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Partial Characterization of a Bovine Group A Rotavirus with a Short Genome Electropherotype†

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A group A rotavirus (ID isolate) recovered from a diarrheic beef calf possessed a short genome electropherotype. This short genome electropherotype was a stable characteristic of the ID isolate as it remained unchanged through 3 passages in gnotobiotic calves or through 19 passages in MA104 cell cultures. Subgroup analysis with monoclonal antibodies in an enzyme-linked immunosorbent assay established that the isolate was a subgroup 1 rotavirus. Neutralization tests demonstrated that this isolate was a distinct serotype from the human group A rotavirus S2 isolate (short genome electropherotype) and the turkey group A rotavirus 174 isolate (semi-short genome electropherotype). The ID isolate was pathogenic for 5- to 21-day-old gnotobiotic calves, inducing diarrhea within 48 h postinoculation.

Rotaviruses are a common, widespread cause of enteric illness in the newborn of many mammalian species (7) and are the single most important etiologic agent of acute gastroenteritis requiring hospitalization of infants and young children in developed countries (13). At least five distinct rotavirus serogroups exist, and the originally recognized rotaviruses, which are the most frequently detected, belong to group A (17, 18). Antigenic differences among the group A rotaviruses were first detected by complement fixation, immunoelectron microscopy, enzyme-linked immunosorbent assay (ELISA), and neutralization assays (28, 37, 38). A later study, however, unexpectedly revealed that serotype differences among group A rotaviruses detected by neutralization assays did not always correlate with antigenic differences detected by other methods (12). Based on these findings, it was proposed to redefine the antigenic specificities among the group A rotaviruses, with serotype used to designate neutralization specificity and subgroup used to indicate differences detected by the complement fixation, immunoelectron microscopy, ELISA, and immune adherence hemagglutination assays. Two subgroups, 1 and 2 (SG1 and SG2), were recognized.

The group A rotaviral genome comprises 11 double-stranded RNA (dsRNA) segments that separate upon electrophoresis in polyacrylamide gels to produce a distinctive migration pattern (genome electropherotype), an attribute that has been exploited extensively in studies on the epidemiology of these viral infections in humans (5, 6, 20). It was soon noted that some human group A rotavirus isolates had short genome electropherotypes in which the smallest two segments (segments 10 and 11) appeared to migrate slower than usual (5, 6, 20). These isolates had the SG1 antigen, whereas those with long genome electropherotypes had the SG2 antigen (1, 11, 14, 20, 27, 31, 33). On the other hand, no correlation was found between the genome electropherotype pattern and the subgroup specificity of group A rotaviruses recovered from other mammalian species (8, 9). In fact, the only animal group A rotaviruses with short genome electropherotypes resembling those of the human group A rotaviruses have been isolated from rabbits (29; T. N. Tanaka, M. E. Conner, D. Y. Graham, and M. K. Estes, Arch. Virol., in press).

This report describes the isolation of a bovine group A rotavirus with a short genome electropherotype and describes some of its characteristics.

MATERIALS AND METHODS

Viruses. The original fecal specimen containing the ID isolate of bovine group A rotavirus was from a diarrheic calf in a beef herd located in southwestern Montana (kindly provided by L. Myers, Bozeman, Mont.). Plaque-purified bovine group A rotavirus (ID isolate) at cell culture passage 19 was obtained from this fecal specimen as described below and used in the neutralization assays. The cell culture-passaged group A porcine (OSU and Gottfried isolates) and human (Wa isolate) rotaviruses have been described previously (4). The group A turkey rotaviruses were isolated in MA104 cell cultures from the intestinal contents of turkeys in Ohio (174 isolate) and Wisconsin (Q isolate) by previously described procedures (26). The genome electropherotypes of the Q and 174 isolates (26; unpublished observation) are typical of avian group A rotaviruses (30) and in this report are called semi-short genome electropherotypes. The cell culture-passaged human group A rotavirus (S2 isolate), which has a short genome electropherotype (23), was kindly provided by M. Ripenhoff-Talty (Buffalo, N.Y.). The bovine group A rotavirus (SF isolate) was isolated from a diarrheic dairy calf in Ohio and serially passaged in MA104 cell monolayers by a previously described roller tube technique (26). At cell culture passage 11, this isolate was plaque purified by a previously described method (4) except that trypsin (type IX; Sigma Chemical Co., St. Louis, Mo.) was added to the overlay medium instead of pancreatin. Plaque-purified virus was then passaged three more times in roller tube monolayers. This 14th cell culture passage was used throughout these studies. The bovine group A rotavirus (NCDV isolate) was isolated in MA104 cell cultures from a fecal specimen collected from an experimentally inoculated, hysterectomy-derived, colostrum-deprived calf. This isolate was serially passaged and plaque purified as described above for the SF isolate. The WA, S2, Gottfried, OSU, and NCDV isolates are serotype 1, 2, 4, 5, and 6 group A rotaviruses, respectively (10, 34).

