

Characterization of rotavirus and norovirus strains: a 6-year study (2004-2009)

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Abstract

Objective: To monitor rotavirus (RV) and norovirus (NoV) infections in hospitalized children ≤ 5 years with acute gastroenteritis in the state of São Paulo, Brazil, during a 6-year period (2004- 2009).

Methods: This retrospective study was conducted with 61 medical centers with convenient surveillance fecal specimens, investigated by enzyme-linked immunosorbent assay, sodium dodecyl sulfate polyacrylamide gel electrophoresis, reverse polymerase chain reaction and sequencing to genotype characterization.

Results: RV and NoV infections were detected in 29.6% (144/487) and 29.2% (26/89) of the samples, respectively. The most frequent RV genotypes detected were G9P[8] in 2004; G1P[8] in 2005; G9P[8] in 2006; and G2P[4] during 2007, 2008, and 2009. Detection rate declined from 36.3% (33/91) in 2004 to 4.2% (4/95) in 2009. NoV genogroup GII was found in 61.6% (16/26) of the samples, and GI in 11.5% (3/26). Mixed NoV-RV infections were observed in 2.2% (2/89) of the samples, involving GI+G9P[8] and GI+G2P[4] strains.

Conclusions: Genotype distribution varied according to collection year, accompanied by a reduction in detection rate. Use of RV vaccine requires implementation of post-marketing surveillance to monitor RV strain diversity and its efficacy against possible new emerging genotypes. NoVs have been increasingly identified as relevant etiological agents among hospitalized children and play an important role in the viral etiology of pediatric acute gastroenteritis in the state of São Paulo.

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Introduction

Viral pathogens are the most common causes of gastroenteritis in communities and other settings, including semi-closed institutions and hospitals.¹ In infancy, group A rotavirus (RV) is considered the most important etiological agent of acute non-bacterial gastroenteritis, including outbreaks and sporadic cases, independent of improvements in basic sanitation and hygiene procedures.²

RVs are the major etiological agent of acute diarrhea in children, responsible for more than 600,000 deaths each year,³ in addition to the significant economic burden of RV disease.⁴ In Brazil, RV infections were estimated to cause ~ 3.5 million diarrhea episodes, 655,853 outpatient visits, 92,453 hospitalizations, and 850 deaths of children ≤ 5 years each year before RV vaccine introduction.⁵

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In 2006 an attenuated G1P[8] vaccine was included in the Brazilian National Immunization Program, preventing severe RV gastroenteritis, and inducing significant reduction in the frequency of RV detection in children with gastroenteritis.⁶ In fact, the RIX4414 vaccine efficacy was evaluated by Araujo et al., showing 53.9-81.5% of protection against severe cases, and 81.2-93% of protection against hospitalization due to RV gastroenteritis in children.⁷

Recently, a high prevalence of G2P[4] was reported in Brazil and linked with this vaccination,⁸ suggesting that this monovalent vaccine possibly created conditions in which G2P[4] could acquire selective advantage over P[8] genotypes. Morillo et al. showed that the G2P[4] genotype was the only strain observed in 2007 during a 5-year surveillance study of RV strains in children < 5 years with acute gastroenteritis from day care centers in the state of São Paulo, Brazil.⁹ However, more detailed investigations concerning the heterologous protection conferred by RV monovalent vaccine are key points to understand its immunogenic behavior.^{7,10}

Noroviruses (NoVs) are the leading cause of acute non-bacterial human gastroenteritis worldwide, responsible for 80-90% of the reported outbreaks.¹¹ Although NoV was the first virus to be clearly associated with acute gastroenteritis, the inability to culture it has caused the underestimation of the disease burden of NoV infection and hampered epidemiological studies. Therefore, the importance of NoV as an etiological agent of acute gastroenteritis in both sporadic cases and outbreaks has been determined after the development of molecular methods for NoV detection.^{12,13}

Globally, NoV outbreaks are mainly caused by genogroup I (GI) and II (GII) strains, and the GII strain is predominant worldwide,^{14,15} including Brazil.^{12,16} NoVs have been increasingly identified as relevant etiological agents among hospitalized children in Europe, Asia and America. The relative importance of NoV may increase with RV vaccination.¹⁷

The aim of this study was to monitor RV and NoV infections among hospitalized children ≤ 5 years with acute gastroenteritis in the state of São Paulo during a 6-year period (2004-2009). The molecular characterization of NoV genogroups and RV genotypes was also undertaken.

