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Epidemiology of Rotavirus Diarrhoea in Iranian Children

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Human rotavirus is the most important cause of severe diarrhoea in infants and young children worldwide. We describe the aetiology of viral diarrhoea and the characteristics of rotavirus infection in Shahrekord, Iran. Two hundred and fifty nine children <5 years old admitted to Hajar Hospital, 245 children with acute diarrhoea attending primary health centres in Shahrekord, and 114 children hospitalised for elective surgery were selected from October 2001 to August 2002. Stool samples were screened for enteric viruses using EM. Rotaviruses were characterised using ELISA, reverse transcription-polymerase chain reaction (RT-PCR), and electropherotyping. One hundred and eighty six viruses were identified, of which 146 (78%) were rotavirus. The second most frequent virus was coronavirus, followed by calicivirus. Rotaviruses exhibited a marked seasonal variation, being most frequently isolated from November to February (50% of rotavirus recovered) and affected mostly children <2 years old. The RT-PCR successfully typed 139 of the 146 (95%) rotavirus G types and 124 (85%) P types. The most frequent P type was, P[8] in 108 (74%), P[4] in 16 (11%), and was P non-typeable in 22 (15%). Among the G types, G1 was identified in 120 (82%), G2 in 19 (13%), and was G-non-typeable in 7 (5%). Our results are the first report of rotavirus genotypes affecting Iranian children. The most frequent G and P types (G1, G2, P[8], and P[4]) are similar to those reported from around the world and will be covered by existing rotavirus vaccines targeting G types G1–G4. ***J. Med. Virol. 73:309–312, 2004.***

KEY WORDS: rotavirus; epidemiology; diarrhoea; children; Iran

INTRODUCTION

Diarrhoea is one of the most common diseases in children resulting in the death of more than 5 million children every year. The greatest morbidity and mortality is seen among children less than 2 years of age [Carroll and Reimer, 2000; Hart, 2003].

Human Rotavirus is responsible for a large proportion of these deaths and 20–52% of acute diarrhoea episodes [Cunliffe et al., 2002a; Hart, 2003]. Rotavirus is a double stranded RNA virus with a genome comprising 11 linear segments, each encoding one or two virus proteins (VPs 1, 2, 3, 4, 6, 7). VP4 and VP7, the outer capsid proteins encoded by respectively genome segments 4 and 9 are both involved in attachment to and entry into enterocytes and are the major rotavirus-neutralizing antigens. Epitopes on VP7 define the G (for glycoprotein) types whereas those on VP4 define the P (for protease-sensitive) types. Currently 14 G and 20 P types have been described. Globally, G1–G4 are the most common G types although there is a tremendous diversity as, for example, while G8 has caused 30% of gastro-enteritis in Malawi [Cunliffe et al., 2001a], G5 was the G type most often isolated in South America [Gouvea et al., 1994], and other novel serotypes (e.g., G9) have been reported worldwide recently [Ramachandran et al., 2000; Cunliffe et al., 2002b].

Diarrhoea is still one of the main health problems in Iranian children. There is no information on the pathogens most often involved, nevertheless many hospitals beds are still occupied by children with acute diarrhoea. This study describes the aetiology of viral diarrhoea and the characteristics of rotavirus in children with acute diarrhoea in Shahrekord, Iran.

MATERIALS AND METHODS

Children under 5 years old admitted to Hajar Hospital (inpatients) or attending three primary health centres (outpatients) in Shahrekord, Iran with a clinical diagnosis of acute diarrhoea were selected systematically

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during working days from October 2001 to August 2002. In addition, a group of children hospitalised for elective surgery in Hajar Hospital (surgical controls) were selected for comparison. After informed consent, participants were interviewed to ascertain their demographic and social background and clinical history. For the purpose of this study, acute diarrhoea was defined as the presence of three or more liquid or semi-liquid stools or a single watery stool per day of less than 14-days duration. A minimum of five children were enrolled every week to obtain a representative sample of the pathogens for the duration of the study.

