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

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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 semi-close 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 Bogotá, 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 Bogotá 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 Bogotá were tested with a commercial astrovirus antigen-detection enzyme immunoassay (EIA) (IDEIA Astrovirus; Dako Diagnostics, Ltd., Ely, United Kingdom). Positive samples were retested 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 Bogotá. 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).

three lineages, showing at least 7% sequence diversity, among the type 1 samples isolated could be identified. One lineage, represented by samples 526, 509, and 418, which showed identical sequences, also included samples from Australia; another lineage, represented by sample 503, also included samples from Australia and Europe; the third lineage was represented by sample Ven835 alone. The observed nucleotide sequence changes resulted in amino acid substitutions in relation to the astrovirus serotype 1 Oxford reference strain: samples Col526, Col509, and Col418 differed at position 191 (Ile→Trp) of open reading frame 2 (ORF 2), while sample Ven835 differed at position 92 (Val→Ile). Thus, sample Ven 835 differed at two positions from samples Col526, Col509, and Col418. The 5 base changes found between sample 503 and the Oxford strain were silent. The Colombian type 2 samples showed only 0.6% (2 bases) sequence variation and comprised one lineage, which includes the Oxford serotype 2 reference strain. Samples Col546 and Col664 showed arginine at position 195 of ORF 2, while sample Col673 showed lysine at that position (data not shown). Type 3 sample Col505 and strains from Europe and Venezuela formed only one lineage. Two lineages were found within type 4 samples, one including samples from Colombia, Australia, and Europe, and another represented only by the Venezuelan specimen.

Despite the limited number of samples analyzed, the prevalence rate and age distribution of astrovirus infections observed in Bogota are comparable to those observed for astrovirus infection in other studies (3–6, 14–18). These results suggest that astroviruses may be an important cause of acute gastroenteritis in Colombia. In addition, a study recently conducted in Venezuela failed to identify an enteric pathogen in 41% of the gastroenteritis cases studied (19); our results also suggest that nearly 10% of these undiagnosed gastroenteritis cases could be astrovirus associated. In this study, the highest infection rates were observed in children 6 to 18 months old. The absence of infections observed during the first 6 months of life may be due in part to the presence of transplacental antibodies to astrovirus. The lower infection rates observed in children older than 18 months of age could be due to active immunity from infection, which might confer protection to symptomatic infection even in the presence of several cocirculating types. The results of seroprevalence studies of antibodies to astroviruses conducted in the United States and The Netherlands are consistent with this notion (7, 10). However, much remains to be learned about protective immunity to astrovirus.

The combination of EIAs for sample screening followed by RT-PCR amplification of the positive samples after cell culture (12) allowed identification of the type of more than 70% of the positive samples. Astrovirus type 1 (40% of all infections) was the most frequent type detected, followed by types 2, 3, and 4. Our data are consistent with the type frequency observed by several studies elsewhere (5). Our data also confirmed the genetic variability previously observed among astrovirus types (13, 14). Type 1 astroviruses could be divided into three lineages, and two lineages were observed for type 4 samples. No geographic clustering of the lineages was observed, since the Colombian isolates did not cluster with the Venezuelan strain but with samples from Australia or Europe. However, the specimens of this study showed genetic diversity with predictable amino acid changes for the capsid protein, unlike the results observed by Palombo and Bishop in the Australian specimens (14), in which all mutations were silent. The epidemiological significance of the genetic variations observed among astrovirus types remains to be determined.

**Nucleotide sequence accession number.** The nucleotide sequences determined in this study have been deposited in GenBank and have been assigned accession no. AF211950 to AF211965.

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**REFERENCES**


