Rotavirus Gastroenteritis in Children in 4 Regions in Brazil: A Hospital-Based Surveillance Study

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Background. Rotavirus is a major cause of gastroenteritis in children. Knowledge of rotavirus genotypes is important for vaccination strategies.

Methods. During 2005–2006, rotavirus surveillance studies were conducted in São Paulo, Salvador, Goiânia, and Porto Alegre, Brazil. Stool samples were collected from children <5 years of age who had diarrhea and were screened by the Rotaclone Enzyme Immunoassay for the presence of rotavirus. Confirmed rotavirus-positive samples were characterized for P and G genotypes by reverse-transcriptase polymerase chain reaction.

Results. A total of 510 stool samples were collected. Of these, 221 (43.3%) were positive for rotavirus. Overall, G9 was the predominant G type, followed by G2, and G1; P[4] and P[8] were the predominant P types. The most frequent G/P genotype combination detected was G2P[4], followed by G9P[8], G9P[4], and G1P[8]. G2P[4] was the predominant type in Goiânia and Salvador; G9P[8] and G1P[8] were predominant in São Paulo and Porto Alegre, respectively.

Conclusions. The prevalence, seasonality, and genotype distribution of rotavirus infection varied in different regions in Brazil. With immunization programs, continuous monitoring of rotavirus types is important to detect novel and emerging strains.

Rotavirus is the leading cause of severe diarrheal illness in infants and young children worldwide. It is a very important contributor to childhood morbidity in developed countries and to childhood morbidity and mortality in developing countries [1].

The rotaviruses comprise the genus *Rotavirus* within the family Reoviridae [1]. Rotavirus has a nonenveloped particle, with a double layer capsid and a core, which contains the viral double-stranded RNA genome. The inner capsid contains the VP6 protein, which is the basis for classification of rotaviruses into 7 serogroups (A-G). Groups A–C are human pathogens, and group A viruses have been most commonly associated with childhood infections. The outermost layer is composed of surface proteins VP7, which defines G serotypes and genotypes, and VP4, which defines P serotypes and genotypes.

At least 15 G and 27 P genotypes of group A rotaviruses have been described [1, 2]. Among them, 5 G serotypes (G1–G4 and G9) constitute >90% of G se-

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rotypes detected globally [3]. Since the 1990s, G9 infections have become more prevalent, and in Brazil, this genotype has been described since 1994 [4]. The geographic and seasonal distributions of rotavirus serotypes have been unpredictable, because predominant serotypes vary from year to year and from region to region in the same country.

Recently, a live attenuated vaccine containing G1P1A[8] (Rotarix; GlaxoSmithKline) and a recombinant vaccine containing 5 human rotavirus serotypes (G1, G2, G3, G4, and P1A[8]; RotaTeq; Merck) have been available in different countries [5–7]. Vaccine-induced cross-immunity against other rotavirus types (eg, G9) has been observed, but the efficacy against other types needs to be evaluated [5]. Vaccination may be associated with replacement events that favor currently minor rotavirus types and emerging rotavirus variants whose serotypes are not represented in the vaccines [8]. Brazil was the first country to introduce the monovalent live attenuated human rotavirus vaccine for use in the public health vaccination network (on 8 March 2006). To date, the pentavalent recombinant vaccine is not available for use in Brazil.

For rotavirus diarrhea control to be successful through vaccination, rotavirus surveillance, including continuous monitoring of the rotavirus types in a region before, during, and after the introduction of a vaccine campaign, is needed to detect uncommon and novel types that might help to explain the impact that vaccines have on the overall reduction in acute gastroenteritis health care use. The objective of this study was to characterize rotavirus genotypes detected over a period of 1 year (August 2005–August 2006) among children <5 years of age who had diarrhea, were hospitalized, and/or required rehydration therapy equivalent to World Health Organization (WHO) plan B or C in 4 different Brazilian cities. Data resulting from this study would contribute toward epidemiological investigation by characterizing the rotavirus genotypes detected in different regions during the same period, reflecting the presence of the virus genotypes in the community.