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Gnotobiotic calves and pigs. Colostrum-deprived gnotobiotic calves were derived by hysterectomy or cesarean section and aseptically transferred into individual sterile isolators in which they remained throughout the experiments. Twice daily, each calf was fed 1.42 liters of infant formula (Similac; Ross Laboratories, Columbus, Ohio). Gnotobiotic pigs were obtained and maintained as previously described (24).

Preparation of hyperimmune antisera. Hyperimmune antisera to the NCDV, 174, and S2 isolates used in the neutralization assays were prepared in gnotobiotic pigs by the following procedure. Gnotobiotic pigs were given an initial intramuscular (i.m.) injection of an emulsion consisting of equal parts of the appropriate cell culture-passaged virus (concentrated 10-fold by ultracentrifugation) and complete Freund adjuvant (Difco Laboratories, Detroit, Mich.). Additional i.m. injections with the same nonadjuvanted, unconcentrated virus were given at weekly intervals during the next 3 to 4 weeks. Serum was collected approximately 1 week after the last injection.

Hyperimmune antiserum to the ID isolate used in the neutralization assays was prepared in a gnotobiotic calf as follows. This calf was experimentally infected by oral inoculation with this isolate after its first passage in a gnotobiotic calf. Five weeks later, a preparation comprising 5 ml of virus-laden fecal specimen collected from this calf on postinoculation day 2 and 20 ml of Eagle minimum essential medium (EMEM) was injected i.m. Serum was collected 6 days after this injection.

Hyperimmune antiserum to the SF isolate used in the neutralization assays was prepared in a gnotobiotic calf as follows. This calf was experimentally infected by oral inoculation with 5 ml of cell culture-passaged plaque-purified virus. Ten days after oral inoculation, a preparation comprising 4 ml of the above virus and 4 ml of complete Freund adjuvant was injected i.m. At 3 and 4 weeks after the adjuvanted injection, 10 ml of nonadjuvanted virus was injected i.m. and serum was collected 5 days after the last injection.

Sera from a noninoculated gnotobiotic calf and a noninoculated gnotobiotic pig served as controls in the neutralization assays.

Anti-NCDV isolate hyperimmune antiserum used as the capture antibody in the subgroup determination ELISA was prepared in a hysterectomy-derived, colostrum-deprived calf as previously described (26).

Experimental inoculation of gnotobiotic calves. A 5% suspension of the original fecal specimen containing the ID isolate was prepared in EMEM and clarified by centrifugation at 600 × *g* for 20 min at 4°C. The supernatant fluid was passed through a filter (0.45-μm pore size), and 8 ml of the resulting bacteria-free filtrate served as the inoculum for gnotobiotic calf 1. This calf was inoculated orally at 5 days of age. Fecal specimens collected on postinoculation days 2 and 4 were processed as above. A 4- to 8-ml volume of filtrate was used to orally inoculate gnotobiotic calves 2 and 3 at 5 and 21 days of age, respectively. A fecal specimen collected from gnotobiotic calf 3 on postinoculation day 2 was processed as above, and 2 ml of this filtrate was used to orally inoculate gnotobiotic calf 4 at 6 days of age. Inoculated gnotobiotic calves were observed for signs of clinical illness for 10 days postinoculation, and fecal specimens were collected daily during the first 5 days postinoculation.

