Breda virus (Toroviridae) infection and systemic antibody response in sentinel calves

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SUMMARY

Enzyme-linked immunosorbent assays were established to detect Breda virus antigen in feces and homologous antibodies of the IgG1, IgM, and IgA isotypes in serum. With the aid of solid-phase immune-electron microscopy, torovirions in fecal material were observed. The course of natural infection was studied in 10 sentinel calves that had been obtained from different farms, and housed together at 1 week of age. They were separated from other cattle until the age of 10 months.

Up to the age of 4 months, all calves regularly excreted Breda virus in the feces. Irrespective of the existence of IgG1 isotype maternal antibodies, all calves had early IgM responses in serum, but lack of IgA seroconversion. In 7 calves, antibody titer decreased below detection, whereas 3 calves had an isotype switch, resulting in persistent IgG1 titer. After introduction into the dairy herd at 10 months of age, all calves had diarrhea, and shedding of Breda virus was observed in 8 of them. Seroconversion for all antibody isotypes was observed, indicating lack of mucosal memory. In contrast, coronavirus infection in the presence of maternal antibodies led to isotype switch in all calves but one, and a memory response was observed after introduction into the dairy herd.

Diarrhea is one of the most important disorders affecting young calves. The pathogens most frequently encountered are rotaviruses, coronaviruses, bovine viral diarrhea virus, *Escherichia coli* K99+, *Salmonella* spp, and *Cryptosporidium* spp.[1-8] In 1986, the Central Veterinary Institute in Lelystad, The Netherlands, reported morbidity between 20 and 80%, and 4% mortality attributable to neonatal calf diarrhea.[1]

Breda virus (BRV) was discovered in 1979 during an episode of neonatal calf diarrhea in Iowa.[9] It was found to differ antigenically from other known bovine viruses, notably from coronaviruses, which it resembles morphologically. Two further isolations of Breda viruses were reported: the Ohio isolate from 5-month-old beef calves[a] and a second Iowa isolate from a 2-day-old calf.[10] With the aid of ELISA, hemagglutination/hemagglutination-inhibition tests, and immune-electron microscopy (IEM), 2 serotypes have been established and are referred to as BRV-1 (the original Iowa isolate) and BRV-2 (represented by the Ohio isolate and the second Iowa isolate).[10] The Breda viruses are antigenically related to Berne virus, which was isolated from a horse in Switzerland in 1972.[11] Berne virus can be grown in cell culture and has been characterized in detail. Its unique structural properties have led to the proposal of a family (Toroviridae) for the Berne/Breda viruses, the former representing the family prototype.[12-14] Breda viruses are pathogenic when experimentally fed to nonimmune calves and may cause severe diarrhea and dehydration.[9,15,16]

More than 88% of adult cattle have circulating antibodies to toroviruses as tested by ELISA[10,17,18] or neutralization assay.[19] Newborn calves generally have high antibody titer, probably of maternal origin, which slowly wanes until at about 4 months of age, when most calves have become seronegative. Between 7 and 12 months of age, most bovids are infected, as evidenced by seroconversion.[17]

The purpose of the study reported here was to monitor a group of 10 sentinel calves for 1 year to study BRV infection under dairy conditions in The Netherlands. The BRV antigen was detected in fecal samples by use of ELISA, and the presence of virus particles was confirmed by use of solid-phase immune-electron microscopy (SPIEM). Isotype-specific BRV-ELISA were established and used to analyze the humoral immune response to BRV, with special attention given to the reactions in newborn calves. In parallel, feces of calves were tested by ELISA for bovine coronavirus (BCV) antigen, and sera were tested for total antibody concentrations and BCV-specific IgM concentration. Active immunization was detected for both viruses (BRV and BCV) in the presence of colostrum-derived circulating antibodies, as has been described for rotavirus, bovine respiratory syncytial virus, and cryptosporidial infections.[20-25]

Materials and Methods

Experimental animals—Ten healthy Friesian dairy calves with measured γ-globulin concentration > 10 g/L were purchased. This concentration was assumed to indicate sufficient intake and absorption of colostral im-

Received for publication Apr 12, 1989.

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The authors thank Drs. D. van Zaane and J. van Oirschot for the monoclonal antibodies to bovine immunoglobulins, and the materials for rotavirus and coronavirus antigen and antibody detection, respectively, Dr. W. Herbst from the Justus-Liebig University in Giessen, Federal Republic of Germany, Dr. J. Calafat, and H. Jansen from the Antoni van Leeuwenhoek Hospital, Amsterdam, for providing facilities for electron microscopy, and A. Westeneng and J. van Dasselaar for technical assistance.

