

## Astrovirus infection in children living in the Central West region of Brazil

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*This study presents data regarding the circulation of astrovirus in Goiânia-GO and Brasília-DF. These viruses were detected in fecal samples from hospitalized children up to five years old with and without acute gastroenteritis. A total of 1244 fecal samples were collected in two periods, 1994 to 1996 (Brasília) and 1998 to 2002 (Goiânia and Brasília), and were analyzed for viral RNA using the reverse transcription-polymerase chain reaction (RT-PCR). Positivity rates of 4.3 and 0.5% for astrovirus were observed in children with acute gastroenteritis and those without gastroenteritis, respectively. Among children with gastroenteritis no statistically significant difference was seen with regards to viral positivity rates in relation to gender and age. However, a higher incidence rate was observed for children from Brasília aged 36 months or more. Overall, astroviruses occurred predominantly from September to March in the two cities, suggesting a seasonal pattern for these viruses which coincides with the highest relative air humidity period. The results of this study highlight the importance of astrovirus as an etiologic agent of acute gastroenteritis in children of the Central West region of Brazil.*

Key words: astrovirus - gastroenteritis - epidemiology - reverse transcription-polymerase chain reaction - Goiás - Brasília - Brazil

Acute gastroenteritis (AG) is considered a significant cause of morbidity and mortality in humans worldwide (Glass et al. 2001). The mortality rate has been estimated in more than three million of persons per year with the majority of deaths occurring in the developing countries (Wilhelmi et al. 2003). Viral enteric pathogens are recognized as important agents related to such a syndrome with major pathogens being *Rotavirus A*, enteric adenovirus, human calicivirus and astrovirus (Wilhelmi et al. 2003).

The astroviruses, family *Astroviridae*, genus *Mastrovirus* include eight types that infect humans (ICTVdB Management 2006). They have been associated with outbreaks of diarrhea involving young children and other age groups in different parts of the world (Matsui & Greenberg 2001). The viruses have a plus-sense, single-stranded RNA (ssRNA) genome with approximately 6800 nucleotides with polyadenylated tail at the 3-end (Matsui & Greenberg 2001). The genome presents three open reading frames: ORF1a, ORF1b, and ORF2, each encoding at the least one polyprotein.

Different methodologies have been employed for the detection and characterization of these viruses including electron- and- immune electron microscopy, enzyme immunoassay, latex agglutination, viral isolation in cell culture, and molecular procedures such as reverse transcription-polymerase chain reaction (RT-PCR) (Silva et al. 2001, Komoriya et al. 2003, Tai et al. 2003, Méndez-Toss et al. 2004). The RT-PCR has been widely utilized for both viral detection and genotyping using primers designed for each one of genomic regions (Noel et al. 1995, Belliot et al. 1997).

The astroviruses are recognized as one of the most common cause of viral gastroenteritis in infants and young children in worldwide (Giordano et al. 2001) and its seasonality is variable according to the geographic region (Naficy et al. 2000, Guix et al. 2002). The main symptom of the astrovirus infections is a watery and mild diarrhea, which generally results in mild diarrheic disease (Matsui & Greenberg 2001), although a recent study has described astrovirus infection related to severe dehydrating gastroenteritis among isolated Brazilian communities (Gabbay et al. 2006).

In Brazil, there are few studies about the occurrence of these viruses which show prevalence rates ranging between 3 and 56% (Stewien et al. 1991, Tanaka et al. 1994, Silva et al. 2001, Gabbay et al. 2006). Considering the Central West region of the country, only one study was carried out which showed astrovirus detection rate of 2.8% (Cardoso et al. 2002). This study presents epidemiological and clinical data regarding the circulation of

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astrovirus in two cities of the Central West region of Brazil which were detected in children with and without AG.