CCIF assay. For the cell culture immunofluorescence (CCIF) assay, monolayers of a fetal rhesus monkey kidney cell line (MA104) were grown in 96-well microtiter plates and prepared for inoculation as described previously (26). Fecal specimens diluted 25-fold in serum-free EMEM were treated with Garamycin (Schering Corp., Kenilworth, N.J.) as described previously (26). Monolayers were inoculated with processed specimens (0.1 ml per well) and incubated for 16 to 18 h at 37°C in a 5% CO_2 atmosphere. After incubation, inoculated monolayers were stained with fluorescein-conjugated anti-bovine group A rotavirus immunoglobulin and examined by immunofluorescence microscopy as described previously (20). In some instances, monolayers were inoculated with cell culture-passaged bovine group A rotavirus (ID isolate) and then stained as above or with fluorescein-conjugated porcine anti-porcine group A rotavirus (OSU isolate) immunoglobulin (24).

Isolation and propagation of ID isolate in cell culture. The original fecal specimen containing the ID isolate was processed as described for the CCIF assay and then inoculated onto MA104 cell monolayers in roller tubes. Maintenance medium was EMEM containing trypsin (type IX) at 2 μg/ml. Subsequent passages were made in roller tube monolayers of MA104 cells at 48- to 72-h intervals with trypsin treatment of the inoculum and trypsin in the maintenance medium as described previously (26). At cell culture passage 11, the isolate was plaque purified by a previously described procedure (4) except that 1 μg of trypsin (type IX) per ml was added to the overlay medium instead of pancreatin. Eight more passages were made in roller tube monolayers as described above.

Virus neutralization assay. The virus neutralization assays were conducted in MA104 cell monolayers grown in 96-well plates as described above. Cell culture-passaged group A rotaviruses were treated at 37°C for 1 h with 60 μg of trypsin (type IX) per ml and then diluted in serum-free EMEM to a concentration of 30 to 300 fluorescence-forming units per 0.1 ml. Diluted virus was mixed with equal volumes of a series of twofold serum dilutions prepared in EMEM and then incubated at 37°C for 1 h. After incubation, 0.2 ml of this mixture was added to each of three wells containing washed monolayers. Inoculated monolayers were incubated at 37°C for 16 h in a 5% CO_2 atmosphere and then fixed and stained as described for the CCIF assay. Neutralizing antibody titers were expressed as the reciprocal of the highest serum dilution to completely neutralize all fluorescence-forming units in each of the three inoculated wells. If the difference between homologous and heterologous reciprocal neutralizing antibody titers was 20-fold or greater, the isolates were considered serotypically distinct; if the difference between the titers was less than 20-fold, the isolates were considered not to be serotypically distinct.

Subgroup analysis. The subgroup antigen of the ID isolate was determined by ELISA by the double antibody-anti-immunoglobulin method and standard procedures (32, 33, 36). Monoclonal antibodies 255/60 and 631/9, which have SG1 and SG2 specificities, respectively (8), were used in this assay. These monoclonal antibodies were kindly provided as myeloma cell culture supernatant fluids by J. Askaa and A. Kapikian, National Institutes of Health, Bethesda, Md. Fecal specimens from gnotobiotic calves orally inoculated with the ID isolate served as the test antigen sources for this assay. A fecal specimen from a hysterectomy-derived, colostrum-deprived calf inoculated with the NCDV isolate and an intestinal contents specimen from a gnotobiotic pig inoculated with the Gottfried isolate served as known SG1 and SG2 rotavirus control antigens (8, 12). Microtiter plate wells coated with a 1:30,000 dilution of anti-NCDV isolate serum were reacted with suspensions of specimens prepared as described for the CCIF assay. Separate groups of wells

containing each bound antigen were then reacted with each monoclonal antibody (diluted fivefold). After incubation with the monoclonal antibodies, all wells were reacted with affinity-purified goat anti-mouse immunoglobulin G antibody (Kirkegaard and Perry Laboratories, Inc., Gaithersburg, Md.) diluted 1:800. The assay was then completed by the addition of the substrate *p*-nitrophenylphosphate, and the optical density (OD) at 405 nm was determined. The criteria for subgroup determination were essentially those of White et al. (33). The ratio between the OD readings of each isolate with the two monoclonal antibodies was determined. An SG1/SG2 ratio of >1.7 indicated SG1 specificity, whereas an SG2/SG1 ratio of >2.0 indicated SG2 specificity.

