

Genomic Diversity of Group A Rotavirus Strains Infecting Humans in Eastern India

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Between 1998 and 2000, a total of 266 samples were found positive for group A rotaviruses by RNA electrophoresis. Samples were collected from patients admitted to two leading hospitals in Calcutta. Serotyping could be done only with 22% of the positive samples, leaving 78% untypeable. The G (VP7 genotypes) and P (VP4 genotypes) types were determined for 159 samples by reverse transcription and multiplex PCR. The predominant genotype was G1P[8] (20%), followed by G2P[4] (15%) and G4P[8] (6%). A number of uncommon genotypes, G1P[4] (4%), G2P[8] (2.5%), G2P[6] (0.6%), G4P[4] (2.5%), and G4P[6] (1.25%), were also detected during this study period. Twenty two percent of specimens showed mixed infections, 38 (24%) of the total samples remained untypeable for either VP7 or VP4, while only 4 (2.5%) of the samples were untypeable for both genes. Eleven specimens collected from Manipur were also genotyped and revealed a very high degree of genomic reassortment.

Group A rotaviruses are the major cause of severe dehydrating gastroenteritis among human infants and young of a wide variety of mammalian and avian species (17), and it has been estimated that nearly a million deaths occur every year (9, 20), predominantly in developing countries.

Fifteen G serotypes of rotaviruses (27) are recognized, depend on the molecular characterization of VP7 (glycosylated outer capsid protein). However, G1 to G4 are the most predominant genotypes in humans. Moreover, a number of unusual genotypes, G5, G8, and G9, have also been reported recently from various countries (8). Thirteen P serotypes (22) and 21 P genotypes are recognized on the basis of VP4 (protease-sensitive outer capsid protein), but the most common P serotypes infecting humans are P1A and P1B, corresponding to the P[8] and P[4] genotypes, respectively. Although the role of VP4 protein in protective immunity is not very well established, information on G and P typing is important for identifying unusual or new virus strains circulating in different populations (19).

The first human rotavirus vaccine (a human-animal tetravalent vaccine) was licensed in the United States in 1998; however, the same vaccine has not been tried extensively in other countries. Due to strain diversity in different parts of the world, knowledge of molecular epidemiology and antigenic diversity of rotaviruses in circulation is imperative for the development of a suitable, efficacious vaccine to combat rotaviral diarrhea. Therefore, in this study we report the characterization of dif-

ferent G and P types of rotavirus strains circulating in a particular area of India.

MATERIALS AND METHODS

Specimen collection. The specimens were collected from children less than 4 years old at B. C. Roy Children's Hospital and from patients of all age groups admitted to the Infectious Diseases Hospital, Kolkata, between 1998 and 2000. Some samples were also collected from the Regional Medical College and field study areas in Manipur between 1990 and 1992. A total of 2,114 samples were screened for rotaviruses by RNA electrophoresis as described by Herring et al. (15) and Follet et al. (10).

ELISA for serotyping. Rotavirus G serotyping enzyme-linked immunosorbent assay (ELISA) was done with a 10% fecal suspension in phosphate-buffered saline according to the method described by Coulson et al. (6). The monoclonal antibodies (MAbs) for VP7 groups A (MAb 101BC5C7), G1 (RV4:2), G2 (RV5:3), G3 (RV3:1), and G4 (ST3:1) were received as a gift from Ruth Bishop of Royal Children's Hospital Research Foundation, Melbourne, Victoria, Australia.

Extraction of dsRNA for RT-PCR. The fecal specimens were processed for the extraction of double-stranded RNA (dsRNA) suitable for reverse transcription (RT)-PCR for the amplification of VP4 and VP7 genes. Rotavirus dsRNA was extracted by the method of Gentsch et al. (11) with some modifications as described by Wu et al. (30).

RT of viral RNA. RT of viral RNA for the amplification of VP4 and VP7 genes was carried out as described by Gentsch et al. (11) and Gouvea et al. (14).

cDNA synthesis by using random primers. Recently, the RT reaction was modified (23) and improved by adding 200 ng of random hexamers (GIBCO-BRL) to the RT mixture, which facilitated the simultaneous amplification of different genes with appropriate primers.

Genotyping. G and P typing of positive samples was carried out by nested and multiplex PCR by using consensus and type-specific primers as described by Taniguchi et al. (28), Gouvea et al. (13), and Wu et al. (30).

(i) **G typing.** Briefly, for G typing, consensus primers C1 and C2 were used in the first-round PCR to amplify 1,062 bp of the full-length VP7 gene. In the second PCR, primers specific for G1 to G4, G6, and G8 to G10 were used as described by Taniguchi et al. (28). The untypeable samples were subjected to another nested PCR by using G5- and G11-specific primers (14). The G12-specific primer was designed by us on the basis of human rotavirus sequences deposited in GenBank (accession no. M58290 and M36396; 5' TAT AGA TTC TTA CTA GTT TTT GTT ATC 3'; 27-mer; nucleotides 151 to 177).

