

*Full Length Research Paper*

# **Epidemiology of astrovirus infection in young children hospitalized with gastroenteritis in Iran, over a period of seven years, using reverse Transcriptase-polymerase chain reaction (RT-PCR)**

**Saied Maham<sup>1</sup>, Neda Marhamati<sup>2</sup>, Fatemeh Fallah<sup>1</sup>, Raheleh Sadat Sajadi Nia<sup>3</sup> and Fatemeh Atashrazm<sup>1\*</sup>**

<sup>1</sup>Pediatric Infection Research Center, Shahid Beheshti Uni University of Medical Sciences, Mofid Children's Hospital, Tehran, Iran.

<sup>2</sup>Shahid Beheshti University of Medical Sciences, Mofid Children's Hospital, Tehran, Iran.

<sup>3</sup>Shahid Beheshti University of Medical Sciences, Tehran, Iran.

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**Human astroviruses have been increasingly identified as important agents of diarrheal disease in children. Outbreaks of diarrhea due to astrovirus have frequently been reported and astroviruses have also been associated with nosocomial infections in hospitals. A 7-year study involving 2,490 gastroenteritis samples was conducted to determine the prevalence and age distribution of human astrovirus infection as well as the seasonality pattern in 5 different cities of Iran using reverse Transcriptase-polymerase chain reaction (RT-PCR). Astrovirus was detected in 40 of 2490 specimens tested by RT-PCR, and astrovirus infection was confirmed by Southern hybridization. Detection rates were higher in winter, although astrovirus infections also occurred in summer months. The overall incidence of astrovirus was found to be 1.6%. The mean age of infected children was 14.7 months, and the median age was 15 months. Majority of the infected children were less than 2 years of age making up 36 (90%) infected children, only 4 cases of infected children were more than 2 years of age (10%). The difference between the two age groups was statistically significant ( $P < 0.02$ ). Our findings provide evidence that astrovirus can be a leading cause of viral gastroenteritis infections and highlight the need to implement astrovirus detection assays in association with rapid rotavirus and adenovirus detection enzyme immunoassays (EIAs) for the clinical diagnosis and nosocomial prevention of viral gastroenteritis infections in pediatric departments.**

**Key words:** Astrovirus, reverse Transcriptase-polymerase chain reaction (RT-PCR), gastroenteritis, pediatric, prevalence.

## **INTRODUCTION**

Astroviruses are non-enveloped single-stranded RNA viruses that were first detected in 1975 by electron microscopy in stool specimens from children with acute gastroenteritis (Madeley and Cosgrove, 1975). The astrovirus genome contains three open reading frames

(ORFs): ORF1a and ORF1b, which encode the viral protease and polymerase, respectively, and ORF2, which encodes the capsid precursor.

Human astroviruses have been increasingly identified as important agents of diarrheal disease in children and the elderly. The main symptom of infection is watery diarrhea, which is often associated with vomiting, fever, and abdominal pain (Matsui and Greenberg, 1996). Outbreaks of diarrhea due to astrovirus have frequently

\*Corresponding author. E-mail: [rahelehsajadinia@yahoo.com](mailto:rahelehsajadinia@yahoo.com).

**Table 1.** Primers used for astrovirus detection using RT- PCR.

Primer	Gene	Location	Polarity	Sequence (5'-3')
Mon 340	ORF1	1182-1203	+	CGTCATTATTTGTTGTCATACT
Mon 348	ORF1	1450-1470	-	ACATGTGCTGCTGTTACTATG

been reported (Belliot et al., 1997; Mitchell et al., 1995; Noel and Cubitt, 1994; Oishi et al., 1994; Taylor et al., 1997), and astroviruses have also been associated with nosocomial infections in hospitals (Shastri et al., 1998; Unicomb et al., 1998). They have also been detected in immunocompromised (Noel and Cubitt, 1994) and AIDS-infected patients (Liste et al., 2000). Astrovirus infections occur worldwide, and their incidence in children with gastroenteritis in both developing and developed countries ranges from 2 to 9% (Bon et al., 1999; Foley et al., 2000; Gaggero et al., 1998; Mustafa et al., 2000; Svenungsson et al., 2000; Walter and Mitchell, 2000), although some studies report prevalences up to 26% (Maldonado et al., 1998).