Extraction and electrophoresis of viral dsRNA. Viral dsRNA was extracted from specimens by CF11 cellulose chromatography (25) and subjected to electrophoresis at 40 mA for 4 to 5 h in Laemmli 7.5% polyacrylamide slab gels as described previously (4).

Polyacrylamide gels were then stained with silver and examined for rotaviral genome electropherotypes.

Electron microscopy. Fecal specimens were prepared for electron microscopic examination by sonication, low-speed centrifugation, and filtration as described previously (26). Virus was pelleted through a 60% sucrose cushion by ultracentrifugation at approximately 178,000 × *g* for 15 min in an air-driven ultracentrifuge (Airfuge; Beckman Instruments, Inc., Palo Alto, Calif.) with a fixed-angle rotor. Pellets were suspended in sterile distilled water and pelleted again by ultracentrifugation as described above. These pellets were suspended in water, negatively stained, and examined with an electron microscope as described previously (26). In certain instances, undiluted cell culture-passaged ID isolate was processed as above except that the filtration steps were omitted.

RESULTS

The original fecal specimen from the diarrheic beef calf in Montana contained group A rotavirus as determined by the CCIF assay. The genome electropherotype of this group A rotavirus, designated the ID isolate, was unusual as the two smallest genome segments migrated considerably slower than expected (data not shown).

All gnotobiotic calves inoculated with the ID isolate developed clinical signs of illness including diarrhea and inappetence. Diarrhea first occurred at about 48 h postinoculation and was characterized by profuse, watery, tan to yellow stools containing curds of undigested milk. These calves remained diarrheic for several days, after which they passed mucoid stools for several days. By 7 to 9 days postinoculation, all calves had returned to normal. Stool specimens collected from each diarrheic calf contained group A rotavirus as determined by the CCIF assay. In addition, numerous rotavirus particles were observed in these specimens by electron microscopy (Fig. 1). Viruses other than rotaviruses were not detected by negative stain electron microscopic examination of stools collected from the diarrheic calves. The group A rotavirus recovered from the inoculated gnotobiotic calves had the short genome electropherotype (Fig. 2). The genome electropherotype of the ID isolate was distinctly different from the genome electropherotypes produced by the bovine group A rotavirus with a typical long pattern, the human group A rotavirus with a typical short pattern, and the turkey group A rotavirus with the typical avian semi-short pattern. The most striking characteristic of this genome electropherotype was the pronounced decreased migration distance of genome segment 10.

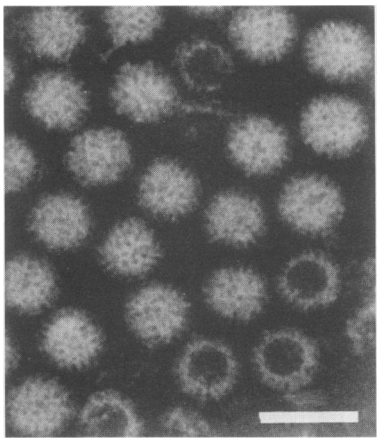

FIG. 1. Negative stain preparation of a fecal specimen collected from diarrheic gnotobiotic calf 3 at 60 h postinoculation with bovine group A rotavirus (ID isolate) from the first passage in a gnotobiotic calf. Typical rotavirus particles, approximately 70 nm in diameter, are apparent. Bar, 100 nm.

