Journal of Medical Virology 86:1065–1072 (2014)

Diversity of Rotavirus Strains Circulating in Northern Brazil After Introduction of a Rotavirus Vaccine: High Prevalence of G3P[6] Genotype

Luana da Silva Soares,* Sylvia de Fátima dos Santos Guerra, Alessilva do Socorro Lima de Oliveira, Fabiola da Silva dos Santos, Euzeni Maria de Fátima Costa de Menezes, Joana d'Arc Pereira Mascarenhas, and Alexandre C. Linhares

Virology Section, Evandro Chagas Institute, Health Surveillance Secretariat, Brazilian Ministry of Health, Brazil

Rotavirus A (RVA) is the most common cause of severe acute gastroenteritis in infants and young children worldwide, causing 453,000 deaths annually. In Brazil, the most frequent genotype identified was G1 during almost three decades in the pre-vaccination period; however, after anti-rotavirus vaccine introduction, there was a predominance of G2 genotype. The aim of this study was to determine the G and P genotypes of rotaviruses isolated from children under 5 years of age with acute gastroenteritis in the Northern region of Brazil, and discuss the emergence of G3P[6] genotype. A total of 783 stool specimens were obtained between January 2011 and March 2012. RVA antigen was detected in 33% (272/783) of samples using a commercial enzyme-linked immunosorbent assay and type-specificity was determined by reverse-transcription polymerase chain reaction. The most common binary combination was G2P[4], representing 41% of cases, followed by G3P[6] (15%), G1P[8] (8%), G3P[8] (4%), G9P[8] (3%), and G12P[6] (2%). G3P[6] strains were analyzed further and phylogenetic analysis of VP7 gene showed that G3 strains clustered into lineage I and showed a high degree of amino acid identity with vaccine strain RV3 (95.1–95.6%). For VP4 sequences, G3P[6] clustered into lineage Ia. It was demonstrated by the first time the emergence of unusual genotype G3P[6] in the Amazon region of Brazil. This genotype shares neither VP7 nor VP4 specificity with the used vaccine and may represent a challenge to vaccination strategies. A continuous monitoring of circulating strains is therefore needed during the post-vaccine era in Brazil. ***J. Med. Virol. 86: 1065–1072, 2014.***

KEY WORDS: diarrhea; Rotarix^{TM}; G3P[6] rotavirus A

INTRODUCTION

Rotavirus A (RVA) is the most common cause of severe acute gastroenteritis in infants and young children worldwide being responsible for 453,000 deaths annually [Tate et al., 2012]. In Brazil, there were approximately 3.5 million cases, 650,000 clinic visits, 100,000 hospitalizations, and 850 deaths caused by rotavirus gastroenteritis each year during the pre-vaccine era [Sartori et al., 2008; Dennehy, 2012].

Currently, two oral RVA vaccines are licensed and widely available: Rotarix^{TM} (GlaxoSmithKline, Rixensart, Belgium) and Rotateq^{TM} (Merck Research, Whitehouse Station, NJ). These vaccines were found to be efficacious against severe rotavirus disease and demonstrated substantial reductions in childhood morbidity and mortality in middle and low-income countries [Munos et al., 2010; Lanzieri et al., 2011; O'Ryan et al., 2011; Dennehy, 2012]. In 2009, the World Health Organization (WHO) recommended the inclusion of rotavirus vaccination into national immunization programs and recently reinforced its implementation as a priority [WHO, 2009, 2013].

Authors' Contributions: L.S.S. was responsible for molecular genetic studies, sequence analysis, and drafted the manuscript. S.F.S.G., A.S.L.O., F.S.S. carried out molecular genetic studies. E.M.F.C.M. contribution to the laboratory methods. J.D.P.M. supervised laboratory work and training. Involved in analysis and provided critical review of manuscript. A.C.L. supervised laboratory work. Contributed to study design and review final versions manuscript. All authors read and approved the final manuscript.

*Correspondence to: Luana da Silva Soares, Seção de Virologia, Instituto Evandro Chagas, Secretaria de Vigilância em Saúde, Ministério da Saúde, Rodovia BR 316-KM 07, S/N, Levilândia, 67030-000 Ananindeua, Pará, Brazil.
E-mail: luanasoares@iec.pa.gov.br

Accepted 29 August 2013

DOI 10.1002/jmv.23797
Published online 17 October 2013 in Wiley Online Library (wileyonlinelibrary.com).

