

Genotyping of group A rotavirus samples from Brazilian children by probe hybridization

D.D.P. Cardoso¹,
M.L. Rácz³,
M.S.P. Azevedo¹,
R.M.B. Martins¹
and C.M.A. Soares²

¹Laboratório de Virologia, Instituto de Patologia Tropical e Saúde Pública, and
²Laboratório de Biologia Molecular, Instituto de Ciências Biológicas,
Universidade Federal de Goiás, Goiânia, GO, Brasil
³Laboratório de Virologia, Instituto de Ciências Biológicas,
Universidade de São Paulo, São Paulo, SP, Brasil

Abstract

The G genotyping of 74 group A rotavirus samples was done by RNA-DNA hybridization (dot-blot) using oligonucleotide probes for the VP7 gene region of the human rotavirus serotypes/genotypes 1, 2, 3 and 4. Thirty-one samples could be genotyped by dot-blot showing the following results: G1 = 16, G4 = 6, G3 = 5, and G2 = 4. The data show circulation of genotypes G1-G4 and the predominance of G1. The knowledge of genotypes provides important information concerning rotavirus circulation in Central Brazil.

Key words

- Rotavirus
- G genotyping
- VP7
- Oligonucleotides

Correspondence

D.D.P. Cardoso
IPTSP/UFG
Rua Delenda Rezende Melo
Esquina com 1ª Avenida
Setor Universitário
74605-050 Goiânia, GO
Brasil
Fax: + 55-62-202-3066
E-mail: divina@netgo.com.br

Research supported by CNPq (No. 800149-89/0) and Fundação de Apoio à Pesquisa-FUNAPE/PRPPG/UFG. Publication supported by FAPESP.

Received July 25, 2000
Accepted February 5, 2001

Human rotaviruses are recognized as the main agents of diarrhea of infants and young children and constitute an important cause of mortality in developing countries. The knowledge of the relative frequency and distribution of rotavirus serotypes/genotypes can help in the analysis of regional differences and variations over the years in population samples (1). The G serotypes/genotypes can be analyzed by several techniques (2-6), including enzyme immunoassay with monoclonal antibodies (EIA-mAb) (7,8). This is the main technique, although it is not able to define all serotypes. It has been proposed that the failure in serotyping by EIA-mAb could be due, for example, to the loss of VP7 (7,9). Nucleic acid hybridization techniques can be used to determine genotypes since the viral double-stranded RNA (dsRNA) is more resistant to degradation than the VP7 protein (10).

The present study describes the data obtained from G genotyping of rotavirus samples

collected from children in Goiânia, GO, Brazil, using the RNA-DNA hybridization technique. We studied 2,158 samples from children up to 10 years of age, with or without diarrhea disease, collected from hospitals, outpatient clinics and day-care centers from March 1986 to June 1995. All fecal samples were previously tested by EIA for group A rotavirus using commercial kits (EIARA, Biomanguinhos, FIOCRUZ, Rio de Janeiro, RJ, Brazil) and RNA polyacrylamide gel electrophoresis (PAGE) (11,12). Two hundred and fifty-four samples were positive for group A rotavirus. Of the 254 rotavirus samples, 233 (91.7%) were from children with diarrhea. It should be pointed out that 226 (95.8%) were hospitalized children. In addition, regarding age, 220 of the 254 positive children were up to two years old. Seventy-four of the positive samples were genotyped by the hybridization technique (dot-blot). Probes complementary to nucleo-