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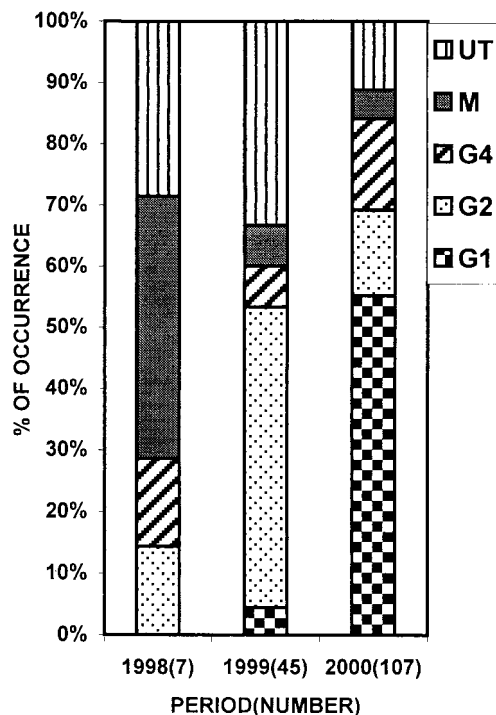


FIG. 1. Yearwise prevalence of different G types in Kolkata. UT, untypeable.

(ii) **P typing.** VP4 typing was carried out by the procedure described by Wu et al. (30). VP4(A) and VP4(B) primers were used in the first-round PCR to synthesize a 1,094-bp product of the partial VP4 gene. In the multiplex PCR, VP4(C), VP4(D), VP4(E), and VP4(F) primers, specific for P[8], P[4], P[6], and P[9] types, respectively, were used.

RESULTS

A total of 1,708 samples were collected from patients of all age groups at the Infectious Diseases Hospital, Kolkata; 125 (7.3%) of these samples were found positive for group A rotaviruses. On the other hand, from among 406 samples collected at B. C. Roy Children’s Hospital (patients less than 4 years old), 141 (34.7%) were positive for group A rotaviruses.

G serotyping by ELISA. A total of 156 samples were serotyped by ELISA by using MAbs specific for serotypes G1 to G4. Only 22% of the rotavirus-positive samples were successfully serotyped; 78% remained untypeable. The most prevalent serotype detected during the study period was G4, found in 21 samples (60%), followed by G1, found in 9 (25.7%), and G2, found in 1 (2.8%); 4 samples (11%) were dually reactive to G1G3 or G1G4.

G types. A total of 159 (60%) samples out of 266 were available in sufficient quantity for further analysis. G types were successfully determined for 130 (82%) of these samples. During the 3-year study period, the incidence of G-type detection was as follows: G1 (38%), G2 (24%), G4 (12.6%), and mixed (7%). Interestingly, G9 was not detected separately but appeared along with the mixed G types. In this study, in 1999 G2 was the most prevalent genotype in Kolkata, followed by G1; however, G1 predominated over G2 in the year 2000 (Fig. 1). A number of mixed G types were isolated during the course

TABLE 1. Detection of mixed rotavirus strains in Kolkata

Yr	Strain	Type ^a		No. of strains
		G	P	
1998	1	G1G4	UT	1
	2	G1G4	P[8]P[4]	1
	3	G1G4	P[8]	1
1999	2	G1G4	P[4]P[8]	1
	3	G1G4	P[8]	1
	4	G1G2	UT	1
	5	G4	P[4]P[8]	2
	6	G2	P[4]P[6]P[8]	1
	7	G2G9	P[4]P[8]	1
	8	G2	P[4]P[8]	5
	9	UT	P[4]P[8]	1
	2000	1	G1G4	UT
3		G1G4	P[8]	2
5		G4	P[4]P[8]	2
8		G2	P[4]P[8]	1
10		G4G9	P[4]	1
11		G4G9	P[8]	1
12		G1	P[4]P[8]	9
13		G2G4	P[4]P[8]	1
14		G1	P[6]P[8]	5

^a UT, untypeable.

of this study (Table 1).

P types. P types were determined for 138 (87%) of 159 rotavirus-positive samples. Only two P types were predominant in Kolkata during the study period, P[8] (37%) and P[4] (26%). The other types observed were P[6] (6%) and mixed P types (19%); 19 samples (13%) were untypeable in this study. The mixed P types consisted of P[4]P[8], P[6]P[8], and P[4]P[6]P[8]. The incidence of each type was observed to vary from year to year. P[6] was the predominant type in the first year; however, P[4] was the most prevalent type in the second year, and in the last year, P[8] occurred most frequently.

Correlation of G and P types. Both G and P types were assigned to 117 (72%) of 159 rotavirus-positive samples by using the above-mentioned procedures. During this study, it was observed that G1 and G4 were mostly associated with the P[8] genotype and that G2 was mostly associated with the P[4] genotype. Moreover, G4 was also detected along with a combination of P[4] or P[6] genotypes, and G2 was detected with P[8] or P[6] genotypes. Overall, G1P[8] was the most prevalent G type, followed by G2P[4]; 20% of the typeable strains were of mixed types (Table 1).