Rotaviruses on classified into 8 major species (A–H) but most human strains belong to group A, although groups B and C have occasionally been associated with human illness [Estes and Kapikian, 2007; Matthijnssens et al., 2012]. Based on the two outer capsid proteins, VP7 and VP4, RVA are classified into 27 G and 37 P types, respectively [Matthijnssens et al., 2011; Trojnar et al., 2013]. Several surveillance and epidemiologic studies have been conducted around the world and the most common strains are G1P[8], G2P[4], G3P[8], G4P[8], and G9P[8], representing approximately 90% of the human RVA strains. Recently, G12 emerged associated with either P[8] or P[6], which is considered the sixth most common global genotype [Santos and Hoshino, 2005; Rahman et al., 2007; Matthijnssens et al., 2010].

In Brazil, the most frequent genotype identified was G1 during almost three decades in the pre-vaccination period, however, after anti-rotavirus vaccine introduction there was a predominance of G2 genotype with a frequency in the average of 74%; however in recent years it was observed a trend for continuous decline of this genotype [Leite et al., 2008; Carvalho-Costa et al., 2011; Oliveira et al., 2012; Soares et al., 2012]. Although rotavirus G3 is an usual genotype in the Northern region of Brazil it has rarely been detected in sporadic cases of gastroenteritis in humans, mostly in combination with P[8] [Oliveira et al., 2012; Soares et al., 2012].

Rotavirus G3 has been detected in several animal hosts, such as cats, dogs, pigs, and birds, mostly combined with P[3] and P[9] VP4 genes and recently a study showed that G3 rotavirus may be associated with severe diarrhea [Martínez-Laso et al., 2009; Martella et al., 2010; Grant et al., 2011; González and Rivero, 2013]. P[6] is in general associated with neonatal rotavirus infections with a wide variety of G-types [Martella et al., 2006; Mascarenhas et al., 2007; Stupka et al., 2009; Lorenzetti et al., 2011; Nordgren et al., 2012].

Recently it has been proposed a new rotavirus classification system based on the molecular characteristics of the 11 genes to achieve a better understanding of the function of each protein and of the evolutionary relationship among species [Matthijnssens et al., 2011]. Three genotype constellations of the non-G and non-P genes have been shown to circulate worldwide among humans: I1-R1-C1-M1-A1-N1-T1-E1-H1 (Wa-like); I2-R2-C2-M2-A2-N2-T2-E2-H2 (DS-1-like); and I3-R3-C3-M3-A3-N3-T3-E3-H3 (AU-1) [Matthijnssens and Van Ranst, 2012]. G3P[6] genotype has been described as a rotavirus with Wa-like genotype constellation and very closely related to attenuated RV3 vaccine strain [Rippinger et al., 2010].

The aim of this study was to characterize the G and P genotypes of RVA isolated from children under 5 years of age with acute gastroenteritis in Northern Brazil, between January 2011 and March 2012, arising from a Brazilian Ministry of Health's nationwide surveillance network to monitor circulating strains. In addition, it was focused on the emergence of the G3P[6] strains, which may suggest a reassortment among common human strains. This may theoretically pose a challenge to current rotavirus vaccination strategies.

MATERIALS AND METHODS

Clinical Specimens

The samples from this study were collected from hospitalized children who presented with symptoms of acute gastroenteritis and were selected from six states in the Northern region of Brazil (Table I). During January 2011 to March 2012 a total of 783 samples were collected and an aliquot of each sample was stored at 2–8°C and transported to Instituto Evandro Chagas, a Brazilian Ministry of Health's National Rotavirus Reference Laboratory.

Ethical Considerations

This study was part of an official Brazilian Ministry of Health's surveillance, therefore there was no need for ethical clearance.

Rotavirus Screening and RNA Extraction

All fecal samples were screened for the presence of RVA by a commercially available enzyme-linked immunosorbent (ELISA) assay according to the manufacturer's instructions (Premier Rotaclone, Meridian Bioscience, Cincinatti, OH). The results were determined by absorbance readings. Viral RNA was extracted using guanidinium isothiocyanate-silica method [Boom et al., 1990]. Polyacrylamide gel

TABLE I. RVA-Positivity Associated With Age Group, Brazilian State, Clinical Characteristics, and Vaccination Status

	RV-A positive/tested (%)
Age group (year)	
0–1	134/407 (33.0)
1–2	63/190 (33.2)
2–5	47/106 (44.3)
>5	19/61 (31.1)
Brazilian state	
Acre	87/189 (46.0)
Amazonas	134/470 (28.5)
Amapá	8/21 (38.0)
Pará	15/57 (26.3)
Rondônia	15/21 (71.4)
Roraima	13/25 (52.0)
Clinical characteristics	
Fever	73/239 (30.5)
Vomiting	132/375 (35.2)
Rotavirus vaccination history (Rotarix™)	
Ineligible	22/61 (36.0)
Unknown	163/398 (41.0)
Unvaccinated	20/66 (30.0)
Vaccinated (received at least one dose)	67/258 (26.0)

electrophoresis (PAGE) was carried out in Tris–glycine buffer and the rotavirus genome profile was determined following electrophoresis of extracted dsRNA through vertical 5% acrylamide bisacrylamide gels [Pereira et al., 1983].

