

RESEARCH ARTICLE

Surveillance of Human Astrovirus Infection in Brazil: The First Report of MLB1 Astrovirus

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Data Availability Statement: All relevant data are within the paper. The nucleotide sequence data reported in this study is freely available in GenBank under the accession numbers: KM269039-KM269070, KM408170-KM408171 and KC294576-KC294577.

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Abstract

Human astrovirus (HAstV) represents the third most common virus associated with acute diarrhea (AD). This study aimed to estimate the prevalence of HAstV infection in Brazilian children under 5 years of age with AD, investigate the presence of recently described HAstV strains, through extensive laboratory-based surveillance of enteric viral agents in three Brazilian coastal regions between 2005 and 2011. Using reverse transcription-polymerase chain reaction (RT-PCR), the overall HAstV detection rate reached 7.1% (207/2.913) with percentage varying according to the geographic region: 3.9% (36/921) in the northeast, 7.9% in the south (71/903) and 9.2% in the southeast (100/1.089) ($p < 0.001$). HAstV were detected in cases of all age groups. Detection rates were slightly higher during the spring. Nucleotide sequence analysis of a 320-bp ORF2 fragment revealed that HAstV-1 was the predominant genotype throughout the seven years of the study. The novel AstV-MLB1 was detected in two children with AD from a subset of 200 samples tested, demonstrating the circulation of this virus both the in northeastern and southeastern regions of Brazil. These results provide additional epidemiological and molecular data on HAstV circulation in three Brazilian coastal regions, highlighting its potential to cause infantile AD.

Introduction

Acute diarrhea (AD) remains a major cause of hospitalization and death in children worldwide, associated with almost 9.9% of the 6.9 million deaths among children under 5 years old in 2011 [1]. Among the AD etiologic agents, viruses play an important role and after rotavirus group A (RVA) and norovirus (NoV), human astrovirus (HAstV) represents the third most common virus found in children with AD, and is thought to be involved in 0.5 to 15% of AD outbreaks [2, 3]. HAstV infections are more frequent in children, the elderly and among immunocompromised patients causing blunting of the tips of the microvilli as well as disruption of the intestinal epithelium [2, 4, 5].

Human astrovirus belongs to the *Astroviridae* family and contains single-stranded, positive-sense, polyadenylated RNA 6.2–7.8 kilobases (kb) in length without an envelope, encased

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within an icosahedral capsid. The genome contains three open reading frames (ORFs) designated ORF1a, ORF1b and ORF2. The two first ORFs encode non-structural proteins, including viral proteinase and RNA polymerase and ORF2 encodes the capsid protein precursor [2, 3].

The *Astroviridae* family contains two distinguished genera: *Mamastrovirus* and *Avastrovirus*. These viruses were originally classified into genera and species based only on the host of origin. Recently, the Astroviruses Study Group, 9th Report ICTV (International Committee on Taxonomy of Viruses), 2011 [6], proposed a classification based on the amino acid sequence, which encodes the capsid polyprotein and represents the most variable region of the genome [2, 3]. In this classification, *Mamastrovirus* genera associated to human disease is subdivided into four divergent species: *MAstV 1* (the classical human astrovirus 1–8), *MAstV 6* (AstV MLB1-3), *MAstV 8* (AstV VA1 and VA3, also known as HMO-C and HMO-B, respectively) and *MAstV 9* (AstV VA2, also Known as HMO-A, and VA4) [2].

In general, HAstV-1 is the most common type found in children, but the predominant genotype can vary with time and location [3, 7, 8]. The recent identification of novel AstV in humans using highly sensitive methods [9–13], highlights the need to analyze the prevalence of these viruses to recognize their actual impact in public health, since they were found to be genetically related to animal viruses and some were isolated from patients with more severe diseases, such as encephalitis [14–16].

This study aimed to associate the HAstV infection to Brazilian children under 5 years old affected with AD in three Brazilian coastal regions in a seven years period (2005–2011), providing epidemiological and molecular characteristics of HAstV genotypes. Recently described strains of HAstV were also studied.

Materials and Methods

Ethical statement

This study, including consent procedures, was approved by the Ethics Committee of Oswaldo Cruz Foundation (CEP 311/06) and is part of an ongoing official Brazilian Ministry of Health surveillance of enteric pathogens to investigate the viral etiology of AD. In this context, stool samples from AD cases were obtained by request of in and out patients attending health centers, including hospitals and public central laboratories, following outbreaks or sporadic cases of AD. For this study, consent was obtained verbally from the parents or relatives guardians on behalf of the children who were enrolled in this study. Data are maintained anonymously.

Clinical samples and studied areas

For this study, stool samples of 2,913 patients under 5 years old with negative diagnosis for RVA and NoV were obtained between January 2005 and December 2011 from three different Brazilian coastal regions (northeast, southeast and south) with distinct demographic and environmental scenarios and were selected for HAstV investigation. The population of the southern and southeastern regions is of a higher income compared to that of the northeastern region, where much of the population do not have access to sanitation, and where AD-related mortality is higher [17]. Acute diarrhea was defined when children presented three or more liquid or semi-liquid evacuations in a 24-h period.

Screening of novel HAstVs from stool samples

To search for novel and recently described HAstVs, 200 stool samples obtained between January and December 2011 from patients under two years old were randomly selected from three

different Brazilian coastal regions (northeast, southeast and south). All samples were tested previously and were negative for RVA, NoV and HAstV1–8.

Statistical analysis

Rates of HAstV positivity and association between the frequency of symptoms and the different age groups were compared using the chi-squared test. Statistical significance was established at $p < 0.05$.

Nucleic acid extraction and detection

Fecal suspension was prepared in a 10% (w/v) Tris/HCL/Ca²⁺ (0,01M, pH 7.2). RNA Extraction was performed using QIAamp Viral RNA extraction kit (Qiagen) methodology according to the manufacturer's instructions. Detection of classic HAstV and complementary DNA (cDNA) was synthesized using the Applied Biosystems High Capacity cDNA Reverse Transcription kit (Foster City, Ca, USA) with a previous denaturation step with dimethylsulfoxide for 7 min at 97°C. For classic HAstV detection, a PCR protocol was performed using a set of specific primers that targeted the ORF2 region capsid (Mon 269/270 [0.4 μM of each primer]) in a final reaction mixture of 50 μl in volume, according to the PCR conditions described previously [18].