MATERIALS AND METHODS

Specimen collection. Stool specimens were obtained from August 2005 through August 2006 from children <5 years of age who had acute diarrhea and lived in 4 different cities in 4 distinct regions in Brazil (Figure 1): Goiânia (Goiás State, Midwest region; ~1.2 million inhabitants), Porto Alegre (Rio Grande do Sul State, South region; ~1.4 million inhabitants), Salvador (Bahia State, Northeast region; ~2.9 million inhabitants), and São Paulo (São Paulo State, Southeast region; ~10.9 million inhabitants). Stool samples were collected from hospitalized children with acute gastroenteritis and/or from children who required rehydration therapy equivalent to WHO plan B (oral rehydration therapy) or plan C (intravenous rehydration therapy) [9]. After collection, samples from São Paulo and Porto Alegre were shipped frozen on dry ice to the Virology Laboratory, Department of Microbiology, Institute of Biomedical Sciences, University of São Paulo (São Paulo), and samples from Goiânia and Salvador were shipped to the Virology Laboratory, Institute of Tropical Pathology and Public Health, Federal University of Goiânia (Goiânia), where the fecal suspensions were prepared and analyzed. Patient data collected included identification, age, date of birth, sex, residence or origin, date of sample collection, date of diarrhea onset, mean number of stools per day, mean number of vomiting episodes per day, and axillary temperature. After vaccine introduction in Brazil in March 2006, information about vaccination was included in the protocol. The study protocol and informed-consent document were approved by ethics review committees at each hospital and at the 2 laboratories involved in the research. The study was conducted in accordance with all Brazilian human experimentation guidelines [10].

Figure 1. Location and climate of cities in Brazil where samples were collected.

Virus strains. Reference rotavirus strains HuRv-A/RV4 (G1P1A[8]), HuRv-A/DS-1 (G2P1B[4]), SiRV-A/SA11 (G3P5B[2]), HuRv-A/ST3 (G4P2A[6]), and HuRv/F45 (G9P1A[8]) were kindly provided by David Snodgrass (Moredun Research Institute; Edinburgh, Scotland) and Enzo Palombo (WHO Collaborating Centre for Research on Human Rotaviruses, Royal Children's Hospital, Melbourne, Australia) and were cultivated in MA104 cells [11].

Preparation of fecal suspensions. Fecal suspensions (20% weight per volume) were prepared in Tris-calcium buffer (0.1 mol/L Tris–hydrochloric acid and 1.5 mmol/L calcium chloride; pH, 7.3). Suspensions were kept for 30 min at room temperature, with periodic vortexing, and were then centrifuged (Eppendorf model 5417-C) for 15 min at 5900 *g*. Supernatants were stored at 20°C.

Table 1. Distribution of Rotavirus Infection by Age Group among Children with Gastroenteritis in Goiânia, Porto Alegre, Salvador, and São Paulo, Brazil, 2005–2006

	No. of rotavirus-positive samples/no. of total samples (%)			
Age group, years	Goiânia	Porto Alegre	Salvador	São Paulo
0–6	2/17 (11.8)	1/7 (14.3)	4/8 (50.0)	11/23 (47.8)
7–12	9/25 (36.0)	5/24 (20.8)	6/27 (22.2)	54/80 (67.5)
13–24	24/40 (60.0)	3/17 (17.6)	16/34 (47.1)	34/66 (51.5)
>24	6/16 (37.5)	3/8 (37.5)	30/78 (38.5)	13/40 (32.5)
All	41/98 (41.8)	12/56 (21.4)	56/147 (38.1)	112/209 (53.6)

NOTE. For Goiânia, $P = .006$; for Porto Alegre, $P = .712$; for Salvador, $P = .197$; and for São Paulo, $P = .003$ (by Fisher's exact test of association between age group and rotavirus positivity).