tides 315-339 of the VP7 gene from the human rotavirus serotypes/genotypes 1, 2, 3, and 4 were synthesized as described by Sethabutr et al. (10). The primers were labeled with [γ ³²P]-ATP according to Sambrook et al. (13). dsRNA was extracted from the samples as described by Pereira et al. (11). Briefly, fecal samples were suspended in 1X PBS, pH 7.4 (0.14 M NaCl, 0.02 M KCl, 0.08 M Na₂HPO₄, 0.01 M KH₂PO₄), extracted with phenol-chloroform and precipitated with ethanol. Hybridization was performed by the method of Sethabutr et al. (10). Briefly, dsRNA was supplemented with the same volume of 20X SSC (20X SSC = 3 M NaCl plus 0.3 M sodium citrate)/37% formaldehyde (3 parts of SSC and 2 parts of formaldehyde) and heated at 65°C for 15 min. The denatured dsRNA was spotted on nitrocellulose membranes in a total volume of 25 μ l. The membranes were heated for 2 h at 80°C and pre-hybridized in a solution containing 50% formamide, 1 mg/ μ l salmon DNA, 50% Denhardt's solution (Ficoll/polyvinylpyrrolidone/bovine serum albumin), and 1 M sodium phosphate buffer. The pre-hybridization and hybridization temperatures were 38°C for genotypes with 2 and 3 oligonucleotides and 42°C for genotypes with 1 and 4 oligonucleotides. Pre-hybridization was carried out for 4 h, followed by hybridization for 18 h in the same solution with 0.1% SDS and the labeled oligonucleotide added. The membranes were washed three times with

solution containing 5X SSC and 0.1% SDS at room temperature, dried and exposed to an X-ray film for 24 h at -70°C.

Genotyping was considered positive when the signal for one genotype was unique or significantly higher than for the other genotypes (at least two times the intensity). Samples that presented the same signal intensity for more than one genotype were considered undetermined. The prototypes Wa (G1), SA-11 (G3) and ST-3 (G4) were used as positive controls. We also used one sample (9800) characterized by EIA-mAb as a positive control of genotype G2.

Table 1 shows the results of genotyping. Of 74 samples analyzed, 31 (41.9%) were assigned to genotypes G1 to G4 by dot-blot. Indices from 52.9 to 91.1% were described for genotyping using molecular techniques (10,14,15). Thirty-four samples (45.9%) were not genotyped by dot-blot analysis. The presence of dsRNA was observed by PAGE in 33 of 34 dot-blot-negative samples (data not shown), a fact excluding the possibility of the failure of genotyping being related to the absence of dsRNA, as suggested by Bingnan et al. (1). Negative results could be related to the failure of hybridization with the oligonucleotides due to the presence of other VP7 genes not included in this investigation, as well as mutation in the analyzed region (14,16,17). Bingnan et al. (1), in Bangladesh, observed a higher genotyping index (66.7%) using three additional oligonucleotides for other rotavirus genotypes. This result supports the suggestion of the occurrence of samples other than G1-G4 among the samples analyzed. In agreement, other serotypes have been described. The G5 serotype that infects mainly swine has been detected in Brazil (18,19). Oligonucleotides complementary to other VP7 regions, as well as oligonucleotides for other rotavirus serotypes should be useful to answer this question. Nine samples hybridized with more than one oligonucleotide probe. The significance of this result will be the subject of a future study. Our data

Table 1 - Distribution of rotavirus G genotypes detected by probe hybridization in the State of Goiás.

Genotype	No.	%
G1	16	21.6
G2	4	5.4
G3	5	6.8
G4	6	8.1
Reactive to more than one probe	9	12.1
Not genotyped	34	45.9
Total	74	100.0

support the need for continuous monitoring of rotavirus serotypes/genotypes at different times in different world regions.

Acknowledgments

The authors would like to thank Adriana Crispim Azevedo for helpful technical assistance.

