International Scholars Journals

Advanced Journal of Microbiology Research ISSN 2241-9837 Vol. 13 (4), pp. 001-006, April, 2019. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Full Length Research Paper

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

Mohammad Kargar [1]* Akram Najafi[1] and Keivan Zandi[2] and Zahra Hashemizadeh[3]

[1]Department of Microbiology, Jahrom Branch, Islamic Azad University, Jahrom, Iran.
[2]Department of Virology, School of Medicine, Bushehr University of Medical Science and Health Services, Bushehr, Iran.
[3]Department of Bacteriology and Virology, Shiraz Unversity Of Medical Science, Shiraz, Iran.

Accepted 12 March, 2019

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

*Corresponding author. E-mail: microkargar@gmail.com. Tel: +98 – 917 – 314 – 9203. Fax: +98 – 711 – 647 – 6101.

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 l 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 H_2SO_4 (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 l 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 l. 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 l 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 l 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

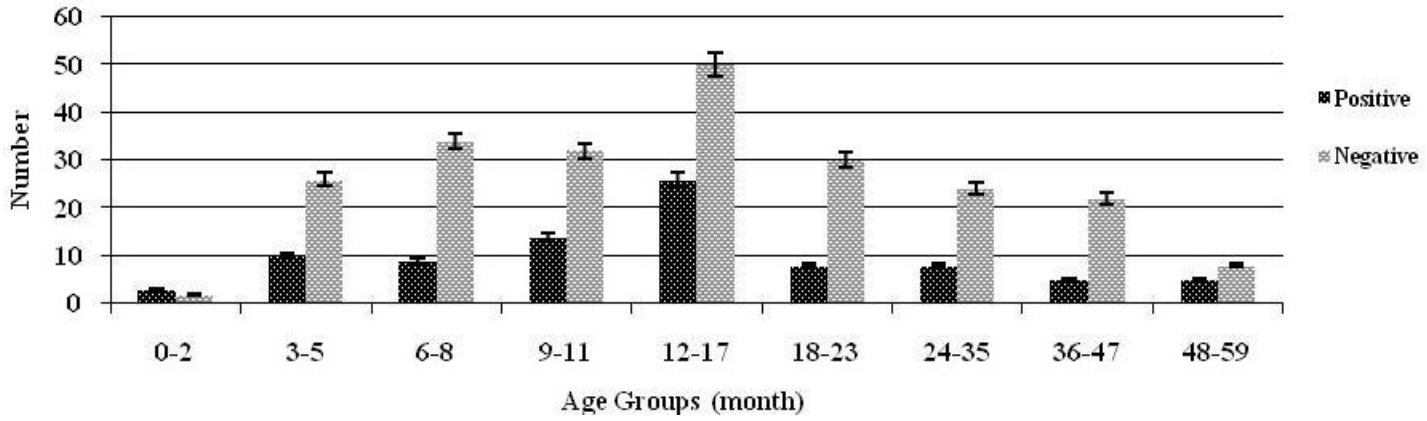

Figure 1. Age distribution of rotavirus infection during study period from October 2009 to September 2010.