For novel AstV detection, screening was carried out by amplifying a 409 bp segment of the ORF1b (RNA polymerase) region of the AstV genome with consensus primers SF0073/SF0076 designed with a bias for detection of a highly divergent AstV (Classic HAstV 1–8, MLB1-3 and VA1-3) [13]. Positive samples were sequenced and submitted to BLAST (software) [19] searching and those that indicated MLB1 homology were then subjected to amplification and sequencing of the 402 bp segment of the MLB1 ORF2 specific genome region using primers SF0053 and SF0061 for characterization [13]. All amplifications were performed using SuperScript III One-Step RT-PCR System (Invitrogen), according to the manufacturer's instructions.

For all PCR reactions, final primers concentrations were of 0.2 μM for both forward and reverse primers, in a final reaction volume of 25 uL. The products were analyzed in a 2% agarose gels and visualized by ethidium bromide staining.

Molecular sequencing

For the molecular characterization of HAstV strains, PCR products were sequenced in both directions using the ABI Prism 3100 Genetic Analyzer and Big Dye Terminator Cycle Sequencing Kit v. 3.1 (Applied Biosystems, Foster City, CA) with the same primers used for the amplification reactions [13,18]. Centri-Sep columns (Princeton Separations, Foster City, CA) were used to purify the sequencing reaction products, according to the manufacturer's instructions.

Phylogenetic analysis

The chromatograms obtained from sequencing reactions were carried out using BioEdit software version 7.1.3.0 [20] for sequence editing. Sequence similarity searches were performed with sequences deposited in GenBank using the basic local alignment search tool (BLAST) software [19]. For phylogenetic analysis, reference sequences available for the same genomic region (ORF2) and size were selected taking into account the standards of each genetic type of human and/or environmental AstV and other similar sequences representing different geographical regions and time periods were also used. Sequences analyzes were performed using MEGA software version 5.1 [21] and multiple sequence alignments were carried out using the

Table 1. Prevalence of HAstV in three Brazilian coastal regions from 2005 to 2011.

Regions	2005	2006	2007	Years 2008	2009	2010	2011	Total
Northeast (%)		2/21 (9.5)	17/124 (13.7)	3/101 (3.0)	5/95 (2.6)	6/235 (2.6)	3/245 (1.2)	36/921 (3.9)
Southeast (%)	19/241 (7.9)	14/133 (10.5)	37/207 (17.9)	8/91 (8.8)	7/147 (4.8)	15/183 (8.2)	0/87 (0)	100/1.089 (9.2)
South (%)	10/72 (13.9)	16/117 (1.7)	13/112 (11.6)	11/163 (6.7)	3/122 (2.5)	16/190 (8.4)	2/127 (1.6)	71/903 (7.9)

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ClustalW program. Phylogenetic trees based on nucleotide (nt) sequences were obtained using the neighbor-joining method with Kimura two-parameter model with the bootstrap probabilities of each node calculated using 2,000 replicates. Values above 70 were considered significant and are represented in the trees. The nucleotide sequence data reported in this study is available in GenBank under the accession numbers: KM269039-KM269070, KM408170-KM408171 and KC294576-KC294577.

Results

Diagnostic and clinical aspects

The overall HAstV positivity rate among RVA and NoV negative AD cases was of 7.1% (207/2.913). Considering the distinct Brazilian coastal regions, HAstV was detected more frequently in the southeastern (9.2% [100/1.089]) and southern (7.9% [71/903]) regions, compared to the northeastern region (3.9% [36/921]); $p < 0.001$ (Table 1).

Human astrovirus was detected in all age groups analyzed: 82/1.224 (6.7%) in infants aged 1–11 months, 64/1.031 (6.2%) in children aged 12–24 months, 48/549 (8.7%) in subjects aged 2–48 months and 13/109 (11.9%) among patients aged 49–60 months. The prevalence rate tended to be higher among children aged 49–60 months ($p = 0.054$; chi-squared test for linear trend), although there was no statistical significance.

Differences in the frequency of some symptoms were observed between HAstV-positive and HAstV-negative patients (Table 2). The presence of mucus in feces was significantly more frequent in HAstV-positive children, particularly in the 1–11-month age group ($p = 0.012$).

Positivity for HAstV was detected throughout the whole year and seasonally was of 30/513 (5.8%) from January to March, 63/857 (7.4%) from April to June, 46/767 (6.0%) from July to September and 68/776 (8.8%) from October to December (Fig 1).

Molecular Characterization

Phylogenetic analysis of a 320-bp nucleotide sequence (ORF2) revealed that almost all HAstV genotypes (except HAstV-5 and HAstV-7) circulated in these three Brazilian coastal regions during the studied period. Considering HAstV-positive sequenced strains, HAstV-1 was the most frequent genotype identified, characterized in 77.7% strains.

In all regions studied, HAstV-1 was detected and represented 25% of the strains observed in the northeastern, 66% in the southeastern and 93% in the southern of Brazil. Other genotypes were also observed, including HAstV-3, which represented 45% of the strains detected in the northeastern, followed by HAstV-6 (25%) and HAstV-2 (8%). In the southeastern, the HAstV genotype-2 (23%) and HAstV-8 (6%) and HAstV-4 (5%) were recorded. In the southern region, besides HAstV-1 (93%), HAstV-8 was the only genotype detected (7%) (Fig 2).

Brazilian HAstV-1 strains grouped into two different genetic clusters (Fig 3A). Cluster 1 strains were closely related to each other (98.4–100% identity at the nt level) and were detected in all studied periods in different geographic regions of Brazil and displayed the highest identity

(97.8–100%) to worldwide 1999–2011 strains. Cluster 2 grouped Brazilian strains detected from 2004–2005 and some Asiatic 2008–2011 strains were restricted to the southeastern region of Brazil. Brazilian HAsV-2 strains also grouped into two different clusters (Fig 3B). Cluster 1 strains were closely related to each other (96.8–100% identity at the nt level) and were detected in 2006–2007 in different geographic regions (south and northeast), and displayed the highest identity (97.8–100%) to worldwide strains. The second HAsV cluster grouped Brazilian strains detected in 2005 and 2007 together with other Brazilian strains, as well as with Italian and Honduran strains, restricted to the southeastern Brazilian region. The Brazilian HAsV-3 strains showed the highest nucleotide identity (>97%) to HAsV-3 strains reported in Japan, Italy, Pakistan and Egypt. Notably, the unique HAsV-4 strain characterized in this study showed the lowest genetic relationship (93.1–93.7%) with Brazilian samples previously described and the highest nucleotide identity observed with worldwide samples (>96%).