RT-PCR and Genotyping

All RVA-positive samples were subjected to reverse transcription-polymerase chain reaction (RT-PCR). First round was performed with consensus primers Beg9/End9 and 4con3/4con2 to amplify VP7 and VP4 genes, respectively. G and P genotyping was performed using seminested type-specific multiplex PCR using specific primers for G (G1, G2, G3, G4, G9, and G12) and P-types (P[4], P[6], P[8], and P[9]), as described previously [Gouvea et al., 1990; Gentsch et al., 1992; Banerjee et al., 2007]. The G and P-types were determined by the specific sizes of the amplicons on agarose gels.

Nucleotide Sequencing and Phylogenetic Analysis

Sequencing of the PCR amplicons for VP7 and VP4 genes of G3 strains were performed using the same primers as those used in the PCR and carried out with a Big Dye Terminator cycle sequencing kit v 3.1 (Applied Biosystems, Foster City, CA). The sequences were collected from an automated ABI Prism 3130*xl* DNA sequencer (Applied Biosystems). Phylogenetic analyses were carried out using MEGA software program version 4.0.1 by the neighbor-joining (NJ) method [Kimura, 1980]. The statistical significance of the genetic relationships was estimated by bootstrap resampling analysis (2,000 replications). The sequences of G3 strains were submitted to GenBank under the accession numbers JX987024–JX987034, JX996189–JX996193, and KC164357– KC164370.

Data Analyses

The frequencies of RVA infection and genotype combinations were calculated using Microsoft Excel software. Comparisons of RVA infection rates in distinct groups were performed using χ^2 test through BioEstat 5.0 [Ayres et al., 2007]. Statistical significance was established at P values <0.05.

RESULTS

Overall samples were screened for RVA antigen by ELISA yielding a positivity of 33% (272/783, range 23–56%). Figure 1 shows the monthly frequencies of RVA detection, with two peaks where RVA rates were over 50%, February and June 2011.

Table I summarizes the RVA positivity associated with major clinical and epidemiologic characteristics of patients. The mean age of patients with rotavirus gastroenteritis was 25 months, with mean age of non-rotavirus acute gastroenteritis of 31 months. A higher RVA positivity (44%) was observed among children aged 2–5 years. Among children who were age-eligible for rotavirus vaccine, 67 (26%, 67/258) were RVA-positive and received Rotarix™, with mean age of 18 months, most of them infected by G2P[4] genotype (46%, 31/67). PAGE was performed in samples, of which 198 (25%) displayed a typical RNA electrophoretic migration pattern. Of these, 41 (21%) and 157 (79%) specimens showed long and short profile, respectively. All G3P[6] rotavirus strains exhibited identical short electropherotypes (RNA pattern not shown).

The most common binary combination was G2P[4], responsible for 41% (106/258) of cases, followed by

