Prevalence of Human Astrovirus Genotypes Associated With Acute Gastroenteritis Among Children in Belém, Brazil


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Worldwide human astroviruses (HAstV) have increasingly been recognized as causative agents of viral gastroenteritis, mainly in infants and young children. The aim of this study was to assess the epidemiology and genotype diversity of HAstVs detected in children who participated in a trial in Belém, Brazil with the rhesus human reassortant rotavirus vaccine tetravalent (RRV-TV). From April/1990 to August/1992, 624 diarrheic stool samples were tested by enzyme immunoassay (EIA) for HAstV, with a positive rate of 4.0%. Reverse transcription-polymerase chain reaction (RT-PCR) was done in 129 samples (25 positive and 104 with twice the optical density (OD) value of negative control by EIA) being 33 positive. The overall positivity yielded by both methods was 5.4% (34/624). Genotyping of the 33 positive samples was done by type-specific RT-PCR and confirmed by sequence analysis. Phylogenetic analysis was performed using a 348-bp fragment of the ORF2 region of the capsid gene. HAstV-1 was the most prevalent, accounting for 45.5% of the isolates, followed by HAstV-2 (27.3%), HAstV-3 (12.1%), HAstV-4 (12.1%), and HAstV-6 (3.0%). The monthly distribution showed that HAstV-1 was predominant in the first year of study (May/1990 to May/1991) with highest prevalence in January/1991. HAstV-2 was predominant from July to November/1991 and HAstV-4 from September to October/1990. At 24 months of age, 30.6% of children had been infected by HAstV. The clinical symptoms registered during HAstV-associated diarrhea were usually mild. These data highlight the circulation of the different HAstV genotypes in Belém during the study period.

KEY WORDS: astroviruses; PCR-genotyping; sequencing; infants; diarrhea

INTRODUCTION

Human astroviruses (HAstVs) were first detected in 1975 in stool samples from children with acute gastroenteritis examined by electron microscopy [Appleton and Higgins, 1975; Madeley and Cosgrove, 1975]. With the improvement of the diagnostic methods, including the use of enzyme immunoassay (EIA) [Herrmann et al., 1991; Lew et al., 1991], and the recently developed molecular techniques [Noel et al., 1995; Sakamoto et al., 2000; Schnagl et al., 2002], a significant amount of data has been gathered highlighting the role of HAstV in the etiology of acute infantile gastroenteritis [Jakab et al., 2004].

Epidemiological studies conducted worldwide, have reported the occurrence of HAstVs infection among children hospitalized with diarrhea and in the context of community-based studies. Overall, prevalence rates have ranged from 2% to 16% in hospital-based studies and from 5% to 17% in community-acquired gastroenteritis [Polombo and Bishop, 1996; Walter and Mitchell, 2000; Méndez-Toss et al., 2004]. HAstVs have also been associated with outbreaks of diarrhea in children [Mitchell et al., 1999] and adults [Belliot et al., 1997]. HAstVs have also been recognized as a common cause of nosocomial diarrhea [Unicomb et al., 1998; Rodriguez-Baez et al., 2002] and was shown to be more severe among patients with HIV infection [Cunliffe et al., 2002].

HAstVs belong to the Astroviridae family, genera Mamastrovirus, and their genome is composed of a single-stranded positive sense RNA molecule containing

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Three open reading frames (ORFs). This comprises ORF1a and ORF1b at the 5′ end of the genome, coding for the nonstructural viral proteins, and the ORF2, at the 3′ end, which encodes the capsid precursor protein used to classify these viruses [Clark and McKendrick, 2003]. HAstVs have been classified into eight genotypes based on the nucleotide sequence of ORF2 region [Noel et al., 1995; Matsui and Greenberg, 2001] and they show a good correlation with the eight serotypes determined according to the reactivity of the capsid proteins with type-specific monoclonal antibodies [Sakamoto et al., 2000; Taylor et al., 2001].

Genotyping surveys have shown that HAstV-1 is the most frequent genotype, followed by HAstV-2, -3, -4, and -5 [Lee and Kurtz, 1994; Glass et al., 1996; Mustafa et al., 2000; Sakamoto et al., 2000]. Studies conducted in Brazil demonstrated a similar pattern, with HAstV-1 being predominant, and other genotypes such as HAstV 2–5 and 8 have been less commonly detected [Silva et al., 2001; Cardoso et al., 2002; Gabbay et al., 2005; Silva et al., 2006].

