Rotavirus Strain Diversity in Rio de Janeiro, Brazil: Characterization of VP4 and VP7 Genotypes in Hospitalized Children

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Summary

Rotavirus strains from 91 patients treated at a children's hospital from 1996 to 1998 in Rio de Janeiro, Brazil, were characterized by electropherotyping, reverse transcription-PCR amplification for P and G genotypes, and Southern hybridization. Results obtained showed that following predominant [P],G type combination: P[4], G2 (21 per cent), P[8], G1 (17 per cent), P[8], G3 (13 per cent), which are prevalent throughout the world. However, an unexpected number of cases were associated with uncommon genotypes: P[8], G2 (13 per cent), P[8], G5 (11 per cent), P[8], G9 (7 per cent), P[8], G10 (4 per cent), P[6], G4 (3 per cent), P[6], G3 (1 per cent), P[4], G9 (1 per cent), and P[6], G9 (1 per cent). Mixed infections with more than one type were identified in only two cases and 16 per cent of the samples were not G and/or P typeable. A subset of G types was confirmed by Southern hybridization and chemiluminescent detection. Rotavirus seasonal distribution was observed between April and July. The contribution of the results obtained in the present investigation corroborates the required epidemiological surveillance for rotavirus infection in Brazil.

Introduction

Rotaviruses A are the single most important etiological agents of severe gastroenteritis and infects children world-wide by the age of 5. Rotavirus infection represent an important public health problem particularly in developing countries where 20–40 per cent of annual hospitalizations occur for childhood diarrhoea, with about 650 000 deaths each year. These deaths occur because of the limited access to rehydration therapy and other medical care in developing countries.[1] In England and Wales, United States, Japan and Australia rotavirus infection is estimated to be responsible for 30–40 per cent of hospitalizations for childhood gastroenteritis, but mortality from rotavirus diarrhoea is extremely rare.[2]

The virus carries a genome of 11 segments of double-stranded RNA (dsRNA) surrounded by a triple-layered capsid consisting of a core, inner capsid and outer capsid layer. The two outer capsid proteins, VP4 and VP7, are responsible for the induction of a neutralizing antibody response and the classification of rotaviruses A into P and G types, respectively.[3] As VP4 and VP7 genes segregate independently, a dual typing system has been established to characterize the strains of rotavirus.[4] The incidence and distribution of rotavirus genotypes varies between geographical areas. Data from molecular and seroepidemiological surveys demonstrated that rotavirus genotypes P[4], G2, P[8], G1, P[8], G3 and P[8], G4 are the most common combinations found in cases of childhood diarrhoea.[4,5]

However, human isolates with uncommon G and P genotypes, such as G5, G8, G9, G10 and P[6], have constituted a significant portion of some epidemiological surveys conducted world-wide.[6–9] Some of these genotypes are present at high frequencies in different areas of the world. For example, genotype G9 that was first described in the United States[10] and later in other localities including developed and developing countries.[11,12] Other recent studies support the increasing importance of serotype G9 as a cause of severe diarrhoea in children around the

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world, including in Brazil.[13–15] Therefore, serotype G9 must be taken into consideration for prospective rotavirus vaccine studies, since this serotype is not constituted in any of the preceding rotavirus vaccines.

Considering the high diversity of rotavirus infecting Brazilian children, we report strain genotyping results from 91 positive rotavirus fecal specimens collected from hospitalized children under the age of 3 with acute gastroenteritis at two public hospitals in the city of Rio de Janeiro. Our results demonstrate that the diversity of strains circulating in Rio de Janeiro exceeds that in many parts of the world, that laboratory surveillance of strains is essential and that vaccines may require additional antigens.

Patients and Methods

Fecal specimens and rotavirus A detection

Between May 1996 and December 1998, a total of 619 fecal samples were collected during the first 8 h of hospitalization from children with gastroenteritis that were in medical care for at least 2 days. Approximately 10 per cent (wt/vol) stool suspensions were prepared in Tris-HCI Ca^{2+} 0.01M (pH 7.2) and rotavirus was detected by a combined enzyme immunoassay for rotavirus and adenovirus following instructions of the manufacturer (Bio-Manguinhos/Oswaldo Cruz Foundation, Ministry of Health of Brazil).[16] Concomitantly, stool suspensions were used for dsRNA extraction by the glass powder method,[17] followed by a polyacrylamide gel electrophoresis (PAGE).[18] Electrophoresed dsRNA segments were visualized by silver staining and defined using classification of eletropherotypes as described previously.[18]

This study was submitted to and approved by the Ethical Committees of the Hospital Municipal Jesus and Hospital Municipal Sales Neto, Rio de Janeiro.

