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CONCISE ARTICLE

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Molecular and Serological Characterization of Group A Rotavirus Isolates Obtained from Hospitalized Children in Goiânia, Brazil, 1998–2000

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Abstract Fecal samples positive for rotavirus group A (n=120) were analyzed by enzyme immunoassay-monoclonal antibody (EIA-MAb) serotyping and/or reverse transcription/multiplex polymerase chain reaction (PCR) amplification to determine the prevalence of the [P] and G genotypes. The most prevalent G genotype/serotype detected was G1 (76.7%), followed by G2 (5.0%). Six samples were characterized as G9 by multiplex PCR, and one sample was characterized as G3 by EIA-MAb. The combinations of [P] and G genotypes found were P[8] and G1 (20.8%), P[6] and G1 (10.8%), P[6] and G9 (4.2%), P[8] and G2 (1.7%), and P[6] and G2 (0.8%). The diversity of rotavirus group A [P] and G genotypes/serotypes reinforces the need for continuous characterization of rotaviruses circulating in populations in Brazil.

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Introduction

Group A rotaviruses constitute the major cause of severe diarrhea in children worldwide and are also responsible for a large number of deaths among children up to 5 years of age [1]. Group A rotaviruses exhibit great genomic and antigenic variation, resulting in several serotypes/genotypes, due to variations in the VP4 and VP7 proteins, designated [P] and G, respectively [2].

The objective of the present study was to detect P and G genotypes of group A rotaviruses from children in the Central West region of Brazil. In this context, this study presents novel data on serotypes/genotypes of group A rotaviruses circulating among children with acute gastroenteritis in Goiânia, Goiás, Brazil.

Materials and Methods

From March 1998 to March 2000, 516 fecal samples were collected from the same number of children up to 5 years of age presenting acute gastroenteritis in two public hospitals in Goiânia, Brazil. Specimens were collected from the children after written consent given by their parent or other legal guardian. This study was approved by the Ethics Committee on Research of the Federal University of Goiás.

Group A rotaviruses were detected in fecal samples [10% fecal suspensions in phosphate-buffered saline (PBS) pH 7.4] by two methods: a combined enzyme immunoassay for rotavirus and adenovirus (EIARA) [3], and polyacrylamide gel electrophoresis [4].

For G serotyping, an enzyme-linked immunoassay (ELISA) technique was used according to the description by Coulson et al. [5], with some modifications: 100 µl of the monoclonal antibodies (MAbs) specific for G serotypes/genotypes (MAbs G1–4) and an MAb specific for group A rotaviruses (Serotec/Rota MA; Serotec Laboratory, Japan) diluted 1:50 and 1:100, respectively, in carbonate/bicarbonate buffer pH 9.6 was added to wells of polystyrene Immuno I microtiter trays (Nunc, Denmark) and incubated for 18 h at 4°C. After washing five times [PBS containing 0.05% (v/v) Tween 20 (PBS-T) (Merck, Germany)], 75 µl of blockade solution [PBS-T containing 0.05% (w/v) bovine serum albumin (BSA) (Sigma Chemical, USA)] was added. After 2 h of incubation, 25 µl of 10% fecal suspension was added to each

well. Each fecal sample was added to wells coated with MAbs G1–4 and the rotavirus group A-specific MAb. After incubation for 18 h at 4°C and washing as described above, 100 µl of preimmune goat serum anti-SA-11 (Bio-Manguinhos/Fiocruz, Brazil) diluted 1:100 in blockade solution (PBS-T/BSA) was added, followed by incubation for 2 h at 37°C and washing. One hundred microliters of a 1:100 dilution in PBS-T/BSA of the conjugate solution (Bio-Manguinhos/Fiocruz) was added, followed by incubation for 1.5 h and washing as described above. One hundred microliters of the substrate solution (Bio-Manguinhos/Fiocruz) was then added, and the reaction was stopped after 15 min. The absorbance value was recorded at 405 nm. In accordance with the manufacturer's instructions, a sample was considered positive for one serotype when the value of optical density of the reaction for that serotype was at least twofold the corresponding value of any other serotype. Simian rotavirus (SA11 serotype 3) was used as a positive control of the reaction.

