VP4 and VP7 Genotyping by Reverse Transcription-PCR of Human Rotavirus in Mexican Children with Acute Diarrhea

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Rotavirus is a worldwide cause of severe diarrhea in young children. Genetic or antigenic differences in VP4 (P) and VP7 (G) capsid proteins have been detected in viral isolates (11). Since VP4 and VP7 genes segregate independently, a binary system is used to classify rotavirus (12). There are certain genotypes that circulate with higher frequencies: P1 to P4 for VP4 and G1 to G4 for VP7 (5, 10, 15, 21). The combinations P1G1, P1G3, P2G2, and P1G4 are the most frequent in Brazil, Israel, Japan, South Africa, and the United States (16, 17, 18, 20). However, relative rates of these combinations are different in countries like India and Egypt, where unusual combinations of P and G alleles are also present (2, 8, 14). Rotavirus strains with different combinations may occur simultaneously during seasonal outbreaks, giving rise to the possibility of mixed infections and genetic rearrangements (3, 6, 9, 22).

In this study we performed dual typing of rotavirus strains gathered from eight laboratories included in the Mexican National Network for Rotavirus Diagnosis. The study encompassed three consecutive rotavirus epidemic seasons from July 1994 to June 1997 in several states of Mexico. Stool specimens from 257 children less than 5 years old were received by the reference center (National Institute of Diagnostics and Epidemiological Reference). VP4 (P1 to P4) and VP7 (G1 to G4) genotyping was carried out independently by heminested reverse transcription-PCRs as previously described (4, 7).

From all 257 samples a 1,062-bp product of the VP7 gene was observed, which corresponds to rotavirus group A (Fig. 1). For VP4, the expected 876-bp amplicon was obtained (not shown). The second amplification products of both genes can be seen in Fig. 1 and 2, in which some examples of mixed infections are also shown. One hundred two samples (39.7%) were of the P1G1 genotype. 49 (19.0%) were P1G3, and 42 (16.3%) were P2G2. Genotypes P4 and G4 were not found. This is the first dual typing of isolates from diarrhea cases in Mexico.

FIG. 1. Amplification products of the VP7 gene. Lanes 1 and 2, first amplification products (1,062 bp); lanes 3 through 5, genotype G1 (749 bp); lanes 6 and 7, genotype G2 (652 bp); lanes 8 through 10, genotype G3 (347 bp); lanes 11 and 12, mixed infections with the G1G3 and G1G2 genotypes, respectively. 6X174 digested with HaeIII (Roche Molecular Biochemicals) was used as the molecular size (M) standard.
increasing prevalence was observed beginning with the second period (Fig. 3). The seasonal shifts in genotype frequencies might be due to different factors, such as genetic variation of rotavirus strains, host range modifications, host immunity, and climate changes (1, 6, 9, 11, 22).

The genetic variation may also be a consequence of simultaneous infection of a single host with different rotavirus strains (3, 6, 19, 22). In this work, 31 samples showed more than one P or G allele, meaning that more than one rotavirus strain was present. The most frequent mixed infection was genotype P1G1G3, which suggests the simultaneous presence of P1G1 and P1G3 rotavirus strains. This result correlates with the high prevalence of those genotypes (Table 1). Mixed infections have also been reported in Brazil (30%), India (6%), the United States (3%), South Africa (3%), and Japan (1%). In this study, Mexico had the second highest percentage (12%) of dual infections reported so far, increasing the likelihood of genetic reassortment, which may yield new gene combinations (2, 3, 9, 15, 20).

Genotypes of rotavirus strains with unusual combinations of P and G alleles were found in lower frequencies as follows: P1G2, 1.9%; P2G1, 3.1%; and P2G3, 1.5% (Table 2). Genotype P2G1 occurred in Yucatan, Colima, Nuevo Leon, and Puebla, while P1G2 was restricted to Colima and Yucatan and P2G3 was present only in Michoacan, Nuevo Leon, and Yucatan. These combinations could have been generated from genetic rearrangements of the most frequent rotavirus genotypes, since they circulated simultaneously in those regions. It is worth mentioning that genotype P2G1 has also been reported at low frequencies (1%) in Brazil and Japan, while P1G2 and P2G3 have not been reported in other countries (8, 10, 19, 20, 21).

Partially untyped samples were also found in this study. In three samples (1.2%), two P1 and one P2 G allele could not be typed, while the P allele was not identified in 13 samples (5%) with the following G alleles: nine G1, one G3, one G1G2 (mixed infection), and two G1G3. This suggests that P alleles different from P1 to P4 segregated with G1 and G3. Thus, there is a larger heterogeneity of P alleles than of G alleles in Mexican field strains. This was also found with monoclonal antibody-based typing (13) and could be due to a higher selective pressure exerted over VP4, since it is the most external viral protein (9, 11). G alleles seemed to be more conserved, because mainly G1, G2, and G3 were found (10, 14, 15, 16, 17, 18).

