

**Original article****Relative frequency of astrovirus in children suffering from gastroenteritis referred to Aboozar hospital, Ahvaz**

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**How to cite this article:**

Mozhgani SHR, Samarbaf-Zadeh AR, Makvandi M, Shamsi-Zadeh A, Parsanahad M, Jalilian S. Relative frequency of astrovirus in children suffering from gastroenteritis referred to Aboozar hospital, Ahvaz. Jundishapur J Microbiol. 2011; 4(Supplement 1): S67-S70.

**Received:** June 2010

**Accepted:** November 2010

**Abstract**

**Introduction and objective:** Human astrovirus (HAstVs), belonging to a family of non enveloped, icosahedral RNA viruses and causes gastroenteritis both in infants and adults. The aim of this study was to determine the relative frequency of viral gastroenteritis caused by astrovirus among children less than five years referred to Ahvaz Aboozar hospital.

**Materials and methods:** Astrovirus infection was detected with Reverse Transcriptase Polymerase Chain Reaction (RT-PCR). The authenticity of PCR products was confirmed by sequencing.

**Results:** Astrovirus infection was detected in 29 cases of 184 (15.77%), 13 positive samples belonged to the children up to six months. The relationship between gender and the prevalence of astroviral gastroenteritis was not significant. Most cases occurred during the coldest months of the year. After the sequencing, genotypes eight and four were the dominant types in this study.

**Conclusion:** It was shown that human astrovirus plays an important role in gastroenteritis in Ahvaz, south west of Iran. The prevalence of the infection was very high. To decrease prevalence of astroviral infection, education and personal hygiene is advised.

**Keywords:** Human Astrovirus; Gastroenteritis; Reverse Transcriptase Polymerase Chain Reaction (RT-PCR)

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Jundishapur Journal of Microbiology, School of Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran, Tel: +98611 3330074; Fax: +98611 3332036; URL: <http://jjm.ajums.ac.ir>; E-mail: editorial office: [jjm@ajums.ac.ir](mailto:jjm@ajums.ac.ir)

**JJM. (2011); 4(Supplement 1): S67-S70.**

## Introduction

Acute gastroenteritis is one of the most common illnesses worldwide and has a significant impact on morbidity and mortality of people [1]. Human astroviruses (HAstV) are members of the *Astroviridae* family, and are identified as an important cause of gastroenteritis not only in children, but also in the adults. Moreover, they can cause severe disease in immunocompromised people [2]. Astroviruses possess an icosahedral capsid and their genomes are single stranded positive sense RNA [3].

The major symptom of astrovirus infection is watery diarrhea that can occur with fever, vomiting, anorexia and abdominal pain [4]. The astrovirus genomes contain three open reading frames (ORFs): ORFs1a and 1b, which encode viral protease and polymerase, and ORF2, which encodes capsid protein [5]. To our best knowledge there is no report about the prevalence of astrovirus infection in Iran and this study is the first one that determines the prevalence of them by using RT-PCR method in our area.

## Materials and methods

Fecal specimens were collected from children less than five years old with gastroenteritis, referred to Ahvaz, Aboozar Hospital from 2008 to 2009. To rule out parasitic and microbial infection, relevant tests were carried out. Fecal samples were examined by microscope to evaluate the WBC status of the specimens. The negative specimens were saved at  $-80^{\circ}\text{C}$ . A hundred eighty five samples were tested for the presence of Astroviral infection.

### *Extraction of viral RNA from fecal suspensions*

Viral RNA was extracted from fecal suspensions using Trizol (Fermentas-Lithuania) according to the manufacturer

instruction. Finally the pellet of RNA was re-suspended in 40 $\mu\text{l}$  DEPC water.

### *Reverse Transcriptase Polymerase Chain Reaction (RT-PCR)*

All of the samples were submitted to RT-PCR using primers Mon 348 (5'ACATGTGCTGCTGTTACTATG3') and Mon 340(5'CGTCATTATTTGTTGT-CATACT3') for ORF1a, The size of amplicon was 289bp [5]. Prior to RT-PCR, denaturation of viral RNA was carried out at  $70^{\circ}\text{C}$  for 5mins, followed by 2mins incubation on ice. The tubes of reverse transcription reaction were incubated at  $42^{\circ}\text{C}$  for 1h. A five microliter sample of the cDNA was used as a template of PCR reaction. PCR conditions were as follow: 35 cycles of amplification ( $94^{\circ}\text{C}$  for 30S,  $50^{\circ}\text{C}$  for 30S,  $72^{\circ}\text{C}$  for 1min) and a final extension at  $72^{\circ}\text{C}$  for 10mins. 10 $\mu\text{l}$  of the final PCR product were subjected to electrophoresis in 2% agar gel, stained by ethidium bromide and which can be visualized on UV transilluminater (Vilber Lourmat, France).

