

Full Length Research Paper

Genotypic distribution of rotavirus strains causing severe gastroenteritis in children under 5 years old in Borazjan, Iran

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Rotaviruses are recognized as the most common causes of severe gastroenteritis and death among children worldwide. The aim of this study was to evaluate the disease burden of rotavirus gastroenteritis and the prevalence of different G genotypes of rotaviruses circulating in children aged <5 years old who were hospitalized for acute gastroenteritis in Borazjan City, Iran. This cross sectional-descriptive study was done on 316 fecal samples collected from children aged <5 years old with acute gastroenteritis. All the stool specimens were tested for rotavirus with enzyme immunoassays (EIA). Rotavirus-positive specimens were genotyped by the Nested reverse transcription polymerase chain reaction (RT-PCR) and using different type specific primers. Out of total collected samples rotavirus infection was detected in 88 (27.85%). Of the rotavirus episodes, 79.54% occurred during the first 2 years of life, with the peak prevalence of severe rotavirus disease occurring in cold seasons. Among the common genotypes, G1 was the most predominant (52.27% of strains) and other identified genotypes included non-typeable, G9 and G4, 40.90%, 4.54% and 2.27% of isolates, respectively. Because of the high frequency of rotavirus infection it is important to continue rotavirus surveillance in the other regions of Iran to determine accurately the burden of rotavirus disease and the emerging new genotypes. This will assist policy makers in decision making on rotavirus vaccine introduction.

Key words: Human rotaviruses, diarrhea, genotypes, children, surveillance.

INTRODUCTION

Diarrhea remains one of the most important global public health challenges in the world (Mandeville et al., 2009). Human rotaviruses are the major etiological agents of acute gastroenteritis in infants and young children worldwide, being associated with 527,000 deaths

annually, 85% of which occur in the low-income countries of Africa and Asia (CDC, 2008). Virtually all children are infected at least once within the first 5 years of life, with the peak incidence widely quoted as occurring between 6 and 24 months of age (Clark et al., 2010; Shim et al., 2010). Rotaviruses belong to the Reoviridae family, non-enveloped and 100 nm diameter, with a genome that consists of 11 segments of double-stranded ribonucleic acid (dsRNA), encoding six structural (VP1–VP4, VP6 and VP7) and six non-structural (NSP1–NSP6) proteins (Estes and Kapikian, 2007). Two structural proteins, VP7 (the glycoprotein or G protein) and VP4 (the protease-cleaved protein or P protein), make up the outer shell and are important targets for vaccine development (Hoshino

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Abbreviations: EIA, Enzyme immunoassays; RT-PCR, reverse transcription polymerase chain reaction; dsRNA, double-stranded ribonucleic acid; OD, optical density; cDNA, complementary deoxyribonucleic acid.

and Kapikian, 1996). To date, seven groups, designated as A-G, have been distinguished in the rotavirus strains on the basis of the antigenicity of the VP6 protein (Ghosh et al., 2010). Among them, group A rotavirus is the single most important cause of acute gastroenteritis in infants and young children worldwide (Parashar et al., 2006). Studies of the genotyping in different regions of the world have been indicated that G1-G4 and G9 genotypes are the most common genotypes detected in children diarrheal disease (Jiraphongsa et al., 2005; Annarita et al., 2010; Tatte et al., 2010). But in recent years, other rare or uncommon rotavirus genotypes, such as G5, G8, G10, G11 and G12, have been reported in many countries (Rahman et al., 2005; Le et al., 2008; Mast et al., 2010; Zuccotti et al., 2010). The directed rotavirus surveillance in all countries is significant to determine precisely the prevalence of rotavirus gastroenteritis, circulating genotypes and assessing the need for general vaccination of children and infants, particularly in the developing regions of the world. The purpose of this study was to monitor the disease burden associated with rotavirus and determine the G genotypes of rotaviruses circulating among children aged <5 years old who were hospitalized for acute gastroenteritis in Borazjan City, Iran.

