Surveillance of rotavirus strains was first established in the United States in 1987, through the analysis of G and P types of isolates obtained in seven sentinel locations, mostly during multiple consecutive rotavirus seasons extending from 1987 to 1993 (4, 14). Subsequently, this initial surveillance was expanded to 37 centers participating in a system of national reporting on year-round rotavirus activity in order to assess geographic and temporal trends, but without monitoring strain types (8, 15).

The composition and distribution of rotavirus types may vary in distinct geographic and socioeconomic regions of the world, as demonstrated in a study that compared data obtained in the United States and in Brazil as models for the epidemiology of rotavirus in temperate, developed and tropical, developing countries, respectively (6). In this context, it was of interest to verify whether the recent data reported by Ramachandran et al. (12) were still in accordance with those obtained by Gouvea et al. (4) and Santos et al. (14) for the United States in the previous decade. Indeed, the major epidemiological characteristics were confirmed: an overwhelming prevalence of conventional strains (83% in the recent season versus 95% in previous seasons) with a predominance of P[8]G1 strains (66 versus 71%) and very low proportions of mixed infections (2.3 versus 3%) were found in the continental United States. Differences in the sensitivities of the assays employed in the two studies might have accounted for the small difference found in the efficiencies of typing rotavirus strains (94.5% in the recent study versus 100% in the old study). Nevertheless, contrary to Ramachandran et al.’s statement, a few strains bearing the supposedly attenuated P[6] specificity had already been identified in association with infant diarrhea in the United States (14). Some of those isolates had been further sequenced and characterized, including the oldest known and cell culture-adapted American P[6] strain, SC2 (13).

Of interest in Ramachandran et al.’s study was the finding of a small percentage (7.2%) of strains of the G9 type in several Midwestern states. Remarkably, however, G9 strains were not detected in the more populated region of Philadelphia, where it had circulated a decade ago (1, 12). Following the first identification of rotavirus type G9 in the developed world (prototype strain WI61 in Philadelphia, Pa., and strain F45 in Japan), it was also recognized as a cause of infant diarrhea in the developing world (strain Mc323 in Chiang Mai, Thailand, and an unnamed strain in Belém, Brazil) (1, 10, 11, 16). Molecular analysis revealed very high genomic identity between strains WI61 and F45, suggesting that they might represent the same emerging virus that had been successfully introduced into the human population (5, 7). Most recently, genetically distinct G9 strains were identified as common human rotaviruses in India and frequent swine pathogens in Southern Brazil (2, 9), thus supporting the suggestion that G9 strains might have emerged in the human population from animal reservoirs by natural reassortment (3, 16).

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lack of routine detection methods for G9 strains. Alternatively, since these strains have been detected in humans from a number of countries, including Japan (6, 10, 11), India (12), Bangladesh (15), Malawi (3), and Thailand (16), and only once before in the United States (2), the new finding that this strain was prevalent in multiple U.S. cities could represent either the introduction of a new strain or the emergence of that earlier isolate. The data presented by Ramachandran et al. (13) were consistent with the emergence of a distinct strain closely related to subgroup I human rotavirus strains with short electrophoretotypes, and consequently, we did not discuss a potential animal origin for these strains, a possibility that was raised in a study of G9 strains from Thailand (16). A more recent study on the antigenic and genetic properties of several of these strains supports the hypothesis that the recent U.S. P[6],G9 isolates are closest genetically to human rotaviruses of the DS-1 genotype (8). Although we did not exhaustively cite all the studies documenting the global importance of serotypes G1 to G4, we did cite representative papers from the United States (9, 18), Japan (17), and Italy (5).

A second conclusion of our study was that strains bearing the P[6] genotype had been detected relatively frequently in multiple U.S. cities for the first time. Previously, this genotype had been detected frequently among infants with diarrhea only in developing countries, for which we cited references from Timenetsky and coworkers from a study in Brazil and from Ramachandran and coworkers in studies from India (12, 14). Regarding the failure to detect G9 strains in Philadelphia, where the first U.S. isolates were identified (2), we are currently involved in targeted studies in Philadelphia examining strains from both an earlier period (1995 to 1996) and a later period (1997 to 1998). G9 strains have been detected during both periods (1, 7), and many of the strains appear to be related to the predominant strain from 1996 to 1997 (13). We anticipate that both of these studies will be submitted for publication in 1999.