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

munoglobulins.[26-28] The study was begun when calves were about 1 week old and had been collected from herds in the province of Utrecht, numbered in order of arrival at the Large Animal Clinic, and were housed there, separate from other cattle, in individual pens. From 4 weeks of age onward, calves were stabled in 2 groups of 5 (No. 1–5 and 6–10). They were fed a commercial milk replacer ad libitum, until weaning at 9 weeks. From the first week onward, hay and concentrates were supplied. After vaccination against lungworm infection, calves were pastured from May (at about 4 months of age) until November, after which they were stabled together with adult cows that were seropositive for BRV. Once or twice daily, calves were examined.

Blood samples were collected at weekly intervals. Rectal fecal samples were obtained every other day for the first 4 months and at weekly intervals thereafter. All samples were stored at −20 C.

Two specific pathogen-free rabbits were immunized by SC inoculation of 8,000 hemagglutinating units (HAU) of gradient-purified BRV[29] in complete Freund adjuvant. Four weeks later, a booster inoculation containing 5,000 HAU of BRV in incomplete Freund adjuvant was administered SC. From serum obtained 1 week later, the immunoglobulins (RaBRV-Ig) were precipitated as described[17] and used as capture antibody in an antigen-detection ELISA. Preimmunization sera that were processed in the same manner served as negative controls.

ELISA for detection of BRV antigen—A modification of the test procedure described by Brown et al.[18] was used. Briefly, polystyrene microtitration plates[b] were coated with negative control serum and RaBRV-Ig, diluted 1:2,000 in 100 m*M* $Na_2CO_3/NaHCO_3$ buffer, pH 9.6 (coating buffer). After overnight incubation at 37 C, plates were washed with a 0.15*M* NaCl solution, containing 0.05% (v/v) Tween 80. Fecal samples were diluted 1:4 in phosphate-buffered saline solution (PBSS), pH 7.3, containing 20% fetal bovine serum (FBS)[c] and centrifugated at 1,000 × g for 20 minutes. The supernatants were added to the plates and were left to incubate for 3 hours at 37 C. After another washing cycle, the hyperimmune bovine anti-BRV serum GC76, conjugated with horseradish peroxidase as described (BaBRV-IgPO),[17] was added at a 1:400 dilution in PBSS containing 0.35*M* NaCl, 1 m*M* EDTA, and 0.05% (v/v) Tween 80 (ELISA buffer). Subsequently, 0.2 m*M* 2.2'-azino-di-(3-ethyl benzthiazoline sulfone-6) diammonium salt (ABTS) in 0.05*M* citric acid, pH 4, was added; 90 minutes later, the reactions were read spectrophotometrically at 405 nm. A difference of > 0.1 absorbance unit between wells coated with pre- and postimmunization sera was considered to be a positive result.

High background reactions were regularly seen with fecal preparations and with ELISA plates coated with preimmune rabbit serum. Heating of the samples (56 C, 30 minutes) diminished nonspecific binding and was used throughout. The specificity (percentage of truly negative reactions) and sensitivity (percentage of truly positive reactions)[30] of the ELISA were assessed by testing gradient fractions of BRV-2 containing fecal preparations from gnotobiotic calves[17] in parallel in ELISA and SPIEM (described later), using the latter as a reference test.

[b] Bioreba ELISA plates, Sanbio, Uden, The Netherlands.
[c] Flow Laboratories, Irvine, Scotland.

Solid-phase immune-electron microscopy (SPIEM)—Carbon-covered grids (300 mesh) were first coated with a 1:200 dilution in PBSS of a calf hyperimmune serum to BRV (GC76) or to cryptosporidia (GC75) for 30 minutes at 20 C. The immunization protocols have been described.[17] An antibody-coated grid was floated for 1 hour on a drop of each fecal preparation, then washed. Finally, grids were stained with 2% phosphotungstic acid, pH 6.8,[31] and examined by electron microscopy[d] at 80 kV for a minimum of 15 minutes.

