JOURNAL OF CLINICAL MICROBIOLOGY, Apr. 1993, p. 917–923
0095-1137/93/040917-07$02.00/0

Vol. 31, No. 4

Restriction Endonuclease Analysis of the vp7 Genes of Human and Animal Rotaviruses

VERA GOUVEA,[1]* CHRISTINA RAMIREZ,[1] BAOGUANG LI,[1] NORMA SANTOS,[1] LINDA SAIF,[2] H. FRED CLARK,[3] AND YASUTAKA HOSHINO[4]

Division of Microbiology, Food and Drug Administration, Washington, DC 20204[1]; Ohio Agricultural Research and Development Center, Ohio State University, Wooster, Ohio 46912[2]; Division of Infectious Diseases, Children's Hospital of Philadelphia, Philadelphia, Pennsylvania 19104[3]; and Laboratory of Infectious Diseases, National Institutes of Health, Bethesda, Maryland 20892[4]

Received 29 September 1992/Accepted 12 January 1993

The vp7 genes of 194 strains of group A rotaviruses representing all known G types were analyzed with three restriction enzymes by direct digestion of amplified cDNA copies or by deduction of the restriction patterns from known sequences. Mammalian rotavirus strains were classified into 28 restriction patterns consisting of combinations of the 6 profiles (s1 to s6) obtained by digestion with *Sau*96I endonuclease, 9 profiles (h1 to h9) obtained with *Hae*III, and 15 profiles (b1 to b15) obtained with *Bst*YI. Digestion with *Sau*96I and *Hae*III identified restriction sites common to all, or almost all, rotavirus strains studied, whereas *Bst*YI was the most discriminating among rotavirus strains. A clear correlation between some restriction patterns or individual profiles and G type and/or host species of origin was found. Several discriminatory restriction sites consisted of type-specific nucleic acid sequences that encoded conserved amino acid residues. Although not directly involved in antigenic diversity, these sites appear to indicate the G type of the isolate. The technique permits rapid comparison of a large number of virus isolates directly from fecal specimens and provides useful markers for investigating the evolution of rotavirus vp7 genes and tracing vaccine virus and interspecies transmission.

Group A rotaviruses are important and widespread agents of gastroenteritis in children and young animals, and great effort has been devoted to the development of an efficient vaccine (16). Lack of absolute host restriction among rotaviruses from different host species has permitted a Jennerian approach to vaccination; candidate animal rotavirus and animal × human reassortants have been developed and evaluated in clinical trials (5).

The rotavirus particle consists of an 11-segment, double-stranded RNA genome enclosed in a double-shelled capsid (6, 16). The inner capsid contains trimers of protein vp6, a potent immunogen that specifies group and subgroup antigens encoded by gene 6. On the outer shell are a major glycoprotein, vp7, encoded by gene 7, 8, or 9 and a minor, protease-sensitive protein, vp4, encoded by gene 4, the viral hemagglutinin which forms the viral spikes. Because both vp7 and vp4 proteins are independently responsible for virus neutralization, a dual classification system has been proposed. In that system a strain would be identified by its vp7 specificity or G type and by its vp4 specificity or P type (6). To date, 14 G types, including the newly described G12 to G14 (3, 24), and at least nine P types have been demonstrated, although P-type classification has not been firmly established. Such a complex and diverse antigenic makeup has made it difficult to interpret results of vaccine trials and to determine the role of antigenic specificity in attempts at immunoprophylaxis.

We recently described coupled reverse transcription (RT) and polymerase chain reaction (PCR) assays (RT-PCR) to amplify double-stranded RNA genomic segments for identification of G-type and P-type human group A rotaviruses, to obtain material for sequencing and cloning, and for identification of group B and C rotaviruses in clinical and veterinary specimens (8–10). In the present report, we describe a restriction endonuclease analysis (REA) of amplified full-length vp7 genes of human and animal strains of group A rotavirus. This method has proved to be extremely convenient for strain differentiation and classification of bacteria and DNA viruses, notably adenoviruses (25), and should be useful for further characterization of rotaviruses.

MATERIALS AND METHODS

Viruses. Several rotavirus strains representing the known human and animal G serotypes were analyzed. The laboratory strains used were obtained from Food and Drug Administration stocks in MA104 cell culture (SA11, NCDV, UK, C486, RRV, Hochi, Wa, MET) or first-passage viruses originally obtained from the National Institutes of Health (YO, 69M, F45, K8, SB1A, FI14, EW, DS1, KUN, H2, H1, CU1, K9, M37); Ohio State University (OSU, U46, EE, B223, Cody, OK, U425, Gottfried [Gott]); Children's Hospital of Philadelphia (WI61, SC2, P, O, CC4, WC3, HCR3); Enzo Palombo, The Royal Children's Hospital, Melbourne, Victoria, Australia (RV5, B37); and Gerald Woode, Texas Veterinary Medical Center, Texas A&M University, College Station (B641, B223).