Enzyme immunoassay. Fecal suspensions were screened by the enzyme immunosorbent assay kit Premier Rotaclone Enzyme Immunoassay (Meridian Bioscience) for the detection of rotavirus antigen in human fecal samples, according to the manufacturer's protocol.

Reverse-transcriptase polymerase chain reaction (RT-PCR) genotyping. All rotavirus group A–positive fecal samples were genotyped for VP7 (G) and VP4 (P) by semi-nested multiplex RT-PCR, as described by Das et al [12] and Gentsch et al [13]. This combined typing scheme was designed to detect VP7 genotypes G1, G2, G3, G4, and G9. For VP4 genotyping, second amplification was done with specific primers to VP4 genotypes P[4], P[6], P[8], P[9], and P[10]. Negative samples were tested for animal G and P genotypes [14, 15].

All mixtures of genotypes identified with the pool of primers were tested with individual primers for the identified genotypes. All stages were performed in 4 separate rooms to avoid cross-contamination of samples, and RT-PCR was performed with viral RNA extracted from reference strains as positive controls and with water as a negative control [16].

Statistical analysis. The prevalence of both rotavirus infection and genotypes observed in the 4 cities was calculated. The association between age group and rotavirus positivity and the presence of rotavirus and specific genotypes were compared among cities by using Fisher's exact test [17]. Statistical significance was assessed at a 2-sided P value of .05.

RESULTS

A total of 510 stool samples were collected. Of these, 221 (43.3%) were positive for rotavirus. The percentage of positive samples varied among the 4 cities: Goiânia (41.8%; 41 of 98 samples), Porto Alegre (21.4%; 12 of 56), Salvador (38.1%; 56 of 147), and São Paulo (53.6%; 112 of 209).

Distribution of rotavirus infection by age group revealed differences across cities. In Goiânia and São Paulo, the association between age group and rotavirus positivity was statistically significant. In Goiânia, the highest percentage of positive samples was found among children aged 13–24 months, and in São Paulo, the highest percentage was found among children

Table 2. Overall Results of Reverse-Transcriptase Polymerase Chain Reaction G and P Genotyping of Rotavirus Strains Circulating in Goiânia, Porto Alegre, Salvador, and São Paulo, Brazil, August 2005–August 2006

	No. (%) of strains					
Genotype[a]	Goiânia (n = 41)	Porto Alegre (n = 12)	Salvador (n = 56)	São Paulo (n = 112)	All (n = 221)	P[b]
G1	13 (31.7)	9 (75.0)	5 (8.9)	7 (6.3)	34 (15.4)	<.001
G2	28 (68.3)	1 (8.3)	33 (58.9)	15 (13.4)	77 (34.8)	<.001
G9	1 (2.4)	2 (16.7)	18 (32.1)	93 (83.0)	114 (51.6)	<.001
G not typed	…	…	8 (14.3)	2 (1.8)	10 (4.5)	.003
P[4]	26 (63.4)	2 (16.7)	30 (53.6)	39 (34.8)	97 (43.9)	.001
P[6]	…	…	…	1 (0.9)	1 (0.5)	>.99
P[8]	15 (36.6)	12 (100.0)	19 (33.9)	51 (45.5)	97 (43.9)	<.001
P[9]	7 (17.1)	…	1 (1.8)	1 (0.9)	9 (4.1)	<.001
P not typed	…	…	7 (12.5)	21 (18.8)	28 (12.7)	.004

[a] Each genotype was counted separately in samples containing mixtures of types.
[b] By using Fisher's exact test.

