Epidemiology of Rotavirus-Norovirus Co-Infection and Determination of Norovirus Genogrouping among Children with Acute Gastroenteritis in Tehran, Iran

Seyed Dawood Mousavi Nasab¹, Farzaneh Sabahi*¹, Manoochehr Makvandi², Siamak Mirab Samiee³, Seyed Alireza Nadji⁴ and Mehrdad Ravanshad¹

¹Department of Virology, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran; ²Department of Medical Virology, School of Medicine, Ahvaz Jundishapur University of Medical Science, Ahvaz, Iran; ³Reference Health Laboratories Research Center, Ministry of Health and Medical Education, Tehran, Iran; ⁴Virology Research Center, National Research Institute for Tuberculosis and Lung Disease (NRITLD), Shahid Beheshti University, Tehran, Iran

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ABSTRACT

Background: Enteric viruses, particularly human rotavirus and norovirus, have been shown to replace bacteria and parasites, as the most common pathogens responsible for acute diarrhea. However, there are still few epidemiological data on the simultaneous occurrence of these viruses in Iran. In this regard, the aim of this study was to assess the useful epidemiological data on the gastroenteritis associated with rotavirus-norovirus mixed infection and to examine the prevalence of norovirus genogrouping among children aged less than five years old in Iran. Methods: A total of 170 stool samples were collected from children under five years of age with the clinical signs and symptoms of acute gastroenteritis, from May 2013 to May 2014. For the detection of both rotavirus and norovirus, total RNA was extracted from all samples, followed by reverse transcription polymerase chain reaction (RT-PCR). For both detected rotaviruses and noroviruses, genogrouping was performed. Results: Of 170 samples, 49 (28.8%) and 15 (8.8%) samples were found to be positive for rotavirus and norovirus infections by RT-PCR. Interestingly, 6 (3.5%) patients were positive for both infections. Among the 15 norovirus-positive patients, 13 (86.6%) and 2 (13.3%) belonged to genogroups GII and GI. Conclusions: The norovirus genogroup GII and rotavirus lead to the serious infections in children with acute gastroenteritis. However, more well-designed studies are needed to further elucidate the role of other enteric viruses in acute gastroenteritis. DOI: 10.22045/ibj.2016.05

Keywords: Gastroenteritis, Rotavirus, Norovirus, Coinfection, Epidemiology

INTRODUCTION

Acute gastroenteritis is a global public health problem caused by bacteria, viruses or various parasites. The disease is occurred among children under five years of age, with an estimated 1.5 billion episodes of diarrhea and 65,000 deaths annually in 22 countries of the Eastern Mediterranean Region¹,². The findings have shown that enteric viruses are the most significant pathogens in acute gastroenteritis, as compared with other pathogens³. The most important viruses involved in outbreaks of enteric gastroenteritis include rotavirus, human norovirus, adenoviruses 40 and 41 and astrovirus⁴,⁵. Rotaviruses have double-stranded RNA (dsRNA) genomes and belong to the family Reoviridae. Based on RNA divergent sequence in VP6 region, rotaviruses are classified into eight groups (A–H)⁶. Rotavirus Group A is predominantly associated with gastroenteritis in infants and young children⁷. The segmented dsRNA genome of rotavirus is enclosed in a
The involvement of rotavirus and norovirus in children with acute gastroenteritis and the epidemiological feature of either rotavirus or norovirus infections has been documented in Iran\(^{[28-30]}\). The present study was conducted to evaluate the association between rotavirus and norovirus infections and to determine norovirus genogroup in children with acute gastroenteritis, which have not been reported yet in Iran.

**MATERIALS AND METHODS**

**Definition of diarrhea**

A day with diarrhea was determined by the occurrence of three or more liquid or semiliquid stools during a 24-h period.

**Fecal sample collection**

A total of 170 diarrheic stool samples (130 outpatients and 40 inpatients) were collected from children (under five years of age) with acute gastroenteritis. The cases were referred to Children’s Medical Centers in Tehran, Iran from May 2013 to May 2014. The patients’ symptoms were accompanied with or without vomiting, fever, nausea, abdominal pain and cramp. Meanwhile, the absence of leukocytes, red blood cells and pus in the stool was confirmed by microscopic examination. The stool samples were then transported on ice to the Virology Department at Tarbiat Modares University (Tehran, Iran) and stored at -70°C until process.