Ninety human fecal specimens were obtained from epidemiological surveys conducted in the United States (11) and abroad during the last 6 years. Twenty veterinary fecal specimens were obtained from field outbreaks of rotavirus diarrhea in calves and piglets in Ohio, Nebraska, Indiana, and Texas. Additional fecal specimens containing viruses were obtained from experimental infections in gnotobiotic calves (B223, B641) (26) and piglets (OSU, EE, Gott).

Viral sequences. Twenty-eight nucleic acid sequences of the vp7 genes of rotavirus were obtained from EMBL/GenBank, 9 were obtained from previous studies (4, 13, 15), and 29 were obtained from a previous study by Nishikawa et

* Corresponding author.

al. (21). The nucleic acid sequences were analyzed by using the DNASTAR software (DNASTAR, Inc., Madison, Wis.); the program uses the isoschizomers *Asu*I for the *Sau*96I endonuclease and *Xho*II for the *Bst*YI endonuclease.

Primers. The primers Beg9 and End9 complementary to the 3′ ends of the vp7 gene of strains Wa and SA11 were previously shown to amplify the full-length gene of human rotaviruses (10). Some strains from animals were poorly amplified by this set of primers, probably because sequence diversity in the region complementary to primer End9 was greater than anticipated. We designed additional End9 primers on the basis of the sequences of bovine UK and porcine CRW8 strains, pooled with Beg9 and End9, and used as a degenerate mixture. The sequences of the primers are as follows: Beg9, 5′-GGCTTTAAAAGAGAGAATTTCCGTC TGG-3′; End9, 5′-GGTCACATCATACAATTCTAATCTA AG-3′; End9 (UK), 5′-GGTCACATCATACAACTCTAAT CT-3′; and End9 (CRW8), 5′-GGTCACATCTTACAGCTTT AACCT-3′.

RT-PCR. Rotavirus double-stranded RNA was extracted from infected cell cultures or from 10 to 50% fecal suspensions and was purified by adsorption to hydroxyapatite as described previously (9). The vp7 gene was reverse transcribed into cDNA and was amplified by a combined RT-PCR technique (10) with the Beg9-degenerate End9 mixture containing the four primers (500 μM each). Five microliters of the PCR product was analyzed by electrophoresis on a 1.2% agarose-ethidium bromide minigel in Tris borate buffer.

REA. Amplified cDNA copies of the entire vp7 genes of rotavirus strains (1,062 bp) were digested with 2 U of *Hae*III (Bethesda Research Laboratories, Rockville, Md.) and *Sau*96I and *Bst*YI (Promega, Madison, Wis.) endonucleases. We had long used *Hae*III to confirm rotavirus PCR products (unpublished data); the other two endonucleases were chosen because of their low but consistent frequency of cleavage. The amount of DNA used for digestion (1 to 15 μl) was adjusted visually on the basis of the intensity of the 1,062-bp PCR product in the ethidium bromide-stained gel. Digestions were performed in accordance with the manufacturer's instructions. After either 1-h or overnight incubation at the recommended temperatures, 5 μl of gel loading buffer (0.125% bromophenol blue, 20% sucrose) was added and the fragments were separated in a 2% NuSieve–1% SeaKem agarose minigel (FMC Products, Rockland, Maine). In a few instances, the PCR amplification generated spurious background bands. In such cases, the nondigested PCR product was loaded side by side with the digested DNA in the agarose gel for comparison. If the spurious bands interfered with the analysis of the restriction profile, the desired 1,062-bp full-length amplified gene was excised from the gel and eluted from the agarose before digestion.

RESULTS

Restriction profiles. Digestion of cDNA copies of the vp7 genes of human and animal rotavirus isolates with *Hae*III, *Sau*96I, and *Bst*YI endonucleases produced a variety of profiles (Fig. 1 to 3). A few additional profiles were obtained through computer analysis of known vp7 gene sequences (Tables 1 to 3).

Deduced REA of the distantly related avian G7 strain Ch2 (20) showed no single restriction site in common with those of mammalian strains, and therefore, it was not analyzed further.

Among the mammalian strains, nine restriction profiles, designated h1 to h9, were obtained upon digestion with *Hae*III endonuclease or predicted by computer analysis (Fig. 1; Table 1). A conserved restriction site at residue 381 was present in all strains analyzed except porcine G11 strain YM. Most strains (73%) possessed this single *Hae*III restriction site, generating profile h1. This group included all human strains except the G9 strains and the unusual strains HCR3 (G3) and A64 (G10). Interestingly, bovine and canine strains produced distinct, non-h1 profiles. Profiles h2 and h3, which were easily recognized in the gel by the 181- to 200-bp doublet, were found exclusively in G6 strains.

