ARTIGO/ARTICLE

G and P rotavirus genotypes in stool samples from children in Teresina, State of Piauí

Genótipos G e P de rotavírus em amostras fecais de crianças de Teresina, Estado do Piauí

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ABSTRACT

A total of 123 stool specimens collected in Teresina, Piauí between 1994 and 1996, from 0 to 2-year-old children with diarrhea, were used for this study. Molecular characterization of the G and P rotavirus genotypes was performed using the reverse transcriptase polymerase chain reaction. The following results were obtained for the P genotypes: P[8] (17.1%), P[1] (4.9%), P[4] (3.3%), P[6, M37] (2.4%) and mixtures (27.6%). The P[1]+P[8] mixture was found in 19.5% of the samples. For the G genotypes, the results were: G1 (25.2%), G5 (13.8%), G2 (2.5%), G4 (2.5%), G9 (0.8%) and mixtures (41.5%). G1+G5 was the mixture most frequently found (12.1%). Our results showed unusual combinations such as P[1]G5 and P[1]+P[8]G5. The high percentage of mixtures and unusual combinations containing mixtures of human and animal rotavirus genotypes strongly suggests the possibility of gene reassortment and interspecies transmission.

Key-words: *Rotavirus. RT-PCR. G genotypes. P genotypes.*

RESUMO

Um total de 123 amostras fecais de crianças de 0 a 2 anos com diarréia, coletadas em Teresina, Piauí, entre 1994 e 1996 foi utilizada neste estudo. Para a caracterização molecular dos genótipos G e P de rotavírus, foram realizadas as reações de transcriptase reversa e reação em cadeia pela polimerase. Os seguintes resultados foram obtidos para o genótipo P: P[8] (17,1%), P[1] (4,9%), P[4] (3,3%), P[6, M37] (2,4%) e misturas (27,6%). A mistura P[1]+P[8] foi encontrada em 19,5% das amostras. Para o genótipo G os resultados foram: G1 (25,2%), G5 (13,8%), G2 (2,5%), G4 (2,5%), G9 (0,8%) e misturas (41,5%). A mistura G1+G5 foi a mais freqüentemente encontrada (12,1%). Nossos resultados mostram combinações não usuais como P[1]G5 e P[1]+P[8]G5. A alta porcentagem de misturas e as combinações não usuais contendo misturas de genótipos de rotavirus humanos e animais sugerem fortemente a possibilidade de rearranjo genético e transmissão interspecies.

Palavras-chaves: *Rotavírus. RT-PCR. Genótipos G. Genótipos P.*

Rotaviruses are a major cause of acute diarrhea in both humans and animals[2 3 17 26 33]. Worldwide, rotaviruses account for more than 125 million cases of child gastroenteritis and nearly one million deaths per year, mainly in developing countries[24].

Rotaviruses are classified in the family *Reoviridae,* genus *Rotavirus*. The *Rotavirus* genus has seven species (*Rotavirus A* to *G*). The mature infectious virion has an overall diameter of approximately 100nm and is made up of three concentric protein layers. The genome is packaged within the innermost protein shell of the particle, or core. The rotavirus genome consists of 11 segments of double-stranded RNA (dsRNA) that encode six structural and six nonstructural proteins[22 38].

Group A rotaviruses have two outer capsid proteins (VP7 and VP4), which are considered independent neutralization antigens and are encoded by different genomic RNA segments. The VP7 serotype is named the G serotype because VP7 is a glycoprotein[9]. Fifteen G serotypes have been recognized to date, and these correlate with all known G genotypes that have been determined by sequence analysis of the VP7 gene[17 30]. The serotype specificity of VP4 is designated by the prefix P because VP4 is protease

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Recebido em: 26/04/07
Aceito em: 12/07/07

sensitive. To date, 15 P serotypes and 27 P genotypes have been described[17 25 32].

G serotypes 1, 2, 3 and 4 are the most commonly detected worldwide[5 7 8 21 27 33] and were the target of a tetravalent rhesus rotavirus vaccine (RRV) that was used in the United States between August 1998 and July 1999, i.e. until it was withdrawn[4]. However, different G-genotype strains, such as G5 and G9, have been found in Brazil[1 11 33 34] and other countries[5 6 22 29 37].

Genotype characterization of rotavirus strains is important for defining the extent of the diversity in strains circulating in different locations[30].