**Ethics statement**

This study was approved by the Ethics Committee of Tarbiat Modares University, and an informed consent was obtained from the mothers of the patients.

**Viral RNA extraction**

To extract RNA, approximately 10% (w/v) suspension of each stool sample was prepared. Briefly, 1 g (pea-sized) or 100 μl stool from each patient was dissolved in 1000 μl phosphate buffered saline and centrifuged at 400 ×g for 20 minutes. Next, 200 μl supernatant from each sample was collected for RNA extraction using the RTP\(^{®}\) DNA/RNA Virus Mini Kit (Stratec Biomedical AG, Germany) according to the manufacturer’s instructions. RNA extracts were stored at -70°C until use.

**Reverse transcription**

Reverse transcription polymerase chain reaction (RT-PCR) was conducted with the RevertAid RT Reverse Transcription Kit (Thermo Fisher Scientific,
Table 1. The sequences of primers used for detection of rotavirus Group A and norovirus genogrouping

| Virus     | Polarity   | Sequence (5'-3')                          | Product size (bp) | Gene   |
|-----------|------------|------------------------------------------|-------------------|--------|
| Rotavirus | Forward    | AAA GGA TGG CCA ACA GGA TCAT             | 569               | VP7    |
|           | Reversed   | GTA TAR AAH ACT TGC CAC CAT              |                   |        |
| Norovirus G1 | Forward | CTG CCC GAATYY GTA AAT GA               | 330               | ORF2   |
|           | Reversed   | CCAACC CARCCAT TRA TAC                  |                   |        |
| Norovirus GII | Forward | CAR GAR BCNATGGTTYAGRTGGATG AG          | 388               | ORF2   |
|           | Reversed   | CCR CCN GCA TRH CCR TTR TAC AT          |                   |        |
| Norovirus GIV | Forward | GCACTCGGCATCATGACAAATTCA                | 995               | ORF1/ORF2 |
|           | Reversed   | GTTTGGGTCCCAATTCCAA                     |                   |        |

USA) according to the manufacturer’s instructions. Briefly, RT reactions were carried out in a final volume of 20 μL containing 4 μL 5× RT buffer, 1 μL 10 mM dNTPs, 1 μL 0.2 U/μl random hexamer, 1 μL 40 U/μl RNase inhibitor, 1 μL 200 U/μl RT enzyme, 6 μl DEPC water (RNase free water) and 6 μl extracted RNA. The reactions were incubated at 42°C for 1 h. To facilitate the reverse transcription of rotavirus dsRNA, the mixture of the primer and template was first denatured by heating at 95°C for 5 min and then snap-chilled on ice for 1 min. The cDNA samples were stored at -20°C until use in PCR reaction.

**Rotavirus detection**

As shown in Table 1, one oligonucleotide primer pair was used to detect rotavirus. The PCR reaction was performed in a final volume of 25 μL containing 4 μL 10× PCR buffer (CinnaGen, Iran), 0.5 μl each 10 pmol/μl primer, 1 μl 10 mM dNTPs (Fermentas, USA), 0.3 μl 500 U/μl Taq DNA polymerase (CinnaGen), 0.5 μl 50 mM MgCl2 (CinnaGen), 11.2 μL H2O and 7.0 μl cDNA template. PCR cycles were carried out in a thermal cycler (Applied Biosystems GeneAmp® verity thermocycler) as follows: an initial denaturation at 95°C for 5 min, 40 cycles of denaturation at 94°C for 3 min, annealing at 55°C for 55 s, elongation at 72°C for 1 min and a final extension step at 72°C for 7 min. The amplification of 569-bp PCR product was considered as positive.

**Detection of genogroups G1, GII and GIV**

To detect genogroups G1, G2 and G1V among patients with acute gastroenteritis, three sets of primers were applied for ORF2 and ORF1/ORF2 regions. The PCR reaction was carried out as above. The sequences of the primers, amplicon and target genes are illustrated in Table 1. The resulting PCR products were analyzed on 2% agarose gels, stained by the GelRed dye (GelRedTM Nucleic Acid Gel Stain) and visualized under UV light. The products were then extracted using a QIA quick PCR Purification Kit (Qiagen, Hilden, Germany). To confirm positive PCR results, the PCR products were sequenced (Macrogen, South Korea). Afterwards, the positive sequences were edited and aligned with those deposited in the GenBank database using the BioEdit software (version 7.0.5.2.) and Clustal X (version 2.0), respectively.