Table 3. Rotavirus G and P Combinations in Goiânia, Porto Alegre, Salvador, and São Paulo, Brazil, 2005–2006

	No. of samples with P genotype							
City, G genotype	P[4]	P[6]	P[8]	P[9]	P[4] + P[8]	P[8] + P[9]	P not typed	Total
Goiânia								
G1	…	…	5	2	…	5	…	12
G2	24	…	1	…	2	…	…	27
G1 + G2	…	…	1	…	…	…	…	1
G9	…	…	1	…	…	…	…	1
Total	24	…	8	2	2	5	…	41
Porto Alegre								
G1	…	…	9	…	0	…	…	9
G2	…	…	1	…	0	…	…	1
G9	…	…	…	…	2	…	…	2
Total	…	…	10	…	2	…	…	12
Salvador								
G2	22	…	4	…	1	…	1	28
G9	3	…	8	…	…	…	1	12
G1 + G2	1	…	…	1	…	…	…	2
G1 + G9	…	…	3	…	…	…	…	3
G2 + G9	2	…	…	…	…	…	1	3
G not typed	1	…	3	…	…	…	4	8
Total	29	…	18	1	1	…	7	56
São Paulo								
G1	1	…	5	…	…	…	1	7
G2	8	…	…	…	…	…	2	10
G9	25	1	44	1	1	…	16	88
G2 + G9	4	…	…	…	…	…	1	5
G not typed	…	…	1	…	…	…	1	2
Total	38	1	50	1	1	…	21	112

aged 7–12 months (Table 1). In Salvador and Porto Alegre, this difference was not statistically significant; therefore, no conclusion could be made about the prevalence of rotavirus infection by age group.

Seasonal distribution of rotavirus infection was also different. In São Paulo, rotavirus infections occurred from April through August, the coolest months of the year, and in Porto Alegre, infections occurred from May through October. In Goiânia, rotavirus infections were detected mainly from May through September, with few positive samples found during December and January. In Salvador, infections were detected during almost all months of the year (Figure 1).

Among the 221 rotavirus strains, G9 was the predominant G genotype (114 [51.6%]), followed by genotypes G2 (77 [34.8%]) and G1 (34 [15.4%]). Among P strains, genotypes P[4] and P[8] were found at the same frequency (43.9%; 97 each) and were the predominant types (Table 2).

With the exception of P[6], frequencies of G and P genotypes were different in each city (Table 2). To examine the rotavirus genotype distribution, mixed infections were considered as independent, with each genotype counted separately. Genotype distribution varied among the cities, and genotype combinations were not uniformly spread across strains from different regions.

We were able to determine both G and P combinations in 188 rotavirus strains (Tables 3 and 4). The most frequent G/P genotype combination detected was G2P[4] (28.7%), followed by G9P[8] (28.2%), G9P[4] (14.9%), and G1P[8] (10.1%). Viruses with G3 or G4 specificity were not detected in any of the cities. Mixtures of G and P genotypes were found in 23 (12.2%) of the 188 fully characterized strains (Table 3). By region, G2P[4] was the predominant combination in strains identified in Goiânia (24 [58.5%] of 41 strains) and Salvador (22 [48.9%] of 45), whereas G9P[8] was the predominant combination in São Paulo (44 [48.9%] of 90) and G1P[8] was the predominant combination in Porto Alegre (9 [75.0%] of 12) (Table 4).

Twenty-one children had previously been vaccinated with the attenuated monovalent G1P1A[8] vaccine: 3 in Porto Alegre, 16 in São Paulo, and 2 in Goiânia. Of the samples from

Table 4. Frequency of Rotavirus G and P Combinations in Goiânia, Porto Alegre, Salvador, and São Paulo, Brazil, 2005–2006