From April/1990 to August/1992, a large placebo-controlled efficacy trial of rhesus-human reassortant rotavirus vaccine tetravalent (RRV-TV) was conducted involving children from Belém, Pará, Brazil. Altogether, 540 infants recruited to participate in this study, received either vaccine doses or placebo at 1, 3, and 5 months of age and were followed-up until 2 years of age. All gastroenteritis episodes were recorded and stool specimens were collected primarily for rotavirus studies. Some of these samples were further used for the purpose of astrovirus studies [Linhares et al., 1996].

The main objective of the present study was to determine the role of HAstVs in the etiology of acute gastroenteritis in children who were enrolled to participate in the RRV-TV trial. We focused mainly on the prevalence of HAstV-related gastroenteritis and used the partial nucleotide sequences of ORF2 to determine the circulating HAstV genotypes.

MATERIALS AND METHODS

Patients and Specimens

The study material consisted of stool specimens collected in episodes of gastroenteritis during a trial with RRV-TV in Belém, North region of Brazil [Linhares et al., 1996] conducted from April/1990 to August/1992. This study was approved by the Ethical Review Committee of the Instituto Evandro Chagas, the Regional Council of Medicine, the Secretary of Public Health of Pará State and the Ministry of Health of Brazil as well as by the Ethical Review Committee of the World Health Organization. The trial was conducted in the outskirts of Belém, in an area of 8 km², on a population of 350,000 inhabitants living under poor sanitation and crowded conditions. Surveillance for gastroenteritis included twice-a-week visits to each child, in order to detect any diarrhea episode, defined as three or more liquid or semi-liquid motions in a 24-hr period. A total of 5,184 episodes were obtained from the 540 children who participated in a vaccine trial in Belém and 3,075 stool samples were collected during the first 2 years of their life. In the present study, around 20% of the specimens obtained per month were randomly selected, and this resulted in a total of 624 samples, of which 293 specimens were from 164 children who had received the vaccine and 331 samples from 167 children who had received the placebo. Using EpiInfo 2000 software for assessing sample size, it could be observed that such a number would be representative of the total cohort.

Enzyme Immunoassay (EIA)

A total of 624 samples were selected and tested for astrovirus by a commercial qualitative enzyme immunoassay kit (IDEIA™ Astrovirus DakoCytomation, Ely, UK), following the recommendations of the manufacturer. Positive samples were defined as those reaching an optical density (OD) of ≥0.150 plus the average of the negative controls using an A450 filter. All samples were previously screened for the presence of rotavirus and adeno-virus by commercial EIA (Premier-Rotaclone™ and Adenoclone™, Meridian Bioscience, Inc., Cincinnati, OH).

RT-PCR Amplification

The viral ssRNA was extracted from fecal suspension using silica powder glass extraction as described by Boom et al. [1990], with modifications by Cardoso et al. [2002].

The RT-PCR was carried out on 129 samples, using primers Mon 269 and Mon 270. These primers targeted the ORF2 region (4526–4974 nt) and the amplification conditions were those described by Noel et al. [1995]. A random hexamer (pd [N]6—50 A 260 units—Amersham Biosciences, Piscataway, NJ) was introduced in the RT reaction to obtain the cDNA [Gabbay et al., 2005]. Briefly, RT was performed by mixing 8 µl of viral ssRNA with 2 µl of the random initiator, denaturation at 80°C for 8 min and ice cooling for 3 min. Thereafter, a volume of 40 µl of the reaction mixture (4 µl of 2.5 mM each of deoxyribonucleoside triphosphate (dNTPs) mixture, 5 µl of tenfold concentrated reaction buffer 10×, 4 µl of MgCl2 50 mM, 1 µl of 10 U ribonuclease inhibitor (Amersham Pharmacia Invitrogen Life Technologies, Carlsbad, CA) 1 µl [10 U] of Superscript™ II Rnase H Reverse Transcriptase (Invitrogen Life Technologies, Carlsbad, CA), and 25 µl of distilled water) was added for a final volume of 50 µl. The cDNA was obtained after incubating the samples at 42°C for 60 min. The PCR reaction mixture was prepared for a final volume of 50 µl, including 10 µl of the cDNA, and 40 µl of the mix (same volume and concentration for dNTPs and reaction buffer; 1.5 µl of MgCl2 50 mM, 1.7 U/µl Taq DNA polymerase, Recombinant (Invitrogen Life Technologies, Carlsbad, CA), and 25 µl of distilled water and 1 µl of 20 µM each primer Mon 269 and Mon 270). PCR was conducted as described by Cardoso et al. [2002]. The RT-PCR products were then resolved on 1% agarose gel electrophoresis and were stained with ethidium
bromide. The visualization of 449 bp specific amplicon of HAstV was made in a Gel Doc 1000 (BioRad, Hercules, CA). Water was used as a negative control in all procedures and recommended manipulations for PCR techniques were strictly followed as a precaution to avoid false-positive results. A HAstV-1 strain was used as positive control.

