Arch Virol (1996) 141: 2365–2374

Archives of Virology

Rotavirus G and P types circulating in Brazil: characterization by RT-PCR, probe hybridization, and sequence analysis

J. P. G. Leite[1,3], A. A. Alfieri[1,2], P. A. Woods[3], R. I. Glass[3], and J. R. Gentsch[3]

[1] Department of Virology, Oswaldo Cruz Institute, Rio de Janeiro, Brazil
[2] Laboratory of Animal Virology, Londrina State University, Parana, Brazil
[3] Viral Gastroenteritis Section, Division of Viral and Rickettsial Diseases, National Center for Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, Georgia, U.S.A.

Accepted July 4, 1996

Summary. We used reverse transcription-polymerase chain reaction (RT-PCR) to determine the P and G genotypes of 130 culture-adapted rotavirus strains isolated from 181 fecal specimens of children under 5 years of age from 9 states and the Federal District of Brazil. The 4 genotypes found most commonly worldwide were also common in Brazil and P[8]G1 was the most prevalent (43%), followed by P[4]G2 (12%), P[8]G3 (6%), and P[8]G4 (6%). However, unusual types P[8]G5, P[6]G2, P[9]G1, P[9]G3, and mixed infections were responsible for 12% and 21% of the cases, respectively. Genotype G5 strains were detected in specimens collected in all 9 areas surveyed from all 4 regions of Brazil. The unusual strain diversity in Brazil suggests that when tetravalent rotavirus vaccines currently being developed are introduced into Brazil, laboratory surveillance will be essential to monitor protection against unusual strains, particularly those of genotype 5, as well as emergence of novel reassortants that may evolve from the large pool of children with mixed infections.

Introduction

Rotavirus is an important etiologic agent of acute gastroenteritis in many species of animals and in humans, and is responsible for approximately 870000 deaths annually among children under five years old, mostly in developing countries. Consequently, rotavirus is a priority for vaccine development [14]. The virus is a member of the family *Reoviridae* and has a genome containing 11 segments of double stranded RNA (dsRNA) enclosed in a triple-layered protein capsid. The middle layer contains one protein, VP6, encoded by the genome segment 6, that carries both group and subgroup antigens [17, 30]. The outer layer is composed of two proteins, VP4 and VP7, encoded by genome segments 4 and 7, 8 or 9,

respectively, that evoke a neutralizing antibody response and form the basis for the current classification of group A rotaviruses into P (VP4) and G (VP7) serotypes [17, 26]. Based on cross-neutralization studies with polyclonal antisera to isolated viruses or against expressed VP4 proteins, four main serotypes have been identified in children with diarrhea: P1A[8]G1, P1B[4]G2, P1A[8]G3, and P1A[8]G4 [3, 6, 23, 48]. Additional serotypes (e.g. P3[9]G1, P1B[4]G12, P4[10]G8) have been identified, but the prevalence of these strains is believed to be low [4, 9, 12, 15, 17, 20, 22, 38, 43, 46].

Because rotavirus diarrhea is equally common among children in developing and developed countries, it seems unlikely that improvements in the water supply and sanitation will reduce disease incidence. Control of rotavirus disease is most likely to be achieved through the development and widespread use of an effective vaccine. Early vaccine trials with single strains showed low or variable efficacy and suggested that protection might be improved if VP7 antigens in the vaccine matched those of strains in the community. Single gene reassortant strains carrying antigens to the major G types are now included in both simian (RRV) and bovine (WC3) vaccines currently being evaluated in field trials with the hope that homotypic protection will make vaccines more efficacious. A recent trial of the RRV-TV vaccine by Linhares in Brazil [28] demonstrated an efficacy of 56% against severe rotavirus diarrhea, less than the 75–85% observed in similar trials in the U.S. [5, 36]. One theoretical explanation of this decreased efficacy could be the presence of unusual strains in circulation that might require inclusion of additional reassortant strains into a vaccine for Brazil.

