Detection of rotavirus and coronavirus shedding in two beef cow herds in Idaho

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Abstract

Fecal samples were taken at the time of pregnancy examinations and at parturition from two beef herds. They were also taken from sick calves at the onset of disease, and from 25% of the healthy calves at 15 days of age. All fecal samples were examined by electron microscopy for viruses.

Four cows in herd A were detected excreting coronavirus, one at the time of the pregnancy examinations and three at parturition. The first cow was removed from the herd and the others calved at the end of the season. There were no sick calves.

No cows in herd B were detected excreting virus at the time of pregnancy checks, but fourteen coronavirus and two rotavirus carrier cows were found at parturition. All but two calves sampled had large numbers of virus particles in their feces. Clinical illness was associated with dams shedding virus and with nightly low temperatures.

Résumé

Excrétion de rotavirus et de coronavirus dans deux élevages de bovins de boucherie dans l'Idaho

Des échantillons de fèces furent prélevés lors de l'examen de gestation et à la parturition dans deux élevages de bovins de boucherie. D'autres échantillons furent aussi prélevés chez des veaux malades, au tout début des symptômes ainsi que chez 25% des veaux en santé à l'âge de 15 jours. Tous les échantillons ont été examinés par microscopie électronique pour la détection de particules virales.

Dans le troupeau A, une vache excrétait le coronavirus au moment de l'examen de gestation, tandis que quatre autres furent identifiées au moment de la parturition. La première identifiée fut retirée du troupeau tandis que les autres ont mis bas normalement à la fin de la saison, sans qu'aucun veau ne devienne malade.

Aucune des vaches de l'élevage B n'a été identifiée positive au moment de l'examen de gestation tandis que 14 porteuses du coronavirus et deux porteuses du rotavirus le furent à la parturition. Tous les veaux, sauf deux, excrétaient un grand nombre de virus dans les fèces. Les signes cliniques de maladie chez les veaux furent associés à l'excrétion virale par les mères ainsi qu'à des températures nocturnes basses.

Can Vet J 1989; 30: 235-239

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Published with the approval of the Director of the Idaho Agricultural Experiment Station, Moscow, Idaho as Research Paper No. 8684.

Supported, in part, by funds from USDA/CSRS/SEA Section 1433 Formula Funds grant #623-2161-36100-FY 1985-86 (728-K281), and research funds from Idaho Agricultural Experiment Station, University of Idaho.

Introduction

According to agricultural statistics, 72,000 calves (10% of all calves born in Idaho) died in 1984 and represented a loss of over $3,000,000 to the livestock industry of the state (1). This does not include cost of extra man-hours for treatment, medications or weight losses. Coronavirus has been one of the most significant agents related to mortality of calves in Idaho (2). In 1978-80 this virus was associated with two-thirds of the incidence of disease in beef calves and one-third in dairy calves under one month of age as diagnosed at the hospital of the Veterinary Teaching Center, University of Idaho (2).

In our experience, control of coronavirus-related disease by use of a commercially available modified-live-virus vaccine used either orally in newborn calves or intramuscularly in preparturient cows has not proved beneficial. This is not surprising because concentrations of coronavirus-specific antibody in colostrum and milk from vaccinated dams were not found to be significantly elevated over those of nonvaccinated cows (3,4).

There is little information concerning the stability of coronavirus in the environment. However, hypothesizing that coronavirus, like rotavirus, is stable in feces for up to nine months (5) and may remain viable in the ground from year to year, calving areas have, in some instances, been moved to virgin ground and increased in size (6). Changes of this type have not consistently reduced the morbidity from this disease. There remains the possibility (7-10) that persistent carrier cows may initially contaminate calving grounds or their calves and thus perpetuate the infection year after year. Studies done with swine suggest that shedding of rotavirus by pregnant sows may be influenced by hormonal levels near parturition as is the shedding of parasitic ova during the same period (5). Shedding of rotavirus by cows with diarrheic calves has also been reported (7). A study in Colorado suggested that dairy cows shed coronavirus particularly during the winter months (8) and other evidence suggested that persistent infection with coronavirus may be very common in cows (9,10). This project was undertaken to determine if beef cows from herds which had experienced rotavirus and coronavirus associated neonatal diarrhea in past years shed rotavirus and/or coronavirus during the pre and periparturient period and if so, whether it was related to clinical disease in the calf of the shedder.

