Association of diarrhea in cattle with torovirus infections on farms

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SUMMARY

An epidemiologic survey was performed to determine the incidence of torovirus infections in 2 disease entities of cattle: diarrhea of replacement calves up to 2 months old, and winter dysentery of adult cattle. Samples were obtained from 187 diarrheal and 115 healthy calves from 15 farms, as well as 149 diarrheal and 67 healthy cows from 27 farms with or without winter dysentery. Enzyme-linked immunosorbent assays for detection of torovirus, rotavirus, and coronavirus antigen in feces, and of torovirus and coronavirus antibodies in serum were used to monitor infections in these groups. Torovirus was detected in 9 of the 15 farms in the study, and in 6% of calves with diarrhea, which was significantly higher than in healthy calves (2%). Seroconversion to torovirus was found significantly more often after winter dysentery episodes than on farms without a disease history; coronavirus seroconversion was less common.

Neonatal calf diarrhea is a major problem encountered in calf rearing and leads to losses attributable to growth retardation and death. Reportedly, the pathogens most frequently involved are rotaviruses and coronaviruses, *Escherichia coli* K99+, *Salmonella* spp, and *Cryptosporidium* spp.[1-5]

Winter dysentery (WD) is an acute gastrointestinal disorder of adult cattle, particularly dairy cattle, characterized by diarrhea and a severe decrease in milk production.[6] Several investigators have described the presence of coronavirus-like particles in the feces of affected cows[7-15]; in a few cases, these findings were confirmed with immunologic tests.[8,14-16]

The Breda viruses (BRV), toroviruses of cattle, were discovered in 1979.[17] Until now, 3 BRV isolates have been described and assigned to 2 serotypes.[18,a] Breda viruses can cause diarrhea in gnotobiotic[17] or conventionally reared calves and in older cattle.[19] They are antigenically related to Berne virus, which has been isolated from a horse in Switzerland in 1972,[20] and to morphologically similar particles in feces from human beings with diarrhea.[21] Only Berne virus has been grown in cell culture and characterized in detail. Its unique properties led to the proposal of a family status for this new group of viruses[22,23]; the family name Toroviridae will be used throughout this study.

More than 88% of dairy cattle in The Netherlands and the Federal Republic of Germany have circulating torovirus antibodies as tested by ELISA.[24] Consequently, young calves generally possess maternal antibodies that, however, do not fully protect them from infection. Torovirus shedding associated with diarrhea and followed by the production of anti-torovirus IgM in serum was observed in 9 of 10 sentinel calves between 1 and 2 months old, and in all 10 calves at 11 months old. Virus shedding was observed in some apparently healthy calves, with no serologic response.[24]

The purpose of the study reported here was to determine the role of torovirus infections in calf diarrhea and WD under field conditions, compared with rotavirus and coronavirus in calf diarrhea, and with coronavirus in WD.

Materials and Methods

Animals—One population consisted of young calves, age < 3 months, from dairy farms that are under regular veterinary surveillance of the ambulatory clinic of the Utrecht Veterinary Faculty. Herds could be selected when they had between 40 and 70 milking cows, when milk production was recorded by the National Milk Recording Service, and when farmers were accustomed to record keeping. Only farms with both cases and controls (≥ 6) were used for this study. Samples were obtained from 302 calves from 15 farms (range, 7 to 39 calves/farm). From November until May, all farms were visited once or twice a week, at which times the calves were examined clinically. Diarrhea was only diagnosed by the research workers and was defined as a change in consistency of the feces ranging from loose feces in apparently healthy calves to

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[a] Saif LJ, Redman DR, Theil KW, et al. Studies on an enteric "Breda" virus in calves (abstr 236), in *Proceedings*. 62nd Annu Meet Res Work Anim Dis 1981; Chicago, Ill.

watery diarrhea in calves with signs of general illness. When diarrhea was observed, fecal samples were collected and stored at −20 C until testing. Farmers were asked to monitor the duration of diarrhea. Control samples were collected from apparently healthy calves. Diarrheal samples (n = 187, mean, 12/farm; range, 2 to 31) and control samples (n = 115, mean, 7/farm; range, 3 to 15) were tested.

The second population consisted of older fattening calves from farms in the same region. Fecal samples were obtained from 10 diarrheal and 197 healthy, individually housed, fattening calves older than 13 weeks from 4 farms.

The third population consisted of animals on farms where episodes of WD had been observed. They were predominantly located in the south of the country in a region controlled by the Animal Health Service in Boxtel. For the diagnosis, the veterinarians used the following working definition: a highly contagious diarrhea with low mortality, primarily affecting lactating cows, occurring during the winter months, and lasting 3 to 4 days. A severe decrease in milk production is common and some animals have dark, bloody feces.[13] Serum samples (≥ 6/farm) were obtained from affected cows (n = 149; 19 farms with 6 to 14 animals each) as soon as possible after onset of the disease, and 3 weeks later. Sera were stored at −20 C until testing. A ≥ fourfold increase in titer was considered significant. Samples were obtained in the same way and in the same time period from cows from 8 farms without a history of WD (n = 67; 8 farms with 6 to 13 animals each).

