animals were responsible for the changes in clinical and pathological findings over time.

Approximately 25% of the PI calves died with gross pneumonic lesions at necropsy, but with no or only mild lesions of mucosal disease. Although previous studies have indicated that PI calves develop secondary infections (9), there have been few, if any studies, indicating the prevalence of mucosal disease lesions versus other lesions among cohorts of PI cattle from the same herd. The relatively high prevalence of respiratory tract lesions in the PI cattle in our study is compatible with the idea that BVDV is involved in the development of respiratory disease (17). However, the role and prevalence of acute versus persistent BVDV infections in cattle with bovine respiratory disease has not previously been determined. Whether respiratory disease in BVDV-infected cattle is due to the direct effects of BVDV on the respiratory system or attributable to secondary infections is also currently unresolved. Cattle with persistent BVDV infections are generally thought to be immunocompromised and therefore at risk of dying from secondary infections (9); however, there is conflicting data regarding the nature and degree of immunosuppression resulting from BVDV infections (18). Additional studies of cattle with naturally acquired persistent BVDV infections may further elucidate these issues.

Two of the PI calves (#3,#4) that died from mucosal disease (#3,#4) had a pronounced hemorrhagic diathesis. The latter syndrome has been associated with acute infections by some strains of BVDV, currently designated as type II BVDVs (19,20). Although the genotype of the BVDV in these calves was not determined, it would have been interesting to determine if the hemorrhagic syndrome in these calves was the result of superinfection or mutation of the persistently infecting strain. Whether or not BVDV had a primary role in the severe aplastic anemia that was the apparent cause of death in calf #11 was also not determined.

Throughout this study, we identified inconsistencies in the results obtained with various diagnostic tests for BVDV. Bovine viral diarrhea virus was not isolated from the plasma of several calves at branding but was subsequently isolated from serum samples collected as they grew older. Maternal antibody present in the plasma of these calves may have interfered with the isolation of BVDV(6).

In several older calves, BVDV was easily isolated from serum samples for many months prior to death, but was not isolated from the tissues collected at necropsy. There are many possible explanations for this, including postmortem degeneration of tissues, failure of the monoclonal antibody used to detect some BVDVs, or minimal BVDV replication in these calves at the time of death. Although not utilized here, polymerase chain reaction (21) might have been successfully employed to detect low levels of BVDV in cases where other techniques yielded negative results.

Isolation of a cytopathic BVDV on several occasions over several months from the serum of calves that eventually died from BVDV-induced mucosal disease was interesting. Recent studies have documented a similar prolonged persistence of cytopathic BVDV in PI cattle (22). Furthermore, it has been proposed that superinfection with a cytopathic BVDV, which, antigenically, only partially matches endogenous noncytopathic BVDV, might cause a chronic form of mucosal disease (22,23), as was seen in the younger calves in this study.

In future, more detailed studies of naturally occurring outbreaks of BVDV infection should further elucidate the ecology of BVDV infection in herds of beef cattle. Such studies should utilize modern microbiological techniques to determine whether changes in the persistently infecting BVDV reflect changes in clinical and pathological findings over time. The results of this study indicate that persistent infection with BVDV should always be considered in calves with chronic ill thrift, chronic enteritis, or respiratory disease.

Acknowledgments

We thank the Hillcrest Hutterite Brethren of Dundurn, Saskatchewan, for their tremendous support. We thank the Golden Hill Cattle Company for accepting the PI calves into their feedlot facility and for their routine handling of these stock. We also thank Drs. J. Van Donkersgoed, E. Clark, A. Berrington, J. Orr, D. Vansconcelos, A. Pocknell, D. Campbell, J. Mills, W. Lusimbo, and G. Tjahjowati for performing the necropsies. CVJ

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COMING EVENTS

ÉVÉNEMENTS À VENIR

CVMA Conventions/ Congrès de l'ACMV

1997
Saskatoon, Saskatchewan
July/juillet 9–12

1998
Toronto, Ontario
July/juillet 8–11

FEBRUARY/FÉVRIER 1997

New Zealand Summer Symposia on Immune Mediated Diseases. February 7 & 14, 1997 at Lake Taupo (North Island) and Blenheim (South Island). Presenters: Dr. Jim Thompson, Dr. Boyd Jones, Dr. Richard Squires. Contact: Christine Allport, Veterinary Continuing Education, Massey University, Palmerston North, New Zealand; tel.: (06) 350 5227; fax: (06) 350 5659.

Cancer and the Role of Nutrition. February 13, 1997 at Colorado State University, Fort Collins, Colorado. Lecture by Dr. Greg Ogilvie on: The metabolic consequences of cancer in companion animals, including emerging nutritional and medical therapies for oncology patients. Contact: Ottawa Academy of Veterinary Medicine c/o Dr. Susan Kilborn, 125 Owl Drive, Ottawa, Ontario K1V 9J5; tel.: (613) 736-7673; fax: (613) 736-9502.

