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Identification of Group B Rotaviruses with Short Genome Electropherotypes from Adult Cows with Diarrhea†

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Two field strains (BB-RVLV and KD) of group B rotaviruses from adult dairy cows with diarrhea displayed short genome electropherotypes. Gnotobiotic calves inoculated with fecal filtrates of each group B rotavirus developed diarrhea, and only group B rotaviruses or antigens were detected in the feces by immunoelectron microscopy and in intestinal epithelial cells by immunofluorescent staining, respectively. The feces or intestinal contents of the cows and inoculated calves were negative for group A and C rotaviruses by enzyme-linked immunosorbent assay, immunoelectron microscopy, or cell culture immunofluorescence assays. Comparison of the genome electropherotypes of the calf-passaged BB-RVLV and KD strains with the original samples and reference bovine group A, B, and C rotaviruses revealed conservation of their short-genome electropherotypes and double-stranded RNA migration patterns characteristic of group B rotaviruses. To our knowledge, our previous study (L. J. Saif, K. V. Brock, D. R. Redman, and E. M. Kohler, Vet. Rec. 128:447–449, 1991) and this report are the first description of bovine group B rotaviruses (in a mixed infection with bovine coronavirus or singly in fecal contents) in adult cows with diarrhea and this is the first report of short-genome electropherotypes among group B rotaviruses.

Rotaviruses are members of the *Reoviridae* family and are important veterinary and medical pathogens (5, 19). The genome of rotaviruses consists of 11 segments of double-stranded RNA (dsRNA) enclosed in a triple-layer capsid (4). Rotaviruses are classified into antigenically distinct groups (A to G) on the basis of a common group antigen on the VP6 protein (5, 11, 15, 21).

Group A rotaviruses are the most frequently detected rotaviruses (5, 19, 21), although recent reports have identified non-group A rotaviruses, especially group B and C rotaviruses, as a cause of diarrheal disease in animals and humans (2, 9, 11, 15, 19, 24). Very limited studies have focused on group B rotaviruses mainly because of an inability to serially propagate these viruses in cell culture (2, 11). During the last decade, group B rotaviruses have been associated with several outbreaks of diarrhea in humans mainly in China (2, 9, 11, 15). Group B rotaviruses have been commonly recognized in lambs in the United Kingdom (3) and more recently in the United States (22). They have also been detected less frequently from diarrheic calves (11, 15). Under experimental conditions, group B rotaviruses cause diarrhea in gnotobiotic calves, pigs, and laboratory rats (2, 11, 15).

Another feature shared by members within a rotavirus group is their similarity in genome electropherotype (i.e., electropherogroup) when the dsRNA is analyzed by polyacrylamide gel electrophoresis (PAGE) (2, 11, 15). The RNA segments can be divided into four size classes (I to IV) on the basis of migration on polyacrylamide gels. Because each strain produces a unique genome profile, the electropherotyping of rotavirus field strains by PAGE is a useful tool not only for diagnosis but also for obtaining important epidemiological information about the origin of field strains and variation and diversity among these strains (2, 11, 15, 21, 23). In addition to the usual "long"-genome electropherotypes, short-genome electropherotypes, in which the smallest two segments appear to migrate more slowly than usual, have been described for group A rotaviruses (1, 7, 8, 10, 20, 23). These short genome electropherotypes are relatively uncommon, have been restricted to group A, and have been described only in humans, rabbits, and calves (19). In this report, we describe for the first time bovine group B rotaviruses displaying short-genome electropherotypes, isolated from adult cows with diarrhea.

The sources of the reference rotaviruses used in this study have been described previously (6, 7, 11, 15, 24). The reference group A and C viruses were grown in rhesus monkey kidney (MA-104) cells in roller bottles and titrated by a plaque assay as previously described (11, 12, 15, 18, 24). The reference bovine group B rotavirus, ATI B strain, was isolated from a diarrheic calf as previously described and displays a long genome electropherotype (11, 15). The bovine group B strains BB-RVLV and KD (short genome electropherotypes) originated from the diarrheic feces of a 2-year-old Holstein dairy cow (Wisconsin) (14) and a 3-year-old Holstein dairy cow (Ohio), respectively. The original BB-RVLV sample (14) also contained bovine coronavirus, which was removed by passage of the original cow fecal filtrate in a bovine coronavirus-immune conventional colostrum-deprived calf (12a).

