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The G and P genotypes of rotavirus stool isolates from 100 children were determined by reverse transcription-PCR and nucleotide sequencing. G1P[4] was the most prevalent genotype (41%), followed by G1P[8] (16%) and G4P[4] (14%). The G genotypes detected were G1 (73%), G4 (17.4%), G9 (6.3%), and G2 (2.8%). The P genotypes were P[4] (71%) and P[8] (29%). Coinfection with more than one G genotype occurred in 12 patients, and coinfection with more than one P genotype occurred in 11 patients.

Rotavirus (RV) is a major cause of gastroenteritis among young children worldwide and is associated with significant morbidity and mortality, particularly in developing countries (3). RV has an 11-segment double-stranded RNA genome and a triple-layer protein capsid. The outer layer of group A RV is composed of two proteins: VP4, which determines the P serotype; and VP7, which determines the G serotype (4). Reverse transcription-PCR (RT-PCR) and/or DNA sequencing has been used to define G and P types (5, 7). P specificity is more conservative than G specificity, with P[8] the most common type worldwide, followed by P[4] and P[6]. P[8] is most frequently associated with serotypes G1, G3, and G4, while P[4] is most frequently associated with G2 (6, 11).

Because antibodies to G protein constitute the major neutralizing antibodies, RV vaccines contained G1, G2, G3, and G4, the most common serotypes worldwide. The first oral RV vaccine (Rotashield) was clinically effective in five global trials (12, 15, 18, 20). However, it was withdrawn because of possible causative relationship to intussusception in infants (14). A new oral pentavalent human-bovine reassortant vaccine was licensed in 2002. Although this vaccine protects against several infections, the increased recognition of new serotypes has led to the requirement for improved surveillance of new RV genotypes. The VP4 genotypes detected were only P[4] (71%) and P[8]

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G3 genotype, one of the most common worldwide, was not detected in any stool sample. P[4] was the most prevalent P genotype. G1P[4], which is reported in the United States and other parts of the world at low frequencies (2, 8, 9, 16), was the most prevalent genotype in our patients (41%).

Recent Centers for Disease Control and Prevention (CDC) surveillance studies showed that the most frequent RV G types in the United States were G1 and G2, followed by G9 (8). G3 and G4 were detected less frequently and sporadically. Detroit was not included in these CDC studies. Our data show that G1 and G4 were the most frequent causative strains (73.4 and 17.4%, respectively). G9 accounted for 6.3%. This was higher than the most recent national average rate of 3%, but was not significantly different ($P = 0.11$) (8). The most common RV strains that have been globally associated with gastroenteritis are G1P[8] (53%), G3P[8] (5.4%), G4P[8] (14.3%), and G2P[4] (10.7%) (6, 11). These strains are significantly under-represented in our area at rates of 16, 0, 1, and 3%, respectively ($P < 0.05$ for all). The G1P[4] genotype has never been reported as the most frequent circulating RV genotype in any other part of the world. The wide geographic area in which our patients lived and the detection of G1P[4] throughout the study period indicate that G1P[4] cases were not the result of horizontal transmission from the same index case. Considering the ethnic diversity in Detroit, it is also possible that G1P[4] reassortment may have emerged through the introduction of G2P[4] strains from other parts of the world.

In general, G9 strains have been associated with P[6], P[8], P[11], or P[19] (1, 8, 19, 21). In our patients, G9 serotypes were associated with P[4] or a combination of P[4] and P[8]. No G9-only serotypes were detected in our patients; all G9 serotypes were identified in combination with G1 serotypes. This indicates that G9 genotype may undergo constant reassortment in nature. Our results support the need for continuous surveillance of rotavirus G and P types. The increased prevalence of G9 in our community raises the possibility that G9 may represent an emerging strain that could escape vaccine-induced immunity and become more prevalent. The efficacy of the new pentavalent vaccine against reassorted virus variants involving G9 and one of the vaccine G types is still to be determined.

### TABLE 1. Frequency of RV G and P genotypes isolated from 100 stool samples

<table>
<thead>
<tr>
<th>Genotype&lt;sup&gt;a&lt;/sup&gt;</th>
<th>% of Stool samples with genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1/P4</td>
<td>41</td>
</tr>
<tr>
<td>G1/P8</td>
<td>16</td>
</tr>
<tr>
<td>G4/P4</td>
<td>14</td>
</tr>
<tr>
<td>G1/P4/P8</td>
<td>7</td>
</tr>
<tr>
<td>G9/G1/P8</td>
<td>3</td>
</tr>
<tr>
<td>G9/G1/P4</td>
<td>3</td>
</tr>
<tr>
<td>G1/G2/P4</td>
<td>3</td>
</tr>
<tr>
<td>G4/P4/P8</td>
<td>2</td>
</tr>
<tr>
<td>G1/G4/P4</td>
<td>1</td>
</tr>
<tr>
<td>G1/G4/P4/P8</td>
<td>1</td>
</tr>
<tr>
<td>G9/G1/P4/P8</td>
<td>1</td>
</tr>
<tr>
<td>G4/P8</td>
<td>1</td>
</tr>
<tr>
<td>G (ND)/P4</td>
<td>3</td>
</tr>
<tr>
<td>G1/P (ND)</td>
<td>4</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>100</td>
</tr>
</tbody>
</table>

<sup>a</sup> ND, not determined.

(29%). Dual P[4] and P[8] genotypes were detected in 11 patients. The distribution of G and P genotypes is shown in Fig. 1. In six samples, a signal for a common VP4 gene was detected, but no product was obtained in a second round of PCR to identify the specific P genotype. All of these samples were confirmed by direct DNA sequencing and BLAST analysis to have the P[8] genotype. In all of these P[8] isolates, similar nucleotide sequences were found in the nucleotide region 339 to 356 (P[8] primer binding site). However, mismatches were found between the P[8]-specific primer (1T-1) and its complementary region of the VP4 cDNA. These mismatches were found at four positions corresponding to nucleotides 342 (G:C), 345 (A:C), 347 (A:C), and 350 (T:C). This would explain the failure to detect the VP4 P[8] genotype by RT-PCR in this study.

Representative PCR products of G and P types including P[4] were verified by nucleotide sequencing. BLAST analysis of all of our genotypes showed 94 to 98% homology with reference strains (data not shown).

Of all RV isolates recovered from the 100 children, G1 was the predominant genotype, followed by G4, G9, and G2. The G3 genotype, one of the most common worldwide, was not detected in any stool sample. P[4] was the most prevalent P genotype. G1P[4], which is reported in the United States and other parts of the world at low frequencies (2, 8, 9, 16), was the most prevalent genotype in our patients (41%).

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![Rotavirus G-types](image1)

![Rotavirus P-types](image2)

**FIG. 1.** Distribution of RV G and P genotypes isolated from 100 stool samples. ND, not determined.
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REFERENCES