ELISA for detection of BRV antibody isotypes—The BRV-specific IgG1 was detected and titrated in an indirect double-antibody sandwich assay, following the method described by van Zaane and IJzerman.[20] Briefly, ELISA plates precoated with RaBRV-Ig were incubated with gradient-purified BRV-2, Iowa isolate[17] (4 HAU/well) in ELISA buffer containing 10% FBS, the test serum, a 1:2,000 dilution of monoclonal antibody (MAB) to bovine IgG1, and finally with rabbit anti-mouse immunoglobulins horseradish-peroxidase conjugate[e] at a 1:1,000 dilution, both in ELISA buffer. The ABTS substrate was then added, and the reactions were read at 405 nm.

For detection of BRV-specific IgM and IgA, antibody-capture assays were used; inter-isotype competition cannot inhibit detection of these less-abundant isotypes.[20] The ELISA plates, coated overnight with a 1:3,000 dilution of MAB to bovine IgM or IgA in coating buffer, were incubated with the test serum, 4 HAU of BRV-2 antigen/well, and a 1:400 dilution of RaBRV-IgPO, all in ELISA buffer containing 10% FBS. All incubation steps were done at 37 C; finally, the ABTS substrate was added and the reactions were read.

Serial twofold serum dilutions starting at 1:10 were made in ELISA buffer; on each plate, hyperimmune calf anti-BRV (GC76) and anti-cryptosporidia serum (GC75) were included as positive and negative controls. Each serum was tested for nonspecific binding and determination of correction factors at a 1:10 dilution in an uncoated well. Titer is given as the reciprocal of the highest serum dilution with an absorbance 1.5 times that of the background. Background absorbance was measured in the rows in which anti-cryptosporidia serum had been tested.

For comparative purposes, an indirect double-antibody sandwich ELISA was used for detection of rotaviral antigen,[32] and a double-antibody sandwich-blocking ELISA was used for assaying BCV antigen and antibodies.[33] For detection of BCV-specific IgM, essentially the same test was used as described for BRV, using BCV antigen and a bovine anti-BCV IgG-horseradish peroxidase conjugate[33] instead.

Results

Torovirus in diarrheal calf feces—Diarrhea started in all calves between 1 and 9 days after arrival at the experimental facility (Fig 1); it lasted between 2 and 13 days. Calves 5, 6, 7, and 9 had signs of dehydration as judged from the skin pinch test, sunken eyes, and PCV values (> 35%) as determined by centrifugation. They were fed rehydration salt solution[f] for 1 to 2 days in addition to the milk diet. Calves 5 and 7 had signs of general

[d] Philips EM-300, Philips, Eindhoven, The Netherlands.
[e] Nordic Immunologic Laboratories, Tilburg, The Netherlands.
[f] Electrol, Aesculaap BV, Boxtel, The Netherlands.