### MATERIALS AND METHODS

**Subject** - The study was performed using 1244 fecal samples collected from hospitalized children at public hospitals in the cities of Goiânia, Goiás (1998 to 2002) and Brasília, Distrito Federal (1994 to 1996 and 1998 to 2002), aged up to five years. Of these, 1041 samples were from children with acute diarrhea (AG) which was defined as three or more liquid or semiliquid evacuations per day, and 203 from non-diarrheic children; 743 samples were from children from Goiânia-GO (699 with AG and 44 without AG) and 501 from children living in Brasília-DF (342 with AG and 159 without AG). All samples from Goiânia were collected "in nature" while of the Brasília samples, 204 samples were collected "in nature" and 297 by rectal swab. Considering the last population census carried out by Instituto Brasileiro de Geografia e Estatística (IBGE 2000), the sampling analyzed in relation to the infantile population was regarded as representative in both cities (95% confidence interval). All samples were primarily screened for rotavirus and adenovirus utilizing a combined enzyme immunoassay for *Rotavirus A* and adenovirus – EIARA – (Pereira et al. 1985) and also by polyacrylamide gel electrophoresis – PAGE (Pereira et al. 1983). Samples were also screened for calicivirus by RT-PCR using primers designed for the RNA polymerase region (Green et al. 1995, Vinjé et al. 1997, Jiang et al. 1999).

The collection of fecal samples was carried out after a written informed consent was provided by parents or legal guardians and the study was approved by the Ethics Committee on Research of the Federal University of Goiás (no. 004/2000).

**Detection of astrovirus** - The astrovirus was detected by RT-PCR using primers designed by Noel et al. (1995). For the procedure, initially, the viral ssRNA was extracted from 300 µl fecal suspension (20% in phosphate buffered saline – PBS – pH 7.4) following methodology described by Boom et al. (1990) with modifications (Cardoso et al. 2002). The reverse transcription was carried out using 16 µl of viral ssRNA added of 2 µl random primer pd(N)<sub>6</sub> (Random Hexamer, Amersham Biosciences) at temperature of 42°C for 60 min with a reaction mixture in a final volume of 50 µl [1X reaction buffer – PCR buffer – (TRIS-HCl 20 mM pH 8.4, 50 mM KCl); 0.4 mM of each dNTP; 4.0 mM of MgCl<sub>2</sub>; 20 U/ml of ribonuclease inhibitor (Invitrogen™/Life Technologies); 120 U/ml of the MMLV reverse transcriptase enzyme (Gibco BRL®/Life Technologies)]. The PCR was carried out using 10 µl of the cDNA and the specific

primers Mon 269-270 (0.4 µM of each primer) (Noel et al. 1995) in a reaction mixture also in a final volume of 50 µl. The reaction mixture contained the same reagents, at same concentrations, except the not utilization of the ribonuclease inhibitor and reverse transcriptase, and the use of 2.5 U/µl of the *Taq* DNA polymerase enzyme (Invitrogen™/Life Technologies) as well as 1.2 mM of MgCl<sub>2</sub>. The amplification was carried out in 40 cycles (94°C – 30 s, 50°C – 30 s, and 72°C – 60 s), following of a final extension at 72°C for 10 min (Eppendorf Mastercycler Personal). The amplified product (449 bp) was visualized from 1.5% agarose gel electrophoresis with ethidium bromide staining (0.5 mg/ml) (Macro Vue UV-20 – Hoefer Scientific Instruments) by comparison with the molecular pattern – 123 bp DNA ladder (Invitrogen™/Life Technologies). In all reactions positive samples were included, provided by Laboratório de Virologia Comparada-Fiocruz, Rio de Janeiro, Brazil, as positive control, and sterile Milli-Q water as negative control. All the stages of the reaction were done in separated places aiming to avoid contaminations.

**Statistical analysis** - The statistical analysis was done using the program Epiinfo version 6.04d. Chi-square test ( $\chi^2$ ) with 95% confidence interval and the Fisher exact test, when necessary, were calculated.

### RESULTS

From 1244 fecal specimens, 46 (3.7%) were positive for astrovirus. The highest viral detection rate was observed in children with AG from Brasília city (7%). Only one child without AG was positive for astrovirus (Table I).