In anticipation of the possible use of a rotavirus vaccine in Brazil, we wanted to characterize the most common strains of rotavirus in circulation to see if these strains matched the antigens included in the current vaccine candidates. In this report, rotavirus strains from children with acute gastroenteritis in 9 states and the Federal District of Brazil were characterized for their G and P genotypes by reverse transcription-polymerase chain reaction (RT-PCR), and probe hybridization with genotype-specific digoxigenin-labeled oligonucleotides. Our results suggest that the diversity of strains circulating in Brazil exceeds that in many parts of the world, that vaccines used in Brazil may require additional antigens, and that laboratory surveillance of strains will be essential after a vaccine is introduced.

Materials and methods

Collection of fecal specimens

To obtain rotavirus specimens, we screened a collection of fecal samples obtained between 1982 and 1994 from children under five years old with acute gastroenteritis. These specimens were collected from 9 states and the Federal District of Brazil as part of an annual rotavirus surveillance project that has been described previously [33]. Specimens positive for rotavirus by EIARA [31] and polyacrylamide gel electrophoresis (PAGE) [32] were sent to the Comparative Virology Laboratory, Oswaldo Cruz Institute, Rio de Janeiro, where they were confirmed and stored at $-20\,^{\circ}C$. Only the specimens with >0.5 grams of feces were used for characterization. Other enteric pathogens were not analyzed in this study.

Culture adaptation of human rotaviruses

The origin of the prototype culture adapted strains used in this study has been previously described [11]. Cultured strains ND25 (P[4]G2, 157C (P[11]G3) and 188A (P[11]G3) were from New Delhi, India [10]. Rotavirus strains were adapted to cell culture in MA-104 (fetal rhesus monkey kidney) or HT-29 (human colon adenocarcinoma) cells obtained from the Biologic Products Section of the Centers for Disease Control and Prevention. The cells were grown in Eagle's minimum essential medium containing antibiotics (MEM) (GIBCO, Long Island, N.Y.) with 10% fetal bovine serum (GIBCO, Long Island, N.Y.) at 37 °C. Fecal suspensions (10–20%) were prepared in MEM and treated with 5 μg/ml of Type IX trypsin (Sigma Chemical Co., St. Louis, MO) for 1 h at 37 °C. 200 μl of the undiluted 10% extracts were then inoculated into two roller tubes each of HT-29 or MA-104 cell monolayers which had previously been overlayered with 2 ml MEM containing 0.5 μg/ml trypsin, and incubated at 37 °C for 1 h in a rolling roller tube wheel. After this time, maintenance MEM medium containing 1 μg of trypsin per ml was added to the tubes, and the tubes rolled at 37 °C. Five days later, the cultures were frozen and thawed three times, and two additional passages were performed as described above. Culture supernatants were assayed for the presence of rotavirus by an enzyme immunoassay (Rotaclone, Cambridge Biotech, Worcester, MA).

RNA extraction and RT-PCR for P and G typing

Five hundred μl of positive supernatant from infected cells was extracted with an equal volume of 1,1,2 trichloro-1,2,2 trifluoro-ethane (Genetron), clarified by centrifugation at 2000 × **g** for 10 min, and the dsRNA extracted using the Boom method [7] except that the silica beads were replaced with a commercial glass powder (RNaid, BIO 101, Inc., LaJolla, CA). P and G genotypes were determined by half-nested RT-PCR as described previously [10, 21, 24, 25]. For the two-step amplification procedure for G-genotyping, we used consensus primers 9Con2 (nt 922–941 of strain Wa VP7 gene: 3′-GTA TAA AAT ACT TGC-CAC CA) and 9Con 1 to generate the PCR product [10]. For P-genotyping, the consensus minus-sense primer Con2 (nt 887–868 of strain Wa VP4 gene: ATT TCG GAC CAT TTA TAA CC), was used in conjunction with Con3 to produce the first amplification RT-PCR product [21]. For P [9] genotyping, the plus-sense primer Con3 was used together with the strain K8-specific primer 4T-1 and the P typing primers described previously [21]. The modified P genotype numbers used here are in accord with recent recommendations of a rotavirus nomenclature working group [16]. Briefly, the most common strains with Wa-like and DS1-like VP4 genes identified by genotyping methods are designated P[8] and P[4] unless they have been characterized by cross-neutralization tests (i.e., the P genotypes of rotaviruses in fecal specimens should be reported as P followed by a number in brackets). In the case of strains that have been characterized by cross-neutralization studies a complete P serotype designation which signifies both serotype and genotype (e.g., strain Wa, P1A[8] or strain DS-1, P1B[4] should be given.