Table 2. The characteristics of positive patients studied

| Parameter     | Rotavirus (n=49) | Norovirus (n=15) | Rotavirus vs. Norovirus | P value | Rotavirus-norovirus (n=6) |
|---------------|------------------|------------------|------------------------|---------|--------------------------|
| Gender        |                  |                  |                        |         |                          |
| Female        | 19/170 11.17     | 5/170 2.9        | Rotavirus (0.237)      |         | 1/6 (16.6%)              |
| Male          | 30/170 17.64     | 10/170 5.9       | Norovirus (0.307)      |         | 5/6 (83.3%)              |
| Age (month)   |                  |                  |                        |         |                          |
| >12           | 15 30.6          | 4 26.7           | Rotavirus (0.005)      |         | 2                        |
| 13-24         | 23 46.9          | 6 40             |                        |         |                          |
| 25-36         | 8 16.3           | 3 20             |                        |         |                          |
| 37-60         | 3 6.1            | 2 13.3           |                        |         |                          |
| Season        |                  |                  |                        |         |                          |
| Spring        | 5 10.2           | 3 20             | Rotavirus (0.0001)     |         | 1                        |
| Summer        | 2 4              | 0 0              |                        |         |                          |
| Autumn        | 10 20.4          | 7 46.7           |                        |         |                          |
| Winter        | 32 65.3          | 5 33.3           |                        |         |                          |
Statistical analysis

Analysis of the data was performed by the SPSS statistical software version 21.0. Chi-square (χ2) test was used to compare the groups, and the test was used for each virus separately. P value less than 0.05 was considered to be statistically significant.

RESULTS

Out of 170 samples, 54.1% were male and 45.9% were female. All the samples were found to be negative for the bacteria and parasites. Demographic parameters, including age, gender and the season of sample collection were recorded for each patient (Table 2).

RT-PCR analyses indicated that among 170 samples, 49 (28.8%) and 15 (8.8%) were positive for rotavirus and norovirus infections, respectively. However, 6 (3.5%) samples were positive for both rotavirus and norovirus (Table 3). Figure 1 shows the positive PCR results on the agarose gel electrophoresis. The authenticity of the PCR product was confirmed by sequencing. A higher rate of rotavirus infection was detected in males than females, which was not significant (95%CI=1.503, 0.764-2.955). In rotavirus-infected group, the highest prevalence was observed in children between 13 and 24 months of age, including 46.9% of all positive cases (95%CI=2.683, 1.337-5.384). Rotavirus was also detected throughout the year. The prevalence of rotavirus gastroenteritis was 10.2% in spring, 4% in summer, 20.4% in autumn and 65.3% in winter. The noroviruses-infected group (n=15) had 3 patients in spring (20%), no patient in summer, 7 in autumn (46.7%) and 5 in winter (33.3%). A significant difference was observed between the incidence of rotavirus in cold season with that of the rest of the year, as demonstrated by the chi-square test (95%CI= 5.465, 2.671-11.182) (P<0.0001).

The frequency of diarrhea for norovirus was significantly higher in males (95%CI=1.780, 0.582-5.451) (P=0.307), as compared to the females. Children less than 24 months of age accounted for 70.83% of the overall norovirus-positive cases with those between 13 and 24 months of age being the most affected (P=0.214). The majority of rotavirus-norovirus dual infections were detected in winter and observed in children with an overall median age of 18 months (Table 2). Among the 15 norovirus-positive children, 13 (86.7%) and 2 (13.3%) belonged to genogroups GII and GI; however, genogroup GIV was not detected in this study (Fig. 2).

Among the 130 outpatients, 39 (30%) and 11 (8.5%) cases were positive for rotavirus and norovirus, and rotavirus-norovirus co-infection was observed only in two cases. Among the 40 hospitalized patients, 10 (25%) and 4 (10%) cases were found to be positive for rotavirus and norovirus and four patients had rotavirus-norovirus (genogroup GII) mixed infections.

DISCUSSION

Diarrhea remains the second leading cause of death due to infections among children under the age of five years worldwide[34]. Many reports have established the importance of rotaviruses and noroviruses as the causes of outbreaks of gastroenteritis[1,2,7,49]. In the present study, we have determined the prevalence of rotavirus and norovirus infections and co-infections among the children with acute gastroenteritis. To our knowledge, this is the first report to determine the human norovirus genogroups (G1, GII and GIV) among children with acute gastroenteritis in Iran.