Materials and methods

Animals — Two beef herds in which severe neonatal diarrhea had occurred for at least four years were chosen for this study. Herd A consisted of a cross-bred beef herd of 200 individually identified cows and 30 heifers. These cows grazed on crested wheat grass range during summer months and were fed predom-

inantly alfalfa hay during the winter. Generally they calved during a 60–90 day period beginning approximately the first of March. Heifers were not separated from the cows for breeding or calving. Calving usually took place on one of several 40 acre alfalfa fields where they had been fed during the winter. The herd remained together before, during and after calving. This herd had also been vaccinated with modified-live rota-coronavirus combined with a killed *Escherichia coli* bacterin (Scour Guard III, Norden, Lincoln, Nebraska 68501) for each of the past two years. Neonatal death losses were at their highest the spring of 1984 with 70% morbidity and a mortality of 50%. Coronavirus had been the only known pathogen found in affected calves each of the past three years.

Herd B consisted of 117 Shorthorn cows and 36 heifers, individually identified by eartag. Management was essentially the same as herd A. The cows had also been vaccinated with a modified-live rota-coronavirus vaccine combined with a killed *E. coli* bacterin (Scour Guard III, Norden, Lincoln, Nebraska 68501). A modified-live rota-corona vaccine (Calf Guard, Norden, Lincoln, Nebraska 68501) had been given to the calves orally at birth the two previous years. During that four-year period the morbidity rate of neonatal diarrhea ranged from 35–50% and the mortality rate from 10–35%. Four to six calves from each herd had been examined each year and the only known enteropathogens found in these calves were coronavirus and occasionally rotavirus as diagnosed by electron microscopy (EM). Rota-coronavirus vaccine was not administered to either herd during the year of the study.

Sample collection — Fecal samples for examination by EM were taken from all cows in both herds at the time of pregnancy examinations approximately four months before calving time. Plastic sleeves were not discarded between sampling of cows, although residual feces were washed off the glove after each sample was taken. Cows that were found to be positive for either rota or coronavirus were removed from the herd when winter feeding began. At calving, a second fecal sample was taken from 100 of the cows in each herd. Calves were not sampled in herd A because there was no clinical disease. Feces from 23 of the 28 diarrheic calves from herd B were sampled on the first day of diarrhea. In addition, feces were collected for EM from 24 clinically normal 15-day-old calves from herd B. Fecal samples were placed in Whirlpak bags (Nasco-West, 1524 Princeton Ave, Modesto, California 95352) identified with the number of the calf and date, refrigerated, and mailed within 24 hours of sampling. Samples were 24–72 h in transit, and were processed within 24 h of receipt by the laboratory.

Electron microscopy — Fecal samples were processed according to standard procedures for the detection of viruses by negative staining (11,12). Two to 3 g of fecal material diluted in 12.5 mL of distilled water were mixed well and centrifuged slowly to separate the heavier sediment. The supernatant was then centrifuged at 100,000 *g* for concentration of all viruses present.

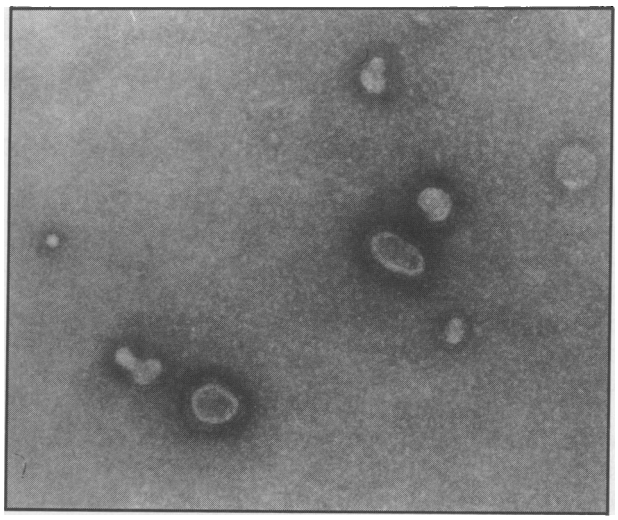

Figure 1. Particles identified as coronavirus. Approximately 80,000 magnification.