Oligonucleotide probes

Six genotype-specific oligonucleotide probes labeled at 5′end with digoxigenin (dig) were designed to have high homology with strains of one serotype and low homology with those of the other serotype (Table 1). Two different probes were designed to confirm genotype G3-specific products based on sequence differences between Brazilian G3 strains and a serotype G3 prototype isolate (Table 1).

Table 1. Sequence of digoxigenin labeled oligonucleotide probes used to characterize G_1 to G_4 and P[9] genotype-specific PCR products of rotavirus strains

Probe	Strain	Nt. position	GenBank accession no.[a]	Sequence[b,c]
G1-2	Wa	152–175	K02033	ATA GAT TTT TGT TGA TTA CTG TAG
G2-2	S2	186–205	M11164	ATT TGT GAG GAC GCA AAA TT
G3-1[d]	107E1B	93–116	U04350	CGT ACT CAA ATC CTT AAC TAG A
G3-2[d]	BR1072	177–196	U40009	ACT GCC ACC ACT CCT TAA TG
G4-1	ST3	111–132	X13609	GTT CGT TCT TGT GAG TTA TAT
P9-1	K8	173–194	D90260	TTG CCT GAT TCT ACA TTG GTG C

[a] These sequences were derived from the following references: strains Wa [37], S2 [8], 107E1B and Br1072 [19], ST3 [35], and K8 [42]

[b] The polarity of all probes is + sense

[c] The temperature for probe hybridization was determined experimentally as 58 °C for G1 to G4, and 62 °C for P[9]

[d] For genotype G3 strains, two probes have been used

Southern hybridization and chemiluminescent detection

Southern hybridization and chemiluminescent detection with genotypes-specific PCR products was carried out according to the protocols and reagents recommended by Boehringer Mannheim Corp. as recently described by Ando et al. [2] and modified by Ramachandran and co-workers for rotavirus [34], with the following modifications: pre-hybridization was performed at 58 °C (G1–G4 probes) or 62 °C (P[9] probe) for 1 h; hybridization was at 58 °C (G1 to G4) or 62 °C (P[9]) for 4 h; and membrane treatment with blocking solution was for 1 h at RT.

G serotyping by enzyme immunoassay (EIA)

Several Brazilian G3 strains which reacted with probe G3-2 but not G3-1 were confirmed to belong to serotype G3 using the methods and monoclonal antibodies (MAbs) of Taniguchi and co-workers [44].

Nucleotide sequencing

Portions of the VP7 gene of four Brazilian G3 strains (nt 102 to 248) and the VP4 gene of three P[9] Brazilian isolates (nt 55 to 384) were sequenced by an automated method as described in the accompanying paper in this issue of *Archives of Virology* [1]. The primers used for sequencing VP7 serotype G3 strains were 9Con1 and 9T-3P, and for the VP4 genotype P[9] strains, Con3 and 4T-1 were used [10, 21]. The sequence data was compared with that of other rotaviruses using the University of Wisconsin Genetics Computer Group package of programs [13].

Accession numbers

The partial VP7 gene sequences of Brazil G3 strains BR1015, BR1071, BR1072, and BR1163 have been assigned accession nos. U40007, U40008, U40009, and U40010, respectively. The partial VP4 gene sequences of Brazil genotype P[9] strains BR1130 and BR1131 have been assigned accession nos. U40011 and U40012, respectively.