Correlation of genotypes with electropherotypes. In contrast to worldwide observations, it was observed during this study that G2 was not always associated with “short” electropherotypes; in some strains, it was associated with “long” electropherotypes. Similarly, G1 was also associated with short electropherotypes in some strains instead of long electropherotypes, as generally believed (Table 2).

G and P typing of the samples from Manipur. Genotyping of 11 samples collected from Manipur showed a high rate of mixed infections. Multiple genotypes were observed in nine specimens (Table 3).

TABLE 2. Electropherotypes and genotypes of Kolkata samples

Sample ID	Collection date (mo/yr)	Type ^a		Electropherotype
		G	P	
Viro196	3/2000	G2	P[8]	Long
Viro226	4/2000	G2	P[4]	Long
Viro263	9/2000	G2	P[8]	Long
Viro264	9/2000	G2	P[8]	Long
DK6	1/1999	G1	P[4]	Short
E01612	9/1999	G1	UT	Short
F17357	9/2000	G1	P[8]	Short
F19452	10/2000	G1	P[8]	Short

^a UT, untypeable.

DISCUSSION

The objective of this study was to identify the G and P types of group A rotaviruses to determine the strain diversity of rotaviruses prevalent in eastern India.

The four predominant rotavirus genotypes, G1P[8], G2P[4], G3P[8], and G4P[8], comprise nearly 83% of all the rotavirus infections in the world (24). In contrast to worldwide observations, in Kolkata no G3 has been detected, and three other predominant genotypes have been associated with only 45% of the rotavirus infections. The uncommon types (G2P[8], G4P[4], G2P[6], and G4P[6]) were observed in 7% of cases in this study; similar observations have been made in other countries, such as Ireland (21), the United Kingdom (7), France (3), Bangladesh (29), and the United States (26), but at very low frequencies. In this study, only four samples were untypeable for both G and P types; on the other hand, nearly 24% remained untypeable for either G or P type. We have used almost all the G-type primers (for G1 to G6 and G8 to G12) and common P-type primers (for P[4], P[8], P[6], and P[9]) in the multiplex PCR. Therefore, these untypeable samples may lie outside of the known types or may represent a new type or subtypes. Further characterization of these unusual strains will reveal their true nature. During this study, a number of mixed G- and P-type strains also appeared in a significant percentage (22%). The combined data for G and P typing are also highly relevant in identifying different strains that might have resulted

from reassortment of genes between animal and human rotaviruses.

We also collected a few rotavirus-positive samples from Manipur, a northeastern state of India, between 1990 and 1992. These samples were collected from the Regional Medical College, Imphal, in Manipur and from field study areas in and around the capital city, Imphal. We genotyped some of those group A rotavirus-positive samples, which are long but subgroup I, similar to the strains reported by Ghosh and Naik (12); some samples are long but subgroup II. For this study, a few samples were genotyped for VP7 and VP4 genes; by combining the data for G and P typing, we observed different combinations, as 9 out of 11 samples showed mixed infections for either the VP7 or the VP4 gene or for both genes. The most common and uncommon G types observed during this study were G1, G2, G3, G4, G6, G8, G9, and G10 within the mixed types with different combinations of P[4], P[8], and P[6]. The electropherotypes of the strains clearly showed 11 bands of dsRNA, and these samples reacted with only one subgroup-specific MAb in an ELISA (data not shown). Further study with these strains will reveal the clear picture of diversity of rotavirus strains and the frequency of reassortment taking place in nature.

India being a vast country, it is essential to study the molecular epidemiology of rotaviruses in different parts of the country to obtain a clear picture of the prevalence of different strains. Studies so far carried out in India have revealed that the predominant strains are G1 to G4 (1, 2, 4, 16, 18, 25), except from northern and central India (5, 25), where G9 has been reported as the predominant G type. Although G9 was not detected separately in this study, it appeared as part of the multiple G types. These multiple G types might have occurred due to reassortment between animal and human rotaviruses, as both animals and humans live in very close proximity to each other in rural India.

It has been observed that limited cross protection occurs among different G serotypes; therefore, in light of the above observations, it may be necessary to include more strains in future vaccine formulations for India. Since multiple G- and P-type strains occur more frequently in this part of the world, a different strategy may be required to develop a successful vaccine for the control of rotavirus diarrhea in India.

TABLE 3. Distribution of different G and P types of rotavirus strains in Manipur^a

Yr	Strain	Type	
		G	P
1990	1	G6G10	P[4]
	2	G2G4	P[4]
	3	G1G4G8G9	P[4]
	4	G4G8	P[8]
	5	G4	P[4]P[8]
	6	G1G3G4G10	P[4]P[8]
	7	G4	P4
	8	G1	P6
1991	3	G1G4G8G9	P[4]
1992	3	G1G4G8G9	P[4]
	9	G1G2	P[4]P[8]

^a One strain of each was found.

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