The objective of the present study was to characterize rotavirus strains circulating among children in Teresina, Piauí, in order to determine for the first time the extent of the diversity of this virus in northeastern Brazil.

MATERIAL AND METHODS

A total of 123 stool specimens collected in a public hospital in Teresina, Piauí, Brazil, between May 1994 and June 1996, from 0 to 2-year-old children with diarrhea, was used for this study. All the samples had previously been tested at the Federal University of Piauí by enzyme immunoassay (EIARA-FIOCRUZ)[29] and were found to be positive for rotavirus.

The samples were frozen and stored at -20°C at the Federal University of Piauí and then shipped frozen on dry ice to the Virology Laboratory in the Department of Microbiology, Institute of Biomedical Sciences, University of São Paulo (ICB/USP) in São Paulo, Brazil, where they were kept at -20°C until used.

Reference samples of rotavirus strains RV-A/Wa, RV-A/DS1, SiRV-A/SA11, RV-A/ST3, PoRV-A/OSU, BoRV-A/NCDV, BoRV-A/UK, BoRV-A/B223, PoRV-A/YM and RV-A/RV4 were kindly provided by Dr. David Snodgrass, Moredun Research Institute, Edinburgh, Scotland, and Dr. Enzo Palombo, World Health Organization Collaborating Centre for Research on Human Rotaviruses, Royal Children's Hospital, Melbourne, Australia, and were cultivated in MA104 cells[31].

Fecal suspensions (20% w/v) were prepared in Tris/calcium buffer (Tris/HCl 0.1M; $CaCl_2$ 1.5mM; pH 7.3). Suspensions were kept for 30 minutes at room temperature with periodic vortex mixing and then centrifuged (Eppendorf, Model 5415-C) for 15 min at 6,000xg. Rotavirus dsRNA was extracted from supernatants using TRIZOL® reagent (Gibco BRL 15,596), in accordance with the technique described by the manufacturer. The RNA suspensions were stored at -20°C until used. The G genotypes were determined by seminested multiplex reverse-transcription polymerase chain reaction (RT-PCR), as described by Gouvea et al[11 13 14] and Das et al[7], and the P genotypes was determined in accordance with Gentsch et al[8] and Gouvea et al[15]. RT-PCR reactions were performed with viral RNA extracted from reference samples as positive controls and water as the negative control. For the first amplification, each extracted dsRNA was used as a template for reverse transcription followed by first-round PCR amplification of the full length VP7 gene (G types) with primers corresponding to highly conserved portions of the 5' and 3' ends of the VP7 genes. PCR products from the first amplification were used as templates for the second amplification round, with a pool of specific VP7 primers. The scheme described above was intended to detect VP7 genotypes G1, G2, G3, G4, G5, G6, G8, G9, G10 and G11. The first round of P genotyping was performed by amplification of the VP8* portion of the VP4 gene, and the second round of amplification was performed with a pool of specific primers for the following P genotypes: P[1], P[4], P[5], P[6], P[7], P[8], P[9], P[10] and P[11].

The samples were then resolved on 1.5% agarose gels to determine the G and P types. The RNA extraction, first amplification, second amplification and gel analysis were performed in four separate rooms to avoid cross-contamination of samples.

RESULTS

Rotaviruses from 108 (87.8%) fecal specimens were typed by RT-PCR for at least one of the G (VP7) or P (VP4) genotypes. The most prevalent G genotype was G1, which was found in 31 (25.2%) samples, followed by G5 (2 samples, 13.8%) and the G1+G5 mixture (15 samples, 12.1%). G2, G4 and G9 were also found. Mixtures of G genotypes were found in 51 (41.5%) samples (Table 1), and the G5 and G1 genotypes were also the most prevalent genotypes in these mixtures (Table 2).

The P[1]+P[8] mixture was the most (19.5%) prevalent P genotype followed by the P[8] genotype (21 samples, 17.1%). P[1], P[4] and P[6, M37] genotypes were also identified. Mixtures of human and animal P genotypes were found in

Table 1 - G and P genotypes of rotavirus strains from children with diarrhea in Teresina, Piauí.