The viral dsRNA was extracted from rotavirus group A-positive fecal samples by the glass powder method [6]. The samples were submitted to G and [P] genotyping by reverse transcription-polymerase chain reaction (RT-PCR) using a pair of consensus primers that correspond to conserved nucleotide sequences of the VP7 [7, 8] or VP4 [9] genes. The resulting amplicons of 904 bp (gene VP7) or 876 bp (gene VP4) were then used as a template in a second PCR, with a mixture of genotype-specific primers complementary to variable regions of the VP4 or VP7 genes (multiplex PCR).

The G typing assays were conducted in accordance with descriptions by Gouvea et al. [7] and Das et al. [8]. The [P] typing was carried out according to Gentsch et al. [9]. Distilled milli-Q water was used as a negative control in all procedures, and proper measures to avoid false-positive results were also taken.

Results and Discussion

In the present study, the prevalence of the G serotype/genotype was 87.5%, which is in agreement with the results reported by other authors [10]. G1 was the most prevalent genotype (76.6%), followed by G2 and G9. Genotypes G1–G4 are considered usual [10, 11, 12], although other G types such as G5, 8, 9 and 10 have also been detected [10].

A certain variation in the prevalence of group A rotaviruses G types has been observed in recent years. In Goiânia, serotype G1 strains were predominant from 1986 to 1988, while G2 was most prevalent from 1989 to 1992 [11]. In the present study, however, serotype G2 was detected in only 5% of the samples typed. Moreover, genotype G9 was detected in 1999, suggesting its emergence as an important diarrheal agent among children in Goiânia, Goiás. These results reinforce the occurrence of shifting incidences of different rotavirus group A serotypes in the region.

The [P] genotypes detected in this study were P[8] and P[6]. The genotypes P[4] and P[8] have been considered the predominant [P] genotypes among the human rotaviruses [12]. The genotype P[6] has been described in association with asymptomatic infections in neonates [13]. A high incidence of P[6] strains among infants with diarrhea also has been reported [14].

The most frequent combinations of [P] and G types, found mainly in children with diarrhea, are P[8] and G1, P[4] and G2, P[8] and G3, and P[8] and G4 [13]. In this study, the most prevalent combination of [P] and G genotypes found was P[8] and G1, whereas the combination P[4] and G2 was not found (Table 1). Combinations such as P[6] and G1, P[6] and G9, P[8] and G2, and P[6] and G2 were also observed in this study; such combinations have been reported previously [12, 15].

Table 1 Combinations of G and [P] types in 120 fecal samples obtained from children in Goiânia, Brazil, 1998–2000

G types	No. (%) of isolates			
	P types		Not P typable	Total
P[8]	P[6]			
G1	25 (20.8)	13 (10.8)	54 (45.0)	92 (76.6)
G2	2 (1.7)	1 (0.8)	3 (2.5)	6 (5.0)
G3	–	–	1 (0.8)	1 (0.8)
G9	–	5 (4.2)	1 (0.8)	6 (5.0)
Not G typable	6 (5.0)	1 (0.8)	8 (6.7)	15 (12.5)
Total	33 (27.5)	20 (16.7)	67 (55.8)	120 (100.0)

According to Iturriza-Gómara et al. [15], P[8] plus G2 is considered an unusual combination of common G and [P] types, P[6] plus G1 is considered a combination of common G types with uncommon [P] types, and P[6] plus G9 is a combination of uncommon G and P types. The authors reported that uncommon associations observed in their study could be related to reassortment between cocirculating rotavirus strains. In light of this, we believe that further studies aimed at obtaining the partial sequences of the P[6] genes of samples G9 and G1 would be an important step to gain further knowledge about the identity of these rotavirus strains.

The data from this study reflect the importance of continual monitoring of rotavirus strains circulating throughout the world due to variations in the occurrence of [P] and G serotypes/genotypes and also to the increasing number of rotavirus strains that were, until recently, considered unusual but that now cause symptomatic gut infections in children from countries such as Brazil.

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