Results

Culture adaptation of rotavirus isolates and RT-PCR P and G typing

From 181 fecal specimens inoculated in MA-104 or HT-29 cells, 130 (72%) were positive for rotavirus antigen by enzyme immunoassay after the third passage. All 130 strains could be P and G genotyped by RT-PCR using either a one or two -step amplification procedure. Strain diversity was high and single infections with strains corresponding to one of the four common genotypes was observed in 67% (N = 86) of the specimens, uncommon strains in 12% (N = 17), and mixed infections in 21% (N = 27) (Table 2). Of the common rotaviruses, genotype P[8]G1 (43%) was the predominant strain in most states, followed by P[4]G2 (12%), P[8]G3 (6%), and P[8]G4 (6%). P[4]G2 was not detected in any of 23 isolates from the Federal District. Interestingly, we detected genotype G5 as 13 single (9%) and 16 mixed (12%) infections circulating in different states/regions and years: AL, RJ/1982, BA, RJ, PR/1983, FD, MG/1985, PE, FD, MG, SP, PR/1986, GO, RJ/1987, BA/1989, and RJ, PR/1994 (Table 1), suggesting that this rotavirus genotype could be endemic in Brazil.The uncommon strains P[6]G1, P[6]G2, P[9]G3, and P[9]G6 were detected in Pernambuco, Rio de Janeiro, Sao Paulo, and Goias states, respectively (Table 2).

Twenty-seven (100% of those tested) of the common genotypes G1 (N = 8), G2(N = 4), G3 (N = 11), and G4 (N = 4) were confirmed by probe hybridization with G type-specific oligonucleotides. Three genotype P[9]G3 strains were also confirmed by hybridization with an oligonucleotide probe homologous to the VP4 gene of strain K8 and with sequence analysis of a fragment of theVP4 gene (nt 55 to 384, predicted aa 16 to 125) of two of these strains. These strains were 95 and 99% identical with the corresponding region of P4[9]G1 prototype strain K8.

Detailed characterization of the G5 isolates detected in this study will be reported elsewhere [1].

Discussion

In the last several years, new methods to detect and characterize genes encoding both the VP4 and VP7 neutralization antigens (P and G genotypes) of human rotavirus strains have advanced our understanding of the importance of strains that could not previously be typed. These methods have been particularly useful for the gene encoding VP4 since MAbs for P serotyping have not been fully developed and applied to clinical specimens. In the current study, 130 culture-adapted rotavirus strains from hospitalized Brazilian children with diarrhea were characterized by RT-PCR P and G genotyping, probe hybridization, and nucleotide sequencing. The most prevalent strains with genotypes P[8]G1, P[4]G2, P[8]G3, and P[8]G4, accounted for two-thirds of the isolates and were the same as those found in previous studies in a variety of countries [18, 27, 39, 40, 47, 49]. However, unusual strains with genotypes P[8]G5, P[6]G2 or P[9]G6, P[9]G3, and mixed infections accounted for one-third of the specimens, indicating that the strain diversity in Brazil is greater than in most

Table 2. G and P genotype of rotavirus strains from children with diarrhea in 9 states of Brazil and the Federal District of Brazil

States	Common serotypes[a]				Others				Mixed infections	
	P[8]G1	P[4]G2	P[8]G3	P[8]G4	P[8]G5	P[6]G2	P[6]G4	P[9]G3	genotypes (N)[b]	total
Northeast										
Pernambuco (PE)	8	1	2	–	1				P[6+8]G1 (1)	13
Alagoas (AL)	1	–	–	5	1				–	7
Bahia (BA)	3	3	–	–	1				P[8]G1 + P[4]G2 (2) P[8]G3 + G1 (1) P[8]G5 + G1 (1)	11
Southeast										
Minas Gerais (MG)	3	2	–	–	2				–	7
Rio de Janeiro (RJ)	13	7	1	2	2	1			P[8]G5 + G3 (1) P[8]G5 + G1(2)	29
Sao Paulo (SP)	7	1	–	–	1			2	P[8]G4 +G3(1) P[8 + 9]G3	13
Central West										
Federal District (FD)	14	–	3	–	2				P[8]G5 + G1 (2) p[8]G5 + G4 (2)	23
Goias (GO)	1	–	1	–	–		1		P[4+8]G5+G1+G2 (2)	5
South										
Parana (PR)	2	1	–	2	3				P[4 + 8]G1 + G2 (1) P[8]G3 + G4 (1) P[4 + 8]G2 + G4 (2) P[8]G5 + G4 (1) P[4 + 8]G1 + G2 + G5 (6)	19
Santa Catarina (SC)	3	–	–	–	–					3
Total	55	15	7	9	13	1	1	2	27	130
%	(43)	(12)	(6)	(6)	(9)	(0.8)	(0.8)	(1.6)	(21)	