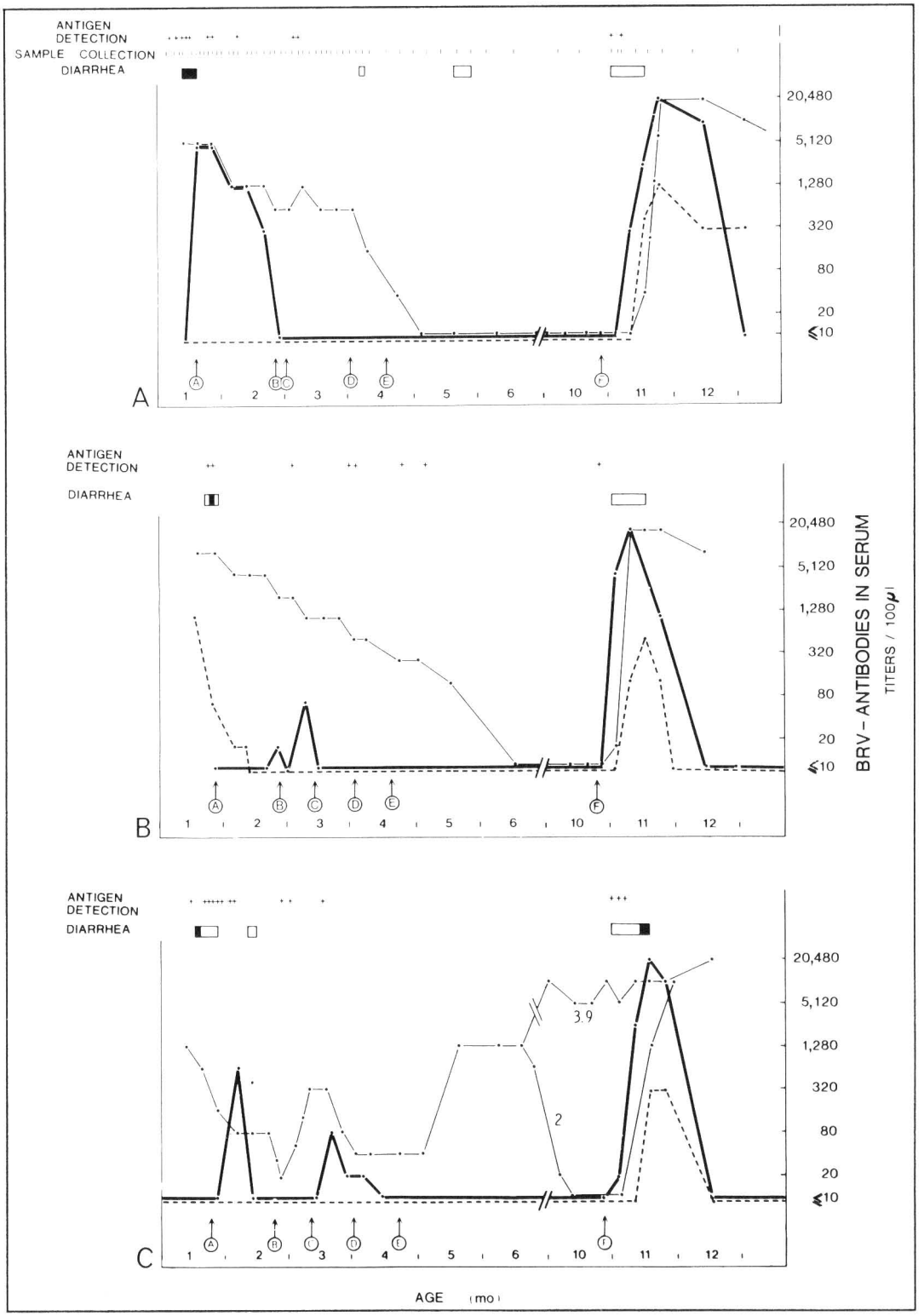

Figure 1—Breda virus (BRV) shedding, diarrheal episodes, and ELISA titers of BRV-specific antibody isotypes in serum of calf 7 (representative also for calves 4, 5, 6, and 10; A), calf 1 (representative also for calf 8; B), and calf 3 (representative also for calves 2 and 9; C). Times of fecal sample collection are indicated by short vertical bars above graph A; sera were obtained at weekly intervals. Arrows attached to circled letters indicate introduction into group housing (A), vaccination against lungworm infection (B), (D), weaning (C), pasturing (E), and introduction into the herd (F). / = IgM; / = IgG1; --- = IgA; ■ = liquid feces; □ = semiliquid feces.

illness; they were shivering, depressed, and anorectic. Rectal temperature remained within the normal range (38.5 to 39.5 C). All calves recovered after fluid administration. When calves were introduced into the clinic's dairy herd at 10 months of age, they all developed diarrhea that lasted for 1 to 3 weeks then stopped without treatment.

In our study, all calves shed BRV several times within

the first 4 months, as detected by ELISA (Fig 1). In all but calf 9, BRV shedding was associated with episodes of diarrhea during the neonatal period. At an older age, calves 1, 2, 4, 5, 6, 8, and 9 were shedding BRV immediately after pasturing, and calves 8 and 9 began shedding again at 9 months of age (results not shown).

During the episode of neonatal diarrhea, we also detected rotavirus in fecal samples from calves 1, 2, 3, 5, 7, 8, and 9 and BCV in those of all calves. We compared the percentages of virus detection in diarrheal (from 2 days before onset to 2 days after recovery) and normally formed feces (Table 1) obtained during the first month of the study.

At 10 months of age, another bout of diarrhea was noticed in calves of the experimental herd; again BRV could be detected in fecal samples (with the exception of calves 6 and 10). Calves 3 and 7 shed rotavirus, and calf 1 shed BCV.

Sixty-seven fecal samples of known ELISA reactivity (22 positive, 45 negative) were selected from our study and were tested by use of SPIEM. When this method was used for reference, the sensitivity and specificity of the BRV-ELISA were 88 and 93%, respectively (Table 2); overall agreement of 95% was found between the 2 tests. An example of SPIEM, using an ELISA-positive specimen, is seen in Figure 2.

BRV-specific immunoglobulin isotypes in calf sera—During initial testing, all calves had anti-BRV IgG1 titer between 40 and 20,480, which slowly decreased to low titer. Later, the serotiter in the calves could be accommodated in 3 patterns (Fig 1).