### *Nucleotide sequencing stage*

Ten positive samples were sent to Milogen Company (France) for sequencing.

## Results

One of the samples missed during the survey. The minimum age of patients was one month and the maximum age was five years. The average of the patients' age was almost eight months. Astrovirus infection was detected in 29 cases out of 184(15.17%). Fourteen samples (48.3%) were obtained from male cases and 15 specimens (51.7%) belonged to the female ones. There were no relationship between gender and the prevalence of the disease. The samples can be categorized as in the following: thirteen samples were six month infants, five were 6-12 month infants, and

11 samples were 1-2 year children. We could not find any positive result for the children between 2-5 years. The highest prevalence of the infection was found to be in winter (51.72%) and the lowest was in summer (6.89%). The authenticity of PCR products were confirmed by the sequencing. The sequencing revealed that genotypes eight were responsible for 90% of astrovirus gastroenteritis. Ten percent of the cases of gastroenteritis were due to genotype four of astroviruses.

### Discussion

Acute gastroenteritis is one of the most common infectious diseases worldwide, and causes significant morbidity in infant [6]. Four major viral pathogens are responsible for gastroenteritis especially in children (rotavirus, sapovirus, astrovirus, and enteric adenoviruses) [6]. Astroviruses have been identified as important agents of acute gastroenteritis both in children [7] and adults [8]. Traditionally, diagnosis of astrovirus infection is performed by electron microscopy [9], but currently ELISA and molecular methods such as RT-PCR are applied to diagnose this viral agent. RT-PCR is a promising technique since it is more sensitive in the detection of astroviral gastroenteritis [10]. Because of the high sensitivity of RT-PCR assay, in this report, detection of Astrovirus was performed by RT-PCR method. According to the literature, the percentage of Astroviral infection in the world varies from 2-9% [11].

To our best knowledge there is no report about the prevalence of astrovirus gastroenteritis in Iran. In this study, 15% of samples were found to be positive to astrovirus. This prevalence is much higher than the prevalence of astrovirus infection in most of the countries in the world [11]. Transmission of this virus occurs by fecal-oral rout [11]. The high prevalence of this

viral infection may show contamination of food and drink water and poor personal hygiene. New born infection might occur during feeding of them by adults. As other studies [5], no relationship was found between gender and prevalence of viral infection. The prevalence of this virus among men was 48.72% and among women was 51.72%. Therefore, there was no relationship between sexuality and the prevalence of this virus.

According to a study reported by Bhattacharya *et al.* [5] in Kolkata, India, 50% of astrovirus positive cases were found among children of age group 6-12 month and the prevalence of astroviral infection among the children between 1-5 year old was low. In this study, the highest prevalence was observed among children up to six months and there were no positive samples in children between 2-5 years old. Our results are in concordance with the above report as astrovirus infection was most prevalent in children up to one year old and this virus was not detected in 2-5 year old children. We think these children have been infected by this virus earlier.

To evaluate this hypothesis we recommend to carry out an ELISA test for the negative astroviral cases in the community to monitor probable former astrovirus infection. Although astroviral infection shows seasonal distribution, but most of astroviral gastroenteritis are found during the winter in temperate climate, and during the rainy season, in tropical countries [12]. Ahvaz is classified as a warm and humid area. In our study in Ahvaz the peak of astrovirus infection was during the winter and the lowest prevalence of this virus was during the summer. These reports are in concordant with other studies [12]. According to the reports, the most prevalent type of Astrovirus is types 2-4, but types 5-7 are less common [13-16]. Type eight has only recently been identified

[13-16], but in our area we found that type eight was the most common type of astrovirus.

### Conclusion

Altogether, the prevalence of this virus in Ahvaz was very high; to decrease the prevalence of astroviral infection education for improvement of personal hygiene is advised.

### Acknowledgment

The authors of this article are greatly thankful to Dr. Pierre Pothier for providing positive astrovirus control for the study. Special gratitude also goes to Mrs. Pirmoradi, Mrs Lotfi, Mrs. Neisi and Mr. Najafi from virology department of Ahvaz Jundishapur university of Medical Science.

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