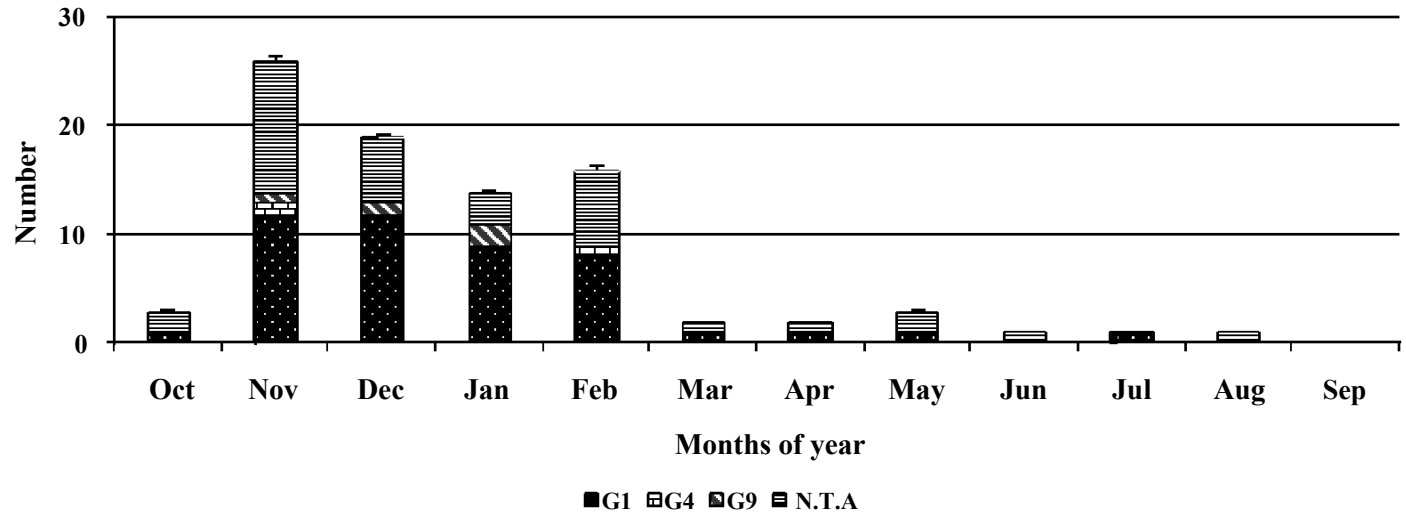

Figure 2. The seasonal distribution of rotavirus genotypes during study period from October 2009 - September 2010.

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

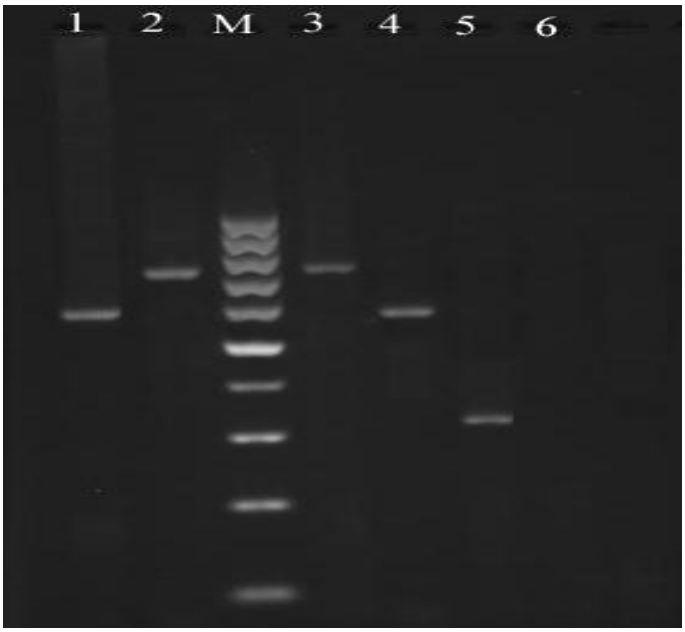

Figure 3. Electrophoresis of Gouvea VP7 genotype amplicons. M(100 bp molecular weight marker), lanes 1,4(G4 genotype), lanes 2,3(G1 genotype), lane 5(G9 genotype) and lane 6 represents the negative control.

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.

ACKNOWLEDGEMENTS

The authors wish to extend their thanks and appreciated to Islamic Azad University of Jahrom branch and Bushehr University of Medical Sciences and Health Services for their financial and executive support of this project.

REFERENCES

Annarita P, Grassi T, Donia D, De Donno A, Idolo A, Alfio C, Alessandri C, Alberto S, Divizia M (2010). Detection and molecular characterization of human rotaviruses isolated in Italy and Albania. J. Med. Virol., 82(3): 510-518.

CDC (2008). Rotavirus Surveillance Worldwide, 2001-2008. MMWR Wkly., 57(46): 1255-1257.

Clark HF, Marcello AE, Lawley D, Reilly M, Dinubile MJ (2010). Unexpectedly high burden of rotavirus gastroenteritis in very young infants. BMC Pediatr., 10: 40.

Cook SM, Glass RI, LeBaron CW, Ho Ms (1990). Global seasonality of rotavirus infections. Bull. World Health Organ., 68(2): 171-177.

De Rougemont A, Kaplon J, Pillet S, Mory O, Gagneur A, Minoui-Tran A, Meritet JF, Mollat C, Lorrot M, Foulongne V, Gillet Y, Nguyen-Bourgain C, Alain S, Agius G, Lazrek M, Colimon R, Fontana C, Gendrel D, Pothier P (2011). Molecular and clinical characterization of rotavirus from diarrheal infants admitted to pediatric emergency units in France. Pediatr. Infect. Dis. J., 30(2): 118-124.