In calves 1, 4, 5, 6, 7, 8, and 10, IgG1 remained undetectable until seroconversion at 11 months of age, 2 to 4 weeks after introduction into the dairy herd. The 2 groups differed in that calves of the latter group had IgA in the serum at 1 week of age.

In calves, 2, 3, and 9, IgG1 titer started to increase at 4 months of age, reaching a plateau 3 weeks later. Calves 3 and 9 remained seropositive, whereas in calf 2, titer gradually decreased after 2 months; at 8 months of age, IgG1 was no longer detectable. This calf seroconverted at 10 months of age, 1 week after introduction into the dairy herd.

Table 1—Viruses in diarrheal (D) and normal (N) fecal samples during the episode of diarrhea in young calves. Samples in D column had been obtained between 2 days before and 2 days after onset of diarrheal signs

	Virus in feces	
	D (%)	N (%)
Breda virus	25 (37)	5 (6)
Rotavirus	10 (15)	6 (7)
Coronavirus	25 (37)	19 (23)
Virus not detected	23 (34)	52 (63)
Total	68 (100)	82 (100)

Table 2—Comparison between ELISA results and solid-phase immune-electron microscopy (SPIEM) reactions for detection of Breda virus in feces of calves (n = 67)

ELISA results	SPIEM results		
	Positive	Negative	Total
Positive	21	1	22
Negative	3	42	45
Total	24	43	67

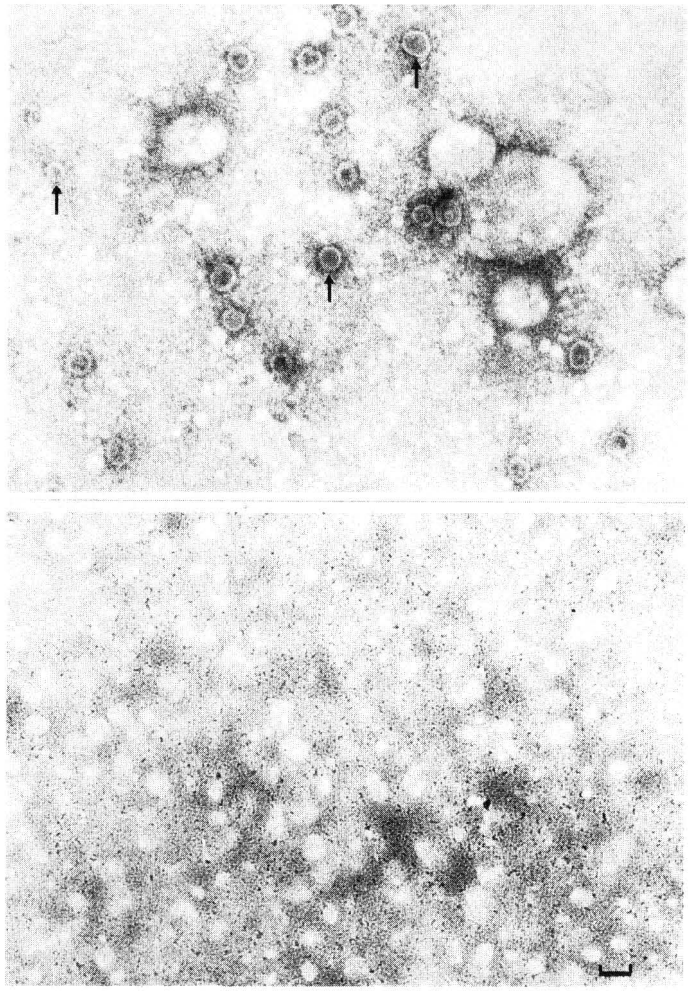

Figure 2—Solid-phase immune-electron microscopy (SPIEM) of feces from a gnotobiotic calf infected with BRV (arrows). Grids had been precoated with calf hyperimmune serum against BRV (top) or cryptosporidia (bottom). Bar = 100 nm.

In all calves, IgM antibody was detected within 2 months after the start of the study. It was first detected between 17 and 44 days after initial BRV shedding, and between 16 and 46 days after onset of neonatal diarrhea. It persisted for 1 to 7 weeks and reached maximal titer >320 in 7 calves. There was a linear correlation (r = 0.6848; P = 0.05; least-squares analysis) between duration of diarrhea and onset of IgM production, in the sense that longer duration of diarrhea resulted in earlier detection of IgM. A second IgM peak was observed in all calves within 1 to 3 weeks after first contact with healthy older cattle. The anti-BRV IgM titer exceeded 320 in all calves and persisted for 1 to 3 weeks.