There was no statistically significant difference of positivity rates in relation to gender for children with AG (27/596-4.5%-male; 18/445-4%-female) ( $\chi^2 = 0.05$   $p = 0.8205$ ). Similarly, although a higher positivity rate was observed in children more than 36 months old (7.3%), no statistically significant difference could be noted with respect to the age groups (Table II).

Astroviruses occurred year-round in all years, predominantly from September to March in both cities (Table III).

Diarrhea was observed in all children and analysis of astrovirus positivity in relation to other gastroenteritis symptoms such as vomiting, fever and abdominal pain in association with the diarrhea was performed for children from Goiânia where it was not observed statistically significant difference (Table IV).

Of 46 astrovirus-positive samples, 14 (30.4%) were also positive for other viruses, as follows: seven samples were also positive for *Rotavirus A*, six for calicivirus and another for specie A adenovirus.

TABLE I

Distribution of astrovirus in fecal samples from children with or without acute gastroenteritis (AG) in relation to city of collection

City	With AG		Without AG		Total	
	No.	%	No.	%	No.	%
Goiânia-GO	21/699	3.0	0/44	0.0	21/743	2.8
Brasília-DF	24/342	7.0 <sup>a</sup>	1/159	0.6	25/501	5.0 <sup>b</sup>
Total	45/1041	4.3	1/203	0.5 <sup>c</sup>	46/1244	3.7

a:  $\chi^2 = 8.00$ ,  $p = 0.0046$ ; b:  $\chi^2 = 3.35$ ,  $p = 0.0672$ ; c:  $\chi^2 = 5.96$ ,  $p = 0.0146$ .

TABLE II  
Distribution of astrovirus in fecal samples from children with acute gastroenteritis (AG), considering cities of collection, in relation to age group

Age group (months)	Brasília <sup>d</sup>		Goiânia		Total	
	No.	%	No.	%	No.	%
0-6	6/100	6.0	5/153	3.3 <sup>b</sup>	11/253	4.3
7-12	4/92	4.3	9/275	3.3	13/367	3.5
13-24	2/87	2.3	4/142	2.8	6/229	2.6
25-36	2/28	7.1	1/63	1.6	3/91	3.3
> 36	5/24	20.8 <sup>a</sup>	1/58	1.7	6/82	7.3 <sup>c</sup>
Total	19/331	5.7	20/691	2.9	39/1022	3.8

a: Fisher exact test  $p = 0.0076$ ; b: Fisher exact test  $p = 0.7849$ ; c: Fisher exact test  $p = 0.1207$ ; d: from 19 children was not known the age and from that, 6 were positive for astrovirus. The child without AG with positivity for astrovirus had 7 months of age.

TABLE III  
Distribution of astrovirus in fecal samples from children with acute gastroenteritis (AG) in relation to the period of year (1994-1996 and 1998-2001)<sup>c</sup>

Climatic season	Brasília		Goiânia		Total	
	No.	%	No.	%	No.	%
Dry (April/August)	0/58	0.0	1/296	0.3	1/354	0.3
Rain (September/March)	24/212	11.3	20/395	5.1 <sup>a</sup>	44/607	7.2 <sup>b</sup>
Total	24/270	8.9	21/691	3.0	45/961	4.7

a:  $\chi^2 = 11.27$ ,  $p = 0.0007$ ; b:  $\chi^2 = 22.78$ ,  $p = 0.0000$ ; c: were not included 80 negative samples collected in dry season in the year of 2002 (8 samples from Goiânia and 72 from Brasília); the positive sample from child without AG was collected in dry season.

TABLE IV  
Distribution of astrovirus in fecal samples from children from Goiânia, Goiás in relation to the clinical symptoms<sup>b</sup>

Symptoms	No.	%
Diarrhea, fever, vomiting, and abdominal pain	6/204	2.9
Diarrhea, fever, and vomiting	5/180	2.8
Diarrhea, vomiting, and abdominal pain	1/49	2.0
Diarrhea and vomiting	7/106	6.6 <sup>a</sup>
Diarrhea	2/61	3.3
Total	21/600	3.5

a: Fisher exact test  $p = 0.0750$ ; b: from 99 children have not the disponibility of complete data in relation to the symptoms presented.