Genotyping by PCR and Sequencing

This was accomplished with all the PCR-positive samples according to the protocol of Sakamoto et al. [2000] with modifications. The first PCR reaction was performed using the previously obtained cDNA and the same technique applied for astrovirus detection, utilizing the primers PreCAP1 and 12Gr (volume of 0.75 μl and concentration of 33 μM each). The second PCR was carried out with 3 μl of the first PCR product for a final volume of reaction mixture of 25 μl using 0.7 μl of the primers end and 0.7 μl of the genotype specific primer AST-S1 to AST-S8 (33 μM each). The reaction was performed under the same concentration condition as the first amplification, except for the Taq DNA polymerase (used 1.25 U). The DNA amplification was carried out as described by Sakamoto et al. [2000]. The amplicons were resolved on 1% or 2% agarose gel electrophoresis, and visualized as described previously. The sequencing was realized as described by Gabbay et al. [2005] by using primers Mon 269 and Mon 270.

Cloning

The cDNA fragment obtained with the primers Mon 269 and Mon270, from one sample (PA-COD329-BR) of this study was cloned using the plasmidial-bacterial system. Purified amplicon was first attached to a cloning PGMT-Easy Vector® (Invitrogen) at lac Z α-peptide gene in order to obtain a recombinant DNA. Plasmids with the inserted viral cDNA were then transformed and amplified in E. coli competent bacteria (JM-109 lineage) and the plasmids were recovered using the SNAP plasmid miniprep kit (Invitrogen, Carlsbad, CA). The nucleotide (nt) sequences of the cloned cDNA were determined by the dideoxy chain termination method using the sequencing ABI PRISM Dye Terminator kit (Applied Biosystems, Foster City, CA) and resolved in an ABI 377 DNA sequencer. Universal primers designed on the basis of plasmid promoter region T7 and SP6 were used to sequence the recombinant DNA in both directions. The phylogenetic analysis was realized as described by Gabbay et al. [2005].

Nucleotide Sequence Accession Numbers

The nucleotide sequences determined in this study have been deposited in the GenBank database and assigned the accession numbers DQ917375 to DQ917407.

RESULTS

From April/1990 to August/1992, a total of 624 fecal specimens were selected and screened for HAstV by EIA, with an overall positivity rate of 4.0% (25/624). The RT-PCR was done in 129 samples: 25 HAstV-positive by EIA and 104 that showed an OD value of at least twice that of the negative control by this technique. Of these, 33 were positive by RT-PCR. Considering both EIA and RT-PCR, the positivity rate for astrovirus was 5.4% (34/624). Twenty-four samples were positive by both techniques, one was positive by EIA only and nine by PCR only. HAstV was detected in 4.8% (14/293) of the samples from the vaccinated children, and in 6% (20/331) of those receiving placebo.

