

## Characterization of rotavirus strains from day care centers: pre- and post-rotavirus vaccine era

Simone G. Morillo<sup>1</sup>, Adriana Luchs<sup>1</sup>, Audrey Cilli<sup>2</sup>, Fernanda F. Costa<sup>2</sup>,  
Rita de Cassia C. Carmona<sup>1</sup>, Maria do Carmo S. T. Timenetsky<sup>3</sup>

### Abstract

**Objectives:** In 2006 the rotavirus vaccine was included in the Brazilian Immunization Program. The aim of this study was to report the results of a 5-year surveillance study of rotavirus strains in children < 5 years with acute gastroenteritis from day care centers in the state of São Paulo, Brazil.

**Methods:** This retrospective study was conducted with 30 day care centers from 2004 to 2008 with convenient surveillance fecal specimens, investigated by ELISA, SDS-PAGE, RT-PCR and gene sequencing to genotype characterization.

**Results:** Rotavirus infection was detected in 28.3% of samples (38/134). The most frequent genotypes detected were G9P[8] and G1P[8] in 2004; G1P[8] in 2005; GNT[NT] in 2006; G2P[4] in 2007; and there were no cases in 2008. Mixed infections were not observed. Detection rate declined from 65.7% (23/35) in 2004 to 50% (9/18) in 2007.

**Conclusions:** Genotype distribution varied according to collection year, accompanied by a reduction in detection rate. Use of rotavirus vaccine requires implementation of post-marketing surveillance to monitor rotavirus strain diversity and its efficacy against possible new emerging genotypes.

*J Pediatr (Rio J). 2010;86(2):155-158: Rotavirus, day care centers, gastroenteritis.*

### Introduction

Viral pathogens are the most common causes of gastroenteritis in communities and other settings, including semi-closed institutions and hospitals.<sup>1</sup> In infancy, group A rotavirus (RVA) are considered to be the most important etiological agent of acute non-bacterial gastroenteritis, including outbreaks and sporadic cases, independent of improvements in basic sanitation and hygiene procedures.<sup>2</sup> RVA are the major etiologic agent of acute diarrhea in

children, responsible for more than 600,000 deaths each year,<sup>3</sup> in addition to the significant economic burden of RVA disease.<sup>4</sup>

In 2006 an attenuated G1P[8] vaccine was included in the Brazilian Immunization Program, preventing severe rotavirus gastroenteritis, and inducing significant reduction in the frequency of RVA detection in children with gastroenteritis.<sup>5</sup> In fact, the RIX4414 vaccine efficacy was evaluated by Araujo

1. Msc. Instituto Adolfo Lutz, Enteric Virus Laboratory, São Paulo, SP, Brazil.
2. Instituto Adolfo Lutz, Enteric Virus Laboratory, São Paulo, SP, Brazil.
3. PhD. Instituto Adolfo Lutz, Enteric Virus Laboratory, São Paulo, SP, Brazil.

Conflicts of interest: Timenetsky MC was the principal investigator during the RIX4414 vaccine in São Paulo, SP, Brazil. Instituto Adolfo Lutz, Secretaria de Vigilância em Saúde and Ministério da Saúde, in a preestablished deal with GSK, received research grants only for expenses with staff, equipment and inputs.

Financial support: Instituto Adolfo Lutz, Department of Health, São Paulo, SP, Brazil.

**Suggested citation:** Morillo SG, Luchs A, Cilli A, Costa FF, Carmona RC, Timenetsky MC. Characterization of rotavirus strains from day care centers: pre- and post-rotavirus vaccine era. *J Pediatr (Rio J)*. 2010;86(2):155-158.

Manuscript submitted Sep 21 2009, accepted for publication Dec 23 2009.

doi:10.2223/JPED.1981

et al.,<sup>6</sup> showing 53.9 to 81.5% of protection against severe cases, and 81.2 to 93.0% of protection against hospitalization due to rotavirus gastroenteritis in children.

Recently, a high prevalence of G2P[4] was reported in Brazil and linked with this vaccination,<sup>7</sup> suggesting that this monovalent vaccine possibly created conditions in which G2P[4] could acquire selective advantage over P[8] genotypes. More detailed investigations concerning the heterologous protection conferred by rotavirus monovalent vaccine are key points to understand its immunogenic behavior.<sup>6,8</sup>

The aim of this study was to report the results of a 5-year surveillance study of rotavirus strains in children < 5 years with acute gastroenteritis from day care center in the state of São Paulo, Brazil, and to evaluate detection trends.