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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 (GpPp) identical to those of prototype strain 116E. A novel strain with a GpPp genotype, representing 13% of the isolates, was identified. These results demonstrate that GpPp and GpPp 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 P9 (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 GpPp (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 allowing the possibility that strains related to New Delhi isolate 116E may be effective as vaccines (1, 2). Previously isolated neonatal rotaviruses belonged to serotypes GpPp (strain M37), GpPp (strain McN13), and GpPp (strain ST3) (9, 12). We subsequently showed by serologic and sequence analyses that prototype strain 116E belongs to serotype Gp and genotype P11, and has not been previously isolated from humans (4). Since the prototype strains for serotypes Gp (human isolate Wf61, serotype GpPp) and P11 (bovine isolate B223, serotype GpPp) 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 μl 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 K9), 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 Wf61); 4 to 8, products amplified from field isolates from Costa Rica possessing serotype G2, to Gp specificities as determined by enzyme-linked immunosorbent assay serotyping with monoclonal antibodies.

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TABLE 1. Electropherotypes, G and P genotypes, and subgroup* of rotaviruses isolated from newborns in New Delhi

<table>
<thead>
<tr>
<th>Hospital</th>
<th>Year(s)</th>
<th>No. of strains tested</th>
<th>G1P1SG1</th>
<th>G1P1SG11</th>
<th>G2P1SG1</th>
<th>G2P1SG11</th>
<th>G3P4SG2</th>
<th>G4P1SG11</th>
<th>DT</th>
<th>Other</th>
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<tr>
<td>A</td>
<td>1986-1988</td>
<td>21</td>
<td>1</td>
<td>18</td>
<td>0</td>
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<td>2</td>
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<tr>
<td>A</td>
<td>1992</td>
<td>7</td>
<td>0</td>
<td>6</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>1992</td>
<td>9</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<td>1</td>
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</tr>
<tr>
<td>B</td>
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<td>7</td>
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<td>4</td>
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<td>0</td>
<td>3</td>
<td>0</td>
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</tr>
<tr>
<td>C</td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

Totals 75 1 49 3 9 1 2 8 2

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

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

** SG1 indicates the isolate could not be subgrouped.

** DT, dual PCR type, i.e., presence of two DNA products, consistent with the presence of two different genotypes. P11, dual types were verified by restriction endonuclease analysis of both products (data not shown).

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

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

geneotypes P2 (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 P11 (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 con1, whose sequence is conserved among the VP7 genes of serotypes G1 to G12 and G45 (GenBank accession numbers, K02033, M11614, 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, 9T3-3, 9T4, and 9T9B are as follows: 9 Con1, nt 37 to 56, Wa, plus sense, and TAGCTCTTITTTAATGTATGG; 9T1-1, nt 176 to 195, Wa, G1, minus sense, and TCTGTGCAAGCAAATAATGT Tag; 9T1-2, nt 262 to 281, S2, G2, minus sense, and GTTTAGAATTGCCTCCTCAG; 9T3-3, nt 484 to 503, 107E1B, G45, minus sense, and GTCCATGCAGCTGTAG; 9T4, nt 423 to 440, ST3, G45, minus sense, and GGTCGATGAAAATCT; 9T9B, nt 131 to 147, 116E, G45, minus sense, and TATAAGGCATTGAC. The expected molecular sizes of the RT-PCR products of the primer pairs consisting of 9 con1 and 9T1-1, 9T1-2, 9T3-3, 9T4, or 9T9B were 158, 244, 466, 403, or 110 bp, respectively. The strain specificities of the P11- and G45-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 G4 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 G9P6 strains found in hospital A between 1992 and 1993. Although the genotype G9 P4 VP7 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 P9 and P11 and G1 and G45) 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 rotavirus strains 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 rotavirus strains 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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