RT-PCR for rotavirus P and G genotyping

The viral dsRNA extracted from rotavirus-positive clarified stool supernatants by the glass powder method[17] was first reverse transcribed (RT) and amplified by PCR (first amplification step) with a pair of consensus primers corresponding to a conserved nucleotide sequences of VP7[19,20] or VP4[21] genes. The amplicons obtained from 904bp (gene VP7) or 876p (gene VP4) were then used as a template in a second PCR, carried out by using 1 µl of the first amplicon and a pool of genotype-specific primers complementary to variable regions of the VP7[20] or VP4[21] genes. Temperature and time conditions for PCR amplifications were performed as originally described.[20,21] Distilled milli-Q water was used as a negative control in all procedures, and recommended manipulations for PCR techniques were carried out as a precaution to avoid false-positive results.

Southern hybridization and chemiluminescent detection

Southern hybridization and chemiluminescent detection of genotype-specific PCR products were carried out according to protocols and reagents described by Leite, *et al*.[6] for G genotyping and Ramachandran, *et al*. [7] for P genotyping.

Results

Ninety-one (15 per cent) out of the 619 fecal samples were positive for rotavirus by enzyme immunoassay and PAGE. A clear segmented dsRNA profile was visualized in all samples and the long electropherotype was predominant in 74 (81 per cent) samples. Table 1 shows the 81 (89 per cent) G genotypes and 83 (91 per cent) P genotypes that were obtained by RT-PCR using two-step amplification procedure. Out of those, 76 (83 per cent) represented a combination of single G and P types, whereas two

TABLE 1

G and P genotypes of 91 rotavirus strains from hospitalized children in the city of Rio de Janeiro, Brazil, from May 1996 to December 1998

Genotypes	Number of G and P genotypes								Total
	G1	G2	G3	G4	G5	G9	G10	G Untypeable	
P[4]	–	16	–	–	–	1	–	–	17
P[6]	–	–	1	2	–	1	–	–	4
P[8]	13	10	10	4	8	5	3	7	60
P[4+8]	–	–	1	1	–	–	–	–	2
P untypeable	2	2	–	–	1	–	–	3	8
Total	15	28	12	7	9	7	3	10	91

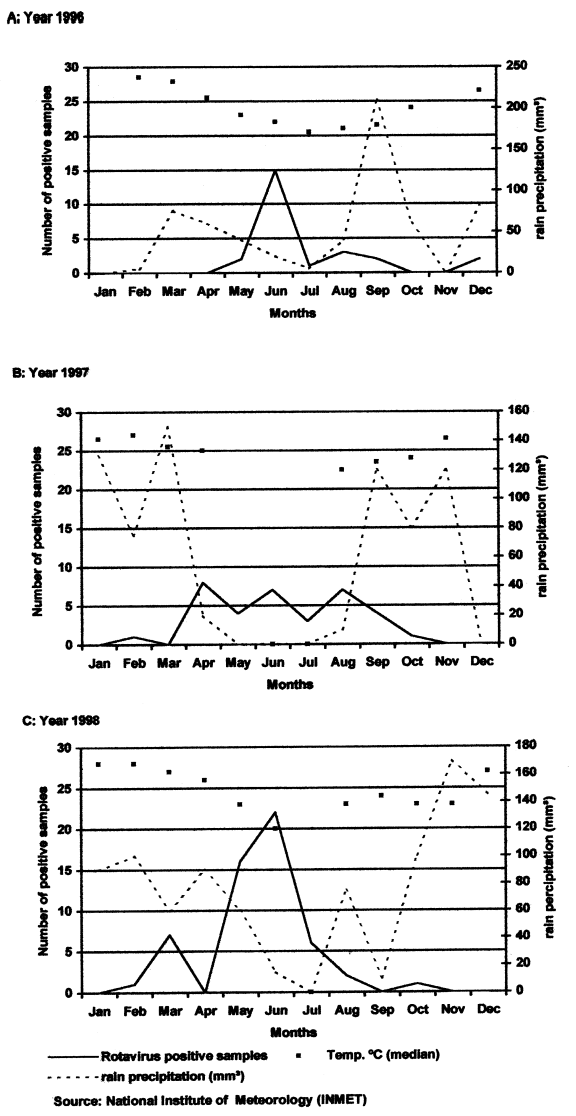

FIG. 1. Seasonal distribution of rotavirus infection among hospitalized children in a 3-year study in Rio de Janeiro, Brazil.