Methods

This retrospective study was conducted with 61 medical centers from 2004 to 2009 with convenient surveillance specimens collected from hospitalized children ≤ 5 years presenting acute gastroenteritis. Stool samples from patients with acute gastroenteritis were sent to the Enteric Diseases Laboratory of Adolfo Lutz Institute, a macro-regional reference center for RV surveillance and a member of the Acute Diarrhea Disease Monitoring Program (ADDMP). This

program aims at early detection of diarrhea outbreaks with national extent and has been previously approved by the Ethics Committee.

The samples studied were part of ADDMP, obtained from a convenient sampling, without inclusion or exclusion criteria, with no characterization of the participating hospitals; therefore, the study may not be representative of the actual epidemiological scenario. The molecular characterization of RV genotypes and NoV genogroups was undertaken. It did not include clinical evaluation, so the study does not allow evaluation of security, immunogenicity or protection provided by vaccination.

A total of 487 stool samples were tested for RV and NoV using commercial immunoenzymatic assays (Premier™ Rotaclone®, Meridian Bioscience Inc., USA, and Norwalk-like virus®, R-Biopharm AG, Germany, respectively), performed according to the manufacturer's instructions. The RV double-stranded RNA (dsRNA) profiles of fecal samples were analyzed by silver-stained polyacrylamide gel electrophoresis (PAGE).¹⁸

NoV single-stranded RNA and RV dsRNA were extracted with Trizol® reagent (Invitrogen, USA), according to the manufacturer's instructions. A reverse transcriptase polymerase chain reaction (RT-PCR) for RV and NoV was performed, according to the protocol previously described.^{12,19-21}

Five RV samples were selected for sequencing in order to obtain additional sequence data and to gain insight into the variability of Brazilian strains. The RT-PCR products of the VP7 gene were purified with PureLink™ purification kit (Invitrogen, USA). Cycle sequencing was carried out using the BigDye™ kit, version 3.1 (Applied Biosystems Inc., USA) with primers Anti-A1 and RVG9.^{19,21} Dye-labeled products were run on an automated sequence analyzer (ABI Prism 377 [Applied Biosystems Inc., USA]) and analyzed with DNASTAR software. Nucleotide sequences were edited and aligned with the BioEdit sequence alignment editor.

The sequences were compared with other sequences available in the GenBank database. The distance tree was constructed with the Clustal method. The sequences determined in this study were deposited in the GenBank database under the following accession numbers: R1114 (HM855236), R1112 (HM855237), R1103 (HM855238), R1102 (HM855239), and R1143 (HM855240).

Results

RV infection was detected in 144 (29.6%) out of 487 specimens. Based on the migration pattern on PAGE, one negative sample for immunoenzymatic assay showed a typical group C RV profile, confirmed by RT-PCR for VP6 gene. RV genotype distribution showed a different profile for each year. The predominant genotypes were G9P[8] (67.8%; 21/31) in 2004, G1P[8] (50%; 14/28) in 2005,

G9P[8] (39.5%; 9/23) in 2006, G2P[4] (100%; 16/16) in 2007, G2P[4] (100%; 31/31) in 2008, and G2P[4] (75%; 3/4) in 2009. Mixed infections (G1+G9P[8]) were observed in 2005 and 2006. The detection rate declined from 36.3% (33/91) in 2004 to 4.2% (4/95) in 2009 (Table 1).

Figure 1 shows the relationship between the VP7 sequences of this study (one G1 strain [R1143], one G2 strain [R1114], and three G9 strains [R1102, R1103, R1112]) and representative strains of genotypes G1 (Wa, M21843), G2 (KO2, AF401754), and G9 (Mc345, D38055). Comparison of the G9 sequences showed 94.6% similarity when compared to Mc345 and 99.7 to 100% of similarity among them. Comparison of the G1 sequence showed 91.2% similarity when compared to Wa, and the G2 sequence showed 98% similarity when compared to KO2.

