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Classification of Rotavirus into G and P Types with Specimens from Children with Acute Diarrhea in New Delhi, India

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Sixty rotavirus-positive stool specimens from children with diarrhea were classified into G and P genotypes. G typing was done by PCR and then by hybridization with G type-specific (G1 to G4) oligonucleotide probes, whereas nested PCR was performed for P typing. Thirty-nine samples could be classified into both G and P types, of which P8G1 and P4G2 (33% each) genotypes were predominant. The P6 genotype was detected in four children with diarrhea.

Group A rotaviruses are major cause of acute gastroenteritis in children and the young of many animal species throughout the world (7). The exact parameters of immunity to rotavirus are not clearly defined; however, it is pertinent to obtain information on epidemiologically important human G and P types to formulate a vaccine strategy (17). The antigenic specificity carried on VP7 determines the G serotype, and that carried on VP4 determines the P serotype (6). Group A rotaviruses have been classified into 14 G serotypes, of which 10 are associated with human infections. Serotypes G1 to G4 are major pathogens of acute diarrhea worldwide (22), whereas G8, G9, and G12 have been occasionally recovered from humans. Serotypes G6 and G10 (bovine serotypes) and G5 (swine rotavirus) have also been reported in cases of diarrhea in children (2, 9, 11). Among 18 known P types, 7 have been identified in human infections, i.e., P8, P4, P6, P9, P10, P11, and HCR3 (5, 6, 16).

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A total of 450 stool specimens were obtained from children of <5 years of age who presented with acute diarrhea during January 1990 to December 1991. Of these, 150 and 300 samples were obtained from Safdarjung Hospital and from Lok Nayak Jai Prakash Narayan Hospital, respectively, New Delhi, India. Rotavirus was detected in fecal samples by enzyme-linked immunosorbent assays (ELISA) (Dakopatts A/S), polyacrylamide gel electrophoresis (PAGE), and reverse transcription (RT)-PCR of gene segment 9. Sixty samples that were positive by RT-PCR and by either of the other two tests, i.e., ELISA or PAGE (14), were further classified into G and/or P genotypes.

Rotaviruses were classified into G types by RT-PCR of the VP7 gene and by its hybridization with serotype-specific radiolabeled oligonucleotide probes. The primers and probes chosen for G typing are as follows. The primers included (i) 5′-GAT CC G AAT GGT TGT GTA ATC CAA T-3′ (nucleotides [nt] 532 to 551) (*Bam*HI linker) and (ii) 5′-AAT TC G CTA CGT TTT CTC TTG G-3′ (nt 824 to 808) (*Eco*RI linker) (the linkers were used to facilitate cloning of the amplicon). The probes included (i) 5′-GTA GAC TCA TTT GAA ATG-3′ (nt 682 to 699) (serotype 1), (ii) 5′-GTA AAC ACA TTT GAG ATT-3′ (nt 682 to 699) (serotype 2), (iii) 5′-ACA AAC ACG TTT GAA GAA-3′ (nt 682 to 699) (serotype 3), and (iv) 5′-ACA GCT ACT TTT GAA ACA-3′ (nt 682 to 699) (serotype 4).

The primers were selected on the basis of the published sequence of the VP7 gene of Nebraska calf diarrhea virus from a conserved region of group A rotaviruses (10). The probes were selected from a region that is conserved within the same serotypes but is divergent from other serotypes. The probes were based on the sequences of the VP7 genes of D (G1), DS1 (G2), P (G3), and ST3 (G4) rotaviruses (12). A 303-bp fragment obtained after RT-PCR was dotted onto a nitrocellulose membrane to prepare four identical blots. Each blot was hybridized with one of the four serotype-specific oligonucleotide probes labeled at the 3′ end with terminal transferase (Boehringer Mannheim) and [α-^{32}P]dCTP (Fig. 1).

To identify P types, seminested PCR for the VP4 gene was used (8). Instead of CON3, a different primer, INT-1 complementary to nt 1 to 20 (5′-GGCATAAAATGGCTCT-3′) was employed. The typing primers 1T-1 to 5T-1 were the same but were short by 1 nt at their 3′ end (Fig. 2).