All 33 RT-PCR positive samples were subjected to genotyping by Nested-PCR and sequencing. Comparing both techniques a concordance into genotype results were observed for HAstV-1 (eight samples), HAstV-2 (two samples), and HAstV-6 (one sample) (Table I). Four strains were positive for HAstV-3 by Nested-PCR, but one of this was HAstV-1 by sequencing. Similarly, four out of eight strains genotyped as HAstV-4 and two strains genotyped as HAstV-7 by Nested-PCR were genotyped by sequencing as HAstV-1 and HAstV-2, respectively (Table I). One case of mixed infection was observed by Nested-PCR, involving HAstV-2 and HAstV-4, but by sequencing only HAstV-2 could be identified (Table I). This sample (PA-COD329-BR) was also cloned using the pair of primers Mon 269 and Mon 270, being positive for HAstV-2 only. Seven (21.2%)

<table>
<thead>
<tr>
<th>Nested-PCR genotyping</th>
<th>HAstV-1</th>
<th>HAstV-2</th>
<th>HAstV-3</th>
<th>HAstV-4</th>
<th>HAstV-6</th>
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<tr>
<td>HAstV-1</td>
<td>8</td>
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<td>3</td>
<td>4</td>
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<td>15</td>
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<tr>
<td>HAstV-2</td>
<td>8</td>
<td>4</td>
<td>1</td>
<td>4</td>
<td>1</td>
<td>9</td>
</tr>
<tr>
<td>HAstV-3</td>
<td>8</td>
<td>1</td>
<td>4</td>
<td>2</td>
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<td>8</td>
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<td>HAstV-4</td>
<td>8</td>
<td>1</td>
<td>4</td>
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<td>HAstV-6</td>
<td>8</td>
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<td>HAstV-7</td>
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<td>HAstV-9+4</td>
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<td>HAstV-NT*</td>
<td>8</td>
<td>1</td>
<td>4</td>
<td>2</td>
<td>1</td>
<td>8</td>
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<td>Total</td>
<td>15</td>
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<td>4</td>
<td>4</td>
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<td>33</td>
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*HAstV-NT—Astrovirus not typed by nested-PCR [Sakamoto et al., 2000].
strains were not typed using the eight specific genotyping primers, however, all of them were genotyped by sequencing as HAstV-1 (two strains), HAstV-2 (four strains), and HAstV-3 (one strain) (Table I).

A phylogenetic tree based on the 348-bp fragment of the ORF2 region was constructed to study the genetic relationship between the 33 HAstV isolated in this study and other published sequences (Fig. 1). The analysis of the nucleotide sequence showed that in Belém five genotypes were circulating. Fifteen isolates (45.5%) clustered into HAstV-1, nine (27.3%) into HAstV-2, four (12.1%) into HAstV-3 and HAstV-4, and one (3.0%) into HAstV-6 (Fig. 1). The group with higher nucleotide diversity was HAstV-2 (6.2%), followed by HAstV-1 (5.8%), HAstV-4 (4.3%), and HAstV-3 (2.3%).

All HAstV-1 strains were classified as 1a according to Medina et al. [2000] and Guix et al. [2002] (Fig. 1). Comparing these samples with the Oxford prototype (HAstV-1) of this lineage, a mean divergence of 2.5% was observed, but with the other lineages difference rates varied from 7.0% to 9.5%. The sample PA-COD379-BR was the most divergent, with an average variation of 3.5% (11 bases) in relation to lineage 1a, and averages ranging from 7.0% to 10.7% in relation to the other HAstV-1 isolates. All HAstV-2 strains were classified as 2b, based on the highest bootstrap value (99%), and the
low divergence rate (2.3%) into this lineage (Fig. 1). The divergence observed among the two lineages of HAstV-2 was the greatest (15%). The HAstV-4 samples were classified as 4b, although mean lineage divergence values were 6.7% (Fig. 1).

Nucleotide sequence changes resulting in amino acid substitutions in relation to the prototypes was observed only in two strains: PA-COD195-BR, genotyped as HAstV-1a, contained an Asp→Val change at amino acid 186 and PA-COD208-BR genotyped as HAstV-4b contained a Val→Asp changed at amino acid 183.