Screening of novel HAsVs

Concerning novel HAsV screening, two samples were positive and characterized as AstV-MLB1 (Fig 4). AstV-VA1 or other unusual HAsV rather than MLB1 were not detected. The two positive MLB1 samples were collected from a one year old child admitted in casualty due to AD in February and November 2011, in two different Brazilian regions: Maranhão State (northeast) and Rio de Janeiro State (southeast).

Table 2. Clinical manifestations of HAsV cases according to age group.

Clinical Characteristics	Age Group				Total Samples/ Studied (%)
	0–11 months Samples/Studied (%)	12–24 months Samples/Studied (%)	25–48 months Samples/Studied (%)	49–60 months Samples/Studied (%)	
Fever					
HAsV negative	716/1233 (58.1)	551/1000 (55.1)	272/505 (53.9)	50/90 (55.6)	1238/2828 (43.7)
HAsV positive	53/85 (62.4)	34/62 (54.8)	21/46 (45.7)	5/9 (55.6)	113/202 (55.9)
p-value	0.438	0.968	0.285	1.000	0.962
Vomit					
HAsV negative	634/1235 (51.3)	551/1009 (54.6)	306/509 (60.1)	46/88 (52.3)	1537/2841 (54.1)
HAsV positive	41/84 (51.2)	31/62 (50.0)	26/47 (55.3)	8/10 (80.0)	108/203 (53.2)
p-value	0.986	0.479	0.521	0.094	0.976
Dehydration					
HAsV negative	190/557 (34.1)	141/498 (28.3)	63/245 (25.7)	12/53 (22.6)	406/1353 (30)
HAsV positive	15/49 (30.6)	10/35 (28.6)	4/29 (13.8)	2/6 (33.3)	31/119 (26.1)
p-value	0.619	0.973	0.157	0.559	0.365
Mucous in feces					
HAsV negative	179/586 (30.5)	132/507 (26.0)	57/251 (22.7)	11/53 (20.8)	379/1397 (27.1)
HAsV positive	25/53 (47.2)	15/38 (39.5)	10/31 (32.3)	1/6 (16.7)	51/128 (39.8)
p-value	0.012	0.071	0.238	0.813	0.002
Blood in feces					
HAsV negative	192/1156 (16.6)	132/948 (13.9)	84/480 (17.5)	6/83 (7.2)	414/2667 (15.5)
HAsV positive	10/81 (12.3)	8/58 (13.8)	4/46 (8.7)	0/10 (0.0)	22/195 (11.3)
p-value	0.315	0.977	0.126	0.379	0.111
Parenteral fluid reposition					
HAsV negative	223/345 (64.6)	165/276 (59.8)	82/144 (56.9)	18/31 (58.1)	488/796 (61.3)
HAsV positive	15/25 (60.0)	15/20 (75.0)	5/12 (41.7)	3/4 (75.0)	38/61 (62.3)
p-value	0.170	0.292	0.521	0.735	0.933

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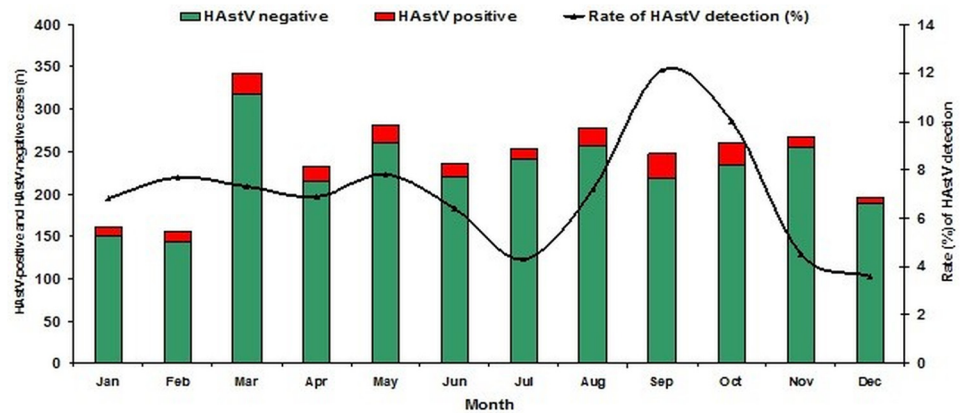


Fig 1. Seasonality of HAstV in cases of acute diarrhea. Laboratory-based HAstV in three Brazilian regions, between 2005 and 2011. The rate of HAstV detection by month and number of HAstV-positive samples. Each month represents cumulative observations.

