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Survey of Rotavirus G and P Types Associated with Human Gastroenteritis in São Paulo, Brazil, from 1986 to 1992

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Rotavirus strains causing gastroenteritis in Brazilian children were characterized by PCR-based typing assays. In addition to strains bearing the major human G and P types, large numbers of strains bearing P3 (M37-like), P6 (HCR3-like), untypeable P and G types, and complex mixtures of P and G types not previously recognized were present in the community.

Group A rotaviruses are the leading cause of acute gastroenteritis in young children, and they are the subject of intense vaccine research (3). Results from vaccine trials have suggested the need for homotypic immunization for protection against rotavirus disease. Therefore, knowledge of the diversity and distribution of rotavirus serotypes circulating in a community is crucial to the formulation of an adequate vaccine and to the evaluation of homotypic and heterotypic protection after vaccination. Rotavirus serotypes are independently defined by the virus outer capsid proteins VP7 (G type) and VP4 (P type). Among the 14 known rotavirus G types, 7 were found in humans (4, 12). Types G1 to G4 are widespread and have frequently been associated with diarrhea in children, whereas types G8, G9, and G12 have rarely been associated with disease. Recently, the two important bovine serotypes, G6 and G10, were recovered in a few cases of diarrhea in children (8, 20). Among the 12 known P types, the following 6 were found in humans: P1 (Wa-like), P2 (DS1-like), P3 (M37-like), P4 (K8-like), P5 (69M-like), and P6 (HCR3-like) (4, 14). In addition, a P type similar to that of the bovine strain B223 was found in strains recovered from asymptomatic neonates in India (6).

In the present study, we analyzed rotavirus-positive stool specimens obtained from children, mostly under 4 years of age, with acute gastroenteritis in the most developed state of Brazil. The specimens were collected at day care centers and from ambulatory and hospitalized patients during a 7-year period. Specimens collected until mid-1991 were from patients living in the São Paulo metropolitan area. Later, additional centers located in 11 other cities in the state joined the survey, and these centers contributed the majority of the specimens collected in 1992. Fecal rotavirus detection was performed by enzyme immunoassay, RNA electropherotyping, and electron microscopy (19). The 139 samples determined to be positive for rotavirus by at least two of these assays were further characterized by the two-step PCR method previously described for G typing (10) and P typing (7, 14).

All specimens produced cDNA segments of the expected sizes for both VP7 and VP4 genes. Nevertheless, a G type could be identified in only 85 (61%) of the specimens and a P type could be identified in 116 (83%) of the specimens in the second amplification-typing assays (Table 1). Analysis of individual specimens demonstrated that only a third of them had a combination of one G type and one P type, thus representing a single rotavirus strain, whereas another third of the specimens had only a G type or a P type identified (Table 2). Seven (5%) additional specimens had neither a G type nor a P type identified.

The four major human G types were represented in this survey (G1, 17% of the specimens; G2, 6%; G3, 22%; G4, 5%), with an apparent shift in the dominant type from G1 in the early years to G3 after 1988. Predominance of G1 in the north Amazon region of Brazil also occurred, from 1984 to 1988 (1, 15). Local variations within the state of São Paulo were observed. An increased number of untypeable samples and an apparent emergence of G2 strains paralleled the broadening of the population study by mid-1991. Type G2 was present in sporadic cases of diarrhea in children in 1991, and it caused a large outbreak in a small city in 1992. Details of this outbreak will be reported elsewhere. G2 strains demonstrated the characteristic short pattern, whereas non-G2 strains were of the long electropherotype.

In this first study of P types in Brazil, an overall predominance of P1 (21%) was observed, consistent with its association with G1, G3, and G4 specificities (7, 17). Predominance of P1 has also been reported in other studies that analyzed the P types of rotaviruses causing diarrhea in children (2, 17, 18). Except in the case of one G1:P2 strain, P2 (8%) was associated with G2 strains, confirming the general correlation between these two specificities. On the other hand, we identified 16 cases of moderate to severe diarrhea associated with single infections with P3 strains. P3 has been primarily associated with asymptomatic neonatal infections in hospital nurseries around the world, and therefore, it has been regarded as a possible attenuation trait (5). In all cases, the presence of exclusively type P3 rotavirus in the patients' specimens was confirmed by the PCR confirmatory test, and in three cases it was also confirmed by partial sequence analysis of the virus VP4 gene (16). In addition to its presence in single infections, P3 was present in 23 mixed infections, indicating a widespread circulation of this P type (13% overall) in the community. Rare cases of P3-related diarrhea have been reported in other studies (2, 16, 18). However, the large number of P3 rotaviruses found in this study raises the possibility that P3 strains, although attenuated in newborns, might still be virulent in infants and young children. This question is relevant because the P3 strain M37 is presently being tested as a vaccine candidate (3).

