

Prevalence and Molecular Genotyping of Group A Rotaviruses in Iranian Children

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Abstract Rotavirus is the leading cause of acute gastroenteritis in worldwide young children. Effective vaccines to prevent rotavirus infection are currently available, although their clinical use is still limited, and rotavirus still causes many episodes of infantile gastroenteritis, mainly during the winter season. The aim of this study was to evaluate the prevalence of rotavirus infection in children aged <5-years-old who were hospitalised for gastroenteritis. One hundred and sixty-three stool samples from hospitalised children (<5-years-old) complicated with severe diarrhoea, in two hospitals in Jahrom City, Iran were collected from 2009 to 2010. Antigenic prevalence of rotavirus group A was distinguished by enzyme immunoassay. The antigen of group A rotavirus was diagnosed by EIA in 75 of 163 collected samples. The genotype of EIA-positive samples was determined by nested RT-PCR. The frequency of rotavirus genotypes G1, G2, G3, G4 and G9 was 17.33, 13.34, 2.67, 30.66 and 2.67 %, respectively. Also, the frequency of mixed and non-typable genotypes was detected in 2.67 and 30.66 %, respectively. G1/G8 mixed infection was the first of these rotavirus genotypes to be reported in Iran. Detection of high prevalence of group A rotavirus infection in hospitalised children with diarrhoea, and determination of circulating rotavirus genotypes in this region of Iran, provide useful data for formulating effective vaccines; especially for infants less than 5-years-old.

Keywords Rotavirus · Prevalence · Molecular genotyping · RT-PCR

Introduction

Diarrhoea is still a major public health problem worldwide, especially in children. In developing countries, 12 or more diarrhoea episodes are estimated to occur per child per year within first 5 years of life. Different aetiological agents, including bacteria, parasites and especially viruses that have been intensively studied in recent years are related to acute diarrhoea [1]. Group A rotaviruses are the one of the most important causes of severe acute diarrhoea in young children worldwide.

Hospital-based studies have revealed that group A rotavirus infection is the cause of acute diarrhoea in 20–70 % of children <5-years-old in developed and developing countries, and leads to death in approximately 800,000 children annually in developing countries [2, 3]. Bishop et al. identified rotavirus for the first time in humans in 1973 when they observed characteristic rotavirus particles in the cytoplasm of duodenal epithelial cells from young children who had been admitted to hospital for acute diarrhoea [1].

The genus Rotavirus belongs to the family of Reoviridae and, according to the antigenic and genetic variants of the VP6 region, can be divided into seven groups named A–G. The majority of human infections are caused by viruses in group A, which can further be differentiated into 23G- and 32P-type, respectively, by VP7 and VP4 antigens [4, 5]. Knowledge of the distribution of G and P genotypes, including detection of emerging genotypes, is crucial to rotavirus vaccination programmes [6]. G1–G4 are the most common worldwide genotypes of rotaviruses [7].

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The capacity of the first-generation of rotavirus vaccines to provide heterotypic protection against other G-types is unclear [8].

The epidemiology of rotavirus infection is complex and varies depending on laboratory setting. Use of newly optimised molecular techniques has increased sensitivity of detection and characterisation of unusual rotavirus strains apart from commonly described genotypes [9]. Results of earlier studies in Iran have shown that rotavirus is a major aetiological agent of acute diarrhoea in infants and young children [10]. Based on earlier findings, the prevalence of different genotypes of group A rotavirus that are circulating in Iran, and detection of uncommon and novel types of rotavirus were evaluated in this study.

Materials and Methods

Sampling

From November 2009 to October 2010, a total of 163 stool specimens were collected from children aged <5-years-old who were hospitalised with gastroenteric symptoms in Motaharri and Paimanie Hospitals in Fars Province, Iran.

Rotavirus antigen detection

Rotavirus antigens were detected in stool specimens by a solid-phase sandwich-type enzyme immunoassay method (IDEIA Rotavirus; Dako, Glostrup, Denmark).

RT-PCR

Rotavirus-positive specimens were categorised into strain G genotype by RT-PCR, as described by Gouvea et al. [11]. Rotavirus dsRNA was extracted from stool specimens by standard phenol–chloroform extraction method [11]. The extracted dsRNA was used in G typing by type-specific primers for RT-PCR. Consensus primers Beg9 and End9 were used in the first round of PCR (30 cycles) to amplify the full-length VP7 gene (1,062 bp). In the second round of PCR, cDNA was used for G typing (25 cycles) with primer sets including: aBT1 (G1), aCT2 (G2), aET3 (G3), aDT4 (G4), cFT5 (G5), aAT8 (G8) and aFT9 (G9) [11, 12]. All PCR products were analysed by electrophoresis in 1.2 % agarose gels that contained 0.5 µg/ml ethidium bromide, and visualised under UV illumination.