Stool samples were collected in plastic containers for light microscopy, rotavirus enzyme immunoassays (EIA), negative stain electron microscopy (EM), rotavirus reverse transcription polymerase chain reaction (RT-PCR), and electropherotyping. EM was performed using a Philip 301 electron microscope (Philips Electron optics UK Division, PYE Unicam, Ltd, Cambridge, UK) [Madeley, 1997] at a screen magnification of 45,000×. Grids were scanned horizontally along the rows of the grid squares from end to end at three different locations. Rotavirus antigens were detected using the Rotaclone EIA kit (Meridian Diagnostics, Cincinnati, OH) as described by the manufacturer. EIA positive samples were suspended in phosphate buffered saline at a concentration of 10% for dsRNA extraction. Suspensions were clarified by centrifugation and dsRNA was extracted using a guanidine isothiocyanate/silica method developed by Boom et al. and modified by Gentsch et al. [1992]. Extracted RNA was eluted in 70 μl of RNase free water and used directly for polyacrylamide gel electrophoresis (PAGE) and RT-PCR. For PAGE, 30 μl of purified dsRNA was heated at 65°C for 10 min and electrophoresed for 120 min at 150 V on 10% polyacrylamide gel. Controls of known long and short electropherotypes were included on each gel. After silver nitrate staining [Herring et al., 1982], the gels were photographed to determine the electropherotypes. Rotavirus G- and P-genotypes were determined using a multiplex RT-PCR as described by Gouvea et al. [1990] and Gentsch et al. [1992].

Amplicons were visualised under UV light after electrophoresis on a 2% agarose gel stained with ethidium bromide. Samples co-migrating with reference strains of known genotypes were assigned a genotype. Samples failing to G or P type were repeated with additional G1 and P[8] typing primers nac 9 and nac 10 respectively [Cunliffe et al., 2001b].

RESULTS

Stool samples were collected from 259 inpatients and 245 outpatients with a clinical diagnosis of diarrhoea and 114 surgical controls. The general characteristics of the participants are shown in Table I. The mean (SD) age of the hospitalised and outpatient children were similar with 15.2 (12) and 15.6 (12.3) months, respectively. Surgical controls however were older with a mean (SD) age of 30 (12.4) months. The gender distribution of the children was comparable with 143 (55%), 133 (54%), and 65 (57%) children being male among the hospita-

TABLE I. Characteristics of Hospitalised and Outpatient Children With Diarrhoea and Surgical Controls

		Children with diarrhoea		
		Inpatients	Outpatients	Surgical controls
Number		259	245	114
Male		143 (55%)	133 (54%)	65 (57%)
Age (months)	1–12	149 (58%)	135 (55%)	7 (6%)
	13–24	76 (29%)	71 (29%)	34 (30%)
	25–59	34 (13%)	39 (16%)	73 (64%)
Age (SD)		15.2 (12.)	15.6 (12.3)	30 (12.4)
Reason for consultation	Diarrhoea	259 (100%)	245 (100)	NA
	Vomiting	196 (76)	54 (23%)	NA
	Fever	33 (13%)	5 (2%)	NA
	Surgery	1 (1%)	0 (0%)	114 (100%)
Birth weight in kg (SD)		3.1 (5.7)	3.1 (5.2)	3.1 (5.1)
Diarrhoea duration (days)		4.3 (3.3)	3.7 (2.7)[a]	NA
Number of episodes in previous 24 hr		7.1 (3.8)	5.7 (2.8)[a]	NA
Consistency	Watery	249 (97%)	225 (92%)	NA
	Semi liquid	128 (49%)	170 (69%)[a]	NA
	Bloody	28 (11%)	7 (3%)[a]	NA
Vomited in previous 24 hr		165 (64%)	66 (27%)[a]	NA
ARI		14 (6%)	5 (2%)[a]	NA
Fever		201 (78%)	127 (54%)[a]	NA
Viruses	Rotavirus	91 (35%)	45 (18%)	10 (8.4%)
	Coronavirus	4 (1.5%)	10 (4%)	2 (1.9%)
	Astrovirus	4 (1.5%)	2 (2%)	0 (0%)
	Calicivirus	3 (1.2%)	5 (2%)	1 (0.5%)
	Adenovirus	3 (1.2%)	5 (2%)	0 (0%)
	Parvovirus	1 (1%)	0 (0%)	0 (0%)