Although astrovirus epidemiological studies have been commonly based on electron microscopy and enzyme immunoassay techniques, during the past few years the number of surveys using molecular techniques, mainly reverse Transcriptase-polymerase chain reaction (RT-PCR), has substantially increased. There is a widespread belief that astrovirus incidence may have been underestimated, since enzyme immunoassay is far less sensitive than RT-PCR (Mitchell et al., 1995; Gaggero et al., 1998). Furthermore, seroprevalence studies indicate that most children acquire astrovirus antibodies during the first years of life (Koopmans et al., 1998; Kriston et al., 1996). Consequently, a new appreciation for the role of astrovirus in diarrheal disease has evolved, and in many cases, astroviruses are regarded as the second most common cause of viral gastroenteritis in children after rotavirus (Glass et al., 1996; Herrmann et al., 1991).

The aim of the present study was to determine the prevalence and age distribution as well as the seasonality pattern of astrovirus infections from children with gastroenteritis in 5 different cities of Iran, during a 7-year period using RT-PCR.

## MATERIALS AND METHODS

### Stool samples

Between May, 2002 and April, 2008, 2,490 fecal samples were collected from infants and children with gastroenteritis who were admitted to one of the hospitals involved in the present clinical study from 5 different cities. Studied patients had a minimum age of 30 days and a maximum of 4 years. Mean age of the studied patients was 48 months. From 2490 fecal samples, 414 were from Bandar Abbas, 394 from Tabriz, 624 from Tehran, 325 from Mashhad and 733 from Shiraz. For astrovirus detection, stools were suspended (10%, w/v) in phosphate-buffered saline containing 2 M NaNO<sub>3</sub>, 1% bovine serum albumin; fraction V, and 0.1% Triton X-

100 (pH 7.2) and pelleted at 1,000 ×g for 5 min, and the resulting supernatant was stored at -70°C for later analysis.

### Astrovirus detection

Astrovirus was detected by RT-PCR after extraction of its RNA and subsequently confirmed by Southern blot hybridization with an internal probe. RNA was purified from 50 µl of fecal supernatant by guanidine thiocyanate extraction, as previously described (Boom et al., 1990). RT-PCR was carried out with primers Mon 340 and 348, which amplify a fragment of ORF1a (Table 1). Five microliters of the extracted RNA was heated to 99°C for 5 min and was immediately placed on ice. First-strand cDNA was synthesized at 42°C for 60 min by adding 1 µM primer Mon 348 and 3 U of reverse transcriptase (Expand; Roche) in 10 µl (final volume) containing 50 mM Tris-HCl (pH 8.3), 40 mM KCl, 5 mM MgCl<sub>2</sub>, 10 mM dithiothreitol, 0.5 mM Tween 20, and 0.2 mM concentrations of each deoxynucleoside triphosphate. Five microliters of the RT product was amplified using 0.5 U of the expand high-fidelity PCR system enzyme mix (Roche) and 0.5 µM (each) primers Mon 340 and 348 in a total volume of 50 µl containing 5 µl of the expand high-fidelity buffer (Roche), 2 mM MgCl<sub>2</sub>, and each deoxynucleoside triphosphate at 0.2 mM. After a denaturation step of 3 min at 95°C, 40 cycles of amplification (94°C, 30 s; 55°C, 30 s; 72°C, 30 s) were performed followed by a final extension of 7 min at 72°C. Ten microliters of the PCR product was analyzed on a 1.5% agarose gel and detected by ethidium bromide staining. PCR products were confirmed by Southern blot hybridization with an internal digoxigenin-labeled probe under stringent conditions (Guix et al., 2002; Mustafa et al., 2000).