In the earliest serum specimens obtained, calves 1 and 8 had detectable IgA. An IgA response was not seen in any calf after the first IgM peak was observed at 1 to 2 months of age. At 11 months, IgA production started in all calves within 1 to 2 weeks after initial IgM detection.

Coronavirus antibodies in calf sera—At first testing, all calves had anti-BCV titer between 200 and 1,600, which

slowly decreased until 4 months of age. Later, antibody titer started to increase in all but calf 1, reaching a plateau within 3 weeks; thereafter, titer gradually decreased. At 10 months of age, 1 week after introduction into the dairy herd, all calves seroconverted again.

In all calves, anti-BCV IgM was detected within 2 months after the start of the study. It was first detected between 14 and 30 days after initial BCV shedding and persisted for 4 to 12 weeks. A second IgM peak was not found after introduction of calves into the dairy herd.

Discussion

Results of this study emphasize the pathogenic importance of toroviruses, which so far has been inferred from either experimentally induced disease in gnotobiotic calves[9] or detection of torovirions or viral antigen in the feces of animals and people during natural episodes of diarrhea.[9,18,34] In our group of calves kept physically separated from adult bovids, but under otherwise conventional condition, neonatal torovirus infection was observed, as evidenced by the detection of antigen and (short-lived) seroconversion. The latter did not result in protective immunity, because a second bout of diarrhea accompanied by virus excretion and seroconversion developed after contact with adult cattle. The source of the virus during the neonatal episode is unknown; for calves that are exposed to older cattle, persistently infected adult cattle are the likely origin.

In all calves, neonatal diarrhea was associated with excretion of torovirus particles; this alone, however, would not prove their etiologic role, because rotaviruses and especially coronaviruses were also detected during the disease episode. Mixed viral infections are frequently seen in young calves with diarrhea.[1,5,7,8] For rotaviruses and coronaviruses, intermittent virus shedding by healthy and diseased calves and cows has been reported.[35-38] Evidence for such viral involvement in disease could be deduced only from the significant difference of shedders between the groups.[4,5] We found BRV in diarrheal feces at greater than fivefold rate (than that in normal feces; Table 1); in view of the unimpressive difference between diarrheal and normal feces for the other viruses, we are led to believe that BRV was the main cause of diarrhea in our calves.

When calves were older, BRV seemed to be the primary cause of diarrhea; BRV shedding was detected in all calves during the episode of diarrhea, whereas rotavirus and BCV were found only sporadically. In human beings, torovirus involvement in gastroenteritis of adults has been documented.[34]

Maternal BRV-specific IgG1 and BCV-specific antibodies were found in all calves at initial testing; the immunoglobulins were inefficient in protecting calves from neonatal infection and did not block the development of serum IgM titer. Circulating antibodies generally have a minor role in protection of villus enterocytes against infection (eg, with rota-, corona-, calici- or astroviruses).[21,39] The observed serum IgM titer increase may prove useful to diagnose BRV infection in the presence of maternal antibodies, as has been described for bovine respiratory syncytial virus infection.[22] A problem arises as to what would be the right moment for testing. In the presence of circulating maternal antibodies, IgM is produced later (≥ 14 days) and at lower maximal titer,[23,39] as seen in our experimental group of cattle.

Also, BCV infection in the presence of circulating maternal antibodies led to a delayed IgM response but, in striking contrast to BRV infection, was followed by an isotype switch and development of mucosal memory; reinfection at 10 months of age triggered a booster antibody response (serum IgM titer staying below detection, and rapid increase of IgG titer). Priming by BRV was followed by IgG production in 3 calves only and did not lead to immunologic memory in any of the calves. In calves with controlled infections, more prolonged diarrhea and virus shedding correlate with greater immune responses,[21] but in our study, BCV was not more frequently associated with diarrhea than was BRV (Table 1). On the other hand, BRV has been reported to infect dome M cells.[40] Because M cells have an important role in local immunity of the gastrointestinal tract, their degeneration might lead to impaired immune response.