strains belonged to single G and mixed P type infections. Strain diversity was high and single infections with strains corresponding to one of the four common genotypes worldwide was observed in 43 (56 per cent) of the samples, where P[4], G2 (21 per cent) was predominant, followed by P[8], G1 (17 per cent), P[8], G3 (13 per cent) and P[8], G4 (5 per cent). Nevertheless, the uncommon genotypes P[8], G2, P[8], G5, P[8], G9 P[8], G10, P[6], G4, P[4], G9, P[6], G9, P[6], G3, P[4+8], G3, and P[4+8], G4 were identified and represented 43 per cent of the samples.

TABLE 2
Clinical symptoms in 91 children hospitalized with rotavirus acute gastroenteritis in Rio de Janeiro, Brazil

Clinical symptoms	Rotavirus-positive cases of diarrhoea	
	Number of cases	(%)
Vomiting	58	64
Fever (37.5–38.5ºC)	50	55
Lack of appetite	40	44
Mucus in faeces	38	42
Cough	34	37
Nasal discharge	26	29
Abdominal pain	24	26
Blood in faeces	6	7
Rash	3	3

The distribution of non-typeable strains for the G or P type or both the G and P types represented 16 per cent of the total.

The mixed P type infections ($n = 2$) and genotypes G5 ($n = 3$) and G9 ($n = 6$) were confirmed by probe hybridization with P and G type-specific oligonucleotide probes, respectively, followed by digoxigenin detection.

The temporal distribution of rotavirus infection in Rio de Janeiro during the 3-year study is represented in Fig. 1. The results show an unvarying pattern characterized by a seasonal peak of infections, predominantly in the months from April to July that correspond to the dry season. The clinical symptoms displayed in Table 2 are related to the 91 hospitalized children, who presented 6 days as the average duration of diarrhoea.

Discussion

Rotavirus is the most common cause of severe gastroenteritis in the world, representing about one-third of all hospitalizations for acute gastroenteritis and one-quarter of all deaths among children with diarrhoeal diseases.[1] Detection of rotavirus in fecal samples and the molecular characterization of G and P genotypes are important in order to identify the circulating strains. Therefore, it is possible to measure the impact of rotavirus vaccines once they represent the best way to restrict the transmission of the disease instead of improvements in sanitation and hygiene.

In the current study we characterized G and P genotypes of rotavirus in 91 fecal specimens from hospitalized children, and a remarkable diversity of strains was found. The most predominant strain was P[4], G2 which differs from surveys reported in Brazil[6,22] and other countries[23,24] where P[8], G1 appears as predominant. Both P[4], G2 and P[8], G1

are included in the four most common genotypes found around the world, as well as P[8], G3 and P[8], G4 detected in the present investigation, although represented in low numbers.

Interestingly, uncommon strains were identified as genotypes P[8], G2, P[8] G5, P[8], G9, P[8], G10, P[6], G4, P[4], G9, P[6], G9, P[6], G3, P[4+8], G3 and P[4+8], G4, indicating the presence of uncommon genotypes in Rio de Janeiro, as previously reported,[9] in São Paulo,[25] Belém[22] and some other Brazilian states.[26,6]

The seasonal distribution exhibited in Fig. 1 for the 3 years indicates that incidence of rotavirus infection varied between April and July, and that for the third year a marked increase was observed. Some studies have demonstrated that a particular seasonality can occur in Brazil's central and southern states with a peak incidence between May and August, but no seasonality is evident in the tropical northern and northeastern areas, with rotavirus cases being detected all year round.[27] Rotavirus infections in Rio de Janeiro appear to occur during the autumn–winter seasons, the dry season, consistent with the findings of numerous reports in other areas of the world.[12,28]

As shown in Table 2, vomiting and fever are the predominating symptoms in cases of rotavirus gastroenteritis as previously reported by authors in Brazil and other countries.[3,29]

Our results reflect the complex distribution of rotavirus genotypes circulating in Rio de Janeiro and encourage further evaluation of rotavirus vaccines, particularly for developing countries where the emergence of uncommon genotypes is frequent and where rotavirus represents the most important cause of death among infants and young children with diarrhoea.

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