### Statistical analysis

T-test was used to evaluate the differences between astrovirus incidence among age groups.

## RESULTS

Over a 7-year period spanning from May, 2002 to April, 2008, a total of 2490 stool specimens collected from children admitted to one of the hospitals involved in this study with acute gastroenteritis were tested for astroviruses. Detection rates were higher in winter (59%), although astrovirus infections also occurred in summer months (8%) (Figure 5). The overall incidence of astrovirus infection was found to be 1.60% (40 of 2490 total samples); 8 positive samples from Bandar Abbas (1.93%), 3 from Tabriz (0.76%), 7 from Tehran (1.12%), 9 from Mashhad (2.77%) and 13 from Shiraz (1.77%). The age distribution of astrovirus infection for the first 4 cities is as shown in Figures 1 to 4. The mean age of infected children was 14.7 months, and the median age was 15 months. Majority of the infected children were less than 2

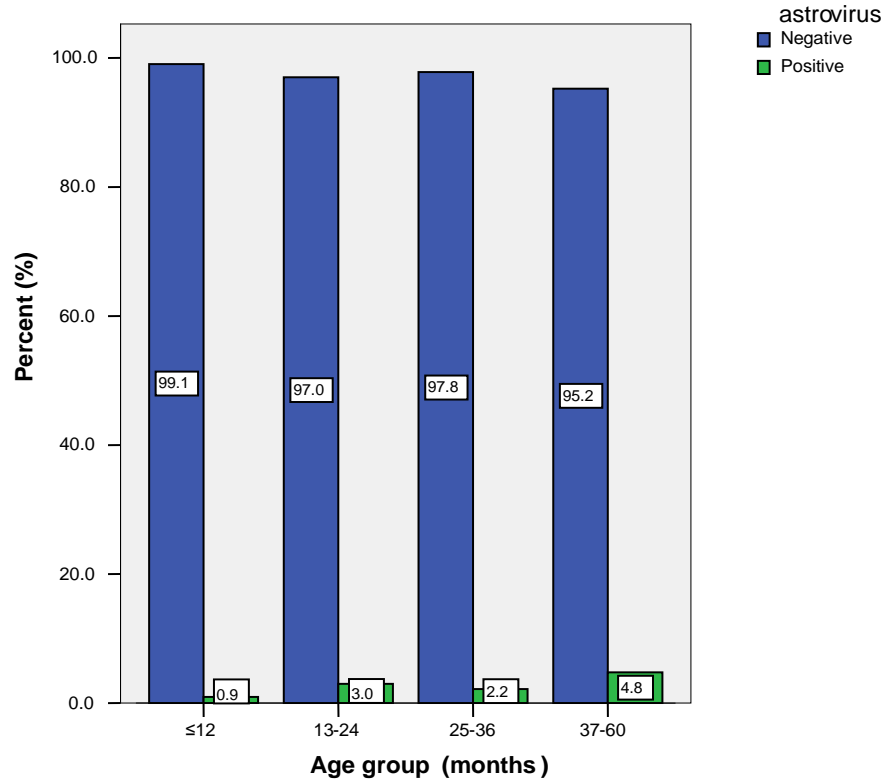


Figure 1. Age distribution of children with astrovirus gastroenteritis from May, 2002 to April, 2008 in Bandar Abbas city.