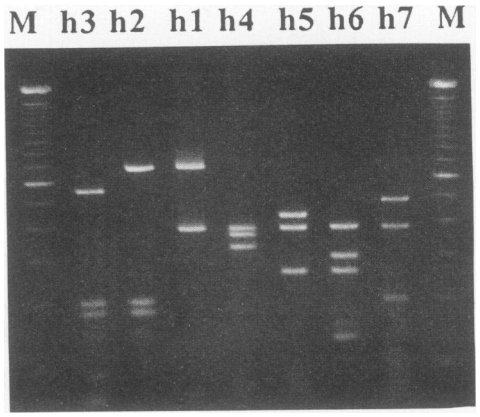

FIG. 1. *Hae*III restriction patterns of the vp7 gene copies of strains WC3 (lane h3), B641 (lane h2), MET (lane h1), OSU (lane h4), CU1 (lane h5), K9 (lane h6), and H2 (lane h7) flanked by 100-bp ladder markers (lanes M).

Digestion with *Sau*96I produced six profiles and revealed an absolutely conserved restriction site at nucleic acid residue 805 (Fig. 2; Table 2). Most of the strains (86%) possessed only this restriction site, resulting in profile s1, whereas the remaining strains possessed an additional restriction site, resulting in profiles s2 to s6. Some profiles correlated well with specific serotypes or species of origin, such as profile s2 with G2 and G5 viruses, s3 with G10, and s4 with the canine strains.

Fifteen restriction profiles, b1 to b15, were obtained by digestion with *Bst*YI endonuclease (Fig. 3; Table 3). Unlike the other two enzymes, no single *Bst*YI restriction site was conserved in all (or almost all) rotavirus strains. Instead, the cDNA copied genes presented either one or two of the nine

TABLE 1. *Hae*III digestion of the vp7 gene copies of rotaviruses

Profile	Restriction site(s) (bp)	Fragment(s) (bp)	Strain
h1	381	381, 681	G1, G2, G3[a], G4, G8, G12, G13, G14
h2	181, 381	181, 200, 681	Some G6
h3	181, 381, 424, 514	181, 200, 43, 90, 548	Some G6
h4	381, 703	381, 322, 359	G9, OSU
h5	381, 807	381, 426, 255	G10, CU1
h6	381, 514, 807	381, 133, 293, 255	K9, A79/10, HCR3
h7	381, 865	381, 484, 197	H2
h8	381, 514	381, 133, 548	cat97
h9		1,062	YM

[a] Except for strains HCR3, H2, and cat97 and the canine strains.

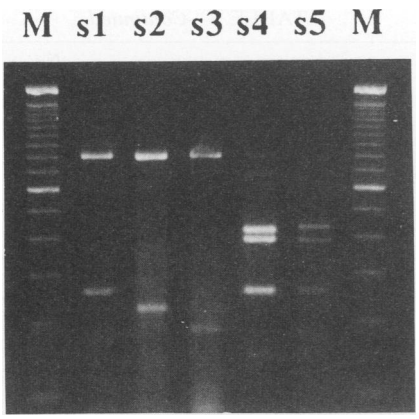

FIG. 2. *Sau*96I restriction patterns of the vp7 gene copies of strains WI61 (lane s1), OSU (lane s2), B223 (lane s3), K9 (lane s4), and M37 (lane s5) flanked by 100-bp ladder markers (lanes M).

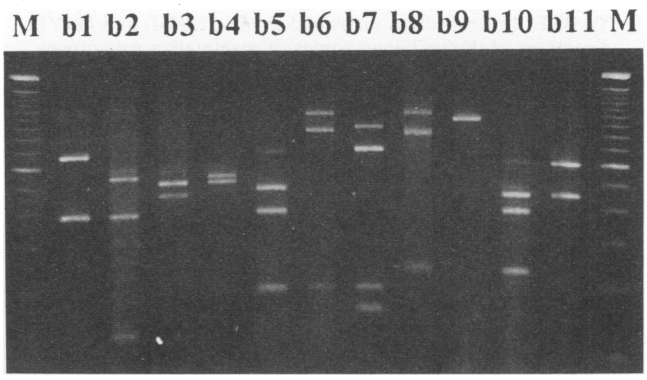

FIG. 3. *Bst*YI restriction patterns of the vp7 gene copies of G3 fecal isolate Rob2 (lane b1), SA11 (lane b2), SC2 (lane b3), G2 fecal isolate CA33 (lane b4), Wa (lane b5), G1 fecal isolate Rob18 (lane b6), G1 fecal isolate W27 (lane b7), Hochi (lane b8), B641 (lane b9), U425 (lane b10), and RRV (lane b11) flanked by 100-bp ladder markers (lanes M). Note patterns compatible with incomplete digestion, represented by the top bands in the lanes for profiles b5 to b8.