## DISCUSSION

Astroviruses are considered important viral enteric pathogens in children with and without AG worldwide (Pennap et al. 2002, Méndez-Toss et al. 2004, Gabbay et al. 2005). In this study, a higher detection rate of astrovirus in cases of AG (4.3%) was observed, as compared to non-diarrheic patients (0.5%) ( $p < 0.05$ ). This result is comparable to those from other studies where viral detection rates in cases of AG ranged from 1.5 to 20% (Mitchell et al. 1999, Giordano et al. 2001, Denno et al. 2005). In contrast, results from children without gastroenteritis are lower than those observed in other studies where detection rates of 1.2 to 5.7% were yielded considering the same population (Pennap et al. 2002, Riechmann et al. 2004).

No significant differences in astrovirus-positivity rate, among children with AG, could be noted in relation to the gender of the children. To our knowledge, a similar analysis was performed only in another study conducted in Rio de Janeiro city, which showed similar results (Miranda 2004, pers. commun.). In children from Goiânia city, it was

observed that astrovirus infections occurred in all age groups, with a higher detection rate in children up to 24 months of age. These results reinforce a previous study conducted in the same area (Cardoso et al. 2002) and are also in accordance with a study carried out by Guix et al. (2002) in Spain. In contrast, a higher positivity rate was observed in children older than 36 months of age in Brasília city. This result shows a different pattern if compared to other studies conducted elsewhere (Silva et al. 2001, Giordano et al. 2004). This result should, however, be analyzed with caution owing to the small number of samples from that age group. Further studies are thus needed to confirm this result.

One objective of this study consisted in the analysis of astrovirus positivity in fecal samples from children with diarrhea alone or in association to other clinical manifestations such as vomiting, fever, and abdominal pain. In the present study the astrovirus positivity was not significantly more related to one or more symptoms other than diarrhea considering the children from Goiânia city. These results agree with those from a study carried

out by López et al. (2000) which analyzed children up to ten years from Spain.

Studies have shown that astroviruses occur preferentially during the colder months of the year (Phan et al. 2004, Galdiero et al. 2005) even though other investigations have shown a higher viral incidence during warmest months (Guerrero et al. 1998, Naficy et al. 2000). Other studies report a year-round occurrence of astrovirus infection (Schnagl et al. 2002, Giordano et al. 2004). In this study, the infection by astrovirus occurred mainly from September to March, considered the rainy season in the region. In accordance with data from the Instituto Nacional de Meteorologia (INMET) – 10<sup>o</sup> Distrito de Meteorologia de Goiás e Tocantins, during the years of the study in Goiânia, there was no significant temperature variation throughout the study period, in contrast to the relative air humidity and rainfall indexes, which showed higher and lower indexes between September and March and April and August, respectively. Therefore, the seasonality of astrovirus infection in our study could be associated with the increase of the relative air humidity resulting from heavier rainfalls in the region. These results agree with those from a previous study (Cardoso et al. 2002) and differ from the pattern of rotavirus occurrence in Goiânia city, which is more prominent during the dry months (April to August) (Cardoso et al. 2003, Costa et al. 2004).

Co-infection of astrovirus with other viruses has also been observed in studies carried out by Román et al. (2003) and Phan et al. (2004) in Spain and Pakistan, respectively. In this study, although the association of astrovirus with AG has been demonstrated, a larger proportion (30.4%) of samples was also positive for other viruses making it difficult to determine the primary viral pathogen.

Anyway, this study highlights that astrovirus is an important agent of acute gastroenteritis in children from the Central West region of Brazil and provides local preliminary epidemiologic features of the disease. Our findings may also be of importance with respect to the implementation of prevention and control measures.

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