Figure 2 showed that the monthly distribution of astrovirus gastroenteritis occurred throughout the study period (from April/1990 to August/1992), with peak incidence rates in the months of January/91, October/91, and November/91 (15%, 17%, and 14%, respectively). The monthly frequency of each genotype is demonstrated in Figure 3. HAstV-1 was predominant during the first year of study (from May/1990 to May/1991, with its highest rate on January/1991). HAstV-4 was detected from September to November/1990, and HAstV-2 from July to November/1991. The comparison of genotypes, according to age groups, showed that HAstV-4 was detected mostly in the age of 3–6 months, HAV-2 in the age of 12–18 months, and HAstV-3 in the age of 18–24 months. HAstV-1 was detected in all age groups with exception of group 18–24 months (Fig. 4).

The age distribution of the astrovirus-positive cases confirmed that children of all ages were infected (Fig. 4), and cumulative incidence demonstrated that in the group aged >24 months 30.6% of the children had already been infected by HAstV (Fig. 5).

The clinical features of 34 astrovirus-related gastroenteritis cases showed that the duration of the symptoms lasted from 1 to 3 days in 50% of the cases, 4 to 6 days in 24%, and ≥7 days in 26% of the cases. Eleven (32%) children developed vomiting, three (9%) had fever of ≥38°C, and three (9%) had mild/moderate dehydration.

**DISCUSSION**

In this study, we took advantage of the stool sample collection obtained during a RRV-TV vaccine trial conducted in Belém, Brazil, to test some of them for the presence of HAstV.

The overall prevalence rate of HAstV infection among these children, using both EIA and RT-PCR, was 5.4%. This positivity was lower than that reported in Finland (9%), where a clinical trial with the RRV-TV vaccine had also been conducted [Pang and Vesikari, 1999]. In studies carried out in outpatient settings, HAstV infection rates were shown to be higher than ours, ranging from 6.8% in Mexico City [Walter et al., 2001] to
17% in Rural Mayan [Maldonado et al., 1998]. In Brazil, most of the studies were conducted in public hospitals, including surveys in Goiás [Cardoso et al., 2002], São Paulo [Tienenetsky et al., 1993], and Rio de Janeiro [Leite et al., 1991], which yielded prevalence rates of 3–5%, which were similar to those reported in Belém. Of note, in Maranhão, Brazil [Gabbay et al., 2005], a prevalence rate of 11% among diarrheic children was recorded being higher than that yielded in our study. Since the same methodology was used in both studies, differences in prevalence rates are likely to be attributable to demographic conditions or even the number of samples gathered in compared studies.

The use of combined techniques, EIA, and RT-PCR, enhanced the HAstV detection. EIA appears to be very useful for the screening of fecal samples, but RT-PCR seems to be more sensitive as demonstrated [Walter and Mitchell, 2000; Dalton et al., 2002; Espul et al., 2004]. Indeed, in our study, 26.5% of HAstV-positive samples were detected by RT-PCR only. This technique was applied to 129 samples that were HAstV-positive by EIA or that yielded an OD value of at least twice that of the negative control.

All HAstV-positive samples were tested by the typespecific RT-PCR [Sakamoto et al., 2000] and sequencing analysis (ORF2) [Noel et al., 1995]. The concordance observed between these two techniques was 69.2%, suggesting that failure of RT-PCR typing could have been due either to mismatching, low RNA yield or possible cross-reactions involving some genotypes, mainly HAstV-4 and HAstV-7 [Matsui et al., 1998]. Studies conducted in a periurban community of Mexico City [Walter et al., 2001] and in Hungary [Jakab et al., 2004] have also utilized these two methodologies and demonstrated a good correlation in the results obtained; however, they used a different set of primers for typing by RT-PCR.

Seven samples were only genotyped by sequencing even though they had been tested individually with each of the eight genotyping primers. The primers Mon 269 and Mon 270 used for sequencing these strains annealed to a conserved region while the primers used in PCR-genotyping annealed to a variable region. This may have influenced on the results obtained in the PCR-genotyping. It is worth mentioning the case of sample PA-COD329-BR that, by PCR-genotyping showed a mixed infection involving HAstV-2 and HAstV-4, and by sequencing HAstV-2 only. To confirm this result this sample was also cloned and only HAstV-2 was detected.