NoV infection was detected in 26 (29.2%) out of 89 samples, using immunoenzymatic and/or RT-PCR assays. Sixteen (16/26; 61.6%) samples were identified as NoV genogroup GII, seven (7/26; 26.9%) as GI, and three (3/26; 11.5%) as GI+GII. Mixed NoV-RV infection was detected in 2.2% (2/89) of the samples, involving GI+G9P[8] and GI+G2P[4] strains.

Discussion

RV is a major causative agent of pediatric gastroenteritis throughout the world; however, with the improved molecular diagnostic techniques, the importance of NoVs is becoming

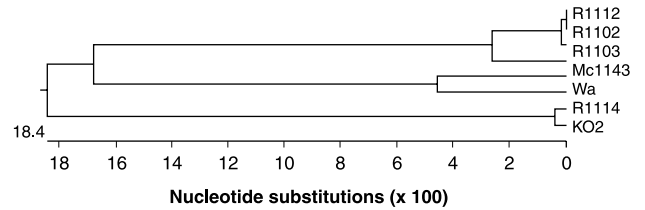


Figure 1 - Dendrogram indicating similarity of nucleotide sequences of human rotavirus strains genotypes G1 (R1143), G2 (R114), and G9 (R1112, R1102, R1103) from medical center children ≤ 5 years with gastroenteritis in the state of São Paulo, Brazil, compared to Wa (G1), KO2 (G2), and Mc345 (G9) strains from GenBank. The scale indicates the number of divergent nucleotide residues

more apparent, and the number of these viruses associated with gastroenteritis continues to increase.⁸ In this study, RV and NoV showed similar association with gastroenteritis requiring hospitalization in children; nevertheless, similar reports usually show NoV as the second cause of hospitalization after RV.^{14,15}

RV genotypes G9 and G1 were the most frequent circulating from 2004 to 2006, corresponding to the results of numerous studies focusing RV G type distribution in many countries, including Brazil.^{9,22-25} The VP7 genes of G9 strains showed identity, indicating that the VP7 genes have the same ancestry.

Table 1 - RV genotyping results of medical center children ≤ 5 years with gastroenteritis in the state of São Paulo, Brazil, 2004-2009

| RV genotype | Year | | | | | |
|----------------------------|---------------|---------------|---------------|---------------|---------------|---------------|
| | 2004 % (n) | 2005 % (n) | 2006 % (n) | 2007 % (n) | 2008 % (n) | 2009 % (n) |
| G1P[4] | - | - | 4.3 (1/23) | - | - | - |
| G1P[8] | 29 (9/31) | 50 (14/28) | 13 (3/23) | - | - | - |
| G2P[4] | - | 3.6 (1/28) | 26 (6/23) | 100 (16/16) | 100 (31/31) | 75 (3/4) |
| G3P[8] | - | - | 4.3 (1/23) | - | - | - |
| G4P[4] | 3.2 (1/31) | - | - | - | - | - |
| G9P[4] | - | 3.6 (1/28) | 4.3 (1/23) | - | - | - |
| G9P[8] | 67.8 (21/31) | 39.2 (11/28) | 39.5 (9/23) | - | - | 25 (1/4) |
| G1+G9P[8] | - | 3.6 (1/28) | 4.3 (1/23) | - | - | - |
| GNTp[8] | - | - | 4.3 (1/23) | - | - | - |
| Total of genotyped samples | 34.1 (31/91) | 36.8 (28/76) | 41.8 (23) | 27.6 (16/58) | 27.7 (31/112) | 4.2 (4/95) |
| PCR negative | 6.1 (2/33) | 9.7 (3/31) | 8.0 (2) | 11.1 (2/18) | 6.1 (2/33) | - |
| Total RV ELISA positive | 36.3 (33/91) | 42.1 (31/76) | 45.5 (25) | 31 (18/58) | 29.5 (33/112) | 4.2 (4/95) |
| Group C RV | - | - | - | - | 0.9 (1/112) | - |
| Total of samples | 91 | 76 | 55 | 58 | 112 | 95 |

NT = non-typeable results; PCR = polymerase chain reaction; RV = rotavirus.