The identification of a large number of infections with P6 strains (11%) constitutes a novelty. This new P type was

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TABLE 1. Temporal distribution of rotavirus G and P types in São Paulo, Brazil, from 1986 to 1992

Year	No. of samples belonging to type:												Total no. of samples
	G1	G2	G3	G4	MixG[a]	G?[b]	P1	P2	P3	P6	MixP[c]	P?[d]	
1986	2	0	0	2	0	4	4	0	3	0	0	1	8
1987	8	0	2	0	0	0	9	0	0	0	1	0	10
1988	2	0	6	1	1	2	5	0	0	2	3	2	12
1989	0	0	2	0	0	3	2	0	0	3	0	0	5
1990	4	0	5	3	2	7	3	0	6	5	5	2	21
1991	3	3	9	0	8	4	0	5	6	1	9	6	27
1992	4	5	7	1	5	34	12	6	3	5	18	12	56
Total	23	8	31	7	16	54	35	11	18	16	36	23	139

[a] MixG, mixture of distinct G types.
[b] G?, G untypeable. Amplified in the reverse transcription PCR assay for the VP7 gene but not in the PCR G-typing assay.
[c] MixP, mixture of distinct P types.
[d] P?, P untypeable.

TABLE 3. Distribution of specimens containing multiple rotavirus strains recovered from patients with diarrhea in São Paulo, Brazil, from 1986 to 1992

Genotype(s)	No. of specimens							
	G1 + G3	G2 + G3	G1 + G2	G1	G2	G3	G?[a]	Total
P1 + P3	3	0	0	0	0	0	6	9
P3 + P6	4	0	0	0	0	2	0	6
P2 + P3	0	1	0	0	0	1	0	2
P2 + P6	0	0	0	0	1	0	1	2
P1 + P6	2	0	0	1	0	1	8	12
P1 + P2 + P3	1	0	0	0	0	0	0	1
P1 + P3 + P6	1	0	0	0	0	1	1	3
P1 + P2 + P6	0	0	0	0	0	0	1	1
P2	0	0	2	0	0	0	0	2
P3	2	0	0	0	0	0	0	2
Total	13	1	2	1	1	5	17	40

[a] G?, G untypeable.

recently identified in HCR3, a strain isolated from a healthy child in Philadelphia (14), and it was later found in the canine strains K9 and A79-10 and the feline strain Cat97 (13). In the present study, we found that rotavirus strains bearing the P6 type can cause diarrhea in children and that they may be widespread in the human population.

A significant proportion (29%) of the specimens had multiple G and/or P types, which is consistent with infections with more than one rotavirus strain (Table 3). The presence of multiple P types in the same specimen was confirmed for all of the specimens by the independent, one-step PCR confirmatory test (7), and in the case of six specimens it was also confirmed by RNA electropherotyping. One potential consequence of the presence of multiple G and/or P types in specimens is a greater than previously suspected chance for reassortment during natural infections.

The finding of a large number of specimens that contained untypeable rotaviruses was rather unexpected. Unlike the inability to type by immunological techniques, the inability to type by PCR typing assay after a successful reverse transcription PCR amplification may only be attributed to significant nucleic acid sequence variations. Although such variations could occur within a given type, they most likely represent a different type. Extended PCR typing assays that identify G and P types commonly found in bovine and porcine strains have been developed (12, 13), and they are currently being applied to those samples that could not be typed by the human PCR typing assays. Initial studies have revealed that about half of these specimens contained rotaviruses bearing G5, a type previously found only in strains recovered from pigs and horses (9).

Although analysis of the untypeable specimens continues, the present study has already demonstrated the existence of unusual genotypic diversity and complexity among the strains recovered from Brazil. In addition to the high proportions of strains bearing P3, P6, and other P and G types distinct from the conventional human types, complex combinations of multiple G and P types not previously described are reported here in association with human gastroenteritis. This situation is in contrast to that found in the United States, where almost all human rotavirus strains were of the conventional G and P types (11, 17). These findings have obvious implications for the development of rotavirus vaccines and immunization programs aimed at providing comprehensive protection against rotavirus disease.

TABLE 2. Distribution of specimens containing single rotavirus strains recovered from patients with diarrhea in São Paulo, Brazil, from 1986 to 1992

Genotype	No. of specimens					
	G1	G2	G3	G4	Total G typeable	G untypeable
P1	11	0	8	5	24	11
P2	1	6	0	0	7	2
P3	1	0	5	1	7	9
P6	3	0	5	0	8	8
Total P typeable	16	6	18	6	46	30
P untypeable	6	1	8	1	16	