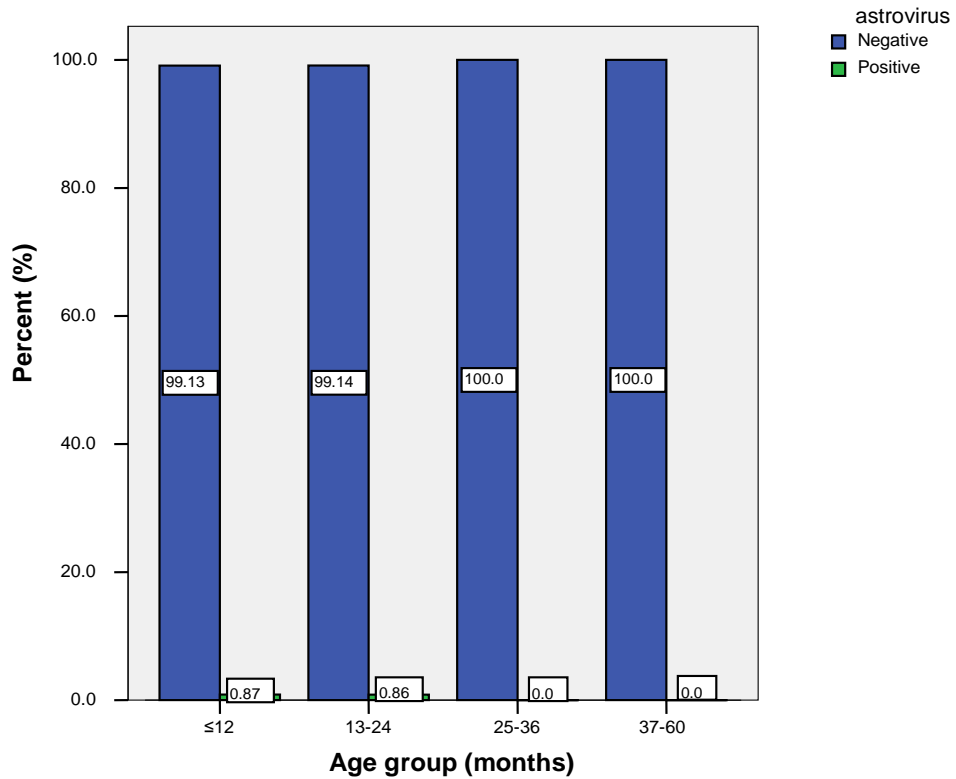
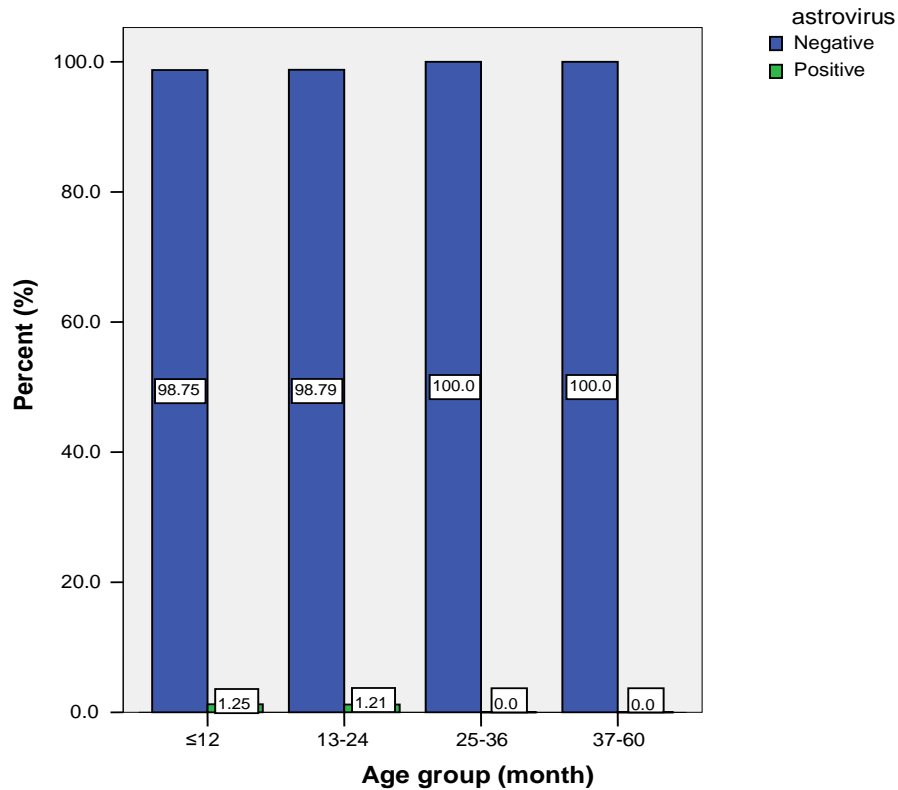
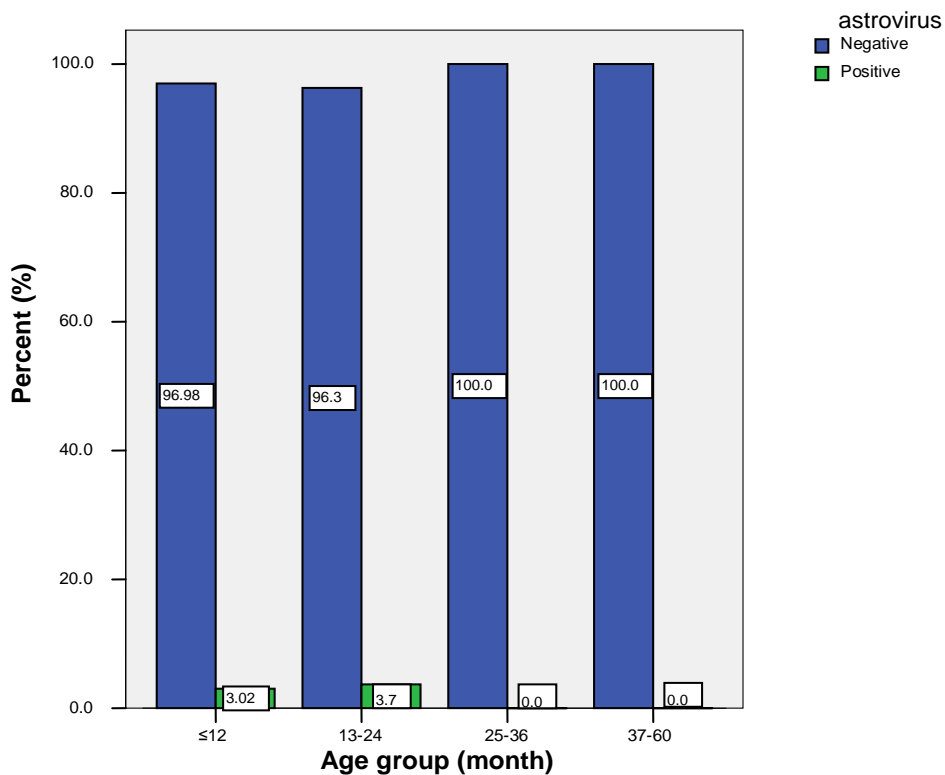