G and P combination	No. (%) of samples				
	Goiânia	Porto Alegre	Salvador	São Paulo	Total
G2 P[4]	24 (58.5)	…	22 (48.9)	8 (8.9)	54 (28.7)
G9 P[8]	1 (2.4)	…	8 (17.8)	44 (48.9)	53 (28.2)
G9 P[4]	…	…	3 (6.7)	25 (27.8)	28 (14.9)
G1 P[8]	5 (12.2)	9 (75.0)	…	5 (5.6)	19 (10.1)
G2 P[8]	1 (2.4)	1 (8.3)	4 (8.9)	…	6 (3.2)
G2 + G9 P[4]	…	…	2 (4.4)	4 (4.4)	6 (3.2)
G1 P[8] + P[9]	5 (12.2)	…	…	…	5 (2.7)
G1 + G9 P[8]	…	…	3 (6.7)	…	3 (1.6)
G2 P[4] + P[8]	2 (4.9)	…	1 (2.2)	…	3 (1.6)
G9 P[4] + P[8]	…	2 (16.7)	…	1 (1.1)	3 (1.6)
G1P[9]	2 (4.9)	…	…	…	2 (1.1)
G1 + G2 P[4]	…	…	1 (2.2)	…	1 (0.5)
G1 + G2 P[8]	1 (2.4)	…	…	…	1 (0.5)
G1 + G2 P[9]	…	…	1 (2.2)	…	1 (0.5)
G1 P[4]	…	…	…	1 (1.1)	1 (0.5)
G9 P[6]	…	…	…	1 (1.1)	1 (0.5)
G9 P[9]	…	…	…	1 (1.1)	1 (0.5)
Total	41 (100)	12 (100)	45 (100)	90 (100)	188 (100)

São Paulo, 7 tested positive for rotavirus. Genotyping results for strains identified in samples from these children were 5 G9 strains, 4 P[8] strains, and 1 P[4] strain.

DISCUSSION

To our knowledge, this is the first study to report the prevalence of G and P genotypes of group A human rotavirus infecting young children in 4 different regions of Brazil (South, Southeast, Midwest, and Northeast) during the same period. Previous studies in Brazil revealed that the frequency of diarrhea associated with rotavirus among hospitalized children <5 years of age ranges from 12% to 46.9% [18–20]. In our study, rotavirus was confirmed in 43.3% of the samples analyzed, with marked differences across different cities. The highest prevalence of rotavirus-associated diarrhea was observed in the Southeast region (São Paulo, 53.6%), and the lowest prevalence was observed in the South region (Porto Alegre, 21.4%). These differences could be the result of the fact that our study was not population based, but was clinic and hospital based. Several potential selection biases, such as help-seeking behavior, access to health care, or differences in socioeconomic status of the families, might have occurred at the study sites and could have affected how representative the study population is of the overall population (although all 4 participating public hospitals are located in large capital cities). Samples were collected from patients without private health insurance and from low-income families.

The age distribution of rotavirus infection varied across cities. In Goiânia, where most of the children tested were <2 years of age (82 of 98 children), the highest percentage of positive samples was found among children aged 13–24 months; this finding differs from a previously published result [18], in which the percentage of rotavirus identification was almost the same among hospitalized children aged 0–6 months, 7–12 months, and 13–24 months. In São Paulo, most of the children tested were <2 years of age, and the frequency of rotavirus infection was highest among children aged 7–12 months. This result was also obtained using samples from the same hospital during 1994–1995 [4], at other locations in Brazil [21], and in other countries [22, 23]. In Salvador, the age distribution of children tested was different from that in the other cities, and most of the infected children (78 [53%] of 147 children) were >24 months of age; however, no conclusion could be made about the prevalence of rotavirus infection by age group.

In Brazil, there are different seasonal patterns of rotavirus infection, depending on the regional climate. Although 90% of the country is in the tropical zone, >60% of the population live in areas where altitude, sea winds, or cold polar fronts moderate the temperature. There are 5 climatic regions in Brazil: tropical, semi-arid tropical, humid coastal, humid equatorial, and humid subtropical (Figure 1). Plateau cities, such as São Paulo, have very mild climates (mean temperature, 19°C). Salvador, located on the coast, has a warmer climate. In southern Brazilian cities, such as Porto Alegre, the subtropical climate is similar to that in parts of the United States and Europe. In Goiânia, temperatures are almost constant, with a