MATERIALS AND METHODS

Specimen collection

From October 2009 to September 2010, a total of 316 stool specimens were collected from children aged <5 years old hospitalized with acute gastroenteritis in 17 shahrivar Hospital in Borazjan City, Iran. These were transported to the laboratory in a cold box and stored at -20°C until ready for analyses. Demographic and clinical data regarding the age, sex, clinical symptoms and temperature for each case were recorded.

Rotavirus detection

All fecal specimens were tested for the presence of group A rotavirus by enzyme immunoassays (EIA) (Generic Assays kit, Germany), according to the manufacturer's instructions. Briefly, 10% stool suspension was added to the well containing the solid phase immobilized polyclonal antibody. Simultaneously 100 µl of a monoclonal antibody conjugated to horseradish peroxidase was added to the well and incubated for 60 min. After washing, 100 µl of substrate was added and incubated for 10 min at the room temperature. The enzymatic reaction that converts the colourless substrate to a blue colour was stopped with H₂SO₄ (1N). The optical density (OD) of the solution was read at 450 nm and specimens having OD values above the cut off value (0.2 + OD of the negative control) were considered positive for rotavirus antigen.

Viral RNA extraction

Rotavirus dsRNA was extracted from 10% stool suspension by

using the RNX-Plus kit (CinnaGen, Tehran, Iran), according to the manufacturers protocol.

Reverse transcription-polymerase chain reaction

Briefly, 5 µl of dsRNA was added with mix of DMSO, 5X RT buffer, dNTPs, primers Beg9, End9, DW, denatured at 97°C for 5 min, then followed by addition of RT enzyme and RNase inhibitor to a final volume of 20 µl. The reverse transcription polymerase chain reaction (RT-PCR) reaction was carried out for 60 min at 42°C to produce the complementary deoxyribonucleic acid (cDNA) used for PCR amplification rotavirus.

Nested multiplex PCR for G genotyping

G genotyping were performed as previously described by Gouvea et al. (1990). Briefly, 10 µl of viral cDNA was added to a mix containing MgCl₂, dNTPs, 10x PCR buffer, Taq DNA polymerase and the forward primer Beg9 and the reverse primer End9 to a final volume of 50 µl. The thermocycle program was carried out at 94°C for 1 min, followed by 30 cycles at 42°C for 2 min, 72°C for 2 min and a final extension at 72°C for 5 min. 5 µl of the resulting amplicons of 1062 bp were then used as a template in the second round of PCR. The multiplex reaction mix also included each of the G-type-specific primers, aBT1 (G1), aCT2 (G2), aET3 (G3), aDT4 (G4), aAT8 (G8), aFT9 (G9), mG10 (G10) and G12), provided by World Health Organization (WHO, 2009). Cycling was done with 20 cycles of the same cycling profile of the first reaction. All PCR products were analyzed by electrophoresis in 2% agarose gel that contained ethidium bromide (10 µg/ml) and visualized under UV illumination (WHO, 2009).

Data analysis

Data were statistically analyzed by SPSS version 17 (SPSS Inc., Chicago, IL, USA), chi-square, Fisher's exact tests. P value <0.05 was considered statistically significant.

RESULTS

Serology of rotavirus infection

Rotavirus was detected in 88 hospitalized patients by EIA, representing 27.85% of total specimens.

Rotavirus and demographic data

The distribution of gender in rotavirus positive cases was 56 (28.14%) in males and 32 (21.77%) in females. No statistically significant differences were observed between gender and rotavirus infection ($P = 0.111$). The frequency of rotavirus acute gastroenteritis was highest in children less than 24 months of age, counting for 79.54% of all cases ($P = 0.044$). Most of the positive samples were detected in children aged 12 to 17 months (Figure 1). A significant difference was found in the frequency of