Gnotobiotic calf experiments were conducted to passage field group B rotaviruses, examine their pathogenicity, and generate large quantities of stock virus (since group B rotaviruses do not replicate in cell culture). Bovine group B rotaviruses were passaged in three newborn gnotobiotic calves, derived, fed, inoculated, and maintained as previously described (7, 16, 17). Calf A was oronasally inoculated with a fecal filtrate of live virulent bovine group B strain BB-RVLV (previously passaged in a conventional colostrum-deprived calf), and calves B and C were oronasally inoculated with a fecal filtrate of the original cow specimen of bovine group B rotavirus KD strain. After inoculation, rectal swabs were collected daily from

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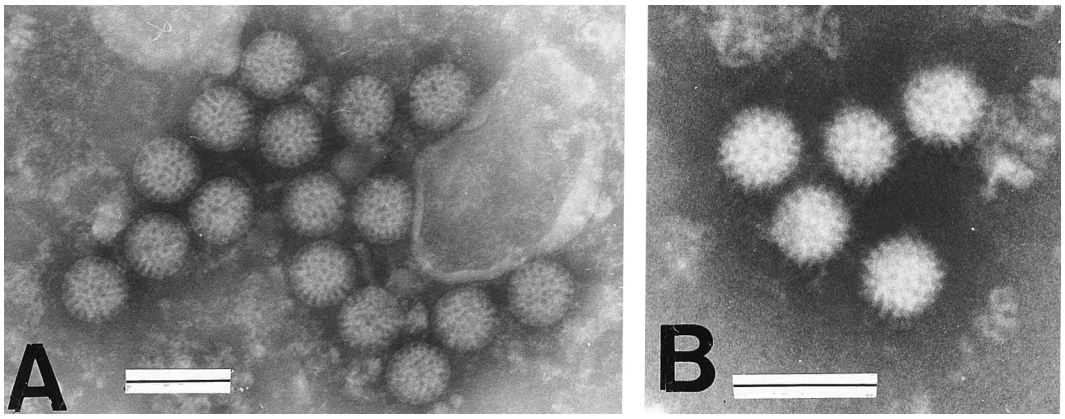

FIG. 1. Aggregates of group B rotaviruses observed by IEM after incubation of fecal specimens from gnotobiotic calves with hyperimmune group B rotavirus antisera. (A) IEM of feces from calf A inoculated with the BB-RVLV strain of group B rotavirus. Note mainly triple-capsid rotavirus particles. (B) IEM of feces from calf B inoculated with the KD strain of group B rotavirus. Note mainly double-capsid rotaviral particles. Bars, 100 nm.

calves A and B for 1 week and every other day for the 2nd week. The swabs were vortexed in 4 ml of diluent containing minimal essential medium and then discarded. The swab fluids were centrifuged at 800 × *g* for 30 min at 4°C. The supernatant was frozen at −20°C for subsequent group A enzyme-linked immunosorbent assay (ELISA) and cell culture immunofluorescence testing (12, 16, 24). Fecal samples were also collected daily during the 2-week sampling period and examined by immunoelectron microscopy (IEM) to detect rotaviruses or other enteric viruses (11–13). These fecal samples were also examined for rotavirus dsRNA electropherotypes by PAGE to confirm the rotavirus electropherogroup and strain shed by the calves (6). Clinical signs were recorded daily and included observations on the color and consistency of stools. The stools were rated as abnormal if they were off-color (bright yellow or green) and of semiliquid to liquid consistency. The calves were euthanized, and segments from the small intestines and colon were removed for immunofluorescent staining (12, 17). Large intestinal contents were collected and examined for virus particles by IEM (13) and for rotavirus dsRNA electropherotypes by PAGE (6).

The fecal samples and intestinal contents from calves infected with group B KD and BB-RVLV strains were processed for ELISA (7, 12), cell culture immunofluorescence (7, 12, 16, 24), and IEM (7, 12, 13) as previously described. Rotavirus genomic dsRNA was extracted from fecal or intestinal specimens (group B) or from cell culture-propagated viruses (groups A and C) by using phenol-chloroform, and samples were analyzed by PAGE to confirm the presence of dsRNA and to examine the genomic electropherotypes (6, 7).

IEM using hyperimmune antisera to group A, B, and C rotaviruses was used to confirm the presence of group B rotavirus particles in the original fecal specimens (BB-RVLV and KD strains) as well as fecal and intestinal samples of infected gnotobiotic calves (11–13). The fecal and intestinal samples were processed for IEM as previously described (13). The processed samples were incubated separately with group A-, B-, or C-specific hyperimmune antisera prepared in gnotobiotic calves (7, 11). The antiserum-sample mixtures were pelleted, negatively stained with phosphotungstic acid, applied to grids, and examined by electron microscopy.