Table 3. The detection rate of rotavirus and norovirus or dual positive infections in inpatient and outpatient stool specimens

| Virus            | Total no. of cases (%) | Inpatient (%) | Outpatient (%) |
|------------------|------------------------|---------------|----------------|
| Rotavirus        | 49 (28.8)              | 10/40 (25)    | 39/130 (30)    |
| Norovirus        | 15 (8.8)               | 4/40 (10)     | 11/130 (8.5)   |
| Norovirus-rotavirus | 6 (3.5)             | 4/40 (10)     | 2/130 (1.5)    |

Fig. 1. Detection of rotavirus and norovirus using PCR. Lane 1, 100 bp ladder; Lanes 2, 3 and 6, the amplified fragment related to rotavirus (570 bp); Lane 4, the amplified fragment of norovirus (330 bp), Lane 5, rotavirus-norovirus co-infection.
In this study, rotaviruses were detected in 28.8% of the patients with acute gastroenteritis, which is consistent with the results reported by Kargar et al.\cite{35} and Parashar et al.\cite{36}. Our findings are also in line with the results of Shoja et al.\cite{13} and Moradi-Lakeh et al.\cite{14}, who reported 11.36% and 79% of rotavirus in two different regions of Iran. The difference between the rate of rotavirus detection among the male (17.64%) and female populations (11.17%) was not significant ($P<0.237$).

The peak of rotavirus infections in Latin America was proclaimed to be in autumn and winter\cite{37}. The findings of the present study also demonstrated that the peak of rotavirus infections occurs in the winter season ($P<0.0001$). Also, the highest rate of rotavirus infection was observed in children between 13 and 24 months of age ($P<0.005$), which was similar to another study\cite{38}.

Norovirus is considered as the second most common cause of viral gastroenteritis with a prevalence rate of 6%-19% globally. In this survey, of 170 patients, 15 (8.8%) cases had positive norovirus infection, which is in agreement with the results of other studies reported in Iran\cite{39,41}. In the present study, noroviruses were detected in spring, autumn and winter but not summer. There was also a considerable increasing trend in the number of cases in the autumn season, which confirms the previous studies\cite{42,43}. The highest rate of norovirus infection was observed in children under 24 months (40%), which is in accordance with the studies reported from Iran\cite{40}, UK\cite{44} and China\cite{45}. The prevalence of norovirus detection in males (10.8%) and females (6.4%) is the same as the results reported from Japan by Ozawa et al.\cite{45}. Our findings revealed that norovirus accounts for 13% of genogroup GI and 87% genogroup GII in cases with acute gastroenteritis. This shows that norovirus genogroup GI is less common than norovirus GII and similar to a report by Bon et al.\cite{46} from France, norovirus GII is the most dominant genogroup in our region. There are few reports available on detection of norovirus genogroup GIV\cite{46,47}. In contrast to the norovirus genogroups GI and GII, the GIV genogroup was not detected in our study. The coinfection of rotaviruses and noroviruses has been reported by some investigators in different regions of the world. In a study conducted by Sai et al.\cite{14} in China, the frequency of rotavirus and norovirus infections was 34.4% and 10.4%, respectively, and their coinfection was 1%. In Morocco, El Qazoui et al.\cite{48} reported the frequency of rotavirus and norovirus coinfection of rotavirus-norovirus among children less than 24 months of age to be 26.6%, 16.1% and 2.7%, respectively. Oldak et al.\cite{49} studied rotavirus and norovirus among the patients with acute gastroenteritis in Netherland. They reported that 48.6% of the patients were positive for rotavirus and 16.5% for norovirus and 3.8% had rotavirus-norovirus coinfection.

In the present study, the coinfection of rotavirus and norovirus was found to be 3.5% and was mostly detected during the cold season, which is in consistent with the result of Tran et al.\cite{50}. We also indicated that the norovirus genogroup GII and rotavirus lead to serious infections in children less than five years old with acute gastroenteritis. However, more well-designed studies are necessary to further elucidate the role of other enteric viruses in acute gastroenteritis.

**CONFLICT OF INTEREST.** None declared.
REFERENCES

1. Elliott EJ. Acute gastroenteritis in children. *BMJ* 2007; 334(7583): 35-40.

2. Malek MA, Teleb N, Abu-Elyazed R, Riddle MS, Sherif ME, Steele AD, Glass RI, Bresee JS. The epidemiology of rotavirus diarrhea in countries in the Eastern Mediterranean Region. *Journal of infectious diseases* 2010; 202(Suppl): S12-S22.