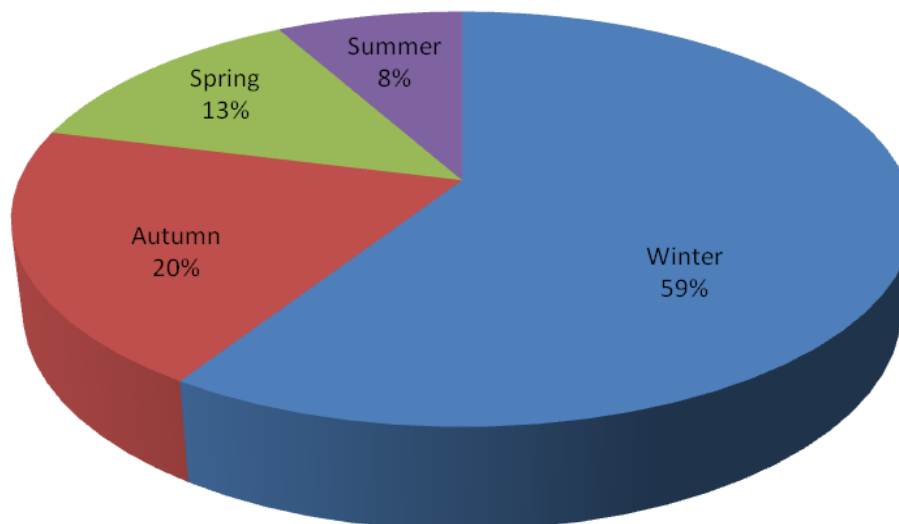
Figure 2. Age distribution of children with astrovirus gastroenteritis from May 2002 to April 2008 in Tabriz city.