In none of the young calves was the isotype switch to BRV-specific IgA detected. Newborn calves are capable of IgA production,[24] which is also suppressed in the presence of maternal antibodies.[23,25,39] Markedly low IgA titer in serum may not have been detected in our testing at weekly intervals; IgA has a serum half-life of only 2 days.[41]

The reinfection of BRV-seropositive calves at 10 months of age again indicates poor correlation between serum IgG1 titer and mucosal protection. Testing for IgG1 in feces might be revealing, because a protective effect can be expected from its presence in the intestinal lumen. In ruminants, selective transport of circulating IgG1 to mucosal surfaces has been described,[41,42] and is similar to IgA transport; in addition, Newby and Bourne[42] reported accumulations of IgG1-producing plasma cells in the lamina propria of the small intestine.

Another explanation for reinfection would be the existence of an antigenically different BRV in the dairy herd. To the authors' knowledge, only 2 BRV serotypes have so far been identified,[10] but more certainly exist. Cross-reacting antigenic determinants have been localized on the peplomer protein of the virus,[29] and the secondary reaction may have been attributable to a heterotypic booster. The question of biological variation in toroviruses is presently under study; >50% of the BRV genome has been sequenced (unpublished data), and heterologous hybridization with BRV has been successful. These developments are expected to result in diagnostic techniques that will be used to determine the role of toroviruses as pathogens in cattle.

References

1. Moerman A, van Zijderveld FG, de Leeuw PW, et al. Neonatal calf diarrhea. *Ann Rep Central Vet Inst, Lelystad* 1986;36–41.
2. Flewett T, Woode GN. The rotaviruses. *Arch Virol* 1978;57:1–23.
3. de Leeuw PW, Ellens DJ, Straver PJ, et al. Rotavirus infections in calves in dairy herds. *Res Vet Sci* 1980;29:135–141.
4. Sharpee RL, Mebus CA, Bass EP. Characterization of a calf diarrheal coronavirus. *Am J Vet Res* 1976;37:1031–1041.
5. Reynolds DJ, Morgan JH, Chanter N, et al. Microbiology of calf diarrhea in southern Britain. *Vet Rec* 1986;119:34–39.
6. WHO scientific working group. Rotavirus and other viral diarrheas. *Bull WHO* 1980;58:183–198.
7. Snodgrass DR, Terzolo HR, Sherwood D, et al. Aetiology of diarrhea in young calves. *Vet Rec* 1986;119:31–34.
8. Zschoeck M, Herbst W, Hamann HP, et al. Elektronenmikros-

kopische und bakteriologische Untersuchungsergebnisse bei der Diarrhoe der Kaelber. *Prakt Tierarztl* 1987;8:5–9.

9. Woode GN, Reed DE, Runnels PL, et al. Studies with an unclassified virus isolated from diarrheic calves. *Vet Microbiol* 1982;7:221–240.

10. Woode GN, Saif LJ, Quesada M, et al. Comparative studies on three isolates of Breda virus of calves. *Am J Vet Res* 1985;46:1003–1010.

11. Weiss M, Steck F, Horzinek MC. Purification and partial characterization of a new enveloped RNA virus (Berne virus). *J Gen Virol* 1983;64:1849–1858.

12. Horzinek MC, Weiss M. Toroviridae: a taxonomic proposal. *Zentralbl Veterinarmed [B]* 1984;31:649–659.

13. Weiss M, Horzinek MC. The proposed family Toroviridae: agents of enteric infections. *Arch Virol* 1987;92:1–15.

14. Horzinek MC, Flewett TH, Saif LJ, et al. A new family of vertebrate viruses: Toroviridae. *Intervirology* 1987;27:17–24.

15. Pohlenz JFL, Cheville NF, Woode GN, et al. Cellular lesions in intestinal mucosa of gnotobiotic calves experimentally infected with a new unclassified bovine virus (Breda virus). *Vet Pathol* 1984;21:407–417.

16. Woode GN. Breda and Breda-like viruses: diagnosis, pathology and epidemiology. In: Bock G, Whelan J, eds. *Novel diarrhoea viruses, CIBA Found Symp 128.* Chichester, England: John Wiley & Sons 1987;175–191.

17. Koopmans M, van den Boom U, Woode GN, et al. Seroepidemiology of Breda virus in cattle using ELISA. *Vet Microbiol* 1989;19:233–243.

18. Brown DWG, Beards GM, Flewett T. Detection of Breda virus antigen and antibody in humans and animals by ELISA. *J Clin Microbiol* 1987;25:637–640.

19. Weiss M, Steck F, Kaderli R, et al. Antibodies to Berne virus in horses and other animals. *Vet Microbiol* 1984;9:523–531.