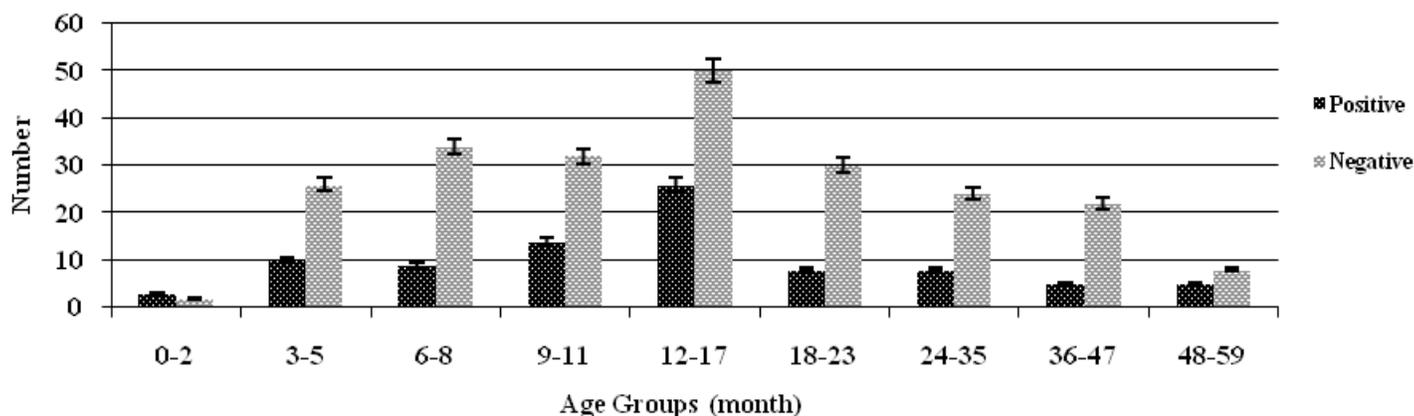


Figure 1. Age distribution of rotavirus infection during study period from October 2009 to September 2010.

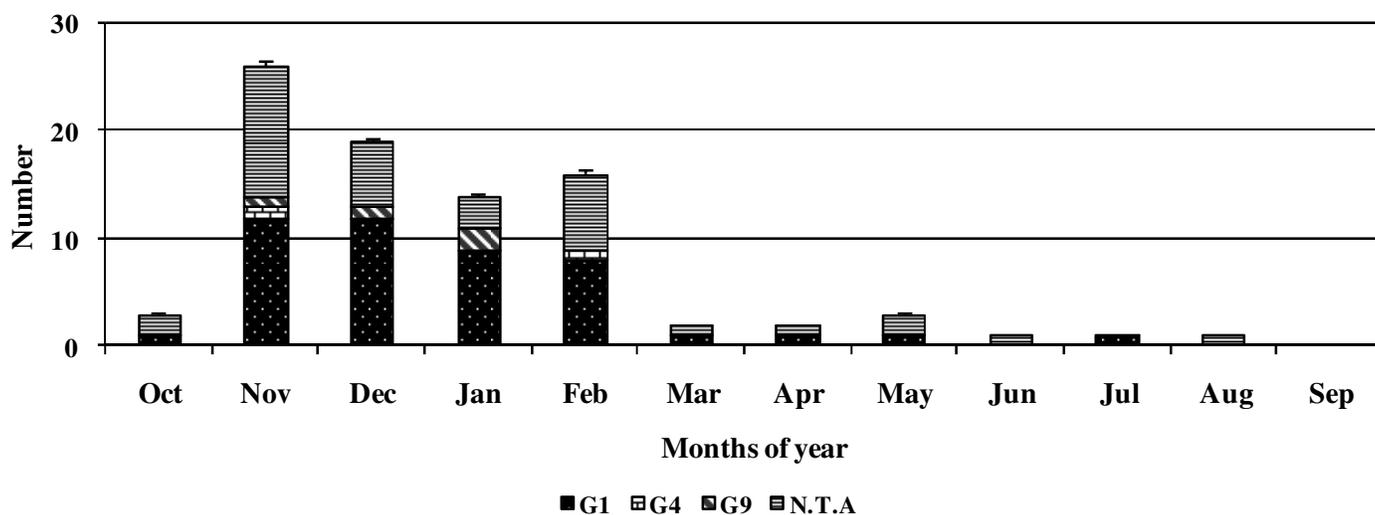


Figure 2. The seasonal distribution of rotavirus genotypes during study period from October 2009 - September 2010.