## Methods

This retrospective study was conducted with 30 day care centers from 2004 to 2008 with convenient surveillance specimens collected from children under 5 years presenting acute gastroenteritis. Stool samples from patients with acute gastroenteritis were sent to the Enteric Virus Laboratory of Instituto Adolfo Lutz (IAL), a regional reference center for rotavirus surveillance and a member of the Acute Diarrheic 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 retrospective sampling, without inclusion or exclusion criteria, with no characterization of the participating day care centers; therefore, the study may not be representative of the actual epidemiological scenario. A molecular characterization of rotavirus genotypes pre- and post-Rotarix® vaccination was performed. It did not include clinical evaluation, so the study does not allow evaluation

of security, immunogenicity or protection provided by vaccination.

A total of 134 fecal samples were tested for RVA using a commercial immunoenzymatic assay (Premier™ Rotaclone®, Meridian Bioscience Inc., USA), performed according to the manufacturer's instructions, and polyacrylamide gel electrophoresis (PAGE). Rotavirus-positive stools samples were typed after reverse transcription followed by nested PCR and sequencing with BigDye™ on an ABI 377 (Applied Biosystems Inc., USA), analyzed with DNASTAR software.

## Results

Rotavirus infection was detected in 28.3% (38/134), and all samples belonged to group A, based on the migration pattern on PAGE. Genotype distribution showed a different profile for each year: 28.5% G9P[8] (10/35), 14.2% G1P[8] (5/35) and 20% GNTP[NT] (7/35) in 2004; 5.4% G1P[8] (2/37) in 2005; 9% GNTP[NT] (4/44) in 2006; 11.1% G2P[4] (2/8) and 38.8% GNTP[NT] (7/18) in 2007. Mixed infections were not observed. Detection rate declined from 65.7% (23/35) in 2004 to 50% (9/18) in 2007, with no cases in 2008 (Table 1).

The relationship between the VP7 sequences of seven G9 strains (R792, R840, R848, R863, R865, R866, R869), one G1 strain (R788), and a representative strain of the G9 genotype (Mc345) and G1 genotype (Wa) was shown as a distance tree constructed using the Clustal method. Comparison of the G9 sequences showed 94.9% similarity when compared to Mc345 and 95.6 to 99.8% of similarity among them. Comparison of the G1 sequence showed 90.3% similarity when compared to Wa.

## Discussion

Our data show the presence of RVA G9P[8], G2P[4], and G1P[8] in agreement with the reported worldwide prevalence

**Table 1** - Overall genotyping results from day care center children with acute diarrhea in the state of São Paulo, Brazil, from 2004 to 2008: pre- and post-rotavirus vaccine era

Year	Total of samples	Number of positive	Rotavirus by genotype (%)			
			G9P[8]	G1P[8]	G2P[4]	NT
2004	35	22	28.5	14.2	0	20
2005	37	2	0	5.4	0	0
2006	44	4	0	0	0	9
2007	18	9	0	0	11	38.8
2008	0	0	0	0	0	0

NT = non-typeable results.

of these three genotypes.<sup>9</sup> As expected, G9 was the most frequent genotype circulating during 2004. This genotype has been previously detected in Brazil, in agreement with global emergence.<sup>10,11</sup> G1 was the second most common G type found, corresponding to the results of numerous studies focusing RVA G type distribution in many countries, including Brazil.<sup>9,11</sup>

The G2P[4] genotype was the only strain observed during 2007. Alternatively to the proposition that the RIX4414 vaccine created conditions in which G2P[4] could have acquired selective advantage, a temporal periodicity, within ~ 10-year cyclic pattern of G2P[4] has been observed in Brazil,<sup>11</sup> and should be considered as an alternative explanation to the increased detection of this genotype since 2006. Although such periodicity could be related to RVA vaccine introduction,<sup>11</sup> G2P[4] genotype was also the most prevalent RVA type detected during 2007 in northwest Portugal, a population naïve for RVA vaccine.<sup>12</sup> Therefore, at least in some settings, this prevalence cannot be explained by the introduction of vaccination and may reflect normal fluctuation of co-circulation RVA genotypes, which is in line with reports from other countries and areas, in non-vaccinated population, showing exactly the same,<sup>13</sup> including South America.<sup>14</sup>