20. van Zaane D, IJzerman J. Monoclonal antibodies against bovine immunoglobulins and their use in isotype-specific ELISAs for rotavirus antibody. *J Immunol Methods* 1984;72:427–441.

21. Saif LJ, Smith KL. Enteric viral infections of calves and passive immunity. *J Dairy Sci* 1985;68:206–228.

22. Westenbrink F, Kimman TG. Immunoglobulin M-specific enzyme-linked immunosorbent assay for serodiagnosis of bovine respiratory syncytial virus infections. *Am J Vet Res* 1987;48:1132–1137.

23. Kimman TG, Westenbrink F, Schreuder BEC, et al. Local and systemic antibody response to bovine respiratory syncytial virus infection and reinfection in calves with and without maternal antibodies. *J Clin Microbiol* 1987;25:1097–1106.

24. Saif LJ. Development of nasal, fecal and serum isotype specific antibodies in calves challenged with bovine coronavirus or rotavirus. *Vet Immunol Immunopathol* 1987;17:425–439.

25. Williams RO. Measurement of class specific antibodies against cryptosporidium in serum and faeces from experimentally infected calves. *Res Vet Sci* 1987;43:264–265.

26. Naylor MN. Colostral immunity in the calf. *Vet Clin North Am [Large Anim Pract]* 1979;1(2):331–361.

27. White DG, Andrews AH. Adequate concentration of circulating colostral proteins for market calves. *Vet Rec* 1986;119:112–113.

28. Kruse PE. The importance of colostral immunoglobulins and their absorption from the intestine of the newborn animal. *Ann Res Vet* 1983;14:349–353.

29. Koopmans M, Ederveen J, Woode GN, et al. Surface proteins of Breda virus. *Am J Vet Res* 1986;47:1896–1900.

30. Bouter LM, van Dongen MCJM. *Epidemiological research.* Utrecht, The Netherlands: Bohn, Scheltema & Holkema, 1988;244–250.

31. Krauss H, Arens M. Die elektronenmikroskopische Untersuchung von Kot- oder Organmaterial als diagnostischer Schnellnachweis bei der Parvovirusinfektion des Hundes. *Prakt Tierarztl* 1981;62:38–41.

32. Ellens DJ, de Leeuw PW. ELISA for diagnosis of rotavirus infections in calves. *J Clin Microbiol* 1977;6:530–532.

33. Ellens DJ, van Balken JAM, de Leeuw PW. Diagnosis of bovine coronavirus infections with hemadsorption-elution-hemagglutination assay (HEHA) and with enzyme-linked-immunosorbent assay (ELISA), in *Proceedings.* 2nd Int Symp Neonatal Diarrhea 1978;321–330.

34. Beards GM, Hall C, Green J, et al. An enveloped virus in stools of children and adults with gastroenteritis that resembles the Breda virus of calves. *Lancet* 1984;2:1050–1052.

35. Crouch CF, Acres SD. Prevalence of rotavirus and coronavirus antigens in the feces of normal cows. *Can J Comp Med* 1984;48:340–342.

36. Collins JK, Riegel BS, Olson JD, et al. Shedding of enteric coronavirus in adult cattle. *Am J Vet Res* 1987;48:361–365.

37. Schwers A, Maenhoudt M, Pastoret PP. Repeated bovine rotavirus infection and excretion in calves. *Vet Rec* 1984;21:411.

38. Laval A, Khelef D, Viso M, et al. Excretion du coronavirus bovin et évolution des anticorps sériques chez les vaches et les veaux des 3 troupeaux francais suivis pendant plusieurs mois consecutifs, in *Proceedings.* 14th World Cong Cattle Dis, 1986;1:348–349.

39. van Zaane D, IJzerman J, de Leeuw PW. Intestinal antibody response after vaccination and infection with rotavirus of calves fed colostrum with or without rotavirus antibody. *Vet Immunol Immunopathol* 1986;11:45–63.

40. Woode GN, Pohlenz JF, Kelso Gourley NE, et al. Astrovirus and Breda virus infection of dome cell epithelium of bovine ileum. *J Clin Microbiol* 1984;19:623–630.

41. Lasceiles AK, Beh KJ, Mukkur TK, et al. The mucosal immune system with particular reference to ruminant animals. In: Morrison WI, ed. *The ruminant immune system in health and disease.* Cambridge, England: Cambridge University Press, 1986;429–457.

42. Newby TJ, Bourne FJ. The nature of the local immune system of the bovine small intestine. *Immunology* 1976;23:475–480.