Statistical Analysis

Data were statistically analysed by SPSS version 14. $P < 0.05$ was considered statistically significant.

Results and Discussion

Serology of Rotavirus Infection

A total of 163 stool samples were collected from children aged < 5-years-old. All children had diarrhoea for a period of 1–5 days before hospitalization. Rotavirus was detected in 75 of 163 (46.02 %) stool specimens analysed for the presence of group A rotavirus antigen by EIA.

Rotavirus and Demographic Data

Rotavirus was detected more often in boys (28.83 %) than in girls (17.18 %). Forty-five of 75 (60 %) patients with rotavirus gastroenteritis were <2 years of age. Detection rate of rotavirus was the highest among infants aged 6–23 months old (Fig. 1).

There was no significant sex difference ($P > 0.05$) between children with or without rotavirus gastroenteritis. Rotavirus was detected throughout the year but relative frequency of rotavirus gastroenteritis was highest in winter. The seasonal distribution of rotavirus infection was as follow: 6.13 % in spring, 4.29 % in summer, 12.88 % in autumn, and 22.69 % in winter. A significant relationship was also found between rotavirus infection and seasonal distribution ($P < 0.05$).

Rotavirus Genotyping

From different studied genotypes of VP7 gene of group A rotaviruses by nested-RT-PCR primer genotyping protocol, the G4 strain of group A rotavirus was the predominant genotype detected in 23 of 75 specimens (30.66 %). The prevalence of G1, G2, G3, G9 and non-typable genotypes of VP7 gene in children with rotavirus gastroenteritis was: 13 (17.33 %), 10 (13.34 %), two (2.67 %), two (2.67 %) and 23 (30.66 %), respectively. The G5 and G8 genotypes were not detected in all rotavirus-infected children. Mixed G1/G8 and G1/G2 genotypes of group A rotavirus were diagnosed in 1.33 % of these patients after confirmation of sequencing. The G1/G8 mixed genotype of rotavirus was reported for the first time in Iran. Detection rate of rotavirus genotypes was highest in January (33.34 %) and lowest in November (13.33 %), compared with other months ($P > 0.05$) (Fig. 2).

Many studies have shown that rotavirus is an important cause of diarrhoea in children in developed and developing countries [13]. Most cases occur in children less than 5 years of age. The prevalence of rotavirus in children with diarrhoea varies from 30 to 50 % [14]. From 2009 to 2010, consecutive surveillance of rotavirus diarrhoea in children under 5 years of age in Jahrom, Iran showed a prevalence of 46 %, which is similar to other

FIG. 1 Age distribution of rotavirus diarrhoea during study period from October 2009–November 2010

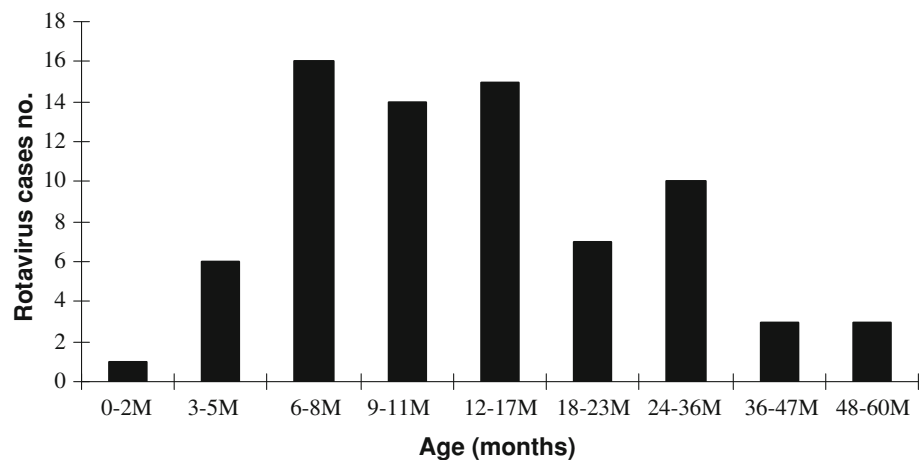
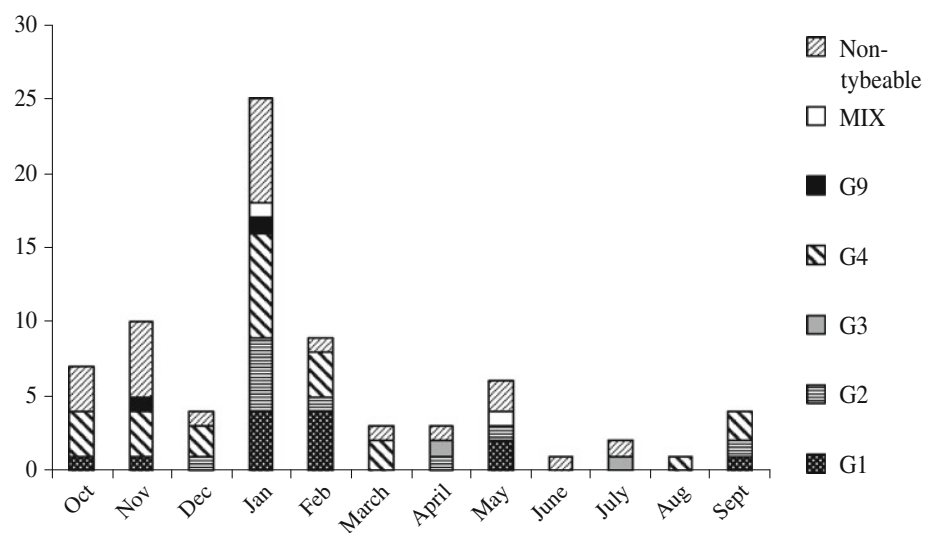