A total of 51 of 60 specimens were subjected to G typing, of which 44 (86.2%) could be classified into G1 to G4 or multiple G types. As for the four major human G types, 17 strains (38.6%) were G1, 13 (29.5%) were G2, 5 (11.3%) were G3, 4 (9.0%) were G4, and 5 (11.3%) were multiple G types. The

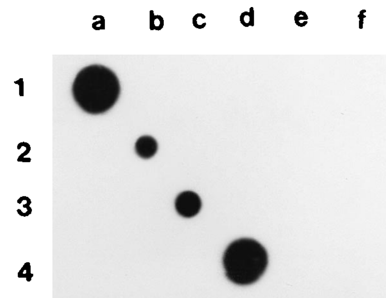

FIG. 1. G genotyping of rotavirus reference strains by RT-PCR and dot hybridization assaying with serotype-specific (G1 to G4) 3′ end-radiolabeled oligonucleotide probes. 1 to 4, four identical membrane strips hybridized with G1 to G4 probes, respectively. a to f, RT-PCR products from rotavirus strains Wa (serotype 1), DS1 (serotype 2), P (serotype 3), ST3 (serotype 4), and SA11 (simian rotavirus [serotype 3]) and total nucleic acid from MA104 cells, respectively. The membranes were exposed to X-ray film with an intensifying screen for 10 h.

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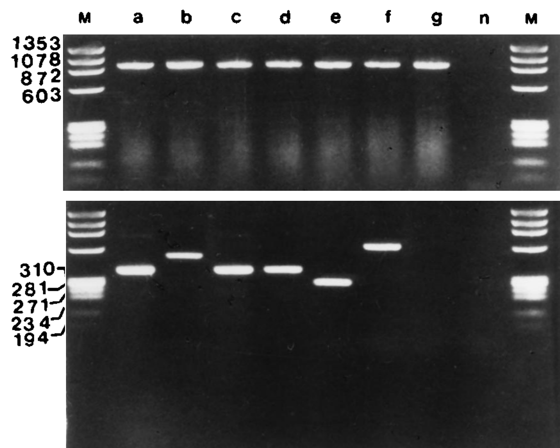

FIG. 2. P genotyping of rotavirus reference strains by RT-PCR. In the first round of amplification, a CON2 and INT-1 primer pair was used to obtain an 887-bp band (lanes a to g, upper panel). In the second round of amplification, 2 μl of 1:5 diluted first-amplification product was reamplified with a pool of five type-specific (nested) primers, 1T-1 to 5T-1, and common primer INT-1 (lanes a to g, lower panel). Lanes: M, *Hae*III-digested ΦX174 marker DNA; a to g, rotavirus strains Wa (P8 [356 bp]), DS1 (P4 [494 bp]), P (P8 [356 bp]), Hochi (P8 [356 bp]), ST3 (P6 [278 bp]), 69M (P10 [594 bp]), and SA11 (P2 [untypeable]), respectively; n, negative control (containing PCR mix but no nucleic acid).

untypeable strains may belong to other G types, for which probes were not included in the panel. The P typing was performed for 57 samples; in the first PCR step, only 43 (75.4%) samples could be amplified, and all of these were typeable with a pool of five type-specific primers by nested PCR. Of these 43 samples, 23 (53.5%) were P8, 14 (32.5%) were P4, 4 (9.3%) were P6, and 2 (4.6%) had multiple P types. In 39 samples, both the G and the P genotypes could be determined. It was observed that P8G1 and P4G2 (13 each) were the predominant types, after which came P8G3 (5 samples) and P8G4 (3 samples), and 1 strain was of the P6G1 genotype. Others were either mixed G types or mixed P types. Two strains could be classified into only P types, and four strains could be classified into only G types. Neither the P9 nor the P10 genotype was detected (Table 1).

The reports on G serotyping of rotaviruses from children with diarrhea in India have shown the presence of all four major serotypes, i.e., G1 to G4, with one or two serotypes being predominant at a particular peak of infection (1, 4). Similarly, in our study G1 and G2 genotypes were the predominant types, after which came G3 and G4. The untypeable strains in our study may belong to the G9 or G10 genotype, both of which have been reported in neonatal infections from India (5). No data on P typing of rotavirus strains from children with diarrhea from India are available, except for one report on P typing of rotaviruses from asymptomatic neonatal infections (5).