restriction sites identified. Another feature observed with *Bst*YI digestion, but not with *Hae*III or *Sau*96I digestion, was the production of a predominant profile on a background of bands consistent with partial digestion. This was particularly common in viruses of the G1 (profiles b5, b6, b7) and G4 (b8) serotypes. Increasing the amount of enzyme and the incubation time to ensure complete digestion failed to alter the results, indicating the existence in the virus population of variants that lacked one or both of the restriction sites for *Bst*YI. The presence of these extra bands, however, did not hamper identification of the predominant profiles. Most *Bst*YI profiles were strongly associated with serotypes, such as profiles b1 and b2 with human G3, b3 and b4 with G2, b8 with G4, and b9 with G6 or with subsets of the G3 serotype, such as profiles b10 and b14 with equine and murine strains, respectively.

Digestion results obtained from blindly assayed fecal specimens strongly supported this association, because complete agreement was demonstrated between b profiles and G serotypes for all 90 human and 3 bovine fecal specimens (Table 4). The remaining bovine and porcine field strains were of undetermined G type; nevertheless, the strains presented profiles typically found in viruses of these animals.

Restriction maps. By grouping all the strains by their individual profiles with each of the three endonucleases, we obtained 28 restriction maps (Table 4). Some profile combinations were favored. This nonrandom combination of individual endonuclease profiles strengthened the overall association of some patterns with a particular G type and/or host of origin. Thus, a clear correlation was found between pattern s1h1b8 and G4 rotavirus from both human and porcine origins, s2h1b3,4 and G2, s1h1b5,6,7 and G1, s1h1b1,2 and human G3, and s1h2,3b9 and G6 rotavirus. Other associations, such as pattern s1h1b10 with equine G3, s1h1b14 with murine G3, and s1h4b15 with G9, were also specific for the limited number of strains examined (two of each). In some cases, associations were more evident with a single profile such as s3 with serotype G10 or a double profile such as s4b15 with the canine group, including strain cat97, rather than a complete pattern obtained with all three endonucleases.

Test reproducibility and virus stability. Our results were obtained from at least duplicate virus RNA preparations and RT-PCR amplifications for each sample. A single inconsistency was found for *Hae*III digestion of two bovine G6

TABLE 2. *Sau*96I digestion of the vp7 gene copies of rotaviruses

Profile	Restriction site(s) (bp)	Fragments (bp)	Association with strain(s):
s1	805	805, 257	G1[a], G3[b], G4, G6, G8, G9, G12 to G14
s2	805, 841	805, 36, 221	G2, most G5
s3	805, 986	805, 181, 76	G10
s4	389, 805	389, 416, 257	Canine strains, cat97
s5	380, 805	380, 425, 257	M37, H2, HCR3
s6	805, 867	805, 62, 195	YM

[a] Except for strain M37.
[b] Except for strains HCR3, H2, and cat97 and the canine strains.

TABLE 3. *Bst*YI digestion of the vp7 gene copies of rotaviruses

Profile	Restriction site(s) (bp)	Fragment(s) (bp)	Characteristics of strains
b1	388	388, 674	Most human G3, some porcine and lapine G3, G13
b2	388, 516	388, 128, 546	Some human G3, SA11
b3	516, 610	516, 94, 452	Some G2
b4	516	516, 546	Some G2
b5	388, 867	388, 479, 195	Some G1
b6	867	867, 195	Some G1, G8, G12, TRF41
b7	154, 867	154, 713, 195	Many G1
b8	229	229, 833	G4, some G5
b9	985	985, 87	G6
b10	388, 610	388, 222, 452	Equine G3
b11	610	610, 452	RRV, R2, F123
b12	388, 435	388, 47, 627	Some G10
b13	435	435, 627	YM, A64
b14	599, 867	599, 268, 195	Murine G3
b15		1,062	G9, canine G3, cat97, some porcine strains

TABLE 4. Restriction maps of *Sau*96I, *Hae*III, and *Bst*YI digestions of the vp7 gene copies of mammalian rotaviruses