A cytopathogenic virus was isolated in roller tube MA104 cell cultures from the original fecal specimen containing the ID isolate. This cell culture-passaged isolate gave characteristic positive immunofluorescence reactions when stained with anti-bovine group A rotavirus or anti-porcine

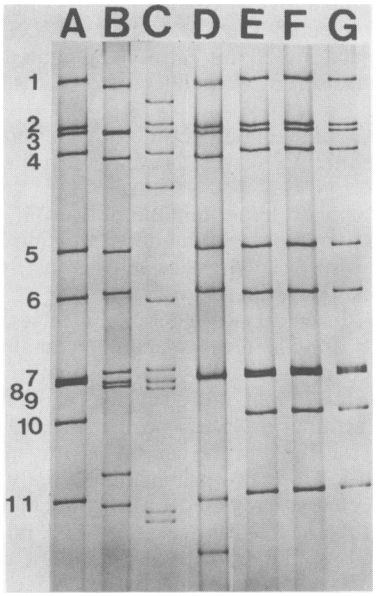

FIG. 2. Comparison of some group A rotavirus genome electropherotypes in the same polyacrylamide gel slab. Migration is from top to bottom. Numbers to the left designate segments of the ID isolate genome. Lanes: A, bovine rotavirus (ID isolate) genome electropherotype (gnotobiotic calf passage 2); B, human rotavirus (S2 isolate) genome electropherotype; C, turkey rotavirus (Q isolate) genome isolate; D, bovine rotavirus (NCDV isolate) genome electropherotype; E, bovine rotavirus (ID isolate) genome electropherotype (gnotobiotic calf passage 3); G, bovine rotavirus (ID isolate) genome electropherotype (cell culture passage 19); F, coelectrophoresis of dsRNA preparations used in lanes E and G.

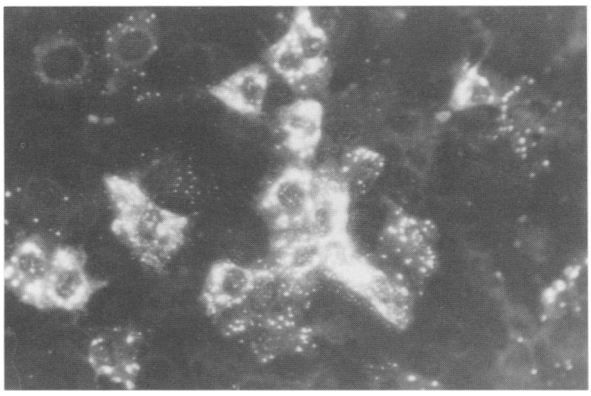

FIG. 3. Immunofluorescent stain for group A rotaviral antigens on MA104 cell monolayer infected with cell culture-passaged plaque-purified ID isolate. Monolayer contains typical group A rotavirus-infected cells. Stain was fluorescein-conjugated porcine anti-porcine group A rotavirus (OSU isolate) immunoglobulin. Magnification, ×200.

group A rotavirus conjugates (Fig. 3). Typical rotavirus particles were detected by negative stain electron microscopy of the cell culture medium harvested from MA104 monolayers infected with the ID isolate. Coelectrophoresis of the dsRNA extracted from the 14th cell culture-passaged plaque-purified ID isolate with the dsRNA extracted from the original fecal specimen demonstrated that these isolates were identical (data not shown). Coelectrophoresis of the dsRNA extracted from the cell culture-passaged plaque-purified ID isolate with the dsRNA extracted from the gnotobiotic calf-passaged ID isolate also showed that these isolates were the same (Fig. 2).

Hyperimmune serum to the ID isolate neutralized the ID virus at high dilutions but did not neutralize the other group A rotaviruses at significantly high titers (Table 1). Hyperimmune antisera to the other bovine group A rotaviruses (NCDV and SF isolates) had higher titers to their homologous viruses than to the ID isolate. Hyperimmune sera to the group A turkey rotavirus (174 isolate) and the human group A rotavirus (S2 isolate) neutralized the homologous viruses but not the ID isolate. Sera from a noninoculated gnotobiotic calf and a gnotobiotic pig did not neutralize any group A rotavirus. Subgroup analysis with monoclonal antibodies in the ELISA indicated that the ID isolate was an SG1 group A rotavirus (Table 2).