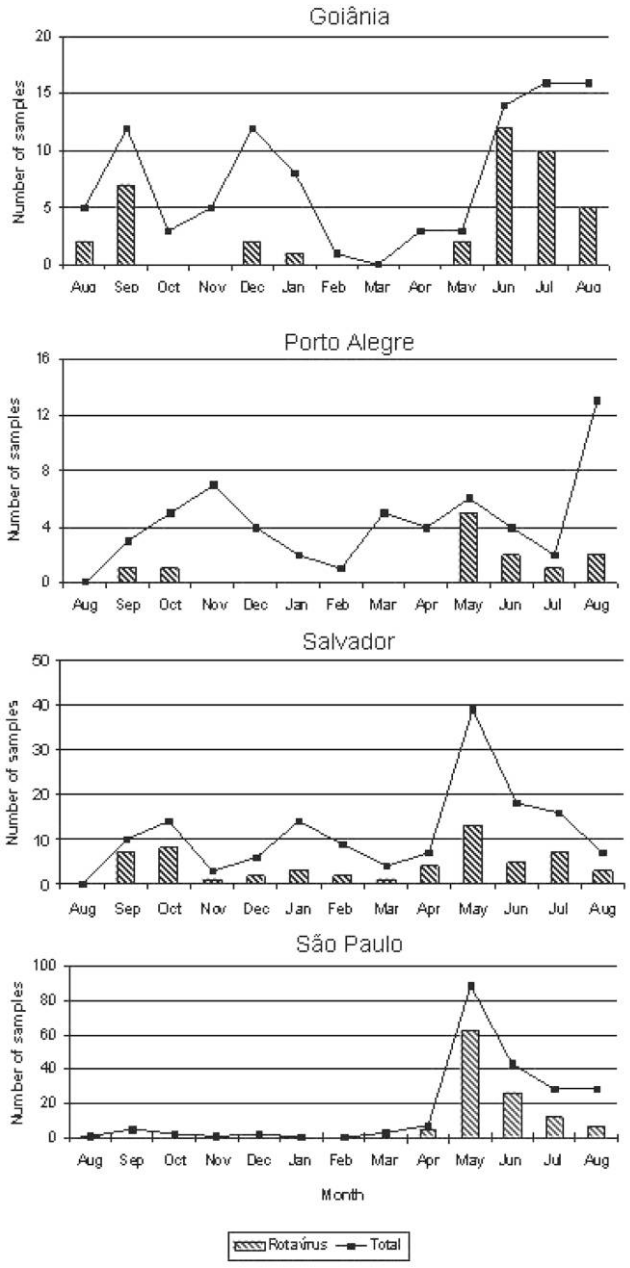

Figure 2. Total number of samples and rotavirus-positive samples, by month of collection, Brazil, 2005–2006.

wet season from October through April, and a dry season from May through September. In the North and Northeast regions, rotavirus infections generally occur during all months of the year. In the South, Southeast, and Midwest regions, a higher frequency of rotavirus infection is found during the dryer months, from May through September [4]. Our results confirm these differences. In Goiânia, Porto Alegre, and São Paulo, rotavirus infections occurred from May through September, the dryer months in these cities, with occasional positive samples found during other months. This was also observed in other South American countries with similar latitudes, such as Paraguay and Argentina [16, 22]. In Salvador, rotavirus was detected year-round (Figure 2), similar to other tropical regions in Brazil [21].

The genotype distribution varied across cities, and genotype combinations were not uniformly spread among strains from different regions. Our data for Goiânia indicated the presence of G2 in 27 (65.8%) of the 41 rotavirus-positive samples, G1 in 12 (29.3%) of 41, and G9 in only 1 (2.4%). The predominant G- and P-type combination was G2P[4] (in 24 [58.5%] of 41 samples). Molecular and serological characterization of group A rotavirus strains was previously performed for hospitalized children in Goiânia (1998–2000). The most prevalent G genotype and/or serotype detected then was G1 (76.7%), followed by G2 (5.0%). G9 was detected in 1.7% of the samples [24]. These results confirm that the circulation of the various rotavirus G genotypes is highly variable for the same region during different periods and that the frequency of genotype G2 in Goiânia is much higher now than it was 5 or 6 years ago.