3. Leshem E, Lopman B, Glass R, Gentsch J, Bányai K, Parashar U, Patel M. Distribution of rotavirus strains and strain-specific effectiveness of the rotavirus vaccine after its introduction: a systematic review and meta-analysis. *The lancet infectious diseases* 2014; 14(9): 847-856.

4. Sai L, Sun J, Shao L, Chen S, Liu H, Ma L. Epidemiology and clinical features of rotavirus and norovirus infection among children in Ji’nan, China. *Virology journal* 2013; 10: 302.

5. Kirby A, Al-Eryani A, Al-Sonboli N, Hafiz T, Beyer M, Al-Aghbari N, Al-Moheri N, Dove W, Cunliffe NA, Cuevas LE. Rotavirus and norovirus infections in children in Sana’a, Yemen. *Tropical medicine and international health* 2011; 16(6): 680-684.

6. Matthijnssens J, Otto PH, Ciarlet M, Desselberger U, Van Ranst M, Johne R. VP6-sequence-based cutoff values as a criterion for rotavirus species demarcation. *Archives of virology* 2012; 157(6): 1177-1182.

7. Santos N, Hoshino Y. Global distribution of rotavirus serotypes/genotypes and its implication for the development and implementation of an effective rotavirus vaccine. *Reviews in medical virology* 2005; 15(1): 29-56.

8. Hoshino Y, Kapikian A. Classification of rotavirus VP4 and VP7 serotypes. Germany: Springer; 1996.

9. Matthijnssens J, Ciarlet M, McDonald SM, Attoui H, Bányai K, Brister JR, Buesa J, Esona MD, Estes MK, Gentsch JR, Iturriaza-Gómez M, Johne R, Kirkwood CD, Martella V, Mertens PP, Nakagomi O, Parreño V, Rahman M, Ruggeri FM, Saif LJ, Santos N, Steyer A, Taniguchi K, Patton JT, Desselberger U, Van Ranst M. Uniformity of rotavirus strain nomenclature proposed by the Rotavirus Classification Working Group (RCWG). *Archives of virology* 2011; 156(8): 1397-1413.

10. Parashar UD, Gibson CJ, Bresee JS, Glass RI. Rotavirus and severe childhood diarrhea. *Emerging infectious diseases* 2006; 12(2): 304-306.

11. Glass RI, Parashar U, Patel M, Gentsch J, Jiang B. Rotavirus vaccines: Successes and challenges. *Journal of infection* 2014; 68(Suppl1): S9-S18.

12. Staat M, Fairbrother G, Edwards K, Griffen M, Szilagyi P, Weinberg G. Delayed onset and diminished magnitude of rotavirus activity–United States, November 2007–May 2008. *Morbidity and mortality weekly report* 2008; 57: 697-700.

13. Shoja Z, Jalilvand S, Mollaei-Kandelous Y, Validi M. Epidemiology of viral gastroenteritis in Iran. *The Pediatric infectious disease journal* 2014; 33(2): 218-220.

14. Moradi-Lakeh M, Shakerian S, Yaghoubi M, Esteghamati A, Shokranef F, Baradaran HR, Mansour Ghanaei R. Rotavirus Infection in Children with Acute Gastroenteritis in Iran: A Systematic review and meta-analysis. *International journal of preventive medicine* 2014; 5(10): 1213-1223.

15. Shoja Z, Jalilvand S, Mokhtari-Azad T, Nategh R. Epidemiology of cocirculating human rotaviruses in Iran. *The Pediatric infectious disease journal* 2013; 32(4): e178-e181.

16. Barclay L, Park G, Vega E, Hall A, Parashar U, Vinjé J, Lopman B. Infection control for norovirus. *Clinical microbiology and infection* 2014; 20(8): 731-740.

17. Gallimore CI, Cubitt DW, Richards AF, Gray JJ. Diversity of enteric viruses detected in patients with gastroenteritis in a tertiary referral paediatric hospital. *Journal of medical virology* 2004; 73(3): 443-449.

18. Bon F, Fascia P, Dauvergne M, Tenenbaum D, Planson H, Petion AM, Pothier P, Kohli E. Prevalence of group A rotavirus, human calicivirus, astrovirus, and adenovirus type 40 and 41 infections among children with acute gastroenteritis in Dijon, France. *Journal of clinical microbiology* 1999; 37(9): 3055-3058.