XV International Symposium of the World Association of Microbiologists, Immunologists and Specialists in Infectious Diseases. February 16–21, 1997 in Nicosia, Cyprus. Theme: Salmonellosis — Brucellosis. Contact: K. Polydorou, V.P.H. Institute, P.O. Box 284, Nicosia, Cyprus; tel./fax: 00357-2-453121.

International Sled Dog VMA Training Seminar for Beginning Sled Dog Race Veterinarians. February 25–27, 1997 at Regal Alaskan, Anchorage, Alaska. Diagnosis, treatment, and prevention of specific disorders afflicting dogs during a race; checkpoint duties and protocols for official race veterinarians; opportunity to participate in pre-Iditarod race activities. CE credits. Deadline: January 25, 1997. Contact: Dr. Tom Young, ISDVMA, P.O. Box 543, Sylvania, Ohio 43560 USA; tel.: (419) 531-5589; fax: (419) 531-5404.

International Speakers Forum — Canadian Farm Animal Genetic Resources at the Crossroads: Crisis or Opportunity? February 27–28, 1997 at Le Chateau Cartier Hotel, 1170 Aylmer Road, Aylmer, Quebec. Registration fee: $45.00. Speakers will include representatives of organizations from around the globe, and those involved in farm animal genetic resources conservation at all levels in Canada. There will also be an exhibit area and a poster session. Contact: Dr. S.K. Ho, Centre for Food and Animal Research, Agriculture and Agri-Food Canada, Research Branch, Ottawa, Ontario K1A 0C6; tel.: (613) 759-1429; fax: (613) 759-1465; email: CFCFAGR@MAGI.COM.

MARCH /MARS 1997

Veterinary Orthopedic Society 24th Annual Conference. March 1–8, 1997 in Big Sky, Montana. Contact: Daman-Nelson Travel, 2 Harrison Street, San Francisco, California 94105; tel.: (800) 899-7669, (415) 247-5500; fax: (415) 247-5510.

American Animal Hospital Association 64th Annual Meeting and Spring Management Conference. March 8–12, 1997 at San Diego Convention Center, San Diego, California. Contact: AAHA Member Service Center, P.O. Box 150899, Denver, Colorado 80215-0899; tel.: (800) 883-6301, or (303) 986-2800.

Association for Equine Sports Medicine 16th Annual Meeting. March 14–17, 1997 in San Antonio, Texas. Topics: muscle disorders and a broad range of sports medicine topics. Contact: Nancy A. Bull, Association for Equine Sports Medicine, P.O. Box 4506, Santa Barbara, California 93140-4506 USA; tel.: (805) 965-1028; fax: (805) 965-0722.

APRIL/AVRIL 1997

The Fourth International Symposium on Ectoparasites of Pets. April 6–8, 1997 at University of California, Riverside, California. Includes: veterinary medicine, parasitology, entomology. Contact: Dr. N.C. Hinkle, Department of Entomology, UCR, Riverside, California 92521 USA; tel.: (909) 787-2422; e-mail: NHinkle@citrus.ucr.edu.

22nd Congress of the German Veterinary Medical Society. April 8–11, 1997 in Bad Nauheim, Germany. Topics on current research results in veterinary and comparative medicine can be presented in German or English. Titles to papers and posters should be submitted to: President of DVG, Prof. Dr. h.c. mult. E. Grunert, Klinik fuer Geburtshilfe, Bischofsholer Damm 15, D-30173 Hannover/Germany; tel.: ++49-511-856-7242; fax: ++49-511-856-7691.

Soft Tissue Surgery — Wound Management and Selected Perineal and Otic Procedures. April 17, 1996 at Ohio State University, Columbus, Ohio. Lecture by Dr. Dan Smeak on: Newer techniques in wound closure and management, plus a potpourri of surgical solutions for perineal and otic diseases, including perineal fistulous tracts. Contact: Ottawa Academy of Veterinary Medicine c/o Dr. Susan Kilborn, 125 Owl Drive, Ottawa, Ontario K1V 9J5; tel.: (613) 736-7673; fax: (613) 736-9502.

Voorjaarsdagen Annual International Veterinary Congress. April 25–27, 1997 in Amsterdam, The Netherlands. Scientific program in English. Sessions: cardiology, ophthalmology, dermatology, neurology, feline respiratory disease, orthopedics, gynecology, diagnostic imaging, urology and nephrology, feline oncology, prostate gland disease, dermatology, reconstructive surgery, perianal surgery. Contact: J.T. Antonie, Secretary, Voorjaarsdagen Committee, P.O. Box 14031, NL-3508 SB, Utrecht, The Netherlands; tel.: +31-(0)30-2510111; fax: +31-(0)30-2511787.