Pattern	Cell-adapted strain(s)	No. of fecal samples	Origin	G type
s1h1b1	P[a], McN[a], M[a], Nemoto[a], WI78[a], AU1[a], Ito[a], ST8[a], AK35[a], MET	22	Human	3
	C11[a], Ala[a]		Lapine	3
	AT/76[b], CRW8[b]		Porcine	3
		2	Porcine	?
	Cat2[a]		Feline	3
	L338[b]		Equine	13
s1h1b2	YO[a], MO[a], 14[a], 15[a]		Human	3
	SA11[b]		Simian	3
s1h1b5	Wa[b], D[a], K8		Human	1
s1h1b6	RV4[b]	15	Human	1
	69M[b], B37[b]		Human	8
	L26[b]		Human	12
	TFR41[b]		Porcine	5
s1h1b7	O	36	Human	1
s1h1b8	Hochi, CC4, ST3[b], VA70[a], VA75[b], VA79[b], PV5257[b], PV5249[b]	12	Human	4
	Gott[b], Ben144[b], BM1[b], SB1A		Porcine	4
	K[b]	9	Porcine	?
s1h1b10	FI14[a], U425		Equine	3
s1h1b11	RRV[b]		Simian	3
	F123[b]		Equine	14
	R2[a]		Lapine	3
s1h1b14	EB[a], EW[a]		Murine	3
s1h2b9	B641[b], OK	3	Bovine	6
		4	Bovine	?
s1h3b9	NCDV[b], UK[b], Rf[b], B60[b], WC3, C486[c], Cody[c]		Bovine	6
s1h4b15	WI61[b], F45[b]		Human	9
s1h5b15	U46	2	Porcine	?
s2h1b3	SC2	3	Human	2
s2h1b4	DS1[a], RV5, KUN, HU5[b], S2[b]	2	Human	2
s2h1b8	H1		Equine	5
s2h1b15	EE		Porcine	5
s2h4b8	OSU[b]		Porcine	5
s3h1b1	61A[b]		Bovine	10
s3h5b12	B223[b], B11[b]		Bovine	10
s3h5b13	A64[b]		Human	10
s4h5b15	CU1[a]		Canine	3
s4h6b15	K9[a], A79/10[a]		Canine	3
s4h8b15	cat97[a]		Feline	3
s5h1b7	M37[a]		Human	1
s5h6b1	HCR3		Human	3
s5h7b10	H2[a]		Equine	3
s6h9b13	YM[b]		Porcine	11

[a] Sequence available but not published.
[b] Sequence deposited in EMBL/GenBank or published previously (4, 13, 15).
[c] Both profiles h2 and h3 were found for these strains.

strains, C486 and Cody, which presented profile h3 in one experiment and profile h2 in another experiment. Nevertheless, the two profiles are characteristic of bovine G6 isolates.

Twenty-two strains with known nucleic acid sequences were assayed, and the results were compared with their corresponding deduced patterns. The test results were consistent with those deduced from sequence data obtained for the same strains by different laboratories over many years, suggesting an overall low rate of mutation for this rotavirus gene.

Analyses of restriction sites. A schematic representation of the restriction sites for *Hae*III (GG ↓ CC), *Sau*96I (G ↓ GNCC), and *Bst*YI (A/G ↓ GATCT/C) digestion of cDNA copies of mammalian rotavirus vp7 genes in relation to the hypervariable regions is shown in Fig. 4. The endonucleases used have four-, five-, or six-base recognition sequences that code for two or three amino acid residues. Those residues varied from being conserved in all strains analyzed to being unique to a certain serotype, animal species, or a given virus strain (Table 5).

Two extremely conserved restriction sites, *Sau*96I at position 805 and *Hae*III at position 381, coded for the amino acid residues that are probably critical for the folding and conformation of the vp7 protein, because both included proline residues. Most restriction sites, however, corresponded to completely, or almost completely, conserved amino acid residues encoded by type-specific or species-specific codons. Few restriction sites were within the hypervariable regions (A to F), and none were at the positions described as potential coding regions for vp7 epitopes in studies on mutants that escaped neutralization (6, 16). Restriction sites 181 (region A) and 424 (region C), characteristic of G6 strains, and 435 (region C), characteristic of G10 and G11 strains, encoded different amino acid residues for distinct G types. However, the site at residue 703, within hypervariable region E, which has been strongly implicated in serotype specificity, coded for semiconserved amino acid residues Val/Ile-Ala in all 66 strains analyzed; only OSU and the G9 strains have the cleavage site, whereas a different modality of the degenerate genetic code is used in the other strains.

DISCUSSION

The vp7 genes of 110 field isolates and 40 cell-grown strains of human and animal group A rotavirus were reverse transcribed, amplified by PCR, and digested with *Hae*III, *Sau*96I, and *Bst*YI endonucleases. In addition, the genes of 66 strains for which nucleic acid sequences were available, including 22 strains that were also tested in the laboratory, were analyzed by computer.