19. Chikhi-Brachet R, Bon F, Toubiana L, Pothier P, Nicolas JC, Flahaut A, Kohli E. Virus diversity in a winter epidemic of acute diarrhea in France. *Journal of clinical microbiology* 2002; 40(11): 4266-4272.

20. Belliot G, Lopman BA, Ambert-Balay K, Pothier P. The burden of norovirus gastroenteritis: an important foodborne and healthcare-related infection. *Clinical microbiology and infection* 2014; 20(8): 724-730.

21. Zheng DP, Ando T, Fankhauser RL, Beard RS, Glass RI, Monroe SS. Norovirus classification and proposed strain nomenclature. *Virology* 2006; 346(2): 312-323.

22. Kroneman A, Vega E, Vennema H, Vinjé J, White PA, Hansman G, Green K, Martella V, Katayama K, Koopmans M. Proposal for a unified norovirus nomenclature and genotyping. *Archives of virology* 2013; 158(10): 2059-2068.

23. Thабane M, Simunovic M, Akhtar-Danesh N, Garg AX, Clark WF, Collins SM, Salvadori M, Marshall JK. An outbreak of acute bacterial gastroenteritis is associated with an increased incidence of irritable bowel syndrome in children. *The American journal of gastroenterology* 2010; 105(4): 933-939.

24. Hoa Tran TN, Trainer E, Nakagomi T, Cunliffe NA, Nakagomi O. Molecular epidemiology of noroviruses associated with acute sporadic gastroenteritis in children: global distribution of genogroups, genotypes and GII.4 variants. *Journal of clinical virology* 2013; 56(3): 185-193.

25. Patel MM, Widdowson M-A, Glass RI, Akazawa K, Vinjé J, Parashar UD. Systematic literature review of role of norovirus in sporadic gastroenteritis. *Emerging infectious diseases* 2008; 14(8): 1224-1231.

26. Wollants E, De Coster S, Van Ranst M, Pottiet A, Monjossen M. Surveillance of norovirus outbreaks in hospitals in Belgium. *Infection, genetics and evolution* 2015; 30: 37-44.

27. Harris JP, Adak GK, O'Brien SJ. To close or not to close? Analysis of 4 year’s data from national surveillance of norovirus outbreaks in hospitals in...
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England. BMJ open 2014; 4: e003919.

28. Sambarfzadeh A, Tehrani EM, Mavandi M, Taremi M. Epidemiological aspects of rotavirus infection in Ahwaz, Iran. Journal of health, population and nutrition 2005; 23(3): 245-249.

29. Mozghani S, Sambarf-Zadeh A, Mavandi M, Kalvandi G, Shamsi-Zadeh A, Jalili Sh, Parsa-nahad M. Astrovirus and rotavirus co-infections in children with gastroenteritis who were referred to Ahvaz Aboozar Hospital, Southern Iran. Jundishapur journal of microbiology 2012; 5(1): 352-354.

30. Fazeli Z, Baghaie N, Khavarinejad RA, Khoramdel M, Sigaroodi A, Nadji SA. Hospital based study of prevalence and genotyping of Noroviruses and Sapoviruses isolated from children with acute gastroenteritis referred to Masih Daneshvari hospital. Gastroenterology and hepatology from bed to bench 2010; 3(2): 91-97.

31. Yan H, Yagyu F, Okitsu S, Nishio O, Ushijima H. Detection of norovirus (GI, GII), Sapovirus and astrovirus in fecal samples using reverse transcription single-round multiplex PCR. Journal of virological methods 2003; 114(1): 37-44.

32. Khamrin P, Okame M, Thongprachum A, Nantachit N, Nishimura S, Okitsu S, Maneekarn N, Ushijima H. A single-tube multiplex PCR for rapid detection in feces of 10 viruses causing diarrhea. Journal of virological methods 2011; 173(2): 390-393.

33. Assis AS, Valle DA, Antunes GR, Tibiriça SH, De Assis RMS, Leite JP, Carvalho IP, Rosa e Silva ML. Rotavirus epidemiology before and after vaccine introduction. Jornal de pediatria 2013; 89(5): 470-476.