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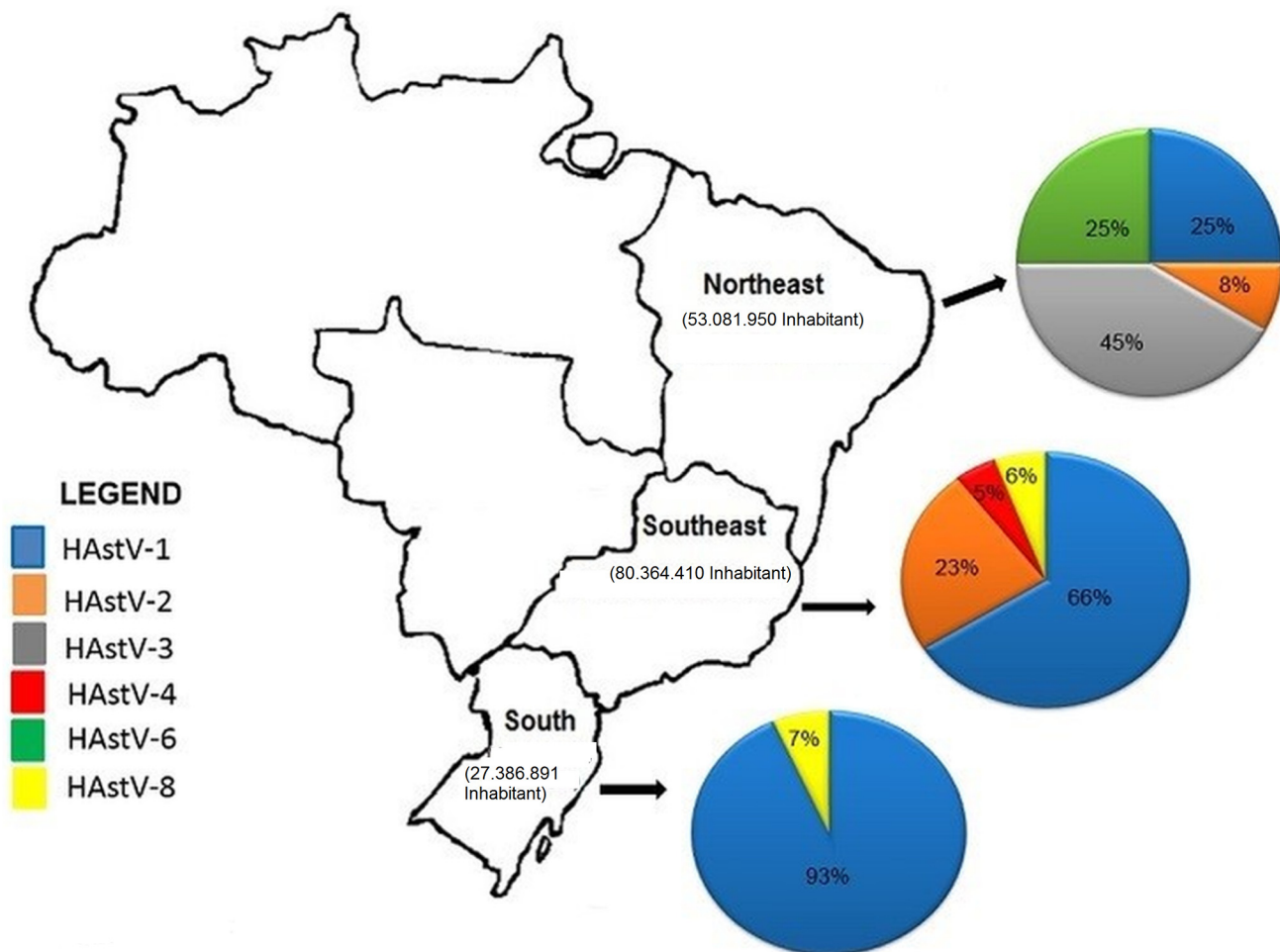


Fig 2. Map of the Brazilian coastal regions, showing the HAstV genotype distributions during 2005–2011.

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Discussion

This study reports an overview of the distribution of HAsTV genotypes in three coast regions of Brazil. It was the first time that the recently novel HAsTV was investigated, increasing