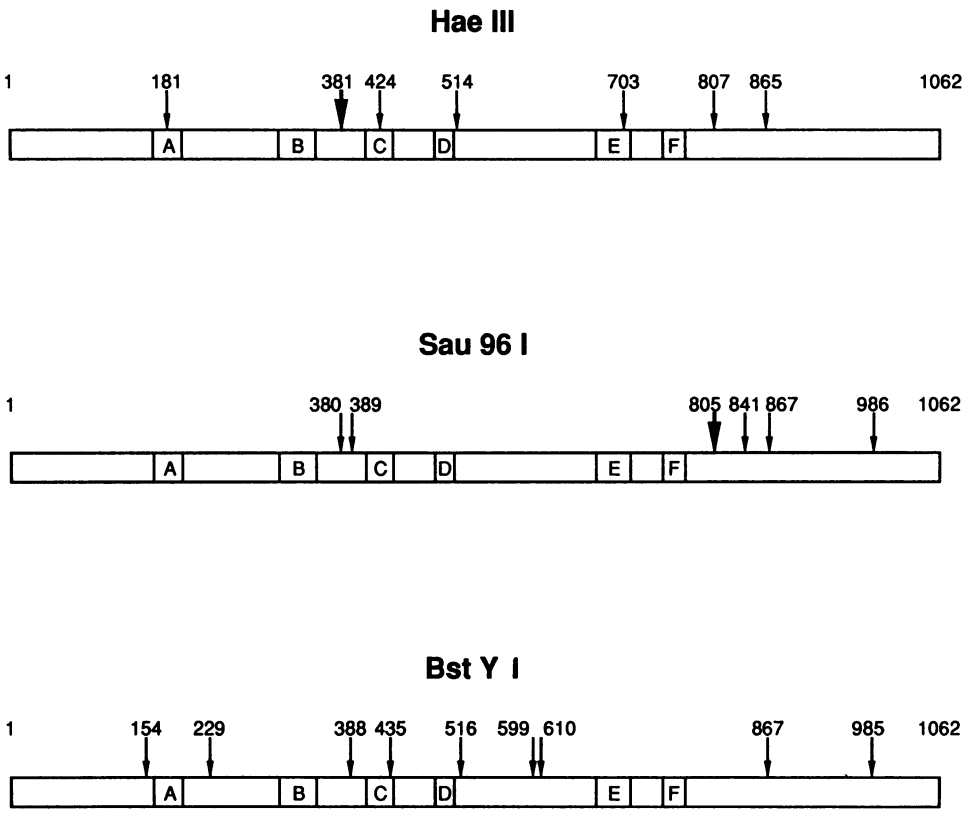

FIG. 4. Schematic representation of rotavirus vp7 gene, its hypervariable regions, and the corresponding *Hae*III, *Sau*96I, and *Bst*YI restriction sites on its cDNA copy.

Some strong associations between restriction patterns and G type or animal species of origin were found. Few of these associations, however, involved restriction sites located within the hypervariable regions which code for amino acid residues that are highly variable among strains of different serotypes but that are conserved within each serotype (14). Those divergent regions have been used to design serotype-specific probes or primers to predict rotavirus serotype by probe hybridization (7, 23) or RT-PCR (10, 19).

Remarkably, many restriction sites that discriminated among G types consisted of nucleic acid regions which encoded conserved amino acid residues. Those regions are not involved in antigenic specificity of the vp7 protein but are probably reminiscent of its G-type ancestor (i.e., restriction sites at residues 985 and 986 differentiated bovine strains G6 and G10, respectively, from the other isolates; the site at residue 229 identified G4; and that at residue 389 identified a subset of G3 formed by strain cat97 and the canine strains). The finding of those type- or species-specific nucleic acid sequences located outside the hypervariable regions and not translated into antigenic diversity had not been described previously. These sequences suffer no selective pressure from neutralizing antibodies; they should remain stable and be valuable for tracing vaccine virus after immunizations and for examining strains for evolutionary and epidemiological studies.

TABLE 5. Characteristics of *Sau*96I, *Hae*III, and *Bst*YI restriction sites on cDNA copies of the vp7 genes of mammalian rotaviruses and their deduced amino acids

Residue		Restriction site(s) (bp)		
Base	Amino acid	*Sau*96I	*Hae*III	*Bst*YI
Conserved	Conserved	805	381[a]	
Variable	Conserved	380, 389, 986	807, 865	229, 985
Variable	Somewhat conserved	867	514, 703[b]	388, 516, 599, 867
Variable	Variable	841	181[b], 424[b]	154, 435[b], 610

[a] Except for one strain (YM).
[b] Within hypervariable regions.

By examining the distribution of REA profiles among the G serotypes, we observed a wide range of relatedness among the vp7 genes of strains belonging to the same G type. Thus, the G4 strains displayed surprising homogeneity, with a single combination of profiles for all strains, suggesting a common ancestral vp7 gene for both human and porcine G4 viruses. Serotypes G2, G6, and G10 formed homogeneous and exclusive categories, whereas serotypes G5 and G1 presented some heterogeneity and sharing of patterns with other G types. In the evolutionary scale, however, serotype G3 was by far the most diverse, with strains dispersed into 10 profile combinations that had probably evolved from divergent ancestors. The genetic diversity of the vp7 genes of G3 strains apparently reflects the extraordinarily broad host range of this serotype and is consistent with results of a previous analysis of their vp7 gene sequences (21). The

limited number of samples available for the other G types did not permit similar speculations on their evolutions. Nevertheless, our study indicated that, after the avian G7 serotype, the G11 serotype, which is represented by porcine strain YM, is the most distantly related group A rotavirus serotype. The lack of a restriction site for *Hae*III was a unique feature of the YM strain, but whether this could be a marker for G11 strains must await examination of YM-like field isolates. The REA technique should be particularly useful for analyzing veterinary specimens that cannot be directly compared by immunological tests because of virus importation restrictions. Also of interest, only the three G3 strains isolated from horses, one in England (H2) and two isolated 8 years later in the United States (FI14, U425), presented profile b10, and only the two G3 strains (strains EW and EB) isolated from mice 25 years apart presented profile b14. Studies of additional equine and murine rotaviruses are needed to verify these associations.