The detection of G2 began to increase in 2006, and this genotype displaced G1 and G9 as the most prevalent during 2007, 2008 and 2009 seasons. Recently, a high prevalence of G2P[4] was reported in Brazil and linked with a universal RV vaccination program using a G1P[8] live oral RV vaccine,^{8,26} suggesting that this monovalent vaccine possibly created conditions in which G2P[4] could acquire selective advantage over P[8] genotypes. Nevertheless, a temporal periodicity, within ~ 10-year cyclic pattern of G2P[4], has been observed in Brazil,²² and should have been considered as an alternative explanation to the increased detection of this genotype since 2006. G2P[4] genotype was also the most prevalent RV type detected during 2007 in northwest Portugal, a population naïve for RV vaccine,²⁷ and in other studies conducted in non-vaccinated populations.^{28,29}

As for pre- and post-vaccination periods, the changes in genotype distribution found are accompanied by a reduction in the detection rate of RV. In fact, this reduction was observed in the number of general notified RV outbreaks in the state of São Paulo, Brazil, which progressively decreased from 35 (10,481 cases) in 2004; 24 (3,144 cases) in 2005; 35 (2,084 cases) in 2006; eight (164 cases) in 2007; one (three cases) in 2008; to none in 2009.³⁰

The frequency of NoV infection in children detected in this work (29.2%) was lower than that observed in a study carried out in Germany (35%), but is similar to the frequency found in Finland during the period of 2001 to 2002 (29%).¹⁴ Previous studies conducted in different states of Brazil showed that NoV prevalence among hospitalized children ranges from 9 to 36%.^{11,31-33}

The data concerning hospitalized pediatric patients in Italy,³⁴ Germany,¹⁴ and Brazil^{2,33} have demonstrated the prevalence of NoV genogroup GII. This study revealed a preponderance of GII, confirming that this specific genogroup is the most frequently associated with NoV illnesses worldwide compared to GI.^{12,14} The epidemiological issue of this finding is still unclear; studies on possible differences in biological properties, such as virulence, transmission routes, or stability of the virus in the environment, might provide explanations.¹⁴

In this study, coinfections have occurred among NoV GI and RV G9/G2. Dual infections involving enteric viruses are relatively common in cases of acute gastroenteritis, indicating a high rate of exposure to a variety of enteric pathogens.¹⁴ However, few reports concerning NoV-RV coinfection are available: 1% in Japan,¹⁵ 2.1% in Germany,¹⁴ and 1.9% in Italy.³⁴ In Brazil, Victoria et al. reported 4% of dual infection due to RV and NoV in hospitalized children in the state of Rio de Janeiro, Brazil.³³ Besides the diversity of viruses and genotypes circulating in the communities, the presence of mixed infections should be expected at higher levels; nevertheless, it seems to be lower in the Brazilian population.

RV vaccine introduction is likely to have been the major influence for the decreasing trends in gastroenteritis hospitalizations since 2006.³⁵ The frequency of NoV infection indicates that NoV is an important factor in the viral etiology of pediatric acute gastroenteritis in the state of São Paulo. A better understanding of the epidemiology of NoV infections will be necessary to assess their role among Brazilian children, and to monitor the impact of RV vaccination program.