Antigen detection in feces—A double-antibody sandwich (DAS) ELISA for detection of torovirus antigen has been described.[19] Gradient-purified BRV-2 (Iowa isolate) from feces of orally infected gnotobiotic calves served as a positive control.[24]

An indirect DAS-ELISA was used for rotavirus[25] detection, and a DAS-blocking ELISA was used for coronavirus antigen.[26] All calves were assayed for the presence of the 3 viruses in duplicate tests. In all tests, a confirmation step was routinely included to verify specificity of the reactions.[19,25,26]

Serologic analysis—Blocking ELISA was used for detection of torovirus and coronavirus antibodies in serum as described.[24] Sera from cattle with WD were tested in ELISA also for antibodies to bovine viral diarrhea virus at the Regional Animal Health Service in Boxtel.

Statistical analysis—The data in population 1 were analyzed by use of crude odds ratios and comparison of proportions (χ^2) for comparison of diarrhea incidence between calves classified according to viral excretion.[27,28] To correct for herd effects, a logistic regression analysis was performed. In this analysis, the probability of having diarrhea was modeled as a function of virus presence, simultaneously correcting for herd effects.[29] Because mixed viral infections were common, their possible influence was also evaluated, leading to the following initial logistic model:

$$\ln[p/(1-p)] = a + b_i \text{ virus}_i + c_j \text{ virus combination}_j + d_k \text{ herd}_k + e$$

where p = probability of a calf having diarrhea.

The age at first infection and duration of diarrhea was analyzed by use of the nonparametric Kruskal-Wallis test.[30] The data in population 2 were analyzed by use of a χ^2 test,[27] to assay the difference in proportion of animals shedding the virus between healthy and diarrheal calves.

The data in population 3 were analyzed by use of crude odds ratios and weighted logistic regression analysis. Weights were the number of samples per farm. The initial regression model was:

$$\ln[p/(1-p)] = a + b_i \text{ \% seroconversion for virus}_i + e,$$

where p = probability that a farm has an episode of WD.

The odds ratio is an important measure of effect in this study and indicates the number of times a virus-shedding animal (herd) is more likely to have diarrhea. An odds ratio of 1 means no effect.[28]

Results

Young calves (population 1)—The diarrhea morbidity of calves < 3 months ranged between 27 and 81%/farm, with a mean of 57% (SD 16.8%). The differences in torovirus and rotavirus incidence between the groups were significant (χ^2, 1 *df*: $P = 0.04$ for torovirus; $P = 0.0001$ for rotavirus), which was not the case for coronavirus (Table 1). The crude odds ratios and their range at a 95% confidence level were calculated for each infection; calves infected with torovirus were 3.9 times more frequently found to be diarrheal than to be healthy (95% confidence interval [CI], 0.9 to 17.6). For rotavirus, the odds ratio was 3.7 (95% CI, 2.0 to 6.8), and for coronavirus the odds ratio was 0.35 (95% CI, 0.2 to 0.6). In total, torovirus alone and in combinations was detected on 9 farms (60%), coronavirus on 14 (93%), and rotavirus on all farms (Fig 1). In the logistic regression model, the presence of torovirus and rotavirus (and their combination) remained significantly associated with diarrhea. The logistic regression coefficients with their SE were torovirus, b = 2.9(1.1) $P = 0.01$, rotavirus, b = 1.5(0.36) $P < 0.001$, torovirus and rotavirus, b = −4.1(1.7) $P = 0.02$, and coronavirus, b = −0.9(0.32) $P < 0.001$. The presence of coronavirus was not positively associated with diarrhea, because coronavirus was present in more healthy calves than in affected calves.

Table 1—Proportion of fecal samples from diarrheal and healthy calves with positive ELISA results for torovirus, rotavirus, or coronavirus

Virus detected	Diarrheal calves No.	%	Healthy calves No.	%	Odds ratio
Torovirus					
Alone	5	2.7	0	0	
Mixed	9	4.8	2	1.7	
Total	12	6.4	2	1.7	3.9
Rotavirus					
Alone	60	32.0	8	7.0	
Mixed	10	5.3	8	7.0	
Total	70	37.4	16	13.9	3.7
Coronavirus					
Alone	20	10.7	34	29.6	
Mixed	12	6.4	9	7.7	
Total	32	17.1	43	37.4	0.35
No virus	89	47.6	63	59.1	
Total	187	100.0	115	100.0	

Odds ratio indicates number of times a virus-shedding animal is more likely to have diarrhea. An odds ratio of 1 means no effect.