**Figure 3.** Age distribution of children with astrovirus gastroenteritis from May, 2002 to April, 2008 in Tehran city.



**Figure 4.** Age distribution of children with astrovirus gastroenteritis from May, 2002 to April, 2008 in Mashhad city.



**Figure 5.** The seasonality pattern of astrovirus gastroenteritis in children from May 2002 to April 2008 in 5 cities of Iran.

years of age making up 36 (90%) of infected children, only 4 cases of infected children were more than 2 years of age (10%). When analyzed by a *t* test, the difference between the two age groups was statistically significant ( $P < 0.02$ ).

## DISCUSSION

This is a new study to use molecular techniques (Southern hybridization and RT-PCR) in a long-term prospective study of astrovirus infection in children hospitalized with acute gastroenteritis. Previous epidemiological studies of astrovirus infection in children have been carried out in a variety of set community-based studies using electron microscopy and enzyme immunoassay. Surveys of the incidence of hospitalization due to astrovirus-induced severe gastroenteritis in developed countries have reported rates of 1.5 to 3% (Carter and Willcocks, 1996). In contrast, a study from Chile found that astroviruses were responsible for up to 20% of hospital admissions (Gaggero et al., 1998) which suggests that the burden of astrovirus disease may be greater in developing countries; however, the incidence of astrovirus infection reported in this study is consistent with developed countries.

In a research by Hamkar et al. (2007), the prevalence of astrovirus infection among children hospitalized with gastroenteritis in 3 cities of Northern Iran was reported to be 2.4% whereas in another study conducted in Ahvaz city a higher rate of incidence (15.17%) has been reported which may be due to poor hygienic condition (Mozhgan et al., 2011).

In this study, the maximum detection rate was observed in children under 2 years of age. Reports from

other countries like Mexico, Thailand, Guatemala, France, Australia, Colombia, and Venezuela have shown higher incidences in younger populations as well (Bon et al., 1999; Gaggero et al., 1998; Mustafa et al., 2000; Herrmann et al., 1991; Cruz et al., 1992; Medin et al., 2000). However, in Guatemala and France (Bon et al., 1999; Cruz et al., 1992), the detection rate at the age of 2 was also high.

Detection rates were higher in winter, although astrovirus infections also occurred in summer months. This seasonal pattern is consistent with other epidemiological studies from temperate regions (Matsui and Greenberg, 1996). However, reports exist which describe high astrovirus incidences during spring and summer (Noel and Cubitt, 1994; Herrmann et al., 1991). Nevertheless, some other long-term studies describe a slightly different pattern without a distinct winter peak in each year (Mitchell et al., 1999). Our study showed a winter peak in each 1-year period.

RT-PCR is the most sensitive test for astrovirus detection as described previously (Guix et al., 2002). In a study by Grote et al. (2011), it was revealed that 30% of fecal samples negative for astrovirus by enzyme-linked immunosorbent assay (ELISA) were found to be positive when tested with RT-PCR; this has been repeated in 2 other studies performed in Saudi Arabia and South Korea (Tayeb et al., 2008; Jeong et al., 2011). These findings emphasize the role of RT-PCR as the most sensitive test for virus detection among infected samples.

Conclusively, our findings provide evidence that astrovirus can be a leading cause of viral gastroenteritis infections and highlight the need to implement astrovirus detection assays in association with rapid rotavirus and adenovirus detection enzyme immunoassays (EIAs) for the clinical diagnosis and nosocomial prevention of viral

gastroenteritis infections in pediatric departments.