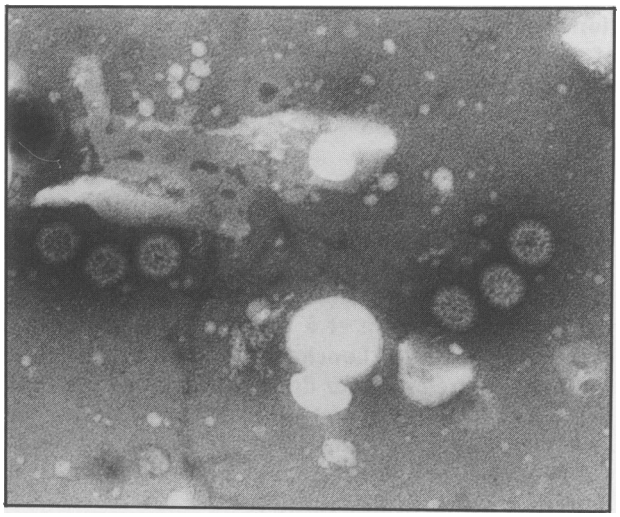

Figure 2. Particles identified as rotavirus. Approximately 80,000 magnification.

The resultant pellet was suspended in distilled water containing 0.2% phosphotungstic acid and 0.05% bovine serum albumin and sprayed on carbon coated, Formvar-covered, 200 mesh, copper grids by use of glass nebulizers. The grids were read at 32,000 magnification in a Zeiss EM 10A transmission electron microscope at 60 kV. Positive samples were quantitated by recording as follows: rare, which indicated an occasional particle seen; 1+, 0–1 particle per field (ppf); 2+, 2–5 ppf; 3+, 5–10 ppf, and 4+, > 10 ppf. Particles resembling those in Figure 1 were identified as coronavirus and those in Figure 2 as rotavirus. If viral particles other than rota- and coronavirus were present, they were also noted.

Samples from both herds were also examined for enterotoxigenic *E. coli*, by use of K99 precipitation test (Coli-Test, Molecular Genetics, Inc. 10320 Bren Rd. East, Minnetonka, Minnesota 55343) and infant mice inoculation (14), for *Cryptosporidia* using acid-fast stained smears (15), and for *Salmonella* and *Campylobacter* by culture using standard laboratory methods (16).

TABLE 1
Clinical fate of calves from coronavirus shedding and nonshedding heifers and cows

Animals	Shedding status of dams: Shedder			Nonshedder		
Calf status:	well	sick	total	well	sick	total
Heifers	1 (17%)	5 (83%)	6	20 (66%)	10 (33%)	30
Cows	5 (54%)	4 (44%)	9	46 (76%)	9 (24%)	55
Total	6 (40%)	9 (60%)	15	66 (77%)	19 (22%)	85

The calving procedure new to both ranches was set up as follows: A designated calving area which had not housed cattle for at least a year was chosen. This area, approximately eight hectares, was maintained free of cows until calving was imminent. Cows expected to calve within a seven to ten day period were brought to the calving ground. Each cow-calf pair was then moved as soon as possible into one of several areas, each filled sequentially, ranging in size from 4 to 16 hectares holding no more than ten cows/hectare. Calves which had clinical signs of disease (depression and/or diarrhea) were moved immediately, with the dam, from the cow-calf area into a hospital pen. In this pen there was a shelter equipped with heat lamps available only to the calves. If the calf recovered, it and the dam were moved into a recovery area separate from the cow-calf area.

Daily low environmental temperatures for the area in which the herd resided during the calving period were recorded by the owners and verified by the National Climatic Data Centre (NOAA, Ashville, North Carolina 28801). Statistical analyses were done using a 2×2 contingency table to test significance of the data displayed in Table 1.

For our purposes, cows with positive fecal samples are referred to as carrier cows although it is realized that negative cows may also have been carriers of less than $10^3 - 10^6$ coronavirus per mL of feces (11–13).

Results

Electron microscopic examination of cow feces collected at the time of pregnancy examinations revealed only one coronavirus-positive cow in herd A. This cow (O42) had been one of the first cows to calve the preceding spring when the 50% morbidity was experienced. In the current year, however, she was separated from the herd before winter feeding. At parturition, three additional cows, which were among the last 15 cows to calve, were positive for coronavirus in their feces.