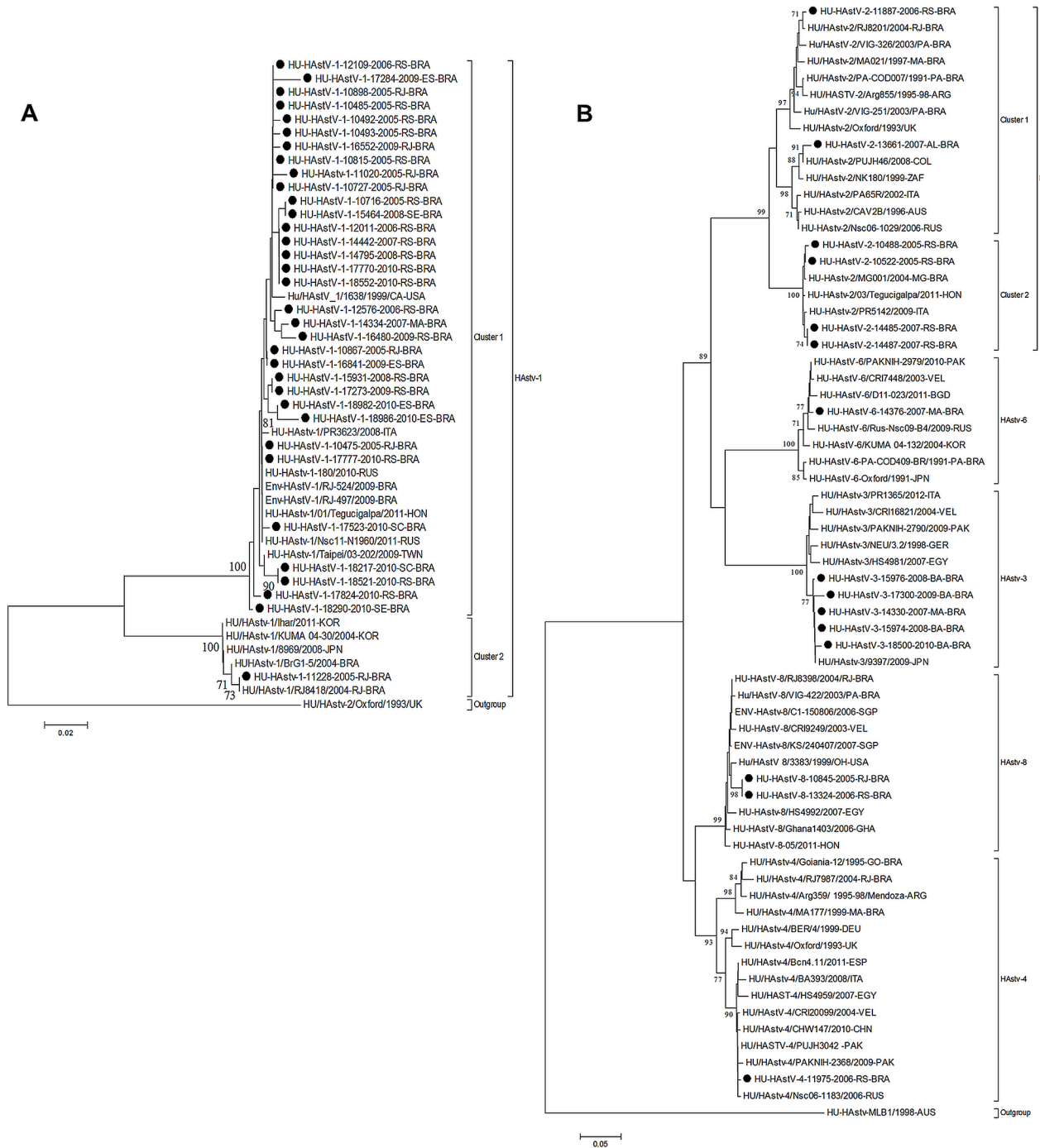


Fig 3. Phylogenetic analyses based on the nucleotide sequences (ORF2 region) of classic HAsTV. (A) Phylogenetic analyzes were based on the 320-bp capsid gene between HAsTV-1 strains detected in this study and HAsTV-1 worldwide strains. (B) Phylogenetic analyzes between strains HAsTV-2-HAsTV-8 detected in this study and HAsTV worldwide strains. The Brazilian strains described in this study are denoted by black circles. The scale bar indicates nucleotide substitutions per site. Bootstrap values (2.000 pseudo-replicates) above 70 are shown. The HAsTV reference strains included in this analysis were obtained from GenBank.

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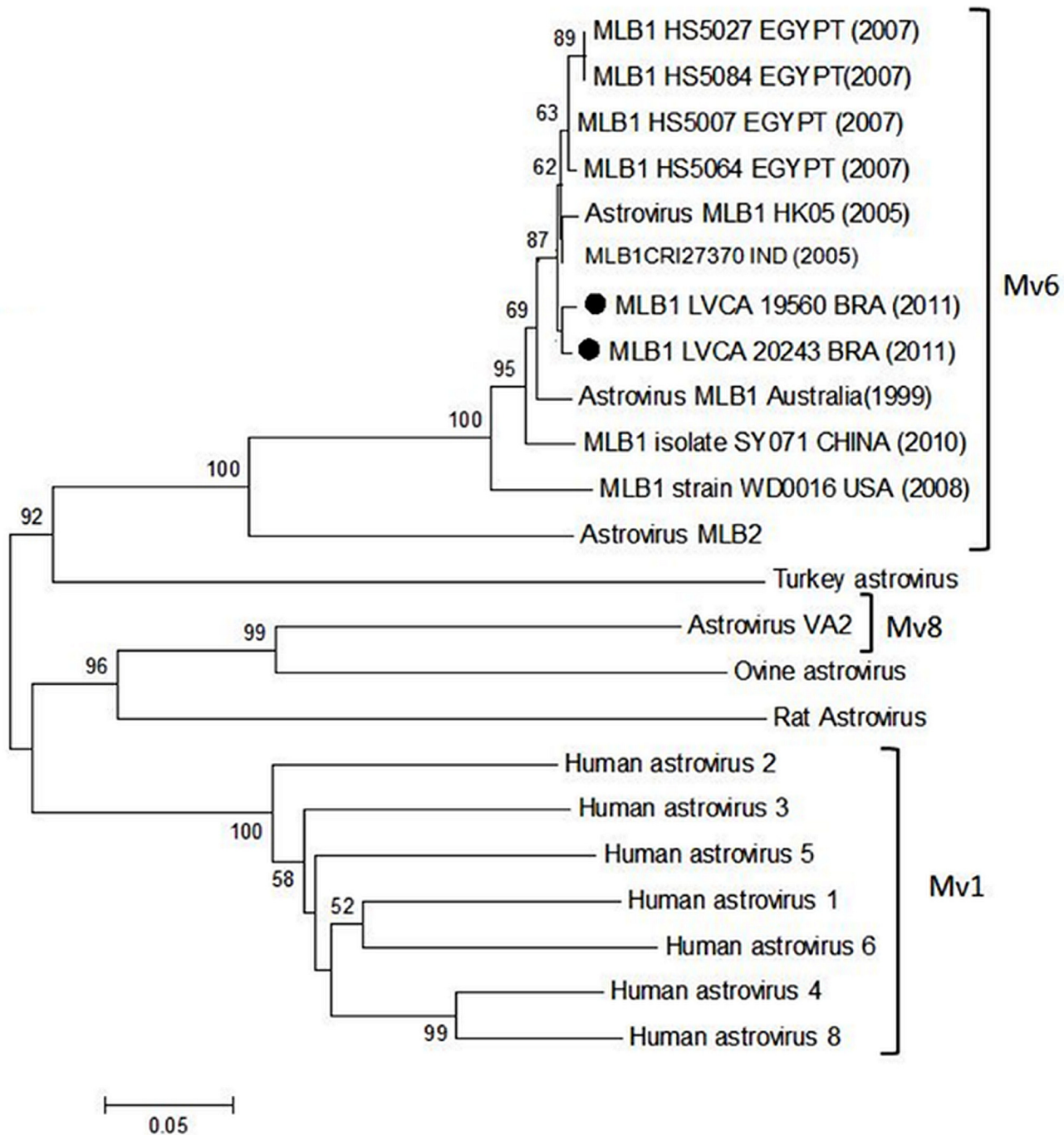


Fig 4. Phylogenetic analyses based on the nucleotide sequences (ORF2 region) of MLB1 Brazilian isolates and representative *Mamastrovirus* species worldwide. The Brazilian strains described in this study are denoted by black circles. The scale bar indicates nucleotide substitutions per site. Bootstrap values (2,000 pseudo-replicates) above 70 are shown. Reference sequences accession number in GenBank Database are cited in order of appearance: NC_011400; FJ402983; NC_014320; JN871245; KC294576; KC294577; HQ674649; HQ674647; HQ674648; HQ674645; JQ086552; NC_016155; GQ502193; NC_002469; NC_001943; L13745; GU732187; DQ070852; DQ028633; GQ901902; AF260508; HM450382; to Turkey AstV (NC_002470).

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knowledge about its characteristics and genotypes circulation. Regarding HAsTV infection prevalence in Brazil, initial studies conducted in different regions yielded positivity rates of

3–5% [22–24], lower than those reported in the present survey. Nevertheless, our data showed similar rates to those in other studies carried out in Brazil [8, 25–27] probably due to the evolution of molecular detection methods with increased sensitivity improving diagnosis. High HAsV positivity rates is normally described in AD outbreaks, such as in a native Brazilian population and in southeastern Mexico, where the prevalence of HAsV infection reached 56% and 28%, respectively [28, 29].