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## REFERENCES

- Belliot G, Laveran H, Monroe SS (1997). Outbreak of gastroenteritis in military recruits associated with serotype 3 astrovirus infection. *J. Med. Virol.* 51:101–106.
- Bon F, Fascia P, Dauvergne M, Tenenbaum D, Planson H, Petion AM, Pothier P, Kohli E (1999). Prevalence of group A rotavirus, human calicivirus, astrovirus, and adenovirus type 40 and 41 infections among children with acute gastroenteritis in Dijon, France. *J. Clin. Microbiol.* 37:3055–3058.
- Boom R, Sol CJA, Salimans MMM, Jansen CL, Wertheimvan Dillen PME, van der Noor daa J (1990). Rapid and simple method for purification of nucleic acids. *J. Clin. Microbiol.* 28:495–503.
- Carter MJ, Willcocks MM (1996). The molecular biology of astroviruses. *Arch. Virol. Suppl.* 12:277–285.
- 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.
- Foley B, O'Mahony J, Morgan SM, Hill C, Morgan JG (2000). Detection of sporadic cases of Norwalk-like virus (NLV) and astrovirus infection in a single Irish hospital from 1996 to 1998. *J. Clin. Virol.* 17:109–117.
- Gaggero A, O'Ryan M, Noel JS, Glass RI, Monroe SS, Mamani N, Prado V, Avendanõ LF (1998). Prevalence of astrovirus infection among Chilean children with acute gastroenteritis. *J. Clin. Microbiol.* 36:3691–3693.
- Glass RI, Noel J, Mitchell D, Herrmann JE, Blacklow NR, Pickering LK, Dennehy P, Ruiz-Palacios G, De Guerrero ML, Monroe SS (1996). The changing epidemiology of astrovirus-associated gastroenteritis: A review. *Arch. Virol. Suppl.* 12:287–300.
- Grote DH, Leung KC, Kesson AM (2011). Investigation of Paediatric Astrovirus Infection : Comparison of an ELISA test and nested RT-PCR assay. The Australian Society For Microbiology. Annual Scientific Meeting (ASM 2011).
- Guix S, Caballero S, Villena C, Bartolome R, Latorre C, Rabella N, Simo M (2002). Molecular epidemiology of astrovirus infection in Barcelona, Spain. *J. Clin. Microbiol.* 40(1):133–139.
- Hamkar R, Yahya Poor Y, Nouruzi M, Jalilvand S, Adibi L, Vaziri S (2007). Prevalence of viral pathogens in children with acute gastroenteritis in Mazindaran province 2004 – 2005. *J. Infec. Dis.* 39(12): 35–40.
- Herrmann JE, Taylor DN, Echeverria P, Blacklow RN (1991). Astroviruses as a cause of gastroenteritis in children. *N. Engl. J. Med.* 324:1757–1760.
- Jeong AY, Jeong HS, Jo MY, Jung SY, Lee MS, Lee JS, Jee YM, Kim JH, Cheon DS (2011). Molecular epidemiology and genetic diversity of human astrovirus in South Korea from 2002 to 2007. *Clin. Microbiol. Infect.* 17: 404–408
- Koopmans MPG, Bijen MHL, Monroe SS, Vinje´ J (1998). Age-stratified seroprevalence of neutralizing antibodies to astrovirus types 1 to 7 in humans in The Netherlands. *Clin. Diagn. Lab. Immunol.* 5:33–37.
- Kriston S, Willcocks MM, Carter MJ, Cubitt WD (1996). Seroprevalence of astrovirus types 1 to 6 in London, determined using recombinant virus antigen. *Epidemiol. Infect.* 117:159–164.
- Liste MB, Natera I, Suarez JA, Pujol FH, Liprandi F, Ludert JE (2000). Enteric virus infections and diarrhea in healthy and human immunodeficiency virus-infected children. *J. Clin. Microbiol.* 38:2873–2877.