Coronavirus-positive cows were not found in herd B at the time of the pregnancy examinations, but of 100 cows examined at parturition there were 13 cows positive for coronavirus, one positive for rotavirus, and one positive for rota- and coronavirus. Fecal samples from 23 of 28 sick calves, and from 24 of 95 normal calves were examined. Of the clinically ill calves sampled, 100% were positive for coronavirus and 19 of the 23 (82.6%) had rotavirus. However, 22 (92%) of the clinically normal calves were also positive for coronavirus and 17 (77%) were positive for rotavirus. No *Salmonella*, *E. coli* K99, *Cryptosporidia* or *Campylobacter* were found. Other viruses were not noted.

Of the 15 cows excreting virus at calving, nine had calves with clinical disease (Table 1) and calves from carrier cows had a significantly higher risk of clinical disease ($\chi^2 = 23.11$, $p < 0.005$). The first two calves to develop clinical illness were the calves of the first two cows found shedding coronavirus at parturition.

Six (40%) of the cows shedding virus were first-calf heifers. Of the 36 heifers, 15 (42%) had calves with clinical disease. Five of the six heifers which were positive for virus had calves which had clinical signs (Table 1). The remaining nine carrier cows shedding virus ranged in age from three to eight years.

Diarrhea in calves occurred as early as four days of age (two calves) and as late as 12 days (one calf). The average time of onset was eight days. The concentration of viruses in the fecal samples from cows as judged by the number seen in the EM examination was usually rare or 1+; whereas, in the samples from calves the concentration was always between 3+ and 4+ regardless of clinical health status. There did not appear to be a difference in the amount of either rota- or coronavirus excreted by the calves which showed signs of disease and those that did not. However, rotavirus was detected in more calves during the first 30 days of the calving season (19 of 24 samples, 80%), than in the last 36 days (eight of 16, 50%). The total number of calves born in herd B as well as the number of calves with clinical illness each week are given (Table 2). As diarrhea generally occurred during the second week of life, the average climatic low temperature for the week that the calves were 6–12 days of age is also shown (Table 2). Although peak calving occurred between March 4 and April 6, the peak morbidity occurred earlier, between February 17 and March 4 during the coldest weather. The incidence of clinical disease dropped sharply when the nightly temperatures rose above 0°C.

All calves with clinical disease were treated with oral fluids (Life Guard, Norden, Lincoln, Nebraska 68501). Nine calves which did not respond were taken to the clinic of a private practitioner and given intraveneous fluids. Three of these died but were not necropsied or sampled further.

Discussion

Calving management for this study was set up in the manner described in an attempt to reduce the amount of virus in the environment of the periparturient cow

TABLE 2
Calves born per week in herd B, number which became sick, and the average low temperature for the week that those calves were 7–14 days of age

Date	Week	Calves born	No. sick (%)	Low temp. °C
Feb 11–16	1	6	1 (16)	−6
Feb 17–23	2	6	4 (66)	−6
Feb 24–Mar 2	3	5	3 (60)	−10
Mar 3–9	4	15	4 (27)	−6
Mar 10–16	5	34	7 (21)	−3
Mar 17–23	6	14	3 (20)	−4
Mar 24–30	7	14	3 (21)	+3
Mar 31–Apr 6	8	15	2 (13)	+3
Apr 7–13	9	15	0 (0)	+3
Apr 14–20	10	15	2 (13)	−2

and the newborn calf. Removal of sick calves from the cow-calf herd was an attempt to reduce the concentration of virus in the environment of subsequent calves. This type of calving management did appear to aid in the control of neonatal enteric disease in these two herds. The removal of the carrier cow in herd A and the fortuitous calving of the other three at the end of the calving season certainly seemed to influence the disease problem in that herd. It is unfortunate that samples from calves were not taken from herd A as it would have been helpful to know if the virus was actually eliminated from the environment in that herd. In herd B the 23% morbidity was the lowest this herd had experienced since 1978.

The fact that the first two calves in herd B to show clinical signs were the offspring of the first two carrier cows to calve suggests the importance of the carrier cow in initiating a disease outbreak. The calves of carrier cows had a 60% chance of developing clinical illness whereas those of noncarriers had only a 22% chance (Table 1). In addition, calves born before the cow-calf area was contaminated, i.e. before the carrier cows calved, remained healthy even though exposed later. Furthermore, one would have expected many more calves of both carriers and noncarriers to show disease as the calving season progressed, particularly since the cow-calf area was probably becoming progressively more contaminated. However, an increase in diarrhea was not observed (Table 2). Actually 68% (19) of the sick calves were seen in the first half of the season when the majority (10) of the carrier cows calved.