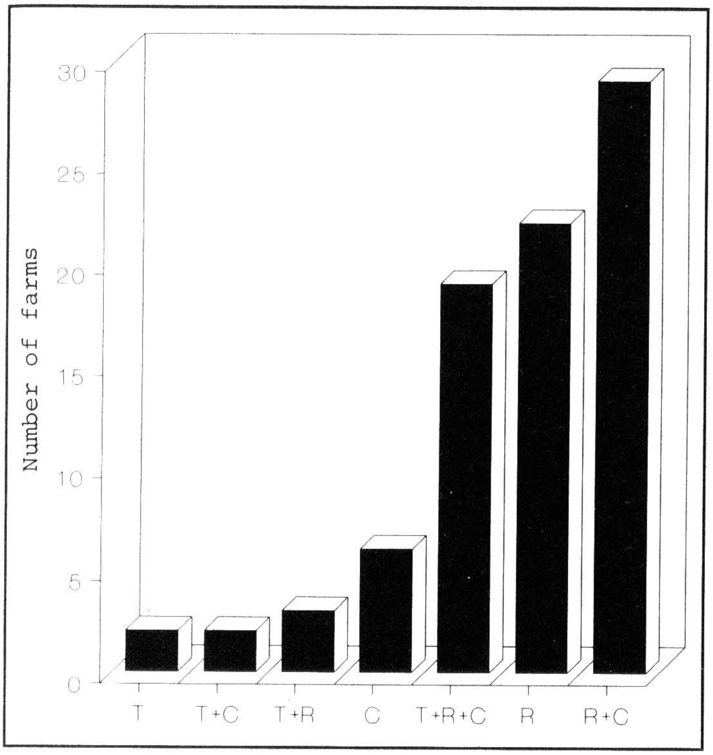

Figure 1—Number of farms on which cattle were infected with torovirus (T), coronavirus (C) and rotavirus (R), alone or in combinations.

Table 2—Age and duration of first diarrhea in calves infected with torovirus, rotavirus, or coronavirus

	Virus detected		
	Torovirus	Rotavirus	Coronavirus
Age			
Mean (d)	11.1	7.7	8.3
SD	8.7	4.9	6.1
Range	1 to 28	3 to 28	1 to 25
No.	12	16	41
Duration			
Mean (d)	9.2	6.8	6.8
SD	5.0	4.7	4.0
Range	2 to 14	1 to 18	1 to 13
No.	10	11	36

Torovirus-associated diarrhea started later than that associated with rotavirus or coronavirus, but the difference was not significant. The mean duration of diarrhea in days was comparable for all 3 viruses (Table 2).

Older calves (population 2)—In calves 4 to 5 months old, diarrhea was observed in 10 calves, 4.8%; n = 207. Two diarrheal calves shed torovirus, 3 calves shed coronavirus. Of the healthy calves (n = 197), 3 (1.5%) were shedding torovirus at the moment of sampling, 1 (0.5%) shed rotavirus and 33 (16.8%) shed coronavirus. The difference in shedding torovirus was significant (χ^2, 1 *df*: $P < 0.001$), the difference in shedding coronavirus was not significant ($P = 0.28$).

Adult cattle (population 3)—The difference in incidence between WD farms and control farms was significant for torovirus (χ^2, 1 *df*; $P = 0.032$), but not for coronavirus ($P > 0.10$; Table 3). For torovirus, the odds ratio (3.5) was significantly different from 1.0 (1.01 to 12.17; 95% CI; Table 3). When computing a weighted logistic regression,

Table 3—Proportion of seroconversions (≥ fourfold increase in titer) to torovirus, coronavirus, and bovine viral diarrhea (BVD) virus in cattle from 19 farms with winter dysentery and six control farms

			Seroconversions		
	NR	N	Torovirus No. (%)	Coronavirus No. (%)	BVD virus No. (%)
Winter dysentery farms	1	10	6(60)	0	0
	2	6	3(50)	2(33)	2(33)
	3	7	3(43)	3(43)	ND
	4	10	3(30)	0	1(10)
	5	6	1(17)	0	ND
	6	6	1(17)	0	0
	7	6	1(17)	3(50)	0
	8	6	1(17)	0	0
	9	10	1(10)	1(10)	ND
	10	14	1(7)	0	0
	11	10	0	2(20)	0
	12	9	0	2(22)	ND
	13	9	0	0	ND
	14	8	0	0	0
	15	7	0	0	0
	16	7	0	0	0
	17	6	0	0	5(80)
	18	6	0	0	0
	19	6	0	0	0
Total		149	21(14)	13(9)	8(6)
Control farms	1	7	1(14)	2(28)	
	2	10	1(11)	0	
	3	9	1(11)	1(11)	
	4	13	0	0	
	5	9	0	0	
	6	7	0	1(14)	
	7	6	0	0	
	8	6	0	0	
Total		67	3(4)	4(6)	ND
Odds ratio			3.5	1.5	

See table 1 for explanation of odds ratio.
NR = Farm number; ND = not done.

a highly ($P = 0.0016$) significant relationship was found between the proportion of BRV seroconversions on a farm and the probability of a WD episode. Using 30% BRV seroconversions as a criterion, BRV infection was associated with episodes of WD on 4 farms of 19.