### Table 1. Distribution of VP4 and VP7 genotypes of human rotaviruses in different States of Mexico

<table>
<thead>
<tr>
<th>State and yr studied</th>
<th>No. of samples</th>
<th>% of each genotype found&lt;sup&gt;a&lt;/sup&gt;</th>
<th>P1G1</th>
<th>P1G2</th>
<th>P1G3</th>
<th>P2G1</th>
<th>P2G2</th>
<th>P2G3</th>
<th>MI&lt;sup&gt;b&lt;/sup&gt;</th>
<th>NT&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colima, 1995–1997</td>
<td>19</td>
<td>47.4</td>
<td>10.5</td>
<td>5.3</td>
<td>10.5</td>
<td>10.5</td>
<td>0</td>
<td>5.3</td>
<td>10.5</td>
<td></td>
</tr>
<tr>
<td>Mexico City, 1995–1997</td>
<td>99</td>
<td>52.5</td>
<td>0</td>
<td>22.2</td>
<td>1.1</td>
<td>5.5</td>
<td>0</td>
<td>11.1</td>
<td>8.0</td>
<td></td>
</tr>
<tr>
<td>Michoacan, 1994–1996</td>
<td>25</td>
<td>68.0</td>
<td>0</td>
<td>20.0</td>
<td>0</td>
<td>4.0</td>
<td>4.0</td>
<td>0</td>
<td>4.0</td>
<td></td>
</tr>
<tr>
<td>Nuevo Leon, 1996</td>
<td>24</td>
<td>8.3</td>
<td>0</td>
<td>8.3</td>
<td>4.2</td>
<td>66.7</td>
<td>8.3</td>
<td>0</td>
<td>4.2</td>
<td></td>
</tr>
<tr>
<td>Puebla, 1994–1996</td>
<td>28</td>
<td>17.9</td>
<td>0</td>
<td>35.7</td>
<td>3.6</td>
<td>10.7</td>
<td>0</td>
<td>32.1</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Quintana Roo, 1997</td>
<td>18</td>
<td>61.1</td>
<td>0</td>
<td>5.5</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>22.2</td>
<td>11.1</td>
<td></td>
</tr>
<tr>
<td>Veracruz, 1995–1996</td>
<td>21</td>
<td>23.8</td>
<td>0</td>
<td>38.0</td>
<td>0</td>
<td>4.8</td>
<td>0</td>
<td>24.8</td>
<td>9.5</td>
<td></td>
</tr>
<tr>
<td>Yucatan, 1997</td>
<td>23</td>
<td>4.3</td>
<td>13.0</td>
<td>0</td>
<td>13.0</td>
<td>60.8</td>
<td>4.3</td>
<td>4.3</td>
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<tr>
<td>Total</td>
<td>257</td>
<td>39.6</td>
<td>1.9</td>
<td>19.0</td>
<td>3.1</td>
<td>16.3</td>
<td>1.5</td>
<td>12.0</td>
<td>6.2</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> Numbers in bold are the highest values for that state.

<sup>b</sup> MI, mixed infection.

<sup>c</sup> NT, not typed.
TABLE 2. Mixed infections and untyped samples found in Mexico between 1994 and 1997

<table>
<thead>
<tr>
<th>Genotype of infecting strain</th>
<th>No. of samplesa</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mixed infection</strong></td>
<td></td>
</tr>
<tr>
<td>P1P2G1</td>
<td>5</td>
</tr>
<tr>
<td>P1P2G2</td>
<td>2</td>
</tr>
<tr>
<td>P1P2G3</td>
<td>7</td>
</tr>
<tr>
<td>P1P2G1G2</td>
<td>1</td>
</tr>
<tr>
<td>P1P2G1G3</td>
<td>1</td>
</tr>
<tr>
<td>P1G1G2</td>
<td>11</td>
</tr>
<tr>
<td>P1G1G2G2</td>
<td>2</td>
</tr>
<tr>
<td>P2G1G2G3</td>
<td>1</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>31 (12.0)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Untyped strainsb</strong></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>P1Gnt</td>
<td>2</td>
</tr>
<tr>
<td>P2Gnt</td>
<td>1</td>
</tr>
<tr>
<td>G1Pnt</td>
<td>9</td>
</tr>
<tr>
<td>G3Pnt</td>
<td>1</td>
</tr>
<tr>
<td>G1G2Pnt</td>
<td>1</td>
</tr>
<tr>
<td>G1G3Pnt</td>
<td>2</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>16 (6.2)</td>
</tr>
</tbody>
</table>

a Numbers in parentheses are percentages of the total samples studied (n = 257).
b nt, not typed.

These data support the need for including a larger set of primers to identify other P and G alleles. In conclusion, our results confirm that there is an important variability among rotavirus strains in Mexico. This is the first report of dual typing, which allowed for identifying one of the main sources of this variability, i.e., the presence of mixed infections.

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Araeili Rodríguez Castillo and Andrés Velasco Villa contributed equally to this work.

REFERENCES