[a] Eight out of 8 genotype G1, 4 out 4 genotype G2, 11 out of 11 genotype G3 and 4 of 4 genotype G4 PCR products were confirmed by hybridization with genotype-specific oligonucleotide probes

[b] Number

other countries studied to date. Another study demonstrating larger than expected strain diversity in the Sao Paulo state of Brazil was reported recently [45], but the current report is the first to demostrate that uncommon strains are widespread in Brazil. Previous studies in Brazil using G serotype-specific MAbs were carried out in Belem (North region) [3, 29] and Sao Luiz (North-East region) [41], and showed that serotype G1 and G3 were predominant, respectively.

Especially interesting was the observation that P[8]G5 strains, which are apparent reassortants between porcine OSU-like (P9[7]G5 and human P[8]G1, P[8]G3 or P[8]G4-like strains, accounted for 9% of single infections and 12% of mixed infections. These strains were detected in 8 of the 9 states plus the Federal District of Brazil and in different years and regions, beginning in 1982 (Maceio/AL, northeast region, Rio de Janeiro/RJ south region) until 1994 (Rio de Janeiro/RJ, southeast region, Londrina/PR, south region). A previous report showed that G_5 was present in RJ, SP, PE, and GO states [24]. Since all of the isolates in the current study were from hospitalized children with diarrhea, it suggests that G5 strains may be epidemiologically important as a cause of rotavirus disease.

Some regions of the world appear to have a higher degree of rotavirus diversity than others. For example, other uncommon strains P[6]G1, P[6]G2, P[6]G3, P[6]G4, and P[6]G9, were recently reported to be present at high frequency in Brazil [45] and India [34]. In India P[6]G9 strains, which had been not detected anywhere, were the most prevalent isolate, while in Brazil strains related to human rotavirus isolates RO1845 and HCR3 (P genotype 3, with serotype G1 or G3 specificity), and to P genotype 6 isolates such as M37, were reported at frequencies of 17% and 15% of typeable single infections [45]. It should be noted, however, that it cannot be excluded that sensitive detection methods such as RT-PCR may increase the ability to detect mixed infections, while the clinical significance of these dual infections is not yet known.

This report provides several insights into rotavirus strain variation and vaccine development. Our finding of a large number of mixed infections as well as strains which may represent reassortants between human and animal rotavirus suggests that the opportunities for new strains to evolve in some regions may be much greater than has been previously expected. Given that vaccines currently being developed are based on the concept that homotypic protection will be greater than heterotypic protection, the ability of these vaccines to protect against endemic strain such as G5 whose antigens are not included in the vaccine remains to be tested under field conditions. If these strains preferentially escape protection by the vaccine, additional reassortant strains might be required for inclusion in a more effective vaccine.

Acknowledgements

J. P. G. Leite and A. A. Alfieri were recipients of fellowships from the Brazilian Research Council (CNPq). We express our gratitude to the Brazilian Study Group on Viral Gastroenteritis, particularly Heilo G. Pereira (in memoria), the mentor of this group; J. M. S. Teixeira, D. P. Cardoso, M. C. Lanna, M. C. de Souza, L. A. Ferreira, C. Houly, and J. A. N.

Candeias for rotavirus samples. We also thank A. M. Fialho for technical assistance, the Centers for Disease Control and Prevention, Biotechnology Core Facility, for the synthesis of primers and probes, and John O'Connor for editing the manuscript.