As expected, HAsV were commonly detected in all age group analyzed. The prevalence rate observed tended to be higher among children aged 40–60 months, although there was no statistical significance between age group and HAsV infection. HAsVs affect predominantly the pediatric population and the age of children infected with classic HAsV varies, ranging from newborns to over 5 years old [2, 8, 30].

HAsV-related AD is generally considered mild and self-limiting, but it can be sufficiently severe to require medical intervention in immunocompromised or malnourished patients [2, 4, 14, 15, 31]. All children enrolled in this study showed signs and symptoms of AD requiring at least one outpatient visit, thus the HAsV detection in AD cases remains of clinical importance. Fever and vomiting were the most common signs and interestingly, mucus in the stool samples were observed and their presence was statistically significant in HAsV AD cases, mainly in infants aged < 1 year. Data were similar to another study conducted in Brazil [8].

Concerning seasonality, it has been proposed that the peak of HAsV detection in temperate climate countries occurs in the colder months and in tropical areas, the maximum incidence of HAsV infections tends to occur in the rainy season (2). The current study was performed in a seven years' time span, and a slight trend towards higher detection rates during the rainy season was observed. Similar to our data, the detection of HAsV in tropical climate countries was more frequent in the rainy season [29, 32]. Previous studies carried out in Brazil showed no seasonality or a higher detection frequency in warmer months [8, 24, 25], but the seasonal HAsV pattern is controversial and depends on the climate, geographical region analyzed and the year of study.

Our data demonstrate the variability of circulating HAsV genotypes and that most of the samples were characterized as HAsV-1, reinforcing data from other surveys from several regions of the world, including Brazil that reported the predominance of HAsV-1 [7, 8, 24–26, 33–40]. Phylogenetic analyses showed that Brazilian strains share high nucleotide identity with global strains described in different countries and continents, demonstrating that the introduction of strains occurs continuously and has a great impact on local and regional epidemiology.

The recent discovery from humans of novel AstV strains that show genetic similarities to animal strains has aroused interest and the perception that these potentially zoonotic agents might have a greater impact on public health and on the etiological profile of AD [9–12, 41]. In Brazil, the diagnosis of novel and classic HAsV are restricted to research center, not available in laboratories routine and their importance as etiological agents of AD in both sporadic cases and outbreaks remains poorly understood.

Our investigation of novel HAsV species was conducted in 200 stool samples from children under two years old. The samples tested were selected at random from 2011 cohorts, with equal numbers tested previously negative for RVA, NoV and classic HAsV 1–8 taken from each Brazilian coastal region. This strategy allowed the identification of AstV-MLB1 in two patients. The unusual AstV-MLB1 strain has been identified in fecal samples of patients in Australia and in countries in North America, Asia, Africa and Europe [9, 10, 42–45]. Temporal and geographical relationships were revealed following phylogenetic ORF2 nucleotide analyzes homology among AstV-MLB1 strains (Fig 4). In this study, no other unusual AstV could be detected. Similar results were observed in other surveillance studies [9].

AstV-MLB1 is not yet a well-established AD agent and its role in human health remains unknown. A recent control case study conducted in India could not determine the association between AstV-MLB1 and AD [43]. Another important point is the fact that a large number of samples remain without an etiologic agent, drawing attention to other pathogens that may be involved. Other enteric viruses such as Adenovirus or Sapovirus, as well as bacteria and parasites may also be responsible. This study reinforces the role of distinct HAsV genotypes in the etiological AD profile in different Brazilian coastal regions, describing the detection of AstV-MLB1 in Brazil for the first time and suggesting potential changes in the etiological AD profile considering new pathogens agents as well as novel AstV.

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Author Contributions

Conceived and designed the experiments: MPTPX EMV FACC JPGL MPM. Performed the experiments: MPTPX MSR JSRA FKBD TRA. Analyzed the data: MPTPX EMV FACC MPM. Contributed reagents/materials/analysis tools: JPGL MPM FACC EMV. Wrote the paper: MPTPX EMV FACC.

References

1. Black RE, Cousens S, Johnson HL, Lawn JE, Rudan I, Bassani DG, et al. (2010) Global, regional, and national causes of child mortality in 2008: a systematic analysis. *Lancet* 375:1969–1987. doi: [10.1016/S0140-6736\(10\)60549-1](https://doi.org/10.1016/S0140-6736(10)60549-1) PMID: [20466419](https://pubmed.ncbi.nlm.nih.gov/20466419/)
2. Bosch A, Pintó RM, Guix S. Human astroviruses. *Clin Microbiol Rev.* (2014) Oct; 27 (4):1048–74. doi: [10.1128/CMR.00013-14](https://doi.org/10.1128/CMR.00013-14) PMID: [25278582](https://pubmed.ncbi.nlm.nih.gov/25278582/)
3. Mendez E, Arias CF, editors (2013) *Astroviruses 6th Ed.* ed. Philadelphia. 609–628 p.
4. Sebire NJ, Malone M, Shah N, Anderson G, Gaspar HB, Cubitt WD (2004) Pathology of astrovirus associated diarrhea in a pediatric bone marrow transplant recipient. *J Clin Pathol* 57: 1001–1003. PMID: [15333670](https://pubmed.ncbi.nlm.nih.gov/15333670/)
5. Nighot PK, Moeser A, Ali RA, Blikslager AT, Koci MD. (2010) Astrovirus infection Induces sodium malabsorption and redistributes sodium hydrogen exchanger expression. *Virology*. Jun 5; 401(2):146–54. doi: [10.1016/j.virol.2010.02.004](https://doi.org/10.1016/j.virol.2010.02.004) PMID: [20219227](https://pubmed.ncbi.nlm.nih.gov/20219227/)
6. Available: <http://ictvonline.org/virusTaxonomy.asp>
7. Gabbay YB, Leite JP, Oliveira DS, Nakamura LS, Nunes MR, Mascarenhas JD, et al. (2007) Molecular epidemiology of astrovirus type 1 in Belém, Brazil, as an agent of infantile gastroenteritis, over a period of 18 years (1982–2000): identification of two possible new lineages. *Virus Res* 129: 166–174. PMID: [17714822](https://pubmed.ncbi.nlm.nih.gov/17714822/)
8. Victoria M, Carvalho-Costa FA, Heinemann MB, Leite JP, Miagostovich MP (2007) Genotypes and molecular epidemiology of human astroviruses in hospitalized children with acute gastroenteritis in Rio de Janeiro, Brazil. *J Med Virol* 79: 939–944. PMID: [17516522](https://pubmed.ncbi.nlm.nih.gov/17516522/)
9. Finkbeiner SR, Le BM, Holtz LR, Storch GA, Wang D (2009) Detection of newly described astrovirus MLB1 in stool samples from children. *Emerg Infect Dis* 15: 441444.
10. Finkbeiner RS, Kirkwood CD, Wang D (2008) Complete genome sequence of a highly divergent astrovirus isolated from a child with acute diarrhea. *Virol J.* 14; 15:117.
11. Finkbeiner SR, Li Y, Ruone S, Conrardy C, Gregoricus N, Toney D, et al. (2009) Identification of a novel astrovirus (astrovirus VA1) associated with an outbreak of acute gastroenteritis. *J Virol* 83: 10836–10839. doi: [10.1128/JVI.00998-09](https://doi.org/10.1128/JVI.00998-09) PMID: [19706703](https://pubmed.ncbi.nlm.nih.gov/19706703/)