[a]$P < 0.05$ when compared to hospitalised children with diarrhoea, NA = not applicable.

lised, outpatients, and surgical controls, respectively. The duration of the diarrhoea episode before consultation was higher in hospitalised than outpatient children with a mean (SD) of 4.3 (3.3) and 3.7 (2.7) days respectively and they had higher stool frequencies before enrolment ($P < 0.05$ for both). Vomiting was the second most frequent complaint and again, hospitalised children were vomiting more frequently than outpatients (76 and 23%, respectively) ($P < 0.05$). Fever was present in the majority of hospitalised children (78%) and acute respiratory symptoms were associated with the diarrhoea episodes in a smaller number (6%).

A total of 186 episodes of virus excretion were identified and rotavirus was identified in 146 (78%). Ten (8.4%) of the rotaviruses were identified in children without diarrhoea as shown in Table I. The second virus detected most frequently was coronavirus (16), followed by calicivirus (all norovirus-like) (9). Other viruses included adenovirus (8), astrovirus (6), and parvovirus (1). Each of the other viruses except rotavirus was recovered from children with diarrhoea.

Rotavirus was identified more frequently in children <2 years old (37 and 19% in hospitalised and outpatient children, respectively) than children >2 years old (23 and 10% for hospitalised and outpatients, respectively), with the highest frequency observed in 1 to 2 year old children followed by children <1 year old. More than half of the rotaviruses identified in hospitalised children were isolated from November to February, with lower numbers occurring during the hot summer months between June and August. Rotavirus infection had a similar pattern in outpatient children as most cases were detected from February to March (the cold winter season) and was rare during the hot summer months between June and August. Rotavirus, however, was responsible for a lower proportion of cases of diarrhoea in the health centres where other viruses (mainly calicivirus and coronavirus) were also identified frequently. Most asymptomatic rotavirus infections identified in surgical controls occurred at the peak of the rotavirus season. Our results did not demonstrate a clear seasonal pattern for other viruses besides rotavirus, as frequencies were too small to show any differences. Astrovirus and adenovirus, however, were mostly identified in the cooler semester of the year.

RT-PCR assigned G types to 139 of 146 (95%) of rotaviruses and P types to 124 (85%) (Fig. 1). The most frequent P type was P[8] in 108 (74%) samples, P[4] in 16 (11%), and 22 (15%) were P-non-typeable. Among the G types, G1 was identified in 120 (82%) samples, G2 in 19 (13%), and 7 (5%) were G-non-typeable. Sixty one strains required the alternative P[8] typing primer nac 10 and 42 strains required the alternative G1 typing primer nac 9. The type designations of strains requiring alternative primers were selectively confirmed by nucleotide sequencing (data not shown).

Electrophoretypes were done for the first 46 rotavirus positive samples. All the short-electrophoretypes were P[4] G2 and all long-electrophoretypes were a combination of P[8]G1and P[8]G2. P[4] had similar monthly