Discussion

Of the young calves under study (population 1), 57% had diarrhea at least once in their first 2 months of life. Similar figures have been published earlier from The Netherlands,[1] England,[2] and Scotland.[3]

The results of antigen detection tests show that toroviruses, although not a major cause of disease, may have a role in calf diarrhea: their incidence in calves with diarrhea was significantly ($P = 0.04$) higher than in healthy animals, although lower than that of rotaviruses or coronaviruses. This type of comparison has been recommended[30] and has made other authors establish the causative role of rotaviruses in diarrhea.[2,3] However, the findings can be influenced by several factors, such as the sensitivity of the assay, the occurrence of serotypes different from the virus used in the assays, duration of virus shedding, and amount of viral particles shed. After experimental infection, large numbers of Breda virus particles were detected immediately after the onset of clinical signs, but for 3 to 4 days only.[17,18] Because collection of samples was done at 3- to 7-day intervals, about half of the calves excreting BRV may have escaped detection. Nevertheless, our results indicate that toroviruses are widespread among farms, which confirms data from serologic surveys.[17,24]

In a recent survey,[31] 7.3% of calves were found by use of electron microscopy (EM) to shed torovirus-like particles. However, these results have not been verified by serologic analysis, and healthy calves were not examined in parallel; Woode et al (1982),[17] using EM and hemagglutination-inhibition tests, found no BRV in 336 diarrheal calves in Iowa. Recently, an ELISA has been used on samples from calves with diarrhea, and 3.9% were reacting; the investigators regarded these results as false-positive reactions.[32] We have routinely included BRV-2 antibody-negative IgG as mock capture antibody to detect false-positive reactions; these incidentally, can be seen with feces as determined by comparing ELISA results with those of solid-phase immune EM (1%, n = 67).[19] Also, compared with a hybridization assay that uses radiolabeled cDNA probes to detect viral RNA in feces, no false-positive reactions were seen (n = 60).[33] An exceptionally high number of animals shedding BRV (16 of 21) has been reported by Lamouliatte.[34]

The incidence figures found for rotaviruses and coronaviruses are in the same range as reported,[1-5,13,34] and rotavirus was confirmed to be a major cause of diarrhea. A significant overall difference in incidence was not found between calves with diarrhea and control calves for coronavirus, as reported by others.[1,2,4]

In older calves (population 2), the incidence of diarrhea was low, but more calves with diarrhea were infected with torovirus than calves without diarrhea.

A virus has been suspected to cause WD since 1957, when MacPherson[35] produced WD-like signs with a 450-nm filtrate of feces from affected cows. For the last decade, attention focused on coronaviruses, but their etiologic role is still undetermined. Several investigators have described coronavirus shedding by cattle with diarrhea,[9-12,15]; others have found the same for apparently healthy animals.[36,37] In epizootics in Japan, a high number of seroconversions (59 of 100) was reported in cattle with diarrhea.[7,16] Van Kruiningen et al[14] detected coronavirus particles and viral antigen in lesions of the large intestine of cattle that had been infected by feeding them feces from animals with WD; no seroconversion to coronaviruses was detected in those animals. In population 3, the overall difference in incidence between farms with and without WD was significant for torovirus, but not for coronavirus. We found a low percentage of seroconversions to coronaviruses and toroviruses in healthy cattle (Table 3). This is not unexpected, because 90% of adult cattle possess antibodies to both viruses, which can be maintained only when booster infections develop. To decide whether the number of seroconversions on a farm exceeds baseline values, the weighted logistic regression analysis was used. A regression curve that can be used to relate the probability of a WD episode on a farm to the number of seroconversions was established. A $\geq$ 90% probability that a farm was in the WD category was seen at $\geq$ 30% seroconversions. The weight was introduced to correct for the differences in sample numbers between farms. Our data indicate that torovirus infections had a role in 4 episodes, coronavirus in 2, and bovine viral diarrhea virus in 1 of 19 farms studied. Thus, a unifying concept of the etiologic agent(s) of WD is still lacking; in fact, it may be inherently elusive. Apart from the possibility that a hitherto unidentified agent causes the condition, infections with notorious enteropathogens may result in the same disease picture.

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