References

1. Alfieri AA, Leite JPG, Nakagomi O, Glass RI, Gentsch JR (1996) Characterization of human rotavirus genotype P[8]G5 from Brazil by probe-hybridization and sequence analysis. Arch Virol 141: 2253–2264
2. Ando T, Monroe SS, Gentsch JR, Jin Q, Lewis DC, Glass RI (1995) Detection and differentiation of antigenically distinct small round-structured viruses (Norwalk-like viruses) by reverse transcription-PCR and Southern hybridization. J Clin Microbiol 33: 64–71
3. Beards G, Desselberger U, Flewett T (1989) Temporal and geographical distributions of human rotavirus serotypes, 1983–1988. J Clin Microbiol 27: 2827–2833
4. Beards G, Xu L, Ballard A, Desselberger U, McCrae MA (1992) A serotype 10 human rotavirus. J Clin Microbiol 30: 1432–1435
5. Bernstein DI, Glass RI, Rodgers G, Davidson BL, Sack DA (1995) Evaluation of rhesus rotavirus monovalent and tetravalent reassortant vaccines in US children. JAMA 273: 1191–1196
6. Bishop R, Unicomb L, Barnes G (1991) Epidemiology of rotavirus serotypes in Melbourne, Australia, from 1973–1989. J Clin Microbiol 29: 862–868
7. Boom R, Sol CJA, Salimans MMM, Jansen CL, Wertheim-Van Dillen PME, Van Der Noordaa J (1990) Rapid and simple method for purification of nucleic acids. J Clin Microbiol 28: 495–503
8. Both GW (1985) Unpublished data
9. Browning GF, Chalmers RM, Fitzgerald TA, Snodgrass DR (1991) Serological and genomic characterisation of L338, a novel equine group A rotavirus G serotype. J Gen Virol 72: 1059–1064
10. Das BK, Gentsch JR, Cicirello HG, Woods PA, Ramachandran M, Gupta A, Ramachandran M, Kumar R, Bhan MK, Glass RI (1994) Characterization of rotavirus strains from newborns in New Delhi, India. J Clin Microbiol 32: 1820–1822
11. Das BK, Gentsch JR, Hoshino Y, Ishida S-I, Nakagomi O, Bhan MK, Kumar R, Glass RI (1993) Characterization of the G serotype and genogroup of New Delhi newborn rotavirus strain 116E. Virology 197: 99–107
12. Das M, Dunn SJ, Woode GN, Greenberg HB, Rao CD (1993) Both surface proteins (VP4 and VP7) of an asymptomatic neonatal rotavirus strain (I321) have high levels of sequence identity with the homologous proteins of a serotype 10 bovine rotavirus. Virology 194: 374–379
13. Devereux J, Haeberli P, Smithies O (1984) A comprehensive set of sequence analysis programs for the VAX. Nucleic Acids Res 12: 387–395
14. De Zoysa I, Feachem RV (1985) Interventions for the control of diarrhoeal diseases among young children: rotavirus and cholera immunization. Bull World Health Organ 63: 569–583
15. Dunn SJ, Greenberg HB, Ward RL, Nakagomi O, Burns JW, Vo PT, Pax KA, Das M, Gowda K, Rao CD (1993) Serotype and genotypic characterization of human serotype 10 rotavirus from asymptomatic neonates. J Clin Microbiol 31: 165–169
16. Estes M (1996) Rotaviruses and their replication. In: Fields B, Knipe DM, Howley PM (eds) Fields virology vol 2, 3rd ed. Lippincott-Raven Press, Philadelphia, pp 1625–1655