DISCUSSION

Group A rotaviruses with short genome electropherotypes, characterized by the decreased migration of segments 10 and 11, are common in humans (5, 6, 20) but have not been isolated previously from domesticated, food-producing animals. Group A rotaviruses isolated from birds and mice, however, have genome electropherotypes in which the mobility of segment 11 is decreased so that it migrates near segment 10 (21, 22, 26, 30); in this report, such genome electropherotypes are referred to as semi-short. More recently, two group A rotaviruses isolated from subclinically infected calves were found to possess genome electropherotypes in which the migration of segment 11 was greatly reduced (19). These genome electropherotypes resemble those of some atypical group A human rotaviruses detected in stool specimens from normal neonates in Johannesburg, South Africa (3), but they are not short genome electropherotypes. Two group A rotaviruses isolated from diarrheic commercially reared rabbits have recently been shown to have short genome electropherotypes (29; Tanaka et al., in press); however, their pathogenicity for rabbits remains to be reported.

A group A rotavirus (ID isolate) recovered from a diarrheic beef calf was found to have a short genome electropherotype. The short genome electropherotype of this isolate was a stable characteristic, as it remained unchanged through 3 passages in gnotobiotic calves or through 19 passages in MA104 cell cultures. Even though both segments 10 and 11 had decreased migration distances, the most striking characteristic of this genome electropherotype was the markedly decreased migration of segment 10. Segment 10, located just beyond segments 7, 8, and 9, migrated considerably less than segment 10 of the short and semi-short genome electropherotypes produced by the human group A rotavirus (S2 isolate) and the turkey group A rotavirus, respectively.

Previous investigations have shown that bovine group A rotaviruses with long genome electropherotypes have the SG1 antigen (8, 9). Our data show that the ID isolate, which has a short genome electropherotype, is also an SG1 group A rotavirus. Thus, SG1 group A rotaviruses isolated from calves can have either long or short genome electropherotypes. This situation differs from that found with SG1 and SG2 porcine group A rotaviruses, which both have long electropherotypes (4, 8, 9), and with SG1 and SG2 lapine group A rotaviruses, which both have short genome electropherotypes (29; Tanaka et al., in press). It also differs from

TABLE 1. Antigenic relationships among selected group A rotaviruses detected by neutralizing antibody assay

Rotavirus	Species of origin	Neutralizing antibody titer of hyperimmune antiserum to rotavirus isolate:						
		Bovine NCDV	Bovine SF	Bovine ID	Human S2	Turkey 174	Bovine control[a]	Porcine control[b]
NCDV	Bovine	6,400[c]	1,600	800	<25	<25	<25	<25
SF	Bovine	400	3,200	25	<25	<25	<25	<25
ID	Bovine	100	<25	102,400	<25	<25	<25	<25
S2	Human	25	<25	<25	12,800	50	<25	<25
Wa	Human	200	<25	200	<25	<25	<25	<25
OSU	Porcine	25	50	25	<25	<25	<25	<25
Gottfried	Porcine	<25	<25	<25	<25	<25	<25	<25
174	Turkey	<25	<25	<25	<25	6,400	<25	<25

[a] Serum from noninoculated gnotobiotic calf.
[b] Serum from noninoculated gnotobiotic pig.
[c] Homologous values are underlined.