Five HAstV types co-circulated in Belém during the study period and HAstV-1 was the most prevalent genotype. This observation is in agreement with
previous studies conducted in different countries of the world, including Brazil [Cardoso et al., 2002; Dalton et al., 2002; Guix et al., 2002; Espul et al., 2004]. In a study conducted in a periurban community of Mexico City, HAstV-2 was found to be the most prevalent [Walter et al., 2001]. In Belém, Brazil, HAstV-2 was considered the second most common genotype by sequencing, similarly to what had been detected in Madrid, Spain [Dalton et al., 2002]. The genotypic complexity of co-circulating strains observed in Belém, has also been reported elsewhere in the world, including Brazil [Naficy et al., 2000; Nadan et al., 2003; Gabbay et al., 2005; Silva et al., 2006].

In light of observations made by some authors [Medina et al., 2000; Guix et al., 2002], HAstV-1 was classified into four lineages (1a to 1d). All HAstV-1 samples from Belém, Brazil were included into 1a lineage, contrasting with the observations of Medina et al. [2000], with strains from Colombia and Venezuela, which were classified into three different lineages (1a, 1b, and 1c). The HAstV-1a samples from Belém, Brazil, were also found in other settings included samples collected in the Amazon region (Col503-Colombia), in the Northeast region (Ma170-São Luís, Maranhão) and West Central region (BrG1-12-Brasília, DF, BrG1-10-Goiânia, Goiás) of Brazil [Medina et al., 2000; Gabbay et al., 2005; Silva et al., 2006]. A sample from Barcelona [Guix et al., 2002] and the HAstV-1 prototype were also included in the HAstV-1a lineage. All samples included in the HAstV-1a tree were collected in the period of 1996–2001, as well as those from Belém, Brazil during 1990–1992. This suggests that strains of such a lineage were circulating for a large length of time.

All samples classified as either HAstV-2 or HAstV-4 belonged to lineages 2b and 4b, respectively. The first type also clustered with samples from Colombia, Brazil (Brasília and São Luís), and the second with strains from Venezuela, Brazil (Brasília, Rio de Janeiro and São Luís) [Medina et al., 2000; Gabbay et al., 2005; Silva et al., 2006; GeneBank AY846635]. With exception of two samples (PA-COD195-BR and PA-COD208-BR), of which the nucleotide sequence changes resulted in amino acid substitutions, the remainder of nucleotide sequence changes, as compared to the prototype, were all synonymous change.

The incidence of HAstV infection peaked twice throughout our study (January/91 and October/91) but low numbers prevent us from drawing firm conclusions on seasonality. The temperature and humidity registered in these 2 months did not differ significantly, but in relation to the rainfall index it was higher in January/91 (392 mm³) than in October/91 (157 mm³). The HAstVs were also detected in another study conducted in the West Central region of Brazil during the period of October–December/98, which corresponds to spring and early summer in this region [Cardoso et al., 2002].

The results obtained in Barcelona, Spain, by Guix et al. [2002] showed an increasing incidence of HAstV-1 during the 3 years of this study, differing from the findings in Belém, where this type prevailed in the first year (May/1990 to April/1991). In our study, HAstV-2 infections clustered from July to November/1991. This temporal distribution pattern differs from that observed by Walter and Mitchell [2000] in a periurban community of Mexico City, where HAstV-2 was detected throughout the entire 2 years study period.

The pattern of age distribution in our study was similar to those observed in Barcelona, Spain, by Guix et al. [2002], except for HAstV-4, which was more often detected among children younger than 3 years. In our study, the highest peaks of detection rate was observed in children aged >3 to 6 (9.0%) and >12 to 18 (7.4%) months of age. These results agree with those from Giordano et al. [2004] in Cordoba, Argentina, who reported a higher positivity rate in these two groups. However, our results differ from those of Barcelona, Spain [Guix et al., 2002], where the peak incidence was observed among older children, aged 2–4 years (9%). In a study conducted in Goiás, Brazil [Cardoso et al., 2002], the overall percentage obtained in children less than 2 years of age was lower, when compared to that for our study. In contrast, in Maranhão, Brazil [Gabbay et al., 2005], the results were similar, as the peak incidence was higher in the age group of 0–6 months (18%). It should be pointed out that 30.6% of the children from Belém had already been infected by HAstV at the age of 24 months.