Dey SK, Hayakawa Y, Rahman M, Islam R, Mizuguchi M, Okitsu S, Ushijima H (2009). G2 strain of rotavirus among infants and children, Bangladesh. Emerg. Infect. Dis., 15(1): 91-94.

Eesteghamati A, Gouya M, Keshtkar A, Najafi L, Zali MR, Sanaei M, Yaghini F, el Mohamady H, Patel M, Klena JD, Teleb N (2009). Sentinel hospital-based surveillance of rotavirus diarrhea in Iran. J. Infect. Dis., 200(Suppl. 1): S244-247.

Estes MK, Kapikian AZ (2007). Rotaviruses. In: Fields BN, Knipe DM, Howley PM, Griffin DE, Lamb RA, Martin MA, Roizman B, Straus SE (eds). Fields Virology, 5th edn, Lippincott Publishers, Philadelphia, pp. 1917-1974.

Fang ZY, Wang B, Kilgore PE, Bresee JS, Zhang LJ, Sun LW, Du ZQ, Tang JY, Hou AC, Shen H, Song XB, Nyambat B, Hummelman E, Xu ZY, Glass RI (2005). Sentinel hospital surveillance for rotavirus diarrhea in the People's Republic of China, August 2001–July 2003. J. Infect. Dis., 192S(uppl 1): S94-99.

Ghosh S, Kobayashi N, Nagashima S, Chawla-Sarkar M, Krishnan T, Ganesh B, Naik TN (2010). Molecular characterization of the VP1, VP2, VP4, VP6, NSP1 and NSP2 genes of bovine group B rotaviruses: Identification of a novel VP4 genotype. Arch. Virol., 155(2): 159-167.

Hoshino Y, Kapikian AZ (1996). Classification of rotavirus VP4 and VP7 serotypes. Arch. Virol. Suppl., 12: 99-111.

Jere KC, Sawyerr T, Seheri LM, Peenze I, Page NA, Geyer A, Steele AD (2011). A first report on the characterization of rotavirus strains in Sierra Leone. J. Med. Virol., 83(3): 540-550.

Jiraphongsa C, Bresee JS, Pongsuwanna Y, Kluabwang P, Poonawagul U, Arporntip P, Kanoksil M, Premsri N, Intusoma U (2005). Rotavirus Surveillance Project Thailand Study Group. Epidemiology and burden of rotavirus diarrhea in Thailand: Results of sentinel surveillance. J. Infect. Dis., 192(Suppl. 1): S87-93.

Kargar M, Akbarizadeh AR (2011). Prevalence and molecular genotyping of group A rotaviruses in Iranian children. Indian J. Virol. (In Press).

Khalili B, Cuevas LE, Reisi N, Dove W, Cunliffe NA, Hart CA (2004). Epidemiology of rotavirus diarrhoea in Iranian children. J. Med. Virol., 73(2): 309-312.

Lacroix L, Galetto-Lacour A, Altwegg M, Egli K, Schmidt M, Gervaix A (2010). Disease burden of rotavirus gastroenteritis in children up to 5 years of age in two Swiss cantons: Pediatrician and hospital-based surveillance. Eur. J. Pediatr., 169(3): 319-325.

Le VP, Kim JY, Cho SL, Nam SW, Lim I, Lee HJ, Kim K, Chung SI, Song W, Lee KM, Rhee MS, Lee JS, Kim W (2008). Detection of unusual rotavirus genotypes G8P[8] and G12P[6] in South Korea. J. Med. Virol., 80(1): 175-182.

Levy K, Hubbard AE, Eisenberg JNS (2009). Seasonality of rotavirus disease in the tropics: A systematic review, meta-analysis. Int. J. Epidemiol., 38(6): 1487-1496.

Lorrot M, Bon F, El Hajje MJ, Aho S, Wolfer M, Giraudon H, Kaplon J, Marc E, Raymond J, Lebon P, Pothier P, Gendrel D (2011). Epidemiology and clinical features of gastroenteritis in hospitalized children: Prospective survey during a 2-year period in a Parisian hospital, France. Eur. J. Clin. Microbiol. Infect. Dis., 30(3): 361-368.

Mandeville KL, Krabshuis J, Ladep NG, Mulder CJJ, Quigley EMM, Khan SA (2009). Gastroenterology in developing countries: Issues and advances. World J. Gastroenterol., 15(23): 2839-2854.