References

- Vernacchio L, Vezina RM, Mitchell AA, Lesko SM, Plaut AG, Acheson DW. *Diarrhea in American and young children in the community setting: incidence, clinical presentation and microbiology*. *Pediatr Infect Dis J*. 2006;25:2-7.
- Xavier MP, Oliveira SA, Ferreira MS, Victoria M, Miranda V, Silva MF, et al. *Detection of calicivirus associated with acute infantile gastroenteritis in Salvador, an urban center in Northeast Brazil*. *Braz J Med Biol Res*. 2009;42:438-44.
- Parashar UD, Gibson CJ, Bresse JS, Glass RI. *Rotavirus and severe childhood diarrhea*. *Emerg Infect Dis*. 2006;12:304-6.
- Afonso A, Antunes H. *Infecção por rotavírus: implicações e custos*. *Acta Pediatr Port*. 2007;38:138-43.
- Sartori AM, Valentim J, de Soárez PC, Novaes HM. *Rotavirus morbidity and mortality in children in Brazil*. *Rev Panam Salud Publica*. 2008;23:92-100.
- Gurgel RQ, Correia JB, Cuevas LE. *Effect of rotavirus vaccination on circulating virus strains*. *Lancet*. 2008;371:301-2.
- Araujo EC, Clemens SA, Oliveira CS, Justino MC, Rubio P, Gabbay YB, et al. *Safety, immunogenicity, and protective efficacy of two doses of RIX4414 live attenuated human rotavirus vaccine in healthy infants*. *J Pediatr (Rio J)*. 2007;83:217-24.
- Nakagomi T, Cuervas LE, Gurgel RG, Elrokhsi SH, Belkhir YA, Abugalia M, et al. *Apparent extinction of non-G2 rotavirus strains from circulation in Recife, Brazil, after the introduction of rotavirus vaccine*. *Arch Virol*. 2008;153:591-3.
- Morillo SG, Luchs A, Cilli A, Costa FF, Carmona Rde C, Timenetsky Mdo C. *Characterization of rotavirus strains from day care centers: pre- and post-rotavirus vaccine era*. *J Pediatr (Rio J)*. 2010;86:155-8.
- Bernstein DI. *RIX4414 (Rotarix): a live attenuated human rotavirus vaccine*. *J Pediatr (Rio J)*. 2007;83:193-5.
- Kirkwood CD, Bishop RF. *Molecular detection of human Calicivirus in young children hospitalized with acute gastroenteritis in Melbourne, Australia, during 1999*. *J Clin Microbiol*. 2001;39:2722-4.
- Morillo SG, Cilli A, Carmona R de C, Timenetsky, M do C. *Identification and molecular characterization of norovirus in São Paulo State, Brazil*. *Braz J Microbiol*. 2008;39:619-22.
- Morillo SG, Luchs A, Cilli A, Ribeiro CD, Calux SJ, Carmona R de C, Timenetsky M do C. *Norovirus 3rd Generation kit: an improvement for rapid diagnosis of sporadic gastroenteritis cases and valuable for outbreak detection*. *J Virol Methods*. 2011;173:13-6.
- Oh DY, Gaedicke G, Schreier E. *Viral agents of acute gastroenteritis in German children: prevalence and molecular diversity*. *J Med Virol*. 2003;71:82-93.
- Nakanishi K, Tsugawa T, Honma S, Nakata S, Tatsumi M, Yoto Y, et al. *Detection of enteric viruses in rectal swabs from children with acute gastroenteritis attending the pediatric outpatient clinics in Sapporo, Japan*. *J Clin Virol*. 2009;46:94-7.
- Morillo SG, Luchs A, Cilli A, Ribeiro CD, Calux SJ, Carmona Rde C, et al. *Large gastroenteritis outbreak due to norovirus GII in São Paulo, Brazil, summer 2010*. *Rev Inst Med Trop São Paulo*. 2011;53:119-20.