References

- Bingnan F, Unicomb L, Rahi Z, Banu NN, Podder G, Clemens J, Van Lonn FPL, Rao MR, Malek A & Tzipori S (1991). Rotavirus-associated diarrhea in rural Bangladesh: Two-year study of incidence and serotype distribution. *Journal of Clinical Microbiology*, 29: 1359-1363.
- Gerna G, Passarani N, Battaglia M & Percivalle E (1984). Rapid serotyping of human rotavirus strains by solid-phase immune electron microscopy. *Journal of Clinical Microbiology*, 19: 273-278.
- Green KY, Sears JF, Taniguchi K, Midthun K, Hoshino Y, Gorziglia M, Nishikawa K, Urasawa S, Kapikian AZ, Chanock RM & Flores J (1988). Prediction of human rotavirus serotype by nucleotide sequence analysis of the VP7 protein gene. *Journal of Virology*, 62: 1819-1823.
- Greenberg HB, Wyatt RG, Kapikian AZ, Kalica AR, Flores J & Jones R (1982). Rescue and serotypic characterization of noncultivable human rotavirus by gene reassortment. *Infection and Immunity*, 37: 104-109.
- Lin M, Imai M, Ikegami N, Bellamy AR, Summers D, Nuss DL, Deibel R & Furuichi Y (1987). cDNA probes of individual genes of human rotavirus distinguish viral subgroups and serotypes. *Journal of Virological Methods*, 15: 285-289.
- Poncet D & Cohen J (1989). A plaque hybridization assay for rotaviruses. *Journal of Virological Methods*, 26: 27-33.
- Thouless ME, Beards GM & Flewett TH (1982). Serotyping and subgrouping of rotavirus strains by the ELISA test. *Archives of Virology*, 73: 219-230.
- Gusmão RH, Mascarenhas JD, Gabbay YB, Lins Lainson Z, Ramos FL, Monteiro TA, Valente AS, Fagundes Neto U & Linhares AC (1999). Rotavirus subgroups, G serotypes, and electrophoretotypes in cases of nosocomial infantile diarrhoea in Belém, Brazil. *Journal of Tropical Pediatrics*, 45: 81-86.
- Mohammed KA, El Assouli SM & Banjar ZM (1994). Human rotavirus subgroups and serotypes in children with acute gastroenteritis in Saudi Arabia from 1988 to 1992. *Journal of Medical Virology*, 44: 237-242.
- Sethabutr O, Unicomb LE, Holmes IH, Taylor DN, Bishop RF & Echeverria P (1990). Serotyping of human group A rotavirus with oligonucleotide probes. *Journal of Infectious Diseases*, 162: 368-372.
- Pereira HG, Azeredo RS, Leite JPG, Barth OM, Suttmoller F, Farias V & Vidal MNP (1983). Comparison of polyacrylamide gel electrophoresis (PAGE), immunoelectron microscopy (IEM) and enzyme immunoassay (EIA) for the rapid diagnosis of rotavirus infection in children. *Memórias do Instituto Oswaldo Cruz*, 78: 483-490.
- Pereira HG, Azeredo RS, Leite JPG, Andrade ZP & De Castro L (1985). A combined enzyme immunoassay for rotavirus and adenovirus (EIARA). *Journal of Virological Methods*, 10: 21-28.
- Sambrook J, Fritsch EF & Maniatis T (1989). *Molecular Cloning: A Laboratory Manual*. 2nd edn. Cold Spring Harbor, New York, 11.31-11.32.
- Nirdnoy W, Sethabutr O, Sakulkaipera T, Harikul S, Hoge CW & Echeverria P (1995). Typing of human group A rotavirus with alkaline phosphatase-labeled oligonucleotide probes or a monoclonal enzyme immunoassay in unfrozen stools of children with diarrhea in Bangkok. *Journal of Medical Virology*, 45: 117-120.
- Adah MI, Rohwedder A, Olaleye OD, Durojalye OA & Werchau H (1997). Serotype of Nigerian rotavirus strains. *Tropical Medicine and International Health*, 2: 363-370.
- Steele AD, Nierkerk MC & Mphahlele MJ (1995). Geographic distribution of human rotavirus VP4 genotypes and VP7 serotypes in five South African geographic regions. *Journal of Clinical Microbiology*, 33: 1516-1519.
- Adah MI, Rohwedder A, Olaleye OD & Werchau H (1997). Nigerian rotavirus serotype G8 could not be typed by PCR due to nucleotide mutation at the 3'-end of the primer binding site. *Archives of Virology*, 142: 1881-1887.
- Gouvea V, Castro L, Timenetsky MC, Greenberg H & Santos N (1994). Rotavirus serotype G5 associated with diarrhea in Brazilian children. *Journal of Clinical Microbiology*, 32: 1408-1409.
- Leite JPG, Alfieri AA, Woods PA, Glass RI & Gentsch JR (1996). Rotavirus G and P types circulating in Brazil: characterization by RT-PCR, probe hybridization, and sequence analysis. *Archives of Virology*, 141: 2365-2374.