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REFERENCES

1. **Beards, G. M., U. Desselberger, and T. H. Flewett.** 1989. Temporal and geographical distributions of human rotavirus serotypes, 1983 to 1988. J. Clin. Microbiol. **27**:2827–2833.
2. **Bern, C., L. Unicomb, J. R. Gentsch, N. Banul, M. Yunus, R. B. Sack, and R. I. Glass.** 1992. Rotavirus diarrhea in Bangladeshi children: correlation of disease severity with serotypes. J. Clin. Microbiol. **30**:3234–3238.
3. **Bishop, R. F.** 1993. Development of candidate rotavirus vaccines. Vaccine **11**:247–254.
4. **Estes, M. K., and J. Cohen.** 1989. Rotavirus gene structure and function. Microbiol. Rev. **53**:410–449.
5. **Flores, J., K. Midthun, Y. Hoshino, K. Green, M. Gorziglia, A. Z. Kapikian, and R. M. Chanock.** 1986. Conservation of the fourth gene among rotaviruses recovered from asymptomatic newborn infants and its possible role in attenuation. J. Virol. **60**:972–979.
6. **Gentsch, J. R., B. K. Das, B. Jian, M. K. Bhan, and R. I. Glass.** 1993. Similarity of the VP4 protein of human rotavirus strain 116E to that of the bovine B223 strain. Virology **194**:424–430.
7. **Gentsch, J. R., R. I. Glass, P. Woods, V. Gouvea, M. Gorziglia, J.**

Flores, B. K. Das, and M. K. Bhan. 1992. Identification of group A rotavirus gene 4 types by polymerase chain reaction. J. Clin. Microbiol. **30:**1365–1373.

8. **Gerna, G., A. Sarasini, M. Parea, S. Arista, P. Miranda, H. Brüssow, Y. Hoshino, and J. Flores.** 1992. Isolation and characterization of two distinct human rotavirus strains with G6 specificity. J. Clin. Microbiol. **30:**9–16.
9. **Gouvea, V., L. de Castro, M. D. C. Timenetsky, H. Greenberg, and N. Santos.** 1994. Rotavirus serotype G5 associated with diarrhea in Brazilian children. J. Clin. Microbiol. **32:**1408–1409.
10. **Gouvea, V., R. I. Glass, P. Woods, K. Taniguchi, H F. Clark, B. Forrester, and Z.-Y. Fang.** 1990. Polymerase chain reaction amplification and typing of rotavirus nucleic acid from stool specimens. J. Clin. Microbiol. **28:**276–282.
11. **Gouvea, V., M.-S. Ho, R. I. Glass, P. Woods, B. Forrester, C. Robinson, R. Ashley, M. Riepenhoff-Talty, H F. Clark, K. Taniguchi, E. Meddix, B. McKeller, and L. Pickering.** 1990. Serotypes and electropherotypes of human rotavirus in the USA: 1987–1989. J. Infect. Dis. **162:**362–367.
12. **Gouvea, V., N. Santos, and M. D. C. Timenetsky.** 1994. Identification of bovine and porcine rotavirus G types by PCR. J. Clin. Microbiol. **32:**1338–1340.
13. **Gouvea, V., N. Santos, and M. D. C. Timenetsky.** 1994. VP4 typing of bovine and porcine group A rotaviruses by PCR. J. Clin. Microbiol. **32:**1333–1337.
14. **Li, B., H F. Clark, and V. Gouvea.** 1993. Nucleotide sequence of the VP4-encoding gene of an unusual human rotavirus (HCR3). Virology **196:**825–830.
15. **Linhares, A. C., Y. B. Gabbay, J. D. P. Mascarenhas, R. B. Freitas, T. H. Flewett, and G. M. Beards.** 1988. Epidemiology of rotavirus subgroups and serotypes in Belém, Pará, Brazil: a three-year study. Ann. Inst. Pasteur Virol. **139:**89–99.
16. **Santos, N., V. Gouvea, M. C. Timenetsky, H F. Clark, M. Riepenhoff-Talty, and A. Garbarg-Chenon.** 1994. Comparative analysis of VP8* sequences from rotaviruses possessing M37-like VP4 recovered from children with and without diarrhoea. J. Gen. Virol. **75:**1775–1780.
17. **Santos, N., M. Riepenhoff-Talty, H F. Clark, P. Offit, and V. Gouvea.** 1994. VP4 genotyping of human rotavirus in the United States. J. Clin. Microbiol. **32:**205–208.
18. **Steele, A. D., D. Garcia, J. Sears, G. Gerna, O. Nakagomi, and J. Flores.** 1993. Distribution of VP4 gene alleles in human rotaviruses by using probes to the hyperdivergent region of the VP4 gene. J. Clin. Microbiol. **31:**1735–1740.
19. **Timenetsky, M. C. S. T., J. J. Kisielius, S. J. F. E. Grisi, A. M. Escobar, M. Ueda, and H. Tanaka.** 1993. Rotavirus, adenovirus, astrovirus, calicivirus and small virus particles in faeces of children with and without diarrhea within the period of 1987 to 1988, in São Paulo. Rev. Inst. Med. Trop. Sao Paulo **35:**275–280.
20. **Urasawa, S., A. Hasegawa, T. Urasawa, K. Taniguchi, F. Wakasugi, H. Suzuki, S. Inouye, B. Pongprot, J. Supawadee, S. Suprasert, P. Rangsiyanond, S. Tonusin, and Y. Yamazi.** 1992. Antigenic and genetic analyses of human rotaviruses in Chiang Mai, Thailand: evidence for a close relationship between human and animal rotaviruses. J. Infect. Dis. **166:**227–234.