34. Lanata CF, Fischer-Walker CL, Olascoaga AC, Torres CX, Aryee MJ, Black RE. Global causes of diarrheal disease mortality in children <5 years of age: a systematic review. PloS one 2013; 8(9): e72788.

35. Kargar M, Zare M, Najafi A. Molecular epidemiology of rotavirus strains circulating among children with gastroenteritis in Iran. Iranian journal of pediatrics 2012; 22(1): 63-69.

36. Parashar UD, Burton A, Lanata C, Boschi-Pinto C, Shibuya K, Steele D, Birmingham M, Glass RI. Global mortality associated with rotavirus disease among children in 2004. Journal of infectious diseases 2009; 200(Suppl 1): S9-S15.

37. Castello AA, Arvay ML, Glass RI, Gentsch J. Rotavirus strain surveillance in Latin America: a review of the last nine years. The Pediatric infectious disease journal 2004; 23(10 Suppl): S168-S72.

38. Phua KB, Tee N, Tan N, Ramakrishnan G, Teoh YL, Bock H, Liu Y. A hospital-based surveillance of rotavirus gastroenteritis in children <5 years of age in Singapore. The Pediatric infectious disease journal 2013; 32(12): e426-e431.

39. Romani S, Mohebbi SR, Hosseini SM, Azimzadeh P, Vahedi M, Derakhshan F, Zali MR. Prevalence of norovirus infection in children and adults with acute gastroenteritis, Tehran, Iran, 2008-2009. Food and environmental virology 2012; 4(1): 1-5.

40. Najafi A, Iranjani N, Najafi S. Epidemiological surveillance of norovirus diarrhea in hospitalized children with acute gastroenteritis in south of Iran. Jundishapur journal of microbiology 2013; 6(4):1-4.

41. Jalilian S, Sambarf-Zadeh AR, Mozghani SHR, Mavandi M, Parsa-Nahad M, Prmormazi R, Shamsi-Zadeh A. Relative frequency of norovirus infection in children suffering from gastroenteritis referred to Aboozar hospital, Ahvaz, Iran. Jundishapur journal of microbiology 2012; 5(1): 355-358.

42. Vinjé J. Advances in laboratory methods for detection and typing of norovirus. Journal of clinical microbiology 2015; 53(2): 373-381.

43. La Rosa G, Poursahan M, Iaconelli M, Muscillo M. Detection of genogroup IV noroviruses in environmental and clinical samples and partial sequencing through rapid amplification of cDNA ends. Archives of virology 2008; 153(11): 2077-2083.

44. Payne DC, Vinjé J, Szilagyi PG, Edwards KM, Staat MA, Weinberg GA, Hall CB, Chappell J, Bernstein DI, Curns AT, Wikswo M, Shirley SH, Hall AJ, Lopman B, Parashar UD. Norovirus and medically attended gastroenteritis in US children. New England journal of medicine 2013; 368(12): 1112-1130.

45. Ozawa K, Ota K, Takeda N, Hamsun GS. Norovirus infections in symptomatic and asymptomatic food handlers in Japan. Journal of clinical microbiology 2007; 45(12): 3996-4005.

46. Topkaya AE, Aksungar B, Özakkafl F, Çapan N. Examination of rotavirus and enteric adenoaviruses in children with acute gastroenteritis. Türk mikrobiyol cem derg 2006; 36(4): 210-213.

47. Kawada JI, Arai N, Nishimura N, Suzuki M, Ohta R, Ozaki T, Ito Y. Clinical characteristics of norovirus gastroenteritis among hospitalized children in Japan. Microbiology and immunology 2012; 56(11): 756-759.

48. El Qazoui M, Oumzil H, Baassi L, El Omari N, Sadki K, Amzazi S, Benhafid M, El Aouad R. Rotavirus and norovirus infections among acute gastroenteritis children in Morocco. BMC infectious diseases 2014; 14: 300.

49. Oldak E, Sulik A, Rozkiewicz D, Liwoch-Nienartowicz N. Norovirus infections in children under 5 years of age hospitalized due to the acute viral gastroenteritis in northeastern Poland. European journal of clinical microbiology and infectious diseases 2012; 31(4): 417-422.

50. Tran A, Talmud D, Lejeune B, Jovenin N, Renou P, Payan C, Leveque N, Andreoletti L. Prevalence of rotavirus, adenoaviruses, norovirus, and astrovirus infections and coinfections among hospitalized children in northern France. Journal of clinical microbiology 2010; 48(5): 1943-1946.