	P genotype						
G genotype	P[1]	P[4]	P[6, M37]	P[8]	mixtures	negative	total
G1	1	2		6	8	14	31
G2		1				1	2
G4			1			2	3
G5	4			2	3	8	17
G9						1	1
Mixtures	1	1	2	12	21	14	51
Negative				1	2	15	18
Total	6	4	3	21	34	55	123

Table 2 - Percentages of single G genotypes and their occurrence in mixtures in rotavirus samples from Teresina, Piauí.

	Single*		Mixture**		Total	
Genotype	nº	%	nº	%	nº	%
G1	31	25.2	37	30.1	68	55.3
G2	2	1.6	21	17.1	23	18.7
G3			6	4.9	6	4.9
G4	3	2.5	15	12.1	18	14.6
G5	17	13.8	45	36.6	62	50.4
G9	1	0.8	8	6.5	9	7.3
G10			3	2.5	3	2.5
G11			1	0.8	1	0.8

* as a single genotype

** genotypes in mixtures

34 (27.6%) samples (Table 3). The percentage of each P genotype, either as a single genotype or in mixtures, is shown in Table 2. An unexpectedly high (44.7%) proportion of the samples could not be typed for P genotypes.

Table 3 - Percentages of single P genotypes and their occurrence in mixtures in rotavirus samples from Teresina, Piauí.

Genotype	Single*		Mixture**		Total	
	n	%	n	%	n	%
P[1]	6	4.9	28	22.8	34	27.7
P[4]	4	3.3	2	1.6	6	4.9
P[6. M37]	3	2.4	8	6.5	11	8.9
P[6. Gott]			3	2.4	3	2.4
P[8]	21	17.1	31	25.2	52	42.3
P[10]	6	4.9	1	0.8	1	0.8

* as a single genotype

** genotypes in mixtures

Common human G and P combinations such as the six P[8]G1 samples (4.8%) and one P[4]G2 sample (0.8%) were observed. However, unusual combinations such as P[1]G1 (0.8%) and P[1]G5 (3.3%) were also found. A variety of combinations of P and/or G mixtures were observed (69.1% of the samples). The overall results from RT-PCR genotyping are summarized in Table 1.

DISCUSSION

The samples used in this study were collected from Teresina, in the State of Piauí in northeastern Brazil. The City of Teresina covers an area of 1,775km^2 [20] and has a population of 655,473 people[18]. In the State of Piauí, 76.7% of the children from 0 to 6 years of age live in homes with inadequate sewerage systems[19].

G1, G2 and G4 rotavirus genotypes are the most predominant genotypes worldwide[9,27] and all of them were detected in this study. The G3 genotype was only found as mixtures in six samples. The G1 genotype, which is considered the most prevalent genotype worldwide and has caused diarrhea among children throughout the world over the last two decades[12,16], was also the most prevalent in this study. This genotype has frequently been associated with the P[8] genotype. In our study, P[8] was found in 17.1% of the samples, but the P[8]G1 genotype was found in only 4.8%. Unusual combinations of G1 with human and animal P genotypes, such as P[1]G1, P[1]+P[8]G1 and P[1][6, M37][8] were found.

G5 was the second most prevalent G genotype (13.8%) and was found in the majority of mixtures. G5 strains are common human pathogens in Brazil, usually in combination with human P[8] specificity[10,34]. Our results show unusual combinations such as P[1]G5 and P[1]+P[8]G5, in addition to the P[8]G5 combination (1.6%).

The unusual combinations described above and other combinations such as P[1]+P[6,M37]+P[6, Gott]G1+G4 and P[1]+P[8]G4+G5, which contain mixtures of human and animal rotavirus genotypes, strongly suggest the possibility of gene reassortment and interspecies transmission.

Mixtures were found in 41.5% of the G genotypes and in 27.6% of the P genotypes. High percentages of mixtures have been found in other studies. In a study in São Paulo, the state with the highest level of development in Brazil, Timenetsky et al[36], found mixtures of G and/or P genotypes in 29% of their samples. Mascarenhas et al[26], studying samples from Belém, in the state of Pará, found that 41% of the P genotypes were mixtures. In a study by Leite et al[23], samples from nine states in southern, southeastern, northeastern and central Brazil were genotyped, and 21% of the genotypes were found to be mixtures.

Our findings in samples from Teresina confirm that the occurrence of mixed infections is characteristic of rotaviruses in Brazil. Furthermore, the existence of unusual genotype diversity and complexity among the strains recovered from Brazil has important implications for rotavirus vaccination strategies.

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