HAstV infection in Belém was in general associated with diarrhea and vomiting (32%) but less often with fever and dehydration (9% each). These findings are similar to those from other community-based studies conducted worldwide [Guerrero et al., 1998; Maldonado et al., 1998].

In comparing the duration of diarrhea with HAstV genotypes, HAstV-4 was associated with a duration (>7 days) longer than that of HAstV-2. Unlike our observation, Walter and Mitchell [2000] in Mexico City had shown that HAstV-2-related episodes lasted longer than those by HAstV-4, but this may have occurred by chance, since we examined a low number of samples.

Of note, the only HAstV-6 infection detected in Belém was clinically severe, since diarrhea episode lasted for more than 7 days, with vomiting (>3 episodes), fever (>38 C) and moderate dehydration. However, conclusions cannot be drawn as a more severe clinical outcome was associated to this specific genotype, based on observations from a single HAstV-6 isolate.

Co-infection involving HAstV and other pathogens (rotavirus, bacteria, and parasites) was not observed in Belém, as it was described elsewhere, including Brazil [Pang and Vesikari, 1999; Guix et al., 2002; Méndez-Toss et al., 2004; Gabbay et al., 2005].

The anti-rotavirus RRV-TV vaccine caused no effect on HAstV gastroenteritis incidence in Belém study, similarly to what has been reported by Pang and Vesikari [1999]. The number of fecal specimens tested in the vaccine group (293 samples) was similar to the placebo group (331 samples), and no statistically significant difference was observed as to HAstV positivity rate (4.8% and 6.0%, respectively).
In summary, our data shows that HAstV accounts for 5.4% of infantile gastroenteritis cases in Belém, Brazil and underscores the importance of HAstV as a potential viral enteropathogen. HAstV-1 was found to predominate in this study but HAstV-2, HAstV-3, HAstV-4, and HAstV-6 were also circulating, though at lower rates. Our findings provide the first epidemiological and molecular information on the HAstV circulation in the Northern Region of Brazil and may be of great importance regarding possible future strategies toward the development of HAstV vaccines. Studies dealing with HAstV infection are of growing importance in view of the recent introduction of rotavirus vaccines in several countries, including Brazil (Linhares and Villa, 2006).

Accession Numbers

The eight prototype sequences used were: HAstV1 (L23513), HAstV2 (L13745), HAstV3 (L38505), HAstV4 (L38506), HAstV5 (U15136), HAstV6 (L38507), HAstV7 (L38508), and HAstV-8 (Z66541). Other strains obtained in GeneBank were used for comparison: BrG1-12 (DQ139826), Bcn1.7 (AF348759), Col503 (AF211964), MA170/BR/99 (DQ071654), MA167/BR/99 (DQ071652), BrG1-10 (DQ139828), Ven835 (AF211956), Bcn1.15 (AF348767), Melb1E (AF175253), Melb1F (AF175254), Bcn1.1 (AF348753), BrG1-5 (DQ139825), Bcn1.3 (AF348755), Bcn1.5 (AF348757), BrG3 (DQ139831), MA027/BR/97 (DQ071649), GO/12/95/BR (DQ070852), Ven35 (AF211952), 929/BR (AY846635), MA177/BR/99 (DQ071655), Bcn4.12 (AF348795), Col237 (AF211957), Melb1C (AF175259), Bcn2.1 (AF348771), Bcn2.2 (AF348772), Melb2A (AF175260), Col673 (AF211959), Col546 (AF211961), BrG2-1 (DQ139830), and MA021/BR/97 (DQ071648).

ACKNOWLEDGMENTS

We gratefully acknowledge the valuable technical support provided by Dr. Olinda Macêdo, Dr. Ivetê V. Costa, Antonia Alves, Maria Silvia de Lucena and Antônio de Moura. Thanks are also due to the field staff and doctors that worked during the RRV-TV rotavirus vaccine trial. Luciana Damascena da Silva, Eieiama L. Cavalcante-Pepino and Liliany S. Nakamura received a grant fellowship from the National Council for the Development of Science and Technology, Brasilia, Federal District, Brazil (CNPq). This study received financial support from SECTAM/FUNTEC/PA, IEC/SVS/MS, IOC/FIOCruz, and CNPq.

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