FIG. 2 The seasonal distribution of different rotavirus genotypes



reports [1, 15]. In the present study, a clear seasonal pattern in rotavirus diarrhoea was seen. Although rotavirus is detected throughout the year, there were obvious seasonal trends and regional variations in the incidence of rotavirus infection in the present study. In general, the incidence peak of rotavirus diarrhoea is in autumn and winter in different regions of countries evaluated [16]. These findings have been reported in Iran, Spain, America, Canada, Japan and Vietnam [1, 17–19]. In common with other investigations [20], the peak season of rotavirus diarrhoea was between November and January in the present study. Also, the highest rate of rotavirus infection was seen in children between 6 and 23 months of age, which was similar to other studies [16, 21]. In the present study, >60 % of human rotavirus infection was cumulatively found in infants younger than 2-years-old. Rotavirus age distribution was related to the peak incidence of infection, decline in maternal antibodies, and immaturity of new passive immune responses. Worldwide genetic diversity of circulating rotavirus strains is associated with

presentation of new emerging strains, which are a cause of variability in the geographical distribution of the virus. The present study confirms the role of human rotavirus as an important enteropathogenic microbial agent and highlights the high prevalence of G1–G4 and G9 strains in Iranian paediatric patients. These findings are similar to epidemiological data described in Australia, Europe and North America, and differ from those observed in developing countries [7, 22, 23].

The G1 and G2 rotavirus genotypes have been detected in other Iranian studies [24]. The highest frequency of G1 strains has been reported in recent studies that have focused on genotyping of rotavirus VP7 gene in Iran and other countries [18]. Also the predominance of G4 strain of rotavirus was seen in the study period, which agrees with the results of VP7 gene typing in other geographical regions including Bangladesh, France and Malaysia [25, 26]. The G3 type of rotavirus was found in only 2.66 % of children with gastroenteritis, which is comparable with the results of previous studies in Iran [18].

In recent years, there has been an increase in research of the importance of G9 genotype in many countries including Iran, United States, Australia, Japan, France and Ireland [18, 27]. In the present study only, one G9 genotype isolate was detected. However, other investigations have demonstrated the need for new generations of rotavirus vaccines to include G9 strains, as a result of the increasing emergence of this type of group A rotavirus. In the present study, the G8 genotype that was first detected in Indonesia [28] was diagnosed in mixed infection with G1 genotype. Furthermore, we documented the first case of G1/G8 mixed infection in Iran. Also, the first reports of G1/G8 mixed infection have been documented in young children with gastroenteritis in Ireland and Nigeria [29, 30]. We found mixed infections with two different rotavirus genotypes in 2.66 % of children, which is similar to previous studies, with a range of 1–26.4 % [31]. These mixed infections occur more frequently in areas with high incidence of rotavirus infection.

The pattern of rotavirus mixed infections detected in the present investigation (G1/G2 and G1/G8) differed from other studies (G9/G3 and G9/G1) [28]. Mixed infection with different rotavirus strains might reflect frequent contamination of water resources with rotavirus strains, and could facilitate generation of novel rotavirus strains through reassortment. Therefore, the frequency of mixed infection of rotaviruses and its influence against efficacy of rotavirus vaccine should be completely investigated. Detection of non-typeable rotavirus strains might be due to the accumulation of segment mutation in the VP7 gene of rotavirus. These rotavirus strains are rarely detected in other investigations [16]. In the present study, the non-typeable strains of rotavirus might represent other genotypes such as G10 and G12.

Conclusion

Diagnosis of high seroprevalence of group A rotavirus infection in hospitalised patients with diarrhoea, and genotyping of single and co-infection rotavirus isolates, provide useful data for designing new effective vaccines, especially for infants aged < 5-years-old.

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