The predominance of the P8 genotype (53.4%) in this study is due to its association with more than one G type specificity, i.e., G1, G3, or G4. Other studies also have reported the predominance of P8 types in rotavirus isolates from children with acute diarrhea (18). All P4 strains were associated with the G2 genotype, except for one strain that had multiple G types (G1 and G2), thus confirming the general association of G2 and P4 genotype specificity. Four P6 strains were identified from patients with diarrhea; one had the G1 genotype, two were not subjected to G typing, and one was G untypeable. P6 strains have been reported from patients with moderate to severe diarrhea in other studies as well (18). Some of the samples had multiple G or P types that may be attributed to mixed infections. In previous studies, mixed G and mixed P types have been reported (20). Coinfection with multiple rotavirus strains can lead to the emergence of reassortants during the course of natural infection.

The RNA migration patterns of all 60 samples were studied (3), and long and short RNA patterns were correlated with genotypes (Table 2). Of the 13 P4G2 strains, 12 had short patterns and one had a long RNA pattern. The G2 genotype is

TABLE 2. Relationship between genotypes and electropherotypes[a]

Genotype	No. of strains	No. with electropherotype		
		Long	Short	−VE
P8G1	13	11	0	2
P8G3	5	5	0	0
P8G4	3	3	0	0
P4G2	13	1	12	0
P6G1	1	1	0	0
P4 + P6G1	1	1	0	0
P8 + P6G (UT)	1	0	1	0
P4G1 + G2	1	1	0	0
P8G1 + G3	2	2	0	0
P (UT)G3 + G4	1	1	0	0

[a] P (UT), P untypeable; G (UT), G untypeable; −VE, RNA bands of rotavirus not visible by PAGE.

TABLE 1. G (VP7) and P (VP4) genotyping of human rotaviruses from children with acute diarrhea

G type	No. with P type										
	P8	P4	P6	P9	P10	P4 + P6	P8 + P6	Total P typeable	P (UT)	ND	Total
G1	13	0	1	0	0	1	0	15	1	1	17
G2	0	13	0	0	0	0	0	13	0	0	13
G3	5	0	0	0	0	0	0	5	0	0	5
G4	3	0	0	0	0	0	0	3	1	0	4
G1 + G2	0	1	0	0	0	0	0	1	0	0	1
G1 + G3	2	0	0	0	0	0	0	2	1	0	3
G3 + G4	0	0	0	0	0	0	0	0	1	0	1
Total G typeable	23	14	1	0	0	1	0	39	4	1	44
G (UT)	0	0	1	0	0	0	1	2	3	2	7
ND	0	0	2	0	0	0	0	2	7	0	9
Total	23	14	4	0	0	0	1	43	14	3	60

[a] P (UT), P untypeable; G (UT), G untypeable; ND, not done.

mostly associated with a short electropherotype, but exceptions having long electropherotypes have also been reported (15). The genotypes P8G1, P8G3, P8G4, and P6G1 had long RNA patterns, as described previously (13). Samples with mixed G types had long electropherotypes, whereas in the case of two samples with mixed P types, one (P4 + P6G1) had a long RNA pattern and one (P8 + P6G [untypeable]) had a short RNA pattern. Two unusual strains, one having the P4G2 genotype and a long electropherotype and the other having the P8+6G(ut) genotype and a short RNA pattern, were observed in this study. Classification of rotaviruses into genotypes along with electropherotypes helps in identifying unusual strains that may arise by reassortment during mixed infections.

Genotyping by RT-PCR has been found to be more efficient than serotyping with monoclonal antibodies (19). In addition, long storage and frequent freeze-thawing of stool samples may denature these antigens on the outer capsid of rotavirus (21). G genotyping by hybridization of RT-PCR products with serotype-specific oligonucleotide probes is better than typing by nested PCR, because the chances of cross-contamination during nested PCR exist even with the utmost care (8).

There is a need for constant monitoring of antigenic diversity among human rotaviruses. This knowledge will be of help in developing an effective vaccine.

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