Non-typeable RVA strains have been reported in almost every epidemiological survey around the world, regardless of the methodology employed. In our study, during 2006 any RVA positive samples from day care centers could be genotyped by multiplex RT-PCR, whereas almost all RVA positive samples of ADDMP from others settings showed G2P[4] specificity. Not only for epidemiological rotavirus strain surveillance but also for effective rotavirus vaccine development, there is a need for sensitive and reliable diagnostic techniques, such as the oligonucleotide microarray hybridization method.<sup>15</sup> Combining the sensitivity of PCR and the specificity of hybridization, it successfully detects and unambiguously identifies such G genotypes.

As for pre- and post-vaccination periods, the changes in genotype distribution found are accompanied by a reduction in the detection rate of RVA from day care center samples sent to IAL starting with 65.7% (23/35) in 2004 toward to 50% (9/18) in 2007, and no cases in 2008. In fact, this reduction was observed in the number of general RVA outbreaks in the state of São Paulo from 35 (10,481 cases) in 2004; 24 (3,144 cases) in 2005; 35 (2,084 cases) in 2006; eight (164 cases) in 2007; to one (three cases) in 2008.<sup>16</sup> On the other hand, analyses of RVA outbreaks of previous year's surveillances<sup>17</sup> suggest a more variable pattern, with a natural fluctuation of RVA cases.

As for other vaccines, implementation of post-marketing surveillance is recommended, especially because diverse RVA strains co-circulate in the human population. It is important not only to monitor RVA diversity, but also its

efficacy, since new emerging genotypes may not share capsid antigen with the vaccine virus. More studies concerning the ecology of rotavirus infections will be important to better understand whether the reduction in RVA detection rate observed in the past few years is a direct consequence of the vaccine implementation or only a cycle pattern.

## 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.
- 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 Brazilian infants*. *J Pediatr (Rio J)*. 2007;83:217-24.
- Nakagomi T, Cuevas LE, Gurgel RG, Elrokhsi SH, Belkhir YA, Abugalia M, et al. *Apparent extinction of non-G2 rotavirus strains from circulation in Recife, Brazil, after introduction of rotavirus vaccine*. *Arch Virol*. 2008;153:591-3.
- Bernstein DI. *RIX4414 (Rotarix): a live attenuated human rotavirus vaccine*. *J Pediatr (Rio J)*. 2007; 83:193-5.
- 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.
- 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.
- 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.
- 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.
- Desselberger U, Wolleswinkel-van den Bosch J, Mrukowicz J, Rodrigo C, Giaquino C, Vesikari T. *Rotavirus types in Europe and their significance for vaccination*. *Pediatr Infect Dis J*. 2006;25: S30-41.
- 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.
- Honma S, Chizhikov V, Santos N, Tatsumi M, Timenetsky MC, Linhares AC, et al. *Development and validation of DNA microarray for genotyping group A rotavirus VP4 (P[4], P[6], P[8], P[9], and P[14]) and VP7 (G1 to G6, G8 to G10, and G12) genes*. *J Clin Microbiol*. 2007;45:2641-8.

16. CVE [website]. São Paulo: Centro de Vigilância Epidemiológica. [http://www.cve.saude.sp.gov.br/htm/hidrica/rotavirus\\_dados.html](http://www.cve.saude.sp.gov.br/htm/hidrica/rotavirus_dados.html). Access: 17/09/2009.
17. CVE [website]. São Paulo: Centro de Vigilância Epidemiológica. [http://www.cve.saude.sp.gov.br/htm/hidrica/hidri\\_estat.html](http://www.cve.saude.sp.gov.br/htm/hidrica/hidri_estat.html). Access: 20/09/2009.

Correspondence:  
Simone Guadagnucci Morillo  
Instituto Adolfo Lutz, Departamento de Virologia,  
Laboratório de Vírus Entéricos  
Avenida Dr Arnaldo, 355  
CEP 01246-902 - São Paulo, SP - Brazil  
Tel.: +55 (11) 3068.2909  
Fax: +55 (11) 3085.3505  
E-mail: sgmorillo@gmail.com