the rotavirus detection and seasonal distribution ($P = 0.001$). The highest prevalence of infection was observed in autumn (54.54%), followed by winter (36.36%), spring (6.82%) and summer (2.28%) respectively. The highest rate of detection of rotavirus genotypes was found in November (29.54%) and the lowest in September, in which no genotype was detected ($P = 0.991$) (Figure 2).

Rotavirus genotyping

Genotyping was performed on 88 rotavirus positive stool samples by using Nested RT-PCR (Figure 3). The most common circulating genotype in the population under surveillance was G1, that being identified in 46 strains out

of 88 samples (52.27%), followed by non-typeable (36/88, 40.91%), G9 (4/88, 4.54%) and G4 (2/88, 2.27%), consecutively. The genotypes G2, G3, G8, G10 and G12 were not found in this study. The most frequent detected genotype was non-typeable in females (50.00%) and G1 in males (58.93%) but this difference was not statistically significant ($P = 0.109$). The most prevalence of rotavirus genotype reported was non-typeable (66.67%) in spring and G1 in the autumn (52.08%) and winter (56.25%) seasons ($P = 0.974$).

DISCUSSION

Rotavirus remains a major cause of acute gastroenteritis

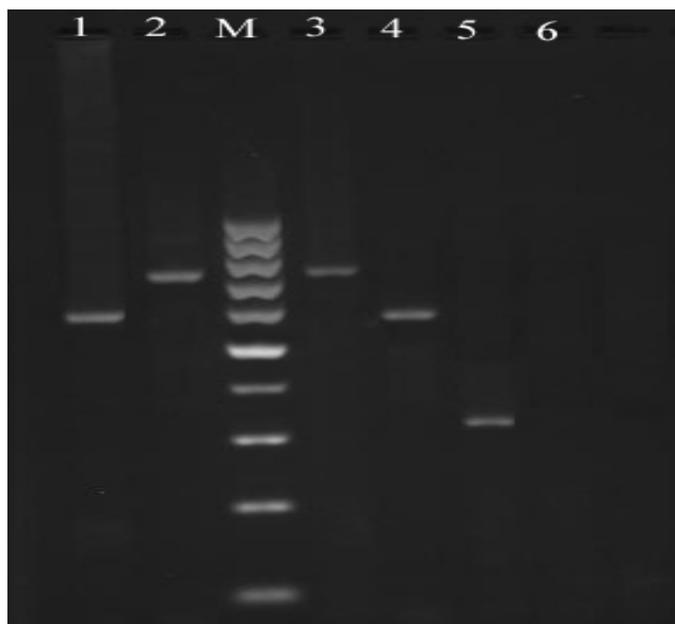


Figure 3. Electrophoresis of Gouvea VP7 genotype amplicons. M(100 bp molecular weight marker), lanes 1,4(G4 genotype), lanes 2,3(G1 genotype), lane 5(G9 genotype) and lane 6 represents the negative control.

among infants and children aged <5 years old, not only in the developing countries like Brazil (Sa'fadi et al., 2010), India (Tatte et al., 2010), Cuba (Ribas et al., 2011), but also in developed countries like Italy (Annarita et al., 2010), Swiss (Lacroix et al., 2010) and France (De Rougemont et al., 2011). In recent studies, it was calculated that the rotaviruses cause approximately 111 million episodes of the gastroenteritis requiring only home care, 25 million clinical visits, 2 million hospitalizations and 352,000-592,000 deaths in children aged <5 years old worldwide, each year (Parashar et al., 2003). The frequency of rotavirus detection in this study (27.85%) was in keeping with numerous other studies conducted in developing and developed countries that have reported rotavirus to be responsible for between 14 and 50% of all cases of gastroenteritis (Nelson et al., 2008; Lacroix et al., 2010; Mladenova et al., 2010; Jere et al., 2011; Lorrot et al., 2011). Extensive molecular epidemiological studies globally have indicated that G1 is the most common circulating genotype. Similarly, the predominance of G1 genotype of rotavirus was observed in our study (52.27%), which is in concordance with the results of genotyping from different parts of the world including: Swiss (Lacroix et al., 2010), Taiwan (Mast et al., 2010), France (Lorrot et al., 2011) and Cuba (Ribas et al., 2011). Although G2 and G3 are among the most prevalent rotavirus genotypes worldwide (Fang et al., 2005; Dey et al., 2009; Wang et al., 2009; Jere et al., 2011),