The gnotobiotic calves which were inoculated with the group B rotaviruses were examined for clinical signs. All the calves displayed abnormal stools after challenge and shed virus. The stools varied from yellow to green and were of liquid consistency. The viral shedding lasted 6 to 7 days, while the abnormal stools were present for 2 to 4 days. The original field fecal samples containing group B rotavirus strains BB-RVLV and KD as well as samples from preexposed and infected gnotobiotic calves were tested for group A rotavirus by ELISA and cell culture immunofluorescence and were negative. The samples were also tested for group A, B, and C rotaviruses by IEM. All the samples contained characteristic larger triple-capsid (Fig. 1A) or smaller double-capsid (Fig. 1B) rotavirus particles, and these particles aggregated only when incubated with group B-specific antiserum (Fig. 1). The number of particles that were detected was variable, but overall small numbers of rotavirus particles were evident, consistent with other studies (2, 11, 15). Other enteric viruses were not detected in these samples by IEM.

The segments from the small intestines and colon of inoculated gnotobiotic calf C were assayed for the presence of group A, B, and C rotavirus antigen by immunofluorescent staining (12, 17) of mucosal impression smears using fluorescein-isothiocyanate-conjugated group A-, B-, and C-specific antisera prepared in gnotobiotic calves (11, 12a). The smears were positive only for group B rotavirus antigens, which were evident in the cytoplasm of epithelial cells in the jejunum and ileum (data not shown).

Bovine group A, B (including BB-RVLV and KD strains), and C samples were analyzed by PAGE to confirm the presence of dsRNA and to examine the genomic electropherotypes as previously described (Fig. 2). For group A bovine rotavirus, the characteristic 4-2-3-2 pattern was observed (Fig. 2, lane A). The group C sample exhibited a 4-3-2-2 pattern characteristic of group C (Fig. 2, lane E). The three group B strains displayed

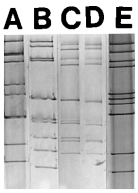

FIG. 2. Electrophoretic genome profiles of bovine group A, B, and C rotaviruses. Lane A, group A NCDV Cody strain; lane B, group B ATI B calf strain; lane C, group B KD strain (short); lane D, group B BB-RVLV strain (short); lane E, group C Shintoku strain. The dsRNA was resolved in polyacrylamide gels and visualized by silver staining. Migration is from top to bottom.

the typical group B 4-2-2-3 genome electropherotype. However, the KD (lane C) and BB-RVLV (lane D) strains displayed short genome profiles compared with the long genome profile of the reference group B ATI B strain (Fig. 2, lane B).

In the present study we have demonstrated the presence of group B rotavirus strains displaying short-genome electropherotypes from adult cows with diarrhea. These findings are important because, to our knowledge, (i) group B rotaviruses have not been previously described to circulate among adult cows except for a prior report from our laboratory describing a mixed infection with bovine group B rotavirus and bovine coronavirus (14), and (ii) there have not been any previous reports of short-genome electropherotypes present among group B rotaviruses.

Genome electropherotyping is an important tool used by rotavirologists for the diagnosis of rotaviruses and for studying the molecular epidemiology of viral infections (11, 12, 15, 19, 21). It provides a rapid means of comparing strains and tentatively predicting their serogroup because each serogroup displays a characteristic electropherogroup (2, 11, 15, 19). For example, most group A rotaviruses display a long-genome electropherotype with a 4-2-3-2 pattern. However, there have been reports of strains displaying short or "supershort" genome profiles among group A rotaviruses (1, 7, 8, 10, 20, 23). Many studies have confirmed that the short genome electropherotype results from rearrangements arising within specific dsRNA segments resulting in a change in their genome profiles (20). The mechanism for the genome rearrangement for one group A rotavirus strain was studied by sequence analysis, and the results indicated the presence of two full-length copies of gene segment 11 which had concatamerized (20). For group B rotaviruses, to our knowledge, our study is the first to describe the presence of short-genome electropherotypes. Additional studies are needed to clarify if these group B rotaviruses with short-genome electropherotypes arise by a mechanism similar to that described for group A rotaviruses. It is possible that the short-genome electropherotype may be the result of slowly migrating gene segments 10 and 11 or a rearrangement in segment 11 might occur, causing it to migrate more slowly and in place of segment 10. The latter may be confirmed by hybridization analysis using a group B-specific gene 10 or gene 11 cDNA probe.

The discovery of group B rotaviruses among adult cows from two different locations is significant because this suggests that this is not a chance occurrence and that group B rotaviruses, previously described only among young calves, may be circulating in the adult cow populations and therefore may represent a potential source of infection for both young and adult animals. Moreover, previous investigators have reported that only 20% of adult cows surveyed in the United Kingdom (n = 118) had antibodies to group B rotavirus whereas 91% had antibodies to group A rotavirus (2), suggesting that many adult cows lack antibodies and may be susceptible to group B rotavirus infections. Additional studies are needed to understand the epidemiological significance of the presence of group B rotaviruses in the U.S. adult cow population.

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