17. Estes MK, Cohen J (1989) Rotavirus gene structure and function. Microbiol Rev 53: 410–449
18. Fang Z-Y, Shangjin J, Shuming Q, Xianfa Z, Di W, Ushijima H, Gentsch J, Yoshikura H, Glass RI (1994) Serotypes of group A rotavirus isolates determined by PCR in Hebei and Henan provinces, China. Chin J Virol 10: 316–320
19. Gentsch JR (1993) Unpublished data
20. Gentsch J, Das BK, Jiang B, Bhan MK, Glass RI (1993) Similiarly of the VP4 protein of human rotavirus strain 116E to that of the bovine B223 strain. Virology 194: 424–430
21. Gentsch JR, Glass RI, Woods P, Gouvea V, Gorziglia M, Flores J, Das BK, Bhan MK (1992) Identification of group A rotavirus gene 4 types by polymerase chain reaction. J Clin Microbiol 30: 1 365–1 373
22. Gerna G, Sarasini A, Parea M, Arista S, Miranda P, Brussow H, Hoshino Y, Flores J (1992) Isolation and characterization of two distinct human rotavirus strains with G6 specificity. J Clin Microbiol 30: 9–16
23. Gorziglia M, Larralde G, Kapikian AZ, Chanock RM (1990) Antigenic relationships among human rotaviruses as determined by outer capsid protein VP4. Proc Natl Acad Sci USA 87: 7 155–7 159
24. Gouvea V, de Castro L, do Carmo Timenetsky M, Greenberg H, Santos N (1994) Rotavirus serotype G5 associated with diarrhea in Brazilian children. J Clin Microbiol 32: 1 408–1 409
25. Gouvea V, Glass RI, Woods P, Taniguichi K, Clark HF, Forrester B, Fang ZY (1990) Polymerase chain reaction amplification and typing of rotavirus nucleic acids from stool specimens. J Clin Microbiol 28: 276–282
26. Hoshino Y, Sereno MM, Midthun K, Flores J, Kapikian AZ, Chanock RM (1985) Independent segregation of two antigenic specificities (VP3 and VP7) involved in neutralization of rotavirus infectivity. Proc Natl Acad Sci USA 82: 8 701–8 704
27. Kaga E, Iizuka M, Nakagomi T, Nakagomi O (1994) The distribution of G (VP7) and P (VP4) serotypes among human rotaviruses recovered from Japanese children with diarrhea. Microbiol Immunol 38: 317–330
28. Linhares AC, Gabbay YB, De Freitas RB, Mascarenhas JDP, Oliveira CS, Bellesi N, Lainson ZL, Ramos FLP, Valente SA (1995) Immunogenicity, safety and efficacy of rhesus-human reassortant rotavirus (RRV-tetravalent) vaccine in Belem, Brazil (1995). Abstract presented at the Fifth Rotavirus Vaccine Workshop, Atlanta, GA, U.S.A., October 16–17, 1995
29. Linhares AC, Gabbay YB, Mascarenhas JDP, Freitas RB, Flewett TH, Beards GM (1988) Epidemiology of rotavirus subgroups and serotypes in Belem, Brazil: a three-year study. Ann Inst Pasteur Virol 139: 89–99
30. Mattion NM, Cohen J, Estes MK (1994) The rotavirus proteins, In: Kapikian AZ (ed) Viral infections of the gastrointestinal tract, 2nd ed. Marcel Dekker, New York, pp 169–250
31. Pereira HG, Azeredo RS, Leite JPG, Andrade ZP, de Castro L (1985) A combined enzyme immunoassay for rotavirus and adenovirus. J Virol Methods 10: 21–28
32. Pereira HG, Azeredo RS, Leite JPG, Candeias JAN, Racz ML, Linhares AC, Gabbay YB, Trabulsi JR (1983) Electrophoretic study of the genome of human rotaviruses from Rio de Janeiro, Sao Paulo and Belem, Brazil. J Hyg 90: 117–125
33. Pereira HG, Linhares AC, Candeias JAN, Glass RI (1993) National laboratory surveillance of viral agents of gastroenteritis in Brazil. Bull Pan Am Health Organ 27: 224–233
34. Ramachandran M, Das BK,Vij A, Kumar R, Bhambal SS, Kesari N, Rawat H, Bahl L, Thakur S, Woods PA, Glass RI, Bhan MK, Gentsch JR (1996) Unusual diversity of human rotavirus G and P genotypes in India. J Clin Microbiol 34: 436–439

