

Characterization of Rotavirus Strains from Newborns in New Delhi, India

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Between 1986 and 1993, 72% of rotavirus strains isolated from newborns at five hospitals in New Delhi, India, had long electropherotypes, subgroup II VP6 antigens, and G and P genotypes (G₉P₁₁) identical to those of prototype strain 116E. A novel strain with a G₉P₆ genotype, representing 13% of the isolates, was identified. These results demonstrate that G₉P₁₁ and G₉P₆ rotavirus strains are common in nurseries in New Delhi.

Serologic characterization of rotavirus strains indicates that outer capsid proteins VP4 and VP7 independently induce type-specific neutralizing antibodies which have been used to classify rotaviruses into G (VP7) and P (VP4) serotypes (11). Among rotavirus strains isolated from children with diarrhea, four major G serotypes, G₁ to G₄, have been shown to be epidemiologically important by enzyme-linked immunosorbent assay with VP7-specific monoclonal antibodies, but until recently, analogous methods to study the P serotypes of common human rotavirus strains (i.e., G serotypes 1 to 4) were not available (16, 17). As a result, nucleic acid-based (genotyping) methods that detect genetically distinct VP4 genes and accurately predict P serotypes have been developed (8). VP4 genes that are distinct at the level of nucleotide and deduced amino acid sequences have been referred to as P types (or P genotypes) (6). Although a nomenclature for corresponding P serotypes has not been agreed upon, some investigators use the same numbering system for genotypes and serotypes and that is the convention used in this report (6, 14). The largest survey of rotavirus field isolates indicated that genotypes P₈ (strain Wa-like VP4 gene) and P₄ (strain RV5-like VP4 gene) are by far the most common among more than 400 strains from several areas of the world (15), while the largest survey in which both G and P genotypes were determined for the same strain indicated that G₁P₈ (strain Wa-like VP7 and VP4 genes) is the most common in Malaysia (13).

We recently demonstrated that asymptomatic neonatal rotavirus infections of children in New Delhi, India, reduced the frequency of subsequent cases of severe rotavirus diarrhea by 46%, essentially confirming the work of Bishop and coworkers and raising the possibility that strains related to New Delhi isolate 116E may be effective as vaccines (1, 2). Previously isolated neonatal rotaviruses belonged to serotypes G₁P₆ (strain M37), G₂P₆ (strain 1076), G₃P₆ (strain McN13), and G₄P₆ (strain ST3) (9, 12). We subsequently showed by serologic and sequence analyses that prototype strain 116E belongs to serotype G₉ and genotype P₁₁ and has not been previously isolated from humans (4). Since the prototype strains for serotypes G₉ (human isolate WI61, serotype G₉P₈) and P₁₁ (bovine isolate B223, serotype G₁₀P₁₁) have P and G types,

respectively, that are different from those of 116E, it was suggested that the latter strain may be a reassortant (7).

We have recently extended these results and shown that strains related to prototype 116E are present in five of six New Delhi hospitals (3). In this report, we describe the complete characterization of rotavirus strains isolated from newborns at six government hospitals in New Delhi between 1986 and 1988 and 1992 and 1993, as well as isolates previously collected in a longitudinal study by using G and P genotyping by reverse transcription (RT)-PCR, electropherotyping, subgrouping analysis, and nucleotide sequencing.

To screen for genotype P₁₁ rotavirus strains, a specific primer, ND2, that is complementary to nucleotides (nt) 116 to 133 of the strain 116E VP4 gene was synthesized and added to a one-amplification RT-PCR system that detects strains with

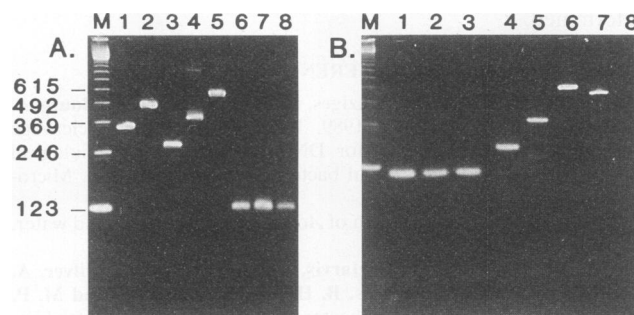


FIG. 1. RT-PCR typing of rotavirus strains. Rotavirus double-stranded RNA was extracted from cell lysates or fecal specimens, and 5 μ l of the eluate was analyzed (8). (A) P typing. Lanes: M, markers (123-bp ladder; Gibco BRL, Long Island, N.Y.; marker molecular sizes are indicated on the left in base pairs); 1 to 5, products amplified from double-stranded RNA from human rotavirus strains possessing P types 8 (lane 1, strain Wa), 4 (lane 2, strain DS-1), 6 (lane 3, strain M37), 9 (lane 4, strain K8), 10 (lane 5, strain M37), and 11 (lane 6, strain 116E); 7 to 8, products from double-stranded RNA extracted from culture-adapted strains (lane 7, strain 113E; lane 8, strain 218D). (B) G typing. Lanes: M, markers (123-bp ladder); 1 to 3, products amplified from double-stranded RNA of human rotavirus G serotype 9 (lane 1, strain 116E; lane 2, strain F45; lane 3, strain WI61); 4 to 8, products amplified from field isolates from Costa Rica possessing serotype G₁ to G₄ specificities as determined by enzyme-linked immunosorbent assay serotyping with monoclonal antibodies.