TABLE 2. Determination of bovine group A rotavirus (ID isolate) subgroup specificity by ELISA with monoclonal antibodies to SG1 and SG2 antigens

Rotavirus	Species of origin	OD at 405 nm with monoclonal antibody to:		OD ratio		Subgroup specificity
		SG1	SG2	SG1/SG2	SG2/SG1	
ID	Bovine	0.82	0.12	6.8	0.15	1
NCDV[a]	Bovine	0.78	0.13	6.0	0.17	1
Gottfried[b]	Porcine	0.17	0.79	0.22	4.7	2

[a] SG1 antigen control.
[b] SG2 antigen control.

the findings with human group A rotaviruses in which isolates with SG1 specificity have short genome electropherotypes, whereas those with long genome electropherotypes have SG2 specificities (1, 11, 20, 27, 31, 33). The recent exception to this dichotomy, an SG1 group A rotavirus with a long genome electropherotype isolated from stool specimen collected from a child with gastroenteritis in Akita, Japan, is suspected of being an animal rotavirus that infected a human (16). Thus, prediction of subgroup specificity based on a genome electropherotype pattern cannot be made. This situation has been even further complicated by the recent recognition of a equine group A rotavirus that has both SG1 and SG2 antigens (9).

Reciprocal cross neutralization tests showed that the ID isolate is serotypically distinct from the serotype 2 human S2 isolate with a short genome electropherotype, the serotype 6 bovine NCDV isolate with a long genome electropherotype, the bovine SF isolate with a long genome electropherotype (unpublished observation), and the group A turkey rotavirus 174 isolate with a semi-short genome electropherotype. Although the two avian group A rotaviruses isolated in Northern Ireland are serotype 7 group A rotaviruses (10), the serotype of the 174 isolate is not known. Nonetheless, the neutralization test data of this study clearly establish that the ID isolate is serotypically distinct from an avian group A rotavirus isolated in the United States. Moreover, hyperimmune serum to the ID isolate also had low or no neutralizing antibody titers to serotype 1, 4, and 5 group A rotaviruses. While these data demonstrate that the ID isolate is distinct from several group A rotavirus serotypes, and probably distinct from several others, the current trend has been to define serotypic relationships among the group A rotaviruses by reciprocal plaque-reduction assays with hyperimmune antisera prepared in guinea pigs by i.m. injections of partially purified virus (10). Thus, a more complete and precise serotypic characterization of the ID isolate will require that additional neutralization assays be conducted with hyperimmune antisera appropriately prepared against all recognized group A rotavirus serotypes.

Except for the newly recognized 69M isolate recovered from a gastroenteritis patient in Indonesia (15), all human group A rotaviruses with short genome electropherotypes belong to serotype 2 (1, 27). Initial serologic studies indicate that the 69M isolate probably is a new serotype of group A rotavirus. The 69M isolate also has an unusual short genome electropherotype characterized by a marked reduced mobility of segment 10, and this genome electropherotype has been designated as super short. The genome electropherotype of the ID isolate closely resembles this super-short genome electropherotype pattern, and further studies should be conducted to determine whether these two viruses belong to the same serotype. Regardless of whether these two viruses are serotypically related or not, the ID isolate joins a small, but growing, list of group A rotaviruses which includes the 69M isolate and the two lapine isolates (29; Tanaka et al., in press) that have short genome electropherotypes but do not belong to serotype 2. Moreover, to our knowledge, this is the first report of a group A rotavirus with a short genome electropherotype being recovered from a domesticated, food-producing animal. Others have detected a rotavirus with a short genome electropherotype in a fecal specimen from diarrheic pigs in Argentina, but were unable to isolate this virus in cell culture to determine whether it had the group A antigen (2). Further, this is the first report to demonstrate that a non-human group A rotavirus isolate with a short genome electropherotype is pathogenic for the host species of origin.

Taken together, the data indicate that the ID isolate is a bovine group A rotavirus with a short genome electropherotype rather than a human group A rotavirus with a short genome electropherotype that has infected a calf. First, this virus was isolated from a beef calf, a domesticated animal that has minimal human contact. Second, the ID isolate infected and induced diarrhea in gnotobiotic calves inoculated between 5 and 21 days of age; others have been unable to induce diarrhea in gnotobiotic calves older than 2 days of age that were inoculated with human rotaviruses (35). Third, the ID isolate is serotypically distinct from the human group A rotavirus S2 isolate with the short genome electropherotype. Finally, it is unlikely that the ID isolate is a human group A rotavirus with the super-short genome electropherotype that has infected a calf as these viruses have not been detected within the human population of the United States.

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