35. Reddy DA, Greenberg HB, Bellamy AR (1989) Nucleotide sequence of St. Thomas 3 rotavirus genomic segment 9: an RNA encoding the major serotypic antigen. Nucleic Acids Res 17: 449
36. Rennels MB, Glass RI, Dennehy PH, Bernstein DI, Pichichero ME, Zito ET (1996) Safety and efficacy of high-dose rhesus-human reassortant rotavirus vaccines: report of the national multicenter trial. Pediatrics 97: 7–13
37. Richardson MA, Iwamoto A, Ikegami N, Nomoto A, Furuichi Y (1984) Nucleotide sequence of the gene encoding the serotype-specific antigen of human (Wa) rotavirus: comparison with the homologous gene from simian SA11 and UK bovine rotaviruses. J Virol 51: 860–862
38. Sereno MM, Gorziglia MI (1994) The outer capsid protein VP4of murine rotavirus strain Eb represents a tentative new P type. Virology 199: 500–504
39. Silberstein I, Shulman LM, Mendelson E, Shif I (1995) Distribution of both rotavirus VP4 genotypes and VP7 serotypes among hospitalized and nonhospitalized Israeli children. J Clin Microbiol 33: 1 421–1 422
40. Steele AD, van Niekerk MC, Mphahlele MJ (1995) Geographic distribution of human rotavirus VP4 genotypes and VP7 serotypes in five South African regions. J Clin Microbiol 33: 1 516–1 519
41. Stewien KE, Mehnert DU, Harsi CM, Stewien ET, Candeias MJG, Tanaka K (1994) Serotypes and electropherotypes of human rotavirus detected in the city of Sao Luis (MA), Brazil. Braz J Med Biol Res 27: 1 355–1 361
42. Taniguchi K, Nishikawa K, Urasawa T, Urasawa S, Midthun K, Kapikian AZ, Gorziglia M (1989) Complete nucleotide sequence of the gene encoding VP4 of a human rotavirus (strain K8), which has unique VP4 neutralization epitopes. J Virol 63: 4 101–4 106
43. Taniguchi K, Pongsuwanna Y, Choonthanon M, Urasawa S (1990) Nucleotide sequence of the VP7 gene of a bovine rotavirus (strain 61 A) with different serotype specificity from serotype 6. Nucleic Acids Res 18: 4 613
44. Taniguchi K, Urasawa T, Morita Y, Greenberg HB, Urasawa S (1987) Direct serotyping of human rotavirus in stools using serotype 1-, 2-, 3-, and 4-specific monoclonal antibodies to VP7. J Infect Dis 155: 1 159–1 166
45. Timenetsky M do C, Santos N, Gouvea V (1994) Survey of rotavirus G and P types associated with human gastroenteritis in Sao Paulo, Brazil, from 1986 to 1992. J Clin Microbiol 32: 2 622–2 624
46. Urasawa S, Urasawa T, Wakasugi F, Kobayashi N, Taniguchi K, Lintag IC, Saniel MC, Goto H (1990) Presumptive seventh serotype of human rotavirus. Arch Virol 113: 279–282
47. Ushijima H, Mukoyama A, Hasegawa A, Nishimura S, Konishi K, Bosu K (1994) Serotyping of human rotaviruses in the Tokyo area (1990–1993) by enzyme immunoassay with monoclonal antibodies and by reverse transcrition and polymerase chain reaction amplification. J Med Virol 44: 162–165
48. Woods PA, Gentsch J, Gouvea V, Mata L, Simhon A, Santosham M, Bai Z-S, Urasawa S, Glass RI (1992) Distribution of serotypes of human rotavirus in different populations. J Clin Microbiol 30: 781–785
49. Wu H, Taniguchi K, Wakasugi F, Ukae S, Chiba S, Ohseto M, Hasegawa A, Urasawa T, Urasawa S (1994) Survey on the distribution of the gene 4 alleles of human rotaviruses by polmerase chain reaction. Epidemiol Infect 112: 615–622

Authors' address: Dr. J. R. Gentsch, Viral Gastroenteritis Section, MS-GO4, Centers for Disease Control and Prevention, 1600 Clifton Road N.E., Atlanta, GA30333, U.S.A.

Received May 17, 1996