Mast TC, Chen PY, Lu KC, Hsu C, Lin HC, Liao WC, Lin DP, Chen HC, Lac C (2010). Epidemiology and economic burden of rotavirus gastroenteritis in hospitals and pediatric clinics in Taiwan, 2005-2006. Vaccine, 28(17): 3008-3013.

Mladenova Z, Korsun N, Geonova T, Iturriza-Gómara M and Rotavirus Study Group (2010). Molecular epidemiology of rotaviruses in Bulgaria: Annual shift of the predominant genotype. Eur. J. Clin. Microbiol. Infect. Dis., 29(5): 555-562.

Nelson EA , Bresee JS, Parashar UD, Widdowson MA, Glass RI (2008). Asian Rotavirus Surveillance Network. Rotavirus epidemiology: The Asian Rotavirus Surveillance Network. Vaccine, 26(26): 3192-3196.

Parashar UD, Gibson CJ, Bresse JS, Glass RI (2006). Rotavirus and severe childhood diarrhea. Emerg. Infect. Dis., 12(2): 304-306.

Parashar UD, Hummelman EG, Bresee JS, Miller MA, Glass RI (2003). Global illness and deaths caused by rotavirus disease in children. Emerg. Infect. Dis., 9(5): 565-572.

Pun SB, Nakagomi T, Sherchand JB, Pandey BD, Cuevas LE, Cunliffe NA, Hart CA, Nakagomi O (2007). Detection of G12 human rotaviruses in Nepal. Emerg. Infect. Dis., 13(3): 482-484.

Rahman M, Matthijnssens J, Nahar S, Podder G, Sack DA, Azim T, Van Ranst M (2005). Characterization of a novel P[25], G11 human group a rotavirus. J. Clin. Microbiol., 43(7): 3208-3212.

Ribas MA, Nagashima S, Calzado A, Acosta G, Tejero Y, Cordero Y, Daynelid Piedra D, Kobayashi N (2011). Emergence of G9 as a predominant genotype of human rotaviruses in Cuba. J. Med. Virol., 83(4): 738-744.

Sa´fadi MA, Berezin EN, Munford V, Almeida FJ, de Moraes JC, Pinheiro CF, Racz ML (2010) . Hospital-Based surveillance to evaluate the impact of rotavirus vaccination in Sao Paulo, Brazil. Pediatr. Infect. Dis. J., 29(11): 1019-1022.

Shim SY, Jung YC, Le VP, Son DW, Ryoo E, Shim JO Lim I, Kim W (2010). Genetic variation of G4P[6] rotaviruses: Evidence for novel strains circulating between the hospital and community. J. Med. Virol., 82(4): 700-706.

Tatte VS, Gentsch JR, Chitambar SD (2010). Characterization of group A rotavirus infections in adolescents and adults from Pune, India: 1993-1996 and 2004-2007. J. Med. Virol., 82(3): 519-527.

Van Damme P, Giaquinto C, Maxwell M, Todd P, Van der Wielen M; REVEAL Study Group (2007). Distribution of rotavirus genotypes in Europe, 2004-2005: The REVEAL Study. J. Infect. Dis., 195(Suppl 1): S17-25.

Wang YH, Kobayashi N, Zhou X, Nagashima S, Zhu ZR, Peng JS, Liu MQ, Hu Q, Zhou DJ, Watanabe S, Ishino M (2009). Phylogenetic analysis of rotaviruses with predominant G3 and emerging G9 genotypes from adults and children in Wuhan, China. J. Med. Virol., 81(2): 382-389.

World Health Organization (2009). Department of Immunization, Vaccines and Biological. Manual of rotavirus detection and characterization methods. CH-1211 Geneva 27, Switzerland. This publication is available on the Internet at: www.who.int/vaccines-documents/.

Zuccotti G, Meneghin F, Dilillo D, Romanò L, Bottone R, Mantegazza C, Giacchino R, Roberto Besana R, Ricciardi G, Sterpa A, Altamura N, Andreotti M, Montrasio G, Macchi L, Anna Pavan A, Paladini S, Zanetti A, Radaelli G (2010). Epidemiological and clinical features of rotavirus among children younger than 5 years of age hospitalized with acute gastroenteritis in Northern Italy. BMC Infect. Dis., 10: 218.