Under experimental conditions, human rotaviruses can infect and produce diarrhea in newborn calves, piglets, and mice (12, 16, 26); immunization of children with strains of bovine and simian origins has produced an opposite situation (5). Recently, a zoonotic origin has been proposed for the emerging group B and C rotavirus infections in humans (22), and similar natural interspecies transmission among group A rotaviruses has been suggested, primarily among G3 strains (2, 17, 18). Thus, a close relationship was found between feline and canine and between feline and human G3 strains by RNA-RNA hybridization (17, 18) and was later confirmed by sequence analysis of their vp7 genes (21). Using REA, we found the same relationships for the feline strains: strain cat97 was classified with the canine group and strain cat2 was classified with the human G3 strains. Interestingly, REA has also placed the unusual human G10 strain A64, which was selected in cell culture from a complex mixture of rotavirus genes (1), in the G10 bovine group and strain HCR3 apart from the common human G3 groups, although it still shared profile b1 with the human G3 strains. HCR3 virus, isolated from a healthy child in Philadelphia, presented several uncommon characteristics and appeared to be more closely related to animal than to human strains (unpublished data). Along with hybridization assays, REA of additional rotavirus strains isolated from a variety of animals should further our understanding of rotavirus transmission.

ACKNOWLEDGMENTS

We thank Gerald Woode and Enzo Palombo for providing the rotavirus strains used in the present study, Blair Rosen and Anil Parwani for testing porcine and bovine strains with serotype-specific probes, Mary Trucksess and John Bond for providing primers, Sebastian Cianci for graphic work, and Lois Tomlinson for editorial assistance.

N.S. is a recipient of a fellowship from CAPES, Brasilia, Brazil.

ADDENDUM IN PROOF

The Russian porcine strain K was described as G4 by sequence analysis (T. A. Akopian et al., Virus Genes **6**(4): 393–396, 1992).