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TABLE 1. Electropherotypes, G and P genotypes, and subgroups^a of rotaviruses isolated from newborns in New Delhi

Hospital	Year(s)	No. of strains tested	No. of strains with the following G genotype, P genotype, and subgroup ^b							
			G ₂ P ₄ SG ₁	G ₉ P ₁₁ SG ₁₁	G ₉ P ₁₁ SG ₇ ^c	G ₉ P ₆ SG ₁₁	G ₉ P ₆ SG ₇ ^c	G ₃ P ₁₁ SG ₁₁	DT ^d	Other ^e
A	1986–1988	21 ^f	1	18	0	0	0	2	0	0
A	1992	7	0	6	1	0	0	0	0	1
A	1993	9	0	0	0	9	1	0	0	0
B	1992	7	0	4	0	0	0	0	3	0
B	1993	3	0	2	1	1	0	0	0	0
C	1993	16	0	12	0	0	0	0	4	0
D	1993	5	0	5	0	0	0	0	0	0
E		7	0	5	1	0	0	0	1	1
Totals		75	1	49	3	9	1	2	8	2

^a Subgroups of rotavirus antigen-positive fecal specimens, as determined by the Rotaclone test (Cambridge Biosciences, Cambridge, Mass.), were determined as described previously, with antibodies provided by H. Greenberg (10).

^b Of the 67 strains tested, 64 had similar long electropherotypes and the lone SG₁ strain had a short electropherotype.

^c SG₇ indicates that the strain could not be subgrouped.

^d DT, dual PCR type, i.e., presence of two DNA products, consistent with the presence of two different genotypes. P₆P₁₁ dual types were verified by restriction endonuclease analysis of both products (data not shown).

^e One strain from hospital A was non-G-nongenotypeable, while the P-typing reaction for one isolate from hospital E was omitted.

^f Culture-adapted strains; all of the other strains tested were isolated directly from fecal specimens.

genotypes P₄ (e.g., strain DS-1 like), P₆ (e.g., strain M37 like), P₈ (e.g., strain Wa like), P₉ (e.g., strain K8 like), and P₁₀ (e.g., strain 69M like). The procedures for RNA extraction and amplification were identical to those described previously, except that 40 PCR cycles were used and the extension time was 3 min at 72°C (8).

For G genotyping (detection of VP7 genes by RT-PCR), a consensus primer, 9 con 1, whose sequence is conserved among the VP7 genes of serotypes G₁ to G₄ and G₉ (GenBank accession numbers, K02033, M11164, U04350, M21650, and L14072) and five genotype-specific primers complementary to variable regions of the VP7 genes of the same serotypes were synthesized and used in an RT-PCR system analogous to that used for P genotyping. The nucleotide positions, strain and serotype specificities, polarities (plus or minus), and sequences (from 5' to 3') of type-specific complementary primers 9T1-1, 9T1-2, 9T-3P, 9T-4, and 9T-9B are as follows: 9 Con 1, nt 37 to 56, Wa, plus sense, and TAGCTCCTTTTAATGTATGG; 9T1-1, nt 176 to 195, Wa, G₁, minus sense, and TCTTGT CAAAGCAAATAATG; 9T1-2, nt 262 to 281, S2, G₂, minus sense, and GTTAGAAATGATTCTCCACT; 9T-3P, nt 484 to 503, 107E1B, G₃, minus sense, and GTCCAGTTGCAGTGT AGC; 9T-4, nt 423 to 440, ST3, G₄, minus sense, and GGGTCGATGGAAAATTCT; 9T-9B, nt 131 to 147, 116E, G₉, minus sense, and TATAAAGTCCATTGCAC. The expected molecular sizes of the RT-PCR products of the primer-pair consisting of 9 con 1 and 9T1-1, 9T1-2, 9T-3P, 9T-4, or 9T-9B were 158, 244, 466, 403, or 110 bp, respectively. The strain specificities of the P₁₁- and G₉-specific RT-PCR primer pairs are shown in Fig. 1A and B.

A summary of the characteristics of 75 strains from newborns is presented in Table 1. The type designations of the strains identified here by genotyping conform to the suggestions of Estes and Cohen (6). G and/or P serotype designations have only been given to strains analyzed by cross-neutralization tests. Nucleotide sequence analysis of variable regions of the VP4 and VP7 genes of two strains from newborns demonstrated that our RT-PCR typing method accurately predicted their G and P genotypes (7).

Recently, we reported the isolation of novel rotavirus strains from newborns with serotype G₉ and genotype P₁₁ specificity (4). These findings have since been extended to five other hospitals in New Delhi where strains related to prototype

strain 116E were detected (3). In this report, we more completely characterized all of the strains isolated in the 1993 study, as well as strains isolated from two of the hospital nurseries between 1986 and 1992. Our results strongly suggest that genotype P₁₁ strains are common in New Delhi. A second novel strain from newborns—genotype G₉P₆—identified at two of the hospitals completely replaced the genotype G₉P₁₁ strains found in hospital A between 1992 and 1993. Although the genotype P₆ VP4 gene of these strains is related to those of previously identified strains from newborns (e.g., Venezuelan strain M37, serotype G₁P₆), genotype G₉P₆ strains have not been isolated before (9). It is possible that these strains arose by reassortment, since we found that isolates with dual types (types P₆ and P₁₁ and G₁ and G₉) were isolated from the same infants in hospital B in 1992, and we subsequently identified genotypes G₉P₆ and G₉P₁₁ cocirculating in 1993 at hospital B. Regardless of their origin, the isolation of three different, unique rotaviruses from newborns in New Delhi and Bangalore suggests that additional surveillance should be conducted to determine the prevalent strains from newborns in other areas of India and to investigate if any of these novel rotaviruses are common in children with diarrhea (3, 5). These studies will be important to assess the possible utility of such strains as vaccine candidates.

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