17. Junquera CG, de Baranda CS, Mialdea OG, Serrano EB, Sánchez-Fauquier A. [Prevalence and clinical characteristics of norovirus gastroenteritis among hospitalized children in Spain](#). *Pediatr Infect Dis J*. 2009;28:604-7.
18. Pereira HG, Azevedo RS, Leite JP, Barth OM, Suttmoller F, de Farias V, et al. Comparison of polyacrylamide gel electrophoresis (PAGE), immuno-electron microscopy (IEM) and enzyme immunoassay (EIA) for the rapid diagnosis of rotavirus infection in children. *Mem Inst Oswaldo Cruz*. 1983;78:483-90.
19. Timenetsky Mdo C, Gouvea V, Santos N, Carmona RC, Hoshino Y. [A novel human rotavirus serotype with dual G5-G11 specificity](#). *J Gen Virol*. 1997;78:1373-8.
20. Gouvea V, Allen JR, Glass RI, Fang ZY, Bremont M, Cohen J, et al. [Detection of group B and C rotaviruses by polymerase chain reaction](#). *J Clin Microbiol*. 1991;29:519-23.
21. Gentsch JR, Glass RI, Woods P, Gouvea V, Gorziglia M, Flores J, et al. [Identification of group A rotavirus gene 4 types by polymerase chain reaction](#). *J Clin Microbiol*. 1992;30:1365-73.
22. Carvalho-Costa FA, Assis RM, Fialho AM, Bóia MN, Alves DP, Martins CM, et al. Detection and molecular characterization of group A rotavirus from hospitalized children in Rio de Janeiro, Brazil, 2004. *Mem Inst Oswaldo Cruz*. 2006;101:291-4.
23. Bányai K, Gentsch JR, Schipp R, Jakab F, Meleg E, Mihály I, et al. Dominating prevalence of P[8], G1 and P[8], G9 rotavirus strains among children admitted to hospital between 2000 and 2003 in Budapest, Hungary. *J Med Virol*. 2005;76:414-23.
24. Carmona RC, Timenetsky Mdo C, Morillo SG, Richtzenhain LJ. [Human rotavirus serotype G9, São Paulo, Brazil, 1996-2003](#). *Emerg Infect Dis*. 2006;12:963-8.
25. Nunes AA, de Mello LM, Parrode RN, Bittar JP, Domingues AL. [Prevalence of rotavirus in acute diarrhea and its association with clinical signs and symptoms](#). *J Trop Pediatr*. 2010;56:212-3.
26. Timenetsky M do C, Carmona R de C, Luchs A, Russo DH, Morillo S, Cilli A, et al. Prevalence of rotavirus genotype G2P4 in Brazil after the rotavirus vaccine introduction. American Society for Virology. 27th Annual Meeting. Cornell University. New York: Ithaca; 2008, 263 p.
27. Antunes H, Afonso A, Iturriza M, Martinho I, Ribeiro C, Rocha S, et al. [G2P\[4\] the most prevalent rotavirus genotype in 2007 winter season in an European non-vaccinated population](#). *J Clin Virol*. 2009;45:76-8.
28. Desselberger U, Wolleswinkel-van den Bosch J, Mrukowicz J, Rodrigo C, Giaquinto C, Vesikari T. [Rotavirus types in Europe and their significance for vaccination](#). *Pediatr Infect Dis J*. 2006;25: S30-41.
29. Ferrera A, Quan D, Espinoza F. Increased prevalence of genotype G2P(4) among children with rotavirus-associated gastroenteritis in Honduras. 17th European Congress of Clinical Microbiology and Infectious Diseases ICC; 2007 Mar 31-Apr 04; Munich. Hoboken (NJ): Wiley-Blackwell; 2007.
30. CVE [Internet]. São Paulo: Centro de Vigilância Epidemiológica. Available from: http://www.cve.saude.sp.gov.br/hm/hidrica/hidri_estat.html. Access: 20/03/2011.
31. Castilho JG, Munford V, Resque HR, Fagundes-Neto U, Vinjé J, Rácz ML. [Genetic diversity of norovirus among children with gastroenteritis in São Paulo State, Brazil](#). *J Clin Microbiol*. 2006;44:3947-53.
32. Borges AM, Teixeira JM, Costa PS, Giugliano LG, Fiaccadori FS, Franco Rde C, et al. Detection of calicivirus from fecal samples from children with acute gastroenteritis in the West Central region of Brazil. *Mem Inst Oswaldo Cruz*. 2006;101:721-4.
33. Victoria M, Carvalho-Costa FA, Heinemann MB, Leite JP, Miagostovich M. [Prevalence and molecular epidemiology of noroviruses in hospitalized children with acute gastroenteritis in Rio de Janeiro, Brazil, 2004](#). *Pediatr Infect Dis J*. 2007;26:602-6.
34. Soares CC, Santos N, Beard RS, Albuquerque MC, Maranhão AG, Rocha LN, et al. [Norovirus detection and genotyping for children with gastroenteritis, Brazil](#). *Emerg Infect Dis*. 2007;13:1244-6.
35. Lanzeri TM, Costa I, Shafi FA, Cunha MH, Ortega-Barria E, Linhares AC, et al. [Trends in hospitalizations from all-cause gastroenteritis in children younger than 5 years of age in Brazil before and after human rotavirus vaccine introduction, 1998-2007](#). *Pediatr Infect Dis J*. 2010;29:673-5.

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