12. Kapoor A, Li L, Victoria J, Oderinde B, Mason C, Pandey P, et al. (2009) Multiple novel astrovirus species in human stool. *J Gen Virol* 90: 2965–2972. doi: [10.1099/vir.0.014449-0](https://doi.org/10.1099/vir.0.014449-0) PMID: [19692544](https://pubmed.ncbi.nlm.nih.gov/19692544/)
13. Finkbeiner SR, Holtz LR, Jiang Y, Rajendran P, Franz CJ, Zhao G, et al. (2009) Human stool contains a previously unrecognized diversity of novel astroviruses. *Virol J*. Oct 8; 6:161. doi: [10.1186/1743-422X-6-161](https://doi.org/10.1186/1743-422X-6-161) PMID: [19814825](https://pubmed.ncbi.nlm.nih.gov/19814825/)
14. Brown JR, Morfopoulou S, Hubb J, Emmett WA, Ip W, Shah D, et al. (2015). Astrovirus VA1/HMO-C: An Increasingly Recognized Neurotropic. *Pathogen in Immunocompromised Patients*. *Clin Infect Dis*. 2015 Mar 15; 60 (6):881–8. doi: [10.1093/cid/ciu940](https://doi.org/10.1093/cid/ciu940) PMID: [25572899](https://pubmed.ncbi.nlm.nih.gov/25572899/)
15. Naccache SN, Peggs KS, Mattes FM, Phadke R, Garson JA, Grant P, et al. (2015). Diagnosis of neuroinvasive astrovirus infection in an immunocompromised adult with encephalitis by unbiased next-generation sequencing. *Clin Infect Dis*. 15; 60(6):919–23. doi: [10.1093/cid/ciu912](https://doi.org/10.1093/cid/ciu912) PMID: [25572898](https://pubmed.ncbi.nlm.nih.gov/25572898/)
16. Quan PL, Wagner TA, Briese T, Torgerson TR, Hornig M, Tashmukhamedova A, et al. (2010) Astrovirus encephalitis in boy with X-linked agammaglobulinemia. *Emerg Infect Dis* 16: 918–925. doi: [10.3201/eid1606.091536](https://doi.org/10.3201/eid1606.091536) PMID: [20507741](https://pubmed.ncbi.nlm.nih.gov/20507741/)
17. Leon S, Ponce AC (2009) Analysis of mortality from diarrheic diseases in under-five children in Brazilian cities with more than 150,000 inhabitants. *Cadernos de Saúde Pública* 25 (5): 1093–1102. PMID: [19488494](https://pubmed.ncbi.nlm.nih.gov/19488494/)
18. Noel JS, Lee TW, Kurtz JB, Glass RI, Monroe SS (1995) Typing of human astroviruses from clinical isolates by enzyme immunoassay and nucleotide sequencing. *J Clin Microbiol* 33: 797–801. PMID: [7790440](https://pubmed.ncbi.nlm.nih.gov/7790440/)
19. Available: <http://blast.ncbi.nlm.nih.gov/>
20. Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucl Acids Symp Ser*: 95–98.
21. Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S (2011) MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol Biol Evol* 28:2731–2739. doi: [10.1093/molbev/msr121](https://doi.org/10.1093/molbev/msr121) PMID: [21546353](https://pubmed.ncbi.nlm.nih.gov/21546353/)
22. Leite JP, Barth OM, Schatzmayr HG (1991) Astrovirus in faeces of children with acute gastroenteritis in Rio de Janeiro, Brazil. *Mem Inst Oswaldo Cruz* 86: 489–490. PMID: [1842447](https://pubmed.ncbi.nlm.nih.gov/1842447/)
23. Silva AM, Leite EG, Assis RM, Majerowicz S, Leite JP (2001) An outbreak of gastroenteritis associated with astrovirus serotype 1 in a day care center, in Rio de Janeiro, Brazil. *Mem Inst Oswaldo Cruz* 96: 1069–1073. PMID: [11784924](https://pubmed.ncbi.nlm.nih.gov/11784924/)
24. Gabbay YB, Luz CR, Costa IV, Cavalcante-Pepino EL, Sousa MS, Oliveira KK, et al. (2005) Prevalence and genetic diversity of astroviruses in children with and without diarrhea in São Paulo, Maranhão, Brazil. *J Med Virol*. 2007 May; 79(5):530–8.
25. Cardoso DDP, Fiaccadori FS, Borges de Lima Dias e Souza M, Bringel Martins RM, Gagliardi Leite JP (2002). Detection and genotyping of astroviruses from children with acute gastroenteritis from Goiânia, Goiás, Brazil. *Med Sci Monit*.; 8(9):CR624–8 PMID: [12218943](https://pubmed.ncbi.nlm.nih.gov/12218943/)
26. Ferreira MS, Xavier Mda P, Tinga AC, Rose TL, Fumian TM, Fialho AM, et al. (2012) Assessment of gastroenteric viruses frequency in a children's day care center in Rio De Janeiro, Brazil: a fifteen year study (1994–2008). *PLoS One* 7(3)
27. Xavier MP, Oliveira SA, Ferreira MS, Victoria M, Miranda V, Silva MF, et al. (2009) Detection of caliciviruses associated with acute infantile gastroenteritis in Salvador, an urban center in Northeast Brazil. *Braz J Med Biol Res* 42: 438–444 PMID: [19377793](https://pubmed.ncbi.nlm.nih.gov/19377793/)
28. Gabbay YB, Linhares AC, Cavalcante-Pepino EL, Nakamura LS, Oliveira DS, da Silva LD, et al. (2007) among children in Belem, Brazil. *J Med. Virol* 79 (5): 530–8. PMID: [17385695](https://pubmed.ncbi.nlm.nih.gov/17385695/)
29. Maldonado Y, Cantwell M, Old M, Hill D, Sanchez ML, Mogan L, et al. (1998) Population-based prevalence of symptomatic and asymptomatic astrovirus infection in rural Mayan infants. *J Infect Dis* 178:334–339. PMID: [9697712](https://pubmed.ncbi.nlm.nih.gov/9697712/)
30. Gabbay YB, Chamone CB, Nakamura LS, Oliveira DS, Abreu SF, Cavalcante-Pepino EL, et al. (2006) Characterization of an astrovirus genotype 2 strain causing an extensive outbreak of gastroenteritis among Maxakali Indians, Southeast Brazil. *J Clin Virol* 37: 287–292. PMID: [16996790](https://pubmed.ncbi.nlm.nih.gov/16996790/)
31. Wunderl W, Meerbach A, Güngör T, Berger C, Greiner O, Caduff R, et al. (2011). Astrovirus infection in hospitalized infants with severe combined immunodeficiency after allogeneic hematopoietic stem cell transplantation. *PLoS One*. 2011; 6(11)
32. Cruz JR, Bartlett AV, Herrmann JE, Caceres P, Blacklow NR, Cano F (1992). Astrovirus-associated diarrhea among Guatemalan ambulatory rural children. *J. Clin. Microbiol*. 30: 1140–1144. Walter JE, Mitchell DK (2003) Astrovirus infection in children. *Curr Opin Infect Dis* 16: 247–253. PMID: [1583111](https://pubmed.ncbi.nlm.nih.gov/1583111/)