nonetheless this strains were not detected in this study. In recent years G4 strain has been detected at relatively high frequency from Germany (Van Damme et al., 2007), Italy (Annarita et al., 2010), South Korea (Shim et al., 2010) and some regions of Iran (Khalili et al., 2004; Eesteghamati et al., 2009; Kargar and Akbarizadeh, 2011). However, G4 was detected only in 2.27% of all children with rotavirus gastroenteritis. Recently, G9 has appeared as the common genotype in Albania (Annarita et al., 2010), Cuba (Ribas et al., 2011) and other countries (Van Damme et al., 2007; Nelson et al., 2008), but in this study only four (4.54%) G9 genotype isolate were identified. The emergence of G9 as an important genotype in developing and industrialized countries necessitates the inclusion of G9 in future rotavirus vaccines. Notably, non-typeable genotypes were present in 40.91% of all the rotavirus positive fecal samples. These rotavirus strains have been detected only rarely in other investigations (Pun et al., 2007; Eesteghamati et al., 2009; Annarita et al., 2010; Kargar and Akbarizadeh, 2011). Non-typeable samples might be the result of a false-positive EIA, the presence of novel strains, the nonuse of specific primers for rare genotypes such as G5, G6, G11 or the failure in RT-PCR technique (WHO, 2009). Because of the appearance of unusual rotavirus strains such as G10, G12 from South Korea (Le et al., 2008), Italy (Zuccotti et al., 2010), France (De Rougemont et al., 2011) and other countries (Pun et al., 2007; Annarita et al., 2010), in this research were used from the specific primers them for the first time in Iran, but none of them were observed in this study. Overall, 79.54% of hospitalizations due to rotavirus gastroenteritis occurred in children younger than 2 years more than in the older age groups, as was found in previous investigations in various countries (Khalili et al., 2004; Pun et al., 2007; Lacroix et al., 2010; Mladenova et al., 2010). The high frequency of rotavirus infection in this age group has important implications for the consideration of strategies for prevention through the routine immunization of children less than 2 years of old.

Epidemiological studies in different regions of the world have indicated that in temperate climates, rotavirus diarrhea occurs predominantly during the cooler months and rarely in the summer months (Cook et al., 1990; Levy et al., 2009; Kargar and Akbarizadeh, 2011). But seasonal patterns in tropical climates have shown rates of rotavirus infection throughout the year with seasonal trends that are less well defined (Cook et al., 1990; Levy et al., 2009; Tatte et al., 2010). Also, in the present study a significant difference was observed between virus isolation and cool seasons as the highest prevalence of infection was detected during the coldest months of year, mainly between November and February. This is distinct from seasonal rotavirus diarrhea in tropical countries. Because of the high burden of rotavirus disease in

developing countries and also many of these countries lie in the tropical belt; further analysis of the seasonal patterns of rotavirus in tropical countries can reveal the epidemiology of this important disease. Therefore the prospective survey of diarrhea illnesses from various part of Iran is necessary to provide a more accurate picture of the proportion of Iranian children with laboratory-provide rotavirus infection. In conclusion, detection of high prevalence of group A rotavirus infection in studied hospitalized children with diarrhea and determination of circulating rotavirus genotypes, provides useful data for formulating new and more effective vaccines, especially for infants aged <5 years old.

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