REFERENCES

1. **Beards, G., A. Ballard, U. Desselberger, and M. A. McCrae.** 1992. A serotype 10 human rotavirus. J. Clin. Microbiol. **30:** 1432–1435.
2. **Birch, C. J., R. L. Heath, J. A. Marshall, S. Liu, and I. D. Gust.** 1985. Isolation of feline rotaviruses and their relationship to human and simian isolates by electropherotype and serotype. J. Gen. Virol. **66:**2731–2735.
3. **Browning, G. F., T. A. Fitzgerald, R. M. Chalmers, and D. R. Snodgrass.** 1991. A novel group A rotavirus G serotype: serological and genomic characterization of equine isolate F123. J. Clin. Microbiol. **29:**2043–2046.
4. **Charpilienne, A., F. Borras, L. D'Auriol, F. Galibert, and J. Cohen.** 1986. Sequence of the gene encoding the outer glycoprotein of the bovine rotavirus (RF strain) and comparison with homologous genes from four bovine, simian and human rotaviruses. Ann. Inst. Pasteur Virol. **137F:**71–77.
5. **Clark, H. F.** 1991. Rotavirus vaccine. Semin. Pediatr. Infect. Dis. **2:**202–206.
6. **Estes, M. K., and J. Cohen.** 1989. Rotavirus gene structure and function. Microbiol. Rev. **53:**410–449.
7. **Flores, J., J. Sears, I. Perez-Shael, L. White, D. Garcia, C. Lanata, and A. Z. Kapikian.** 1990. Identification of human rotavirus serotype by hybridization to polymerase chain reaction-generated probes derived from a hyperdivergent region of the gene encoding outer capsid protein vp7. J. Virol. **64:**4021–4024.
8. **Gentsch, J. R., R. I. Glass, P. Woods, V. Gouvea, M. Gorziglia, J. Flores, B. K. Das, and M. K. Bhan.** 1992. Identification of group A rotavirus gene 4 types by polymerase chain reaction. J. Clin. Microbiol. **30:**1356–1373.
9. **Gouvea, V., J. R. Allen, R. I. Glass, Z.-Y. Fang, M. Bremont, J. Cohen, M. A. McCrae, L. J. Saif, P. Sanarachatanant, and E. O. Caul.** 1991. Detection of group B and C rotaviruses by polymerase chain reaction. J. Clin. Microbiol. **29:**519–523.
10. **Gouvea, V., R. I. Glass, P. Woods, K. Taniguchi, H. F. Clark, B. Forrester, and Z.-Y. Fang.** 1990. Polymerase chain reaction amplification and typing of rotavirus nucleic acid from stool specimens. J. Clin. Microbiol. **28:**276–282.
11. **Gouvea, V., M.-S. Ho, R. I. Glass, P. Woods, B. Forrester, C. Robinson, R. Ashley, M. Rippenhof-Talty, H. F. Clark, and K. Taniguchi.** 1990. Serotypes and electropherotypes of human rotavirus in the USA: 1987–1989. J. Infect. Dis. **162:**362–367.
12. **Gouvea, V. S., A. A. Alencar, O. M. Barth, L. de Castro, A. M. Fialho, H. P. Araujo, S. Majerowicz, and H. G. Pereira.** 1986. Diarrhoea in mice infected with a human rotavirus. J. Gen. Virol. **67:**577–581.
13. **Green, K. Y., Y. Hoshino, and N. Ikegami.** 1989. Sequence analysis of the gene encoding the serotype-specific glycoprotein (vp7) of two new human rotavirus serotypes. Virology **168:**429–433.
14. **Green, K. Y., K. Midthun, M. Gorziglia, Y. Hoshino, A. Z. Kapikian, R. M. Chanock, and J. Flores.** 1987. Comparison of the amino acid sequences of the major neutralization protein of four human rotavirus serotypes. Virology **161:**153–159.
15. **Huang, J., H. S. Nagesha, M. L. Dyall-Smith, and I. H. Holmes.** 1989. Comparative sequence analysis of vp7 genes from five Australian porcine rotaviruses. Arch. Virol. **109:**173–183.
16. **Kapikian, A. Z., and R. M. Chanock.** 1990. Rotaviruses, p. 1353–1404. *In* B. N. Fields, D. N. Knipe, R. M. Chanock, J. L. Melnick, B. Roizman, and R. E. Shope (ed.), Virology. Raven Press, New York.
17. **Nakagomi, O., and T. Nakagomi.** 1991. Genetic diversity and similarity among mammalian rotaviruses in relation to interspecies transmission of rotavirus. Arch. Virol. **120:**43–55.
18. **Nakagomi, O., A. Ohshima, Y. Aboudy, I. Shif, M. Mochizuchi, T. Nakagomi, and T. Gotlieb-Stematsky.** 1990. Molecular identification by RNA-RNA hybridization of a human rotavirus that is closely related to rotaviruses of feline and canine origin. J. Clin. Microbiol. **28:**1198–1203.
19. **Nakagomi, O., H. Oyamada, and T. Nakagomi.** 1991. Experience with serotyping rotavirus strains by reverse transcription and two-step polymerase chain reaction with generic and type specific primers. Mol. Cell. Probes **5:**285–289.
20. **Nishikawa, K., Y. Hoshino, and M. Gorziglia.** 1991. Sequence of the vp7 gene of chicken rotavirus Ch2 strain of serotype 7 rotavirus. Virology **185:**853–856.
21. **Nishikawa, K., Y. Hoshino, K. Taniguchi, K. Y. Green, H. B. Greeberg, A. Z. Kapikian, R. M. Chanock, and M. Gorziglia.** 1989. Rotavirus vp7 neutralization epitopes of serotype 3

strains. Virology **171**:503–515.
22. **Saif, L. J.** 1989. Nongroup A rotaviruses, p. 73–95. *In* L. J. Saif and K. W. Theil (ed.), Viral diarrheas of man and animals. CRC Press, Inc., Boca Raton, Fla.
23. **Sethabutr, O., S. Hanchalay, U. Lexomboon, R. F. Bishop, I. H. Holmes, and P. Echeverria.** 1992. Typing of human group A rotavirus with alkaline phosphatase-labelled oligonucleotide probes. J. Med. Virol. **37**:192–196.
24. **Taniguchi, K., T. Urasawa, N. Kobayashi, M. Gorziglia, and S. Urasawa.** 1990. Nucleotide sequence of vp4 and vp7 genes of human rotaviruses with subgroup I specificity and long RNA pattern: implication for new G serotype specificity. J. Virol. **64**:5640–5644.
25. **Wadell, G.** 1984. Molecular epidemiology of human adenoviruses. Curr. Top. Microbiol. Immunol. **110**:191–220.
26. **Woode, G. N., N. E. Kelso, T. F. Simpsom, S. K. Gaul, L. E. Evans, and L. Babiuk.** 1983. Antigenic relationships among some bovine rotaviruses: serum neutralization and cross-protection in gnotobiotic calves. J. Clin. Microbiol. **18**:358–364.