Calving grounds would become rapidly and heavily contaminated with virus after the onset of calving if, as in herd B, 98% of the calves were incubating and excreting great quantities of virus. The fact that only some calves became ill, although viruses were presumably multiplying at a great rate in the intestinal cells of the unaffected calves also, makes it apparent that neonatal enteric disease results from the effect of multiple circumstances. The presence of a pathogen is merely one of several necessary requisites for clinical disease.

One factor contributing to clinical disease associated with coronavirus in the calf may well be time of exposure, which is suggested by the fact that the first two sick calves were the offspring of the first two carrier cows to calve. In experimental enteropathogenic *E. coli* infections, exposure to the organism before colostral intake resulted in disease despite the fact that colostrum containing specific antibodies against *E. coli* was ingested soon after inoculation of pathogenic organisms (17). The exposure of the calf of a carrier cow to coronavirus could possibly occur before (*in utero*), during (coming in contact with the virus as it came through the birth canal), or soon after birth, before or during suckling. Although all of the calves born earlier were shedding large amounts of virus at 15 days of age, it is reasonable to assume that they became infected with the viruses after the first two carrier cows and their calves were moved into the cow-calf area. Calves of the carrier cows could have been shedding coronavirus and exposing the older calves (which were then two to eight days old) as early as 24 h after their birth (18). Furthermore, since there was no uniform time of moving calves from the calving ground to the cow-calf area, calves could have been moved at any time during their first 24 h, even before they had suckled. It could be speculated that such calves of negative dams may have been exposed to virus in the then contaminated cow-calf area within several hours of birth, or were actually calves of carrier cows which were not identified by the relatively insensitive EM examinations.

Another factor affecting clinical disease in these calves seemed to be age of the dam. First calf heifers appeared to be real liabilities in herd B. Not only were 40% carriers, but calves of the negative heifers also appeared to be more susceptible to disease (Table 1) than calves from older cows. Heifer colostrum is, no doubt, of lesser amount and quality. It was also possible that some of the negative heifers were actually carriers and were not identified due to the insensitivity of the EM examination. Furthermore the heifers were never vaccinated against rotavirus and coronavirus whereas the cows had been in past years. Vaccination did appear to reduce shedding of coronavirus by dairy cows studied in Colorado (8). However, how long vaccination would have an effect upon shedding of virus is unknown.

The study in Colorado also showed an increase in the shedding of coronavirus at parturition (8). Parturition has been reported to influence the shedding of other viruses (5), and is perhaps associated with corti-

TABLE 3
Association of climatic temperature and occurrence of diarrhea in calves from coronavirus-shedding and nonshedding cows

Temp.	Clinical status of calf	Calving cows	
		Shedders	Nonshedders
Below 0°C	Diarrheic	8 (80%)	6 (10.7%)
	Healthy	2 (20%)	50 (89.3%)
	Total calved	10	56
Above 0°C	Diarrheic	1 (20%)	2 (7%)
	Healthy	4 (80%)	27 (93%)
	Total calved	5	29

costeroid increase in the blood of the dam. The increased level of corticosteroid in the blood of the newborn may also influence the clinical manifestation of disease by affecting the susceptibility of the calf to early exposure to the virus.

The shedding of coronavirus by dairy cows in Colorado peaked during the winter months and was not detected during summer months of July–September (8). Colorado, like Idaho, is well known for cold, stressful winters. In our study, 60% of the carriers were found during the period of below-freezing temperatures. Cold weather appeared to affect clinical manifestation of disease in the calves as well. The percentage of calves clinically affected decreased as the nightly low temperature rose, although numbers of calves born and exposed were increasing. The number of calves born during the first three weeks was too small to show a statistically significant relationship with the cold temperatures, however, 80% of the calves of carrier cows born before the nightly temperature rose above freezing showed clinical disease. Even calves of the nonshedder cows had a higher risk of contracting disease during the coldest period (Table 3).

The use of EM to identify carrier animals is probably not a practical method of attempting to control viral neonatal disease of calves, particularly since the low level of shedding at time of pregnancy examination (summer and fall) yields too few viral particles for detection. However, since this study suggests that removal of carrier animals may be an efficient method of controlling this economically important disease, tests on fecal material utilizing serological techniques such as an enzyme-linked immunosorbent assay (ELISA) could be investigated for such a purpose. Such tests would have the advantage of being inexpensive for the examination of many samples and has the probability of being much more sensitive. CVJ

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