In the present study, G2 was also the predominant strain in Salvador (58.9%), followed by G9 (32.1%). In a previous rotavirus surveillance study conducted among hospitalized children in the same city during the 1999, 2000, and 2002 seasons, G9 was reported as the predominant G genotype (85.2%); other strains detected belonged to genotypes G1 (7.1%) and G4 (1.2%) [20].

In a previous study of gastroenteritis in Porto Alegre during a 2-year period (July 1996–June 1998), rotavirus was identified as the causative agent in 42 (13.4%) of 312 patients during the first year and in 55 (18.9%) of 291 patients during the second year. G and P genotype diversity was not characterized [25]. Our study is the first to report the G and P characterization of rotavirus in Porto Alegre and revealed the lowest frequency of rotavirus detection (21.4%) across the 4 cities. G1P[8] was the most detected strain.

Our results reveal that genotype G9 accounted for 83.0% of the rotavirus strains in São Paulo, followed by genotypes G2 (13.4%) and G1 (6.3%). Temporal and geographical variations have been observed in the frequency of the G9 genotype in São Paulo. The first case of rotavirus G9 infection was described in São Paulo in 1994 [4]. The frequency of this genotype has varied since its emergence. An 8-year survey (1996–2003) for rotavirus infection in São Paulo revealed that G1 was the predominant serotype (68.2%), followed by G9 (17.2%), G4 (6.3%), G2 (1.2%), and G3 (1.2%). G9 was reported in 8.7% of the samples in 2001 and in 46.9% in 2002 [19]. In recent years, the G9 genotype has had increased importance in many countries worldwide, including the United States, Australia, Japan, France, Italy, and Ireland [3]. The high prevalence of G9 genotypes in São Paulo is similar to the one observed in Italy (83.9%) [26].

Worldwide, G1P[8], G2P[4], G3P[8], and G4P[8], the 4 predominant G/P rotavirus genotypes, account for >90% of all identified rotavirus strains [3]. In our study, G2P[4] was the most frequent G and P combination and was found mainly in Goiânia (58.5%) and Salvador (48.9%). Recent studies in 2 Brazilian state capitals reported G2P[4] strains predominating in the population after introduction of the monovalent vaccine [8, 27].

Of 188 fully characterized strains, 23 (12.2%) were shown to contain mixed genotypes (Table 4). In developing areas, such as Brazil, mixed rotavirus infections have been shown to occur in high frequency and could generate new rotavirus variants through reassortment [4]. In our results, G9P[4] was the third most frequent combination detected (14.9%), and such a rare strain may be the result of reassortment.

We have also detected 9 strains with P[9] specificity that were associated with G1, G9, or a mixture of G1 and G2 specificities. This genotype is commonly found in cats and, in some instances, in humans. Strains with P[9] specificity were reported in Rio de Janeiro, Brazil, in combination with G10 [28], G3 [30] or both G1 and G3 [29]. This is the first time that this P type has been reported in association with G9 in Brazil. This is also the first time that P[9] has been reported in Goiânia and Salvador. The relative frequency of strains with P[9] specificity represents <2.5% of the global frequency of rotavirus genotypes [31]; however, it was found in 9 (8.0%) of our 112 strains and could be considered to be an emerging P type in Brazil.

Because the prevalence of genotypes may vary significantly from region to region and from year to year, continuous monitoring of rotavirus genotypes is important to detect novel and uncommon strains after the introduction of immunization programs. Consistent with previously published studies, there was considerable geographic and seasonal variation in the distribution of rotavirus infections, reflecting the unpredictable nature of these infections. The results of the present study highlight the necessity for a rotavirus vaccine to provide effective protection against all the major genotypes.

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