- Madeley CR, Cosgrove BP (1975). 28 nm particles in faeces in infantile gastroenteritis. *Lancet* 2:451–452.
- Maldonado Y, Cantwell M, Old M, Hill D, Sanchez ML, Logan L, Millan-Velasco F, Valdespino JL, Sepulveda J, Matsui S (1998). Population-based prevalence of symptomatic and asymptomatic astrovirus infection in rural Mayan infants. *J. Infect. Dis.* 178:334–339.
- Matsui SM, Greenberg HB (1996). Astroviruses. *Fields virol.* 3: 811–824.
- Medin SM, Gutierrez MF, Liprandi F, Ludert JE (2000). Identification and type distribution of astroviruses among children with gastroenteritis in Colombia and Venezuela. *J. Clin. Microbiol.* 38:3481–3483.
- Mitchell DK, Monroe SS, Jiang X, Matson DO, Glass RI, Pickering LK (1995). Virologic features of an astrovirus diarrhea outbreak in a day care center revealed by reverse transcriptase-polymerase chain reaction. *J. Infect. Dis.* 172:1437–1444.
- Mitchell DL, Matson DO, Jiang X, Berke T, Monroe SS, Carter MC, Willcocks MM, Pickering LK (1999). Molecular epidemiology of childhood astrovirus infection in child care centers. *J. Infect. Dis.* 180:514–517.
- Mozhghani SHR, Samarbaaf-Zadeh AR, Makvandi M, Shamsi-Zadeh A, Parsanahad M, Jalilian SH (2011). Relative frequency of astrovirus in children suffering from gastroenteritis referred to Aboozar Hospital, Ahvaz. *JJM.* 4(1):67–70.
- 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.
- Noel D, Cubitt D (1994). Identification of astrovirus serotypes from children treated at the Hospitals for Sick Children, London 1981–1993. *Epidemiol. Infect.* 113:153–159.
- Oishi I, Yamazaki K, Kimoto T, Minekawa Y, Utagawa E, Yamazaki S, Inouye S, Grohmann GS, Monroe SS, Stine SE, Carcamo C, Ando T, Glass RI (1994). A large outbreak of acute gastroenteritis associated with astrovirus among students and teachers in Osaka, Japan. *J. Infect. Dis.* 170:439–443.
- Shastri S, Doane AM, Gonzales J, Upadhyayula U, Bass DM (1998). Prevalence of astroviruses in a children's hospital. *J. Clin. Microbiol.* 36:2571–2574.
- Svenungsson B, Lagergren A, Ekwall E, Evengard B, Hedlund KO, Ka'rnell A, Lo'fdahl S, Svensson L, Weintraub A (2000). Enteropathogens in adult patients with diarrhea and healthy control subjects: A 1-year prospective study in a Swedish clinic for infectious diseases. *Clin. Infect. Dis.* 30:770–778.
- Tayeb HT, Dela Cruz DM, Al-Qahtani A, Al-Ahdal MN, Carter MJ (2008). Enteric viruses in pediatric diarrhea in Saudi Arabia. *J. Med. Virol.* 80:1919–1929
- Taylor MB, Marx FE, Grabow WOK (1997). Rotavirus, astrovirus and adenovirus associated with an outbreak of gastroenteritis in a South African child care centre. *Epidemiol. Infect.* 119:227–230.
- Unicomb LE, Banu NN, Azim T, Islam A, Bardhan PK, Faruque ASG, Hall A, Moe JS, Noel CL, Monroe SS, Albert MJ, Glass RI (1998). Astrovirus infection in association with acute, persistent and nosocomial diarrhea in Bangladesh. *Pediatr. Infect. Dis. J.* 17:611–614.
- Walter JE, Mitchell DK (2000). Role of astroviruses in childhood diarrhea. *Curr. Opin. Pediatr.* 12:275–279.