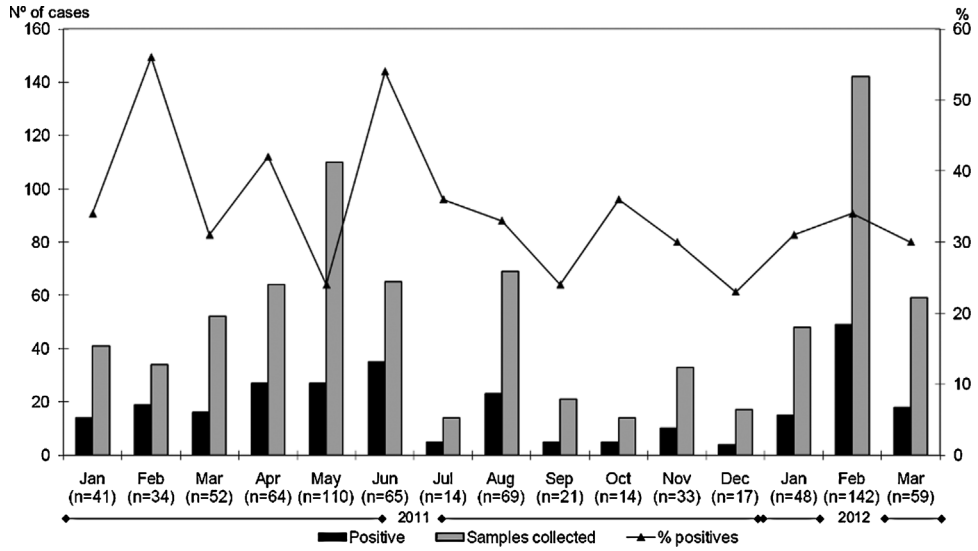

Fig. 1. Temporal distribution of RVA positivity in cases of gastroenteritis in Northern region of Brazil, between January 2011 and March 2012.

G3P[6] (15%, 39/258), G1P[8] (8%, 21/258), G3P[8] (4%, 11/258), G9P[8] (3%, 7/258), and G12P[6] (2%, 5/258). Mixed infections were detected in 31 samples (12%). Forty-one RVA-positive specimens could not be assigned to a specific G or P type. The distribution of G and P RV-A genotypes is shown in Figure 2. It was observed that G2P[4] genotype was identified throughout the study period and G3P[6] genotype was detected initially in November 2011. There was no significant difference ($P = 0.569$) when the rates of G2 genotype were compared among vaccinated and unvaccinated infants.

Sixteen G3 samples, all of which collected during 2012, were subjected to partial sequencing analyses of VP7 and VP4 genes. With regards to VP7 gene, G3 strains formed two distinct groups and clustered into lineage I according with Martínez-Laso et al. [2009]. One group was composed by 14 G3P[6] samples and were highly similar to each other (nt: 99.0–100%) as well as to African samples, ETH44 and BFA, collected in 2009 and 2010, showing with these a nucleotide similarity higher than 99%. Two G3P[8] strains gathered in another cluster with samples from USA, Thailand, Russia, and Spain (nt and aa: 98.5–99.3%). Likewise, a comparison of VP7 sequences of own Brazilian G3 samples clustered with vaccine strain RV3 showing a high degree of amino acid identity (95.1–95.6%; Fig. 3). Brazilian G3 strains showed amino acid substitutions at 96 and 213 positions, both of which from aspartic acid to asparagine, in antigenic regions A and C (data not shown).

With regards to VP4 sequences, G3P[6] clustered into lineage Ia. The degree of nucleotide identity among Brazilian strains was higher than 99% and when compared to the RV3 strain the median of nucleotide identity was 94% (Fig. 4). G3P[8] samples grouped into lineage III (data not shown).

DISCUSSION

In the present study, RVA gastroenteritis was associated with 33% of pediatric inpatients less than 5 years of age, a rate similar to those studies conducted in Latin America countries such as Guatemala, Venezuela, and Chile, where RVA frequency ranged from 20% to 40% [González et al., 2011; Linhares et al., 2011; Cortes et al., 2012; Lucero et al., 2012]. G1P[8] genotype was the most frequent binary combination found before rotavirus vaccine implementation [Santos and Hoshino, 2005; Patton, 2012]. In post-vaccine era, several studies reported the striking increase of G2P[4] circulating strains, mainly in Latin America countries, leading to the hypothesis of vaccine-induced selective pressure. Nevertheless, it cannot be ruled out that such phenomenon just reflect temporal fluctuation of G2P[4] [Gurgel et al., 2007; Linhares et al., 2011; O'Ryan et al., 2011; Assis et al., 2013]. Further long-term surveillance studies are needed to clarify this yet controversial issue.

It was observed a 26% rate of RVA detection among children vaccinated with Rotarix™. Similar results were found in two recent studies performed from 2005 to 2010 in Brazil, where prevalence rates ranged from 23% to 29% [Carvalho-Costa et al., 2011; Soares et al., 2012]. In the present study, G2P[4] genotype occurred in 46% of children who received