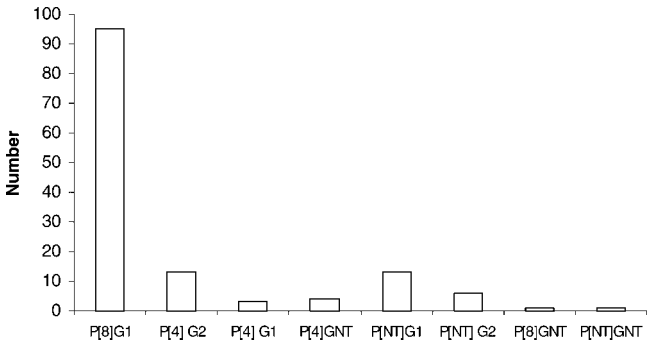

Fig. 1. Distribution of P–G combinations.

distribution patterns to the G and P non-typeable specimens and these were most frequent in the last and first months of the year, the time when rotavirus was most frequent. The variation of P–G combinations seen during the rest of the year suggests that there is wide strain diversity, with some strains predominating in a given year (data not shown). P[8] rotaviruses were seen in all months. P[4] was seen only as a cluster at the end of the year and P-non-typeable strains were mostly seen from January to July.

DISCUSSION

This report confirms that rotavirus is an important cause of paediatric diarrhoea in Southwestern Iran. Our findings are in agreement with reports world wide and a previous study from Iran [Moddares, 1995; Kelkar et al., 1999] which reported that rotavirus is the most important cause of severe diarrhoea in children. In our study, rotavirus was responsible for 35% of diarrhoea episodes in hospitalised children and 18% of episodes in the children attending the health centres. This over representation in hospitalised children is the result of the more severe clinical episode resulting from rotavirus as compared to other viruses and is a well-recognised feature of rotavirus infection globally [el-Sheikh et al., 2001]. The frequency of rotavirus infection was strongly associated with age and more than 90% of rotavirus cases occurred in children <2 years old. The highest frequency was observed in 13–24 month old children and the second most affected group were infants.

Rotavirus has a marked seasonal variation in this region and was isolated most frequently from November to February with 50% of rotaviruses recovered in these months. These results are in agreement with studies reporting a peak of rotavirus in winter months in temperate regions. The peak incidence, however, is different to a previous study from Tehran [Amini et al., 1990], which reported a higher incidence in the spring. Although the peak incidence occurred in the winter, the incidence of rotavirus was still high in the spring. We would need to continue surveillance for rotavirus to elucidate whether the two studies have demonstrated a different seasonal pattern in two towns, or variations in the annual incidence with a higher rotavirus incidence in the year when our study took place.

The frequency of detection of other viruses, besides rotavirus, was higher in outpatient children. This is likely to result from the milder diarrhoea caused by these viruses. Similar to rotavirus, astrovirus and adenovirus were identified mostly in the cooler semester of the year. This is the most frequent season for these viruses in temperate climates and varies from tropical areas, when these viruses often occur during the rainy season [Sethi et al., 1989]. The data, however, did not demonstrate a clear pattern, as the calicivirus and coronavirus frequencies were too small to find statistical differences. In addition, although coronavirus have been reported as a cause of diarrhoeal disease, their role is unclear [Zhang et al., 1994]. Adenoviruses however seemed to coincide with the rotavirus season and calicivirus appeared after this season from May to June. However it is stressed that the prevalence of calicivirus reported here is likely to be an underestimate as EM is not the most sensitive method for detecting the virus [Kirkwood and Bishop, 2001]. Viruses other than rotavirus however did not seem to cluster around any specific month.

The results in the first report on the genotypes of rotavirus affecting Iranian children. It is noteworthy that a large number of rotaviruses could not be G or P typed with the conventional primers described by Gouvea et al. [1990] and Gentsch et al. [1992] and additional primers were required that substantially increased the number of typable strains in our collection. The monthly distribution of the G and P types shows that the G2 and P[4] and non-typeable G and P were most often seen at the last and first months of the year and other P–G combinations were seen during the year, reflecting that there is a large diversity of rotavirus strains during the year. G1, G2, P[8], and P[4] are among the most common global rotavirus serotypes and should be covered by existing rotavirus vaccines [Hoshino and Kapikian, 2000].

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