Identification and Type Distribution of Astroviruses among Children with Gastroenteritis in Colombia and Venezuela

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Astrovirus infections were detected by enzyme immunoassay in 12 (5%) of 251 stool samples from children with gastroenteritis from Bogota, Colombia. In addition, astroviruses were detected by reverse transcription-PCR in 3 (10%) of 29 stool samples negative for other enteric pathogens collected in Caracas, Venezuela, from children with gastroenteritis. Astrovirus type 1 was the most frequently detected virus.

Astroviruses were first associated with infantile gastroenteritis in 1975 by two independent investigators (9). However, it is only recently, after improvements in diagnostic methods, that the role of astroviruses as etiological agents of infantile gastroenteritis has started to be fully appreciated (5). Astroviruses are small nonenveloped viruses with a positive-sense single-stranded RNA genome (9). They have been classified in the family Astroviridae, and other members of the family include viruses of vertebrates and birds (9). In developed countries, astroviruses have been associated with 4 to 10% of endemic diarrheal episodes in children (3, 14, 15, 17); studies of astroviruses in developing countries have found comparable prevalences (4, 6, 16, 18). However, up to 26% of all diarrheal episodes were associated with astroviral infection in a study conducted in a semiclose Mayan community (8). Astroviruses have also been associated with outbreaks of diarrhea in children (11) and in adults (1). In addition, in immunocompromised persons, astroviruses have been reported as the most common viruses detected in patients with diarrhea (5).

Diarrheal infections are among the most common illnesses affecting children under 4 years of age in Colombia and Venezuela. While the importance of rotaviruses as a causal agent of gastroenteritis has been well established in these countries (2, 19), little is known of the importance of other enteric viruses in the etiology of acute diarrhea. In this work, we evaluated the presence of astroviruses in stool samples from children with acute gastroenteritis from Bogota, Colombia, and Caracas, Venezuela, and identified the circulating serotypes.

A total of 251 fecal samples were collected from 251 children with acute diarrhea who sought care in 16 emergency rooms in Bogota between June 1997 and February 1999. All children were under 4 years of age, and samples were collected within 72 h after the onset of symptoms. In addition, 29 selected samples from Caracas, collected from children with acute diarrhea between October 1994 and March 1995, were also tested. These samples were chosen because they were previously known to be negative for rotavirus, enteropathogenic bacteria, and enteropathogenic parasites (19). Samples were stored at −20°C until processed.

All samples collected in Bogota were tested with a commercial astrovirus antigen-detection enzyme immunoassay (EIA) (IDEIA Astrovirus; Dako Diagnostics, Ltd., Ely, United Kingdom). Positive samples were restested at least twice by EIA and further confirmed by reverse transcription (RT)-PCR by using previous cultures of the clarified samples grown in Caco-2 cells for 48 h as described by Mustafa et al. (12). RNA was isolated from cultures (TRIAZOL; Gibco BRL, Gaithersburg, Md.), and the RT-PCR was carried out in one tube with a commercial kit (RT-PCR Access; Promega, Madison, Wis.) with the astrovirus-specific primers Mon 269 and Mon 270 (13). To determine the astrovirus types, the RT-PCR products were sequenced after purification directly with an ABI Prism 377 automatic sequencer, and the sequences were compared to prototype strains in a database. DNA sequences were analyzed by DNAMan version 3.2 (Lynnon Biosoft) to produce homology and phylogenetic trees. Phylogenetic analyses were based on a 348-nucleotide sequence within the 449-bp PCR products (13). Phylogenetic trees were constructed by the neighboring method. Samples collected in Caracas were tested for astrovirus only by RT-PCR. In addition, 50 samples which gave negative results by EIA were chosen at random and tested for astrovirus by RT-PCR. The Chi-square test was used to compare prevalence rates among children in different age groups.

Astrovirus was detected by EIA in 12 (5%) of the 251 samples collected in Bogota. The positive samples were collected in 4 of the 16 emergency rooms studied. Viruses were detected in children 7 to 36 months of age, but prevalence rates were significantly higher (P < 0.01) in children who were 7 to 18 months of age (Table 1). Ten of the samples positive by EIA could be amplified by RT-PCR after cell culture, and the sequence of the RT-PCR products could be determined. Comparison with the sequence of reference astrovirus serotypes in GenBank indicated that five of the Colombian samples could be classified as type 1, three as type 2, and one each as type 3 and type 4 (Fig. 1). From the 29 selected samples from Caracas, 3 (10%) were found to be positive for astrovirus by RT-PCR. Partial sequencing of the RT-PCR products allowed one of the samples to be classified as type 1 (Fig. 1). No positive samples were detected by RT-PCR among the 50 EIA-negative samples chosen at random.

A phylogenetic tree was constructed to study the relationship between the astroviruses isolated in this study and other astroviruses (n = 17) isolated elsewhere (Fig. 1). The Colombian type 1 samples showed up to 7% (23 bases) sequence diversity among themselves and up to 9% sequence diversity with sample Ven835. Based on these sequence variations,
TABLE 1. Age distribution of astrovirus infection in children from Bogota with acute diarrhea

<table>
<thead>
<tr>
<th>Age group (mo)</th>
<th>No. of samples tested</th>
<th>No. (%) of positive samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–6</td>
<td>47</td>
<td>0</td>
</tr>
<tr>
<td>7–12</td>
<td>63</td>
<td>6 (10)</td>
</tr>
<tr>
<td>13–18</td>
<td>20</td>
<td>3 (15)</td>
</tr>
<tr>
<td>19–24</td>
<td>53</td>
<td>1 (2)</td>
</tr>
<tr>
<td>25–36</td>
<td>46</td>
<td>2 (4)</td>
</tr>
<tr>
<td>37–50</td>
<td>22</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>251</td>
<td>12 (5)</td>
</tr>
</tbody>
</table>

* Prevalence rates were significantly higher in the age groups 7 to 12 and 13 to 18 months combined (P < 0.01).

![Phylogenetic tree](image)

FIG. 1. Phylogenetic tree showing the genetic relatedness of astrovirus strains isolated in Colombia and Venezuela to the Oxford astrovirus reference strain of the eight known serotypes and strains from Australia, the United Kingdom, and Venezuela. Strains from the same geographic area diverging less than 1.0% were not included. Bootstrap values, expressed as percentages of 500 replications, are given at the branch points. Strains were arbitrarily named: Oxf, Oxford; Aus, Australia; UK, United Kingdom; Ven, Venezuela. Sequences of strains used for comparison were obtained from the GenBank database (accession no.: Oxford strains, L25513, L13745, L38505, L38506, U51538, L38507, L38508, and Z66541; Australian strains, AF175254, AF175257, U49216, and U49218; United Kingdom strains, S68561 and Z33883; Venezuelan strains, AF211952 and AF211953).

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