33. Mustafa H, Palombo EA, Bishop RF (2000) Epidemiology of astrovirus infection in young children hospitalized with acute gastroenteritis in Melbourne, Australia, over a period of four consecutive years, 1995 to 1998. *J Clin Microbiol* 38: 1058–1062. PMID: [10698996](#)
34. Giordano MO, Martinez LC, Isa MB, Paez Rearte M, Nates SV (2004) Childhood astrovirus-associated diarrhea in the ambulatory setting in a Public Hospital in Cordoba city, Argentina. *Rev Inst Med Trop Sao Paulo* 46: 93–96. PMID: [15141279](#)
35. Verma H, Chitambar SD, Gopalkrishna V (2010) Astrovirus associated acute gastroenteritis in western India: predominance of dual serotype strains. *Infect Genet Evol* 10: 575–579. doi: [10.1016/j.meegid.2010.01.008](#) PMID: [20117249](#)
36. Jeong AY, Jeong HS, Jo MY, Jung SY, Lee MS, Lee JS, et al. (2011) Molecular epidemiology and genetic diversity of human astrovirus in South Korea from 2002 to 2007. *Clin Microbiol Infect* 17: 404–408. doi: [10.1111/j.1469-0691.2010.03263.x](#) PMID: [20491833](#)
37. Malasao R, Khamrin P, Chaimongkol N, Ushijima H, Maneekarn N (2012) Diversity of human astrovirus genotypes circulating in children with acute gastroenteritis in Thailand during 2000–2011. *J Med Virol* 84: 1751–1756. doi: [10.1002/jmv.23396](#) PMID: [22997078](#)
38. Tseng WC, Wu FT, Hsiung CA, Chang WC, Wu HS, Wu CY, et al. (2012) Astrovirus gastroenteritis in hospitalized children of less than 5 years of age in Taiwan, 2009. *J Microbiol Immunol Infect* 45: 311–317. doi: [10.1016/j.jmii.2011.12.017](#) PMID: [22209687](#)
39. Ouyang Y, Ma H, Jin M, Wang X, Wang J, Xu L, et al. (2012) Etiology and epidemiology of viral diarrhea in children under the age of five hospitalized in Tianjin, China. *Arch Virol* 157: 881–887. doi: [10.1007/s00705-012-1235-9](#) PMID: [22318672](#)
40. Afrad MH, Karmakar PC, Das SK, Matthijnsens J, Ahmed F, Nahar S, et al. (2013) Epidemiology and genetic diversity of human astrovirus infection among hospitalized patients with acute diarrhea in Bangladesh from 2010 to 2012. *J Clin Virol* 58: 612618.
41. Chu DK, Chin AW, Smith GJ, Chan KH, Guan Y, Peiris JS, et al. (2010) Detection of novel astroviruses in urban brown rats and previously known astroviruses in humans. *J Gen Virol* 91: 2457–2462. doi: [10.1099/vir.0.022764-0](#) PMID: [20554799](#)
42. Holtz LR, Bauer IK, Rajendran P, Kang G, Wang D (2011) Astrovirus MLB1 is not associated with diarrhea in a cohort of Indian children. *PLoS One* 6(12).
43. Bányai K, Meleg E, Moschidou P, Martella V (2010). Detection of newly described astrovirus MLB1 in stool samples from children. *Emerg Infect Dis* 16: 169; author reply 169–170. doi: [10.3201/eid1601.091563](#) PMID: [20031079](#)
44. Matsumoto T, Wangchuk S, Tshering K, Yahiro T, Zangmo S, Dorji T, et al. (2013) Nishizono A, Ahmed K. Complete Genome Sequences of Two Astrovirus MLB1 Strains from Bhutanese Children with Diarrhea. *Genome Announc*. 25; 1(4).
45. Medici MC, Tummolo F, Calderaro A, Elia G, Banyai K, De Conto F, et al. (2014). MLB1 astrovirus in children with gastroenteritis, Italy. *Emerg Infect Dis*. 20(1):169–70. doi: [10.3201/eid2001.131259](#) PMID: [24377948](#)