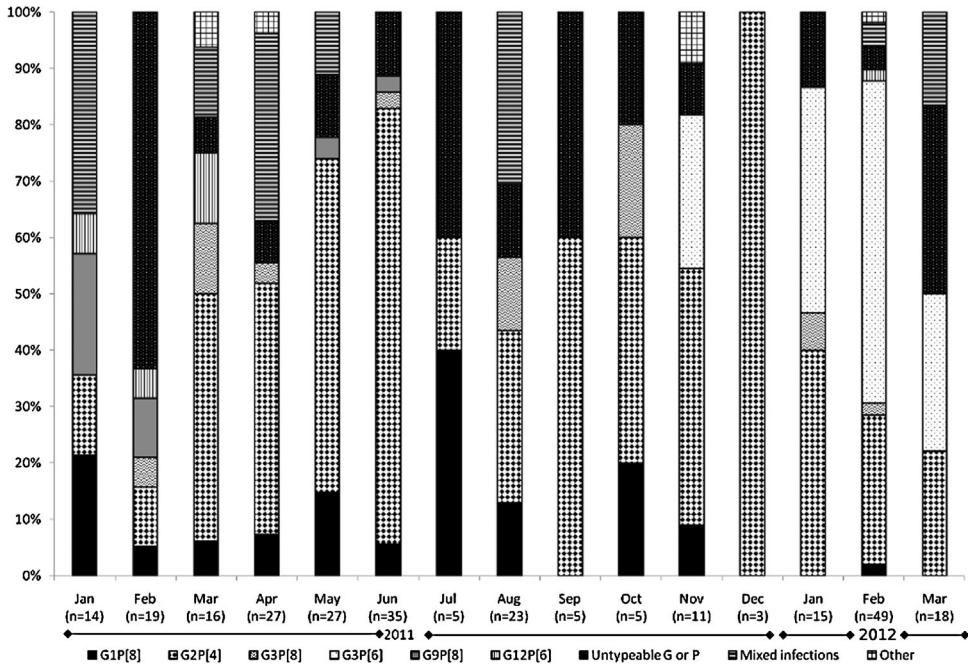

Fig. 2. G and P RVA genotype distribution in Northern region of Brazil, between January 2011 and March 2012.

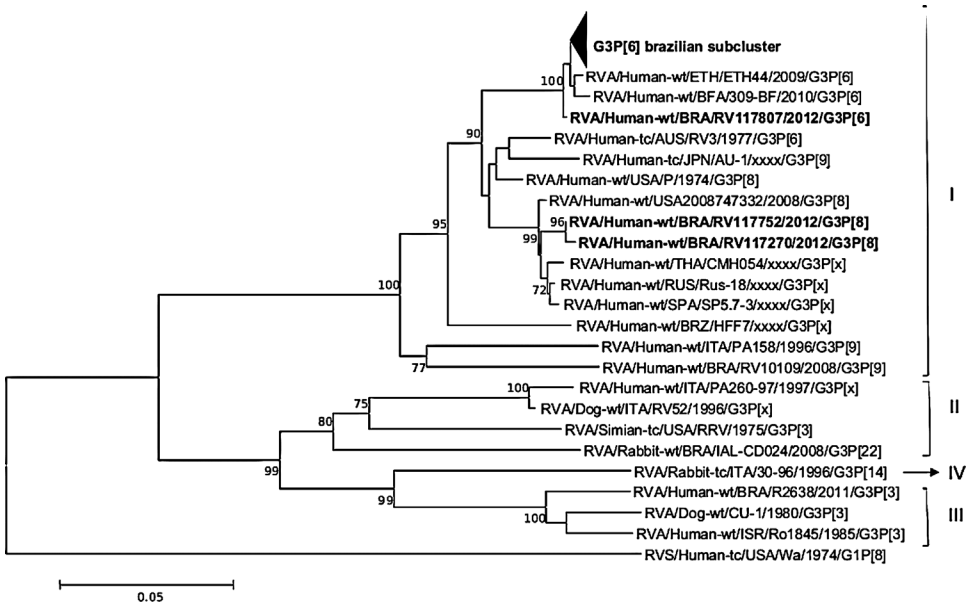

Fig. 3. Phylogenetic analysis of the VP7 protein of circulating G3 Brazilian RVA strains. Neighbor-joining tree was constructed using on the partial nucleotide sequences of VP7 gene (764 bp; nt 112–875; aa 38–291). Bootstrap values above 70% are given at branch nodes. G3 strains analyzed in this study are in bold and condensed into a black triangle.

Rotarix™, a rate slightly lower than that of an investigation conducted in Rio de Janeiro, where this type was associated in 57% of vaccinated patients [Carvalho-Costa et al., 2011]. Even though this genotype possesses distinct antigens compared to Rotarix™, recent studies have shown significant vaccine efficacy against G2P[4] rotavirus [Correia et al., 2010; Justino et al., 2011; Patel et al., 2013]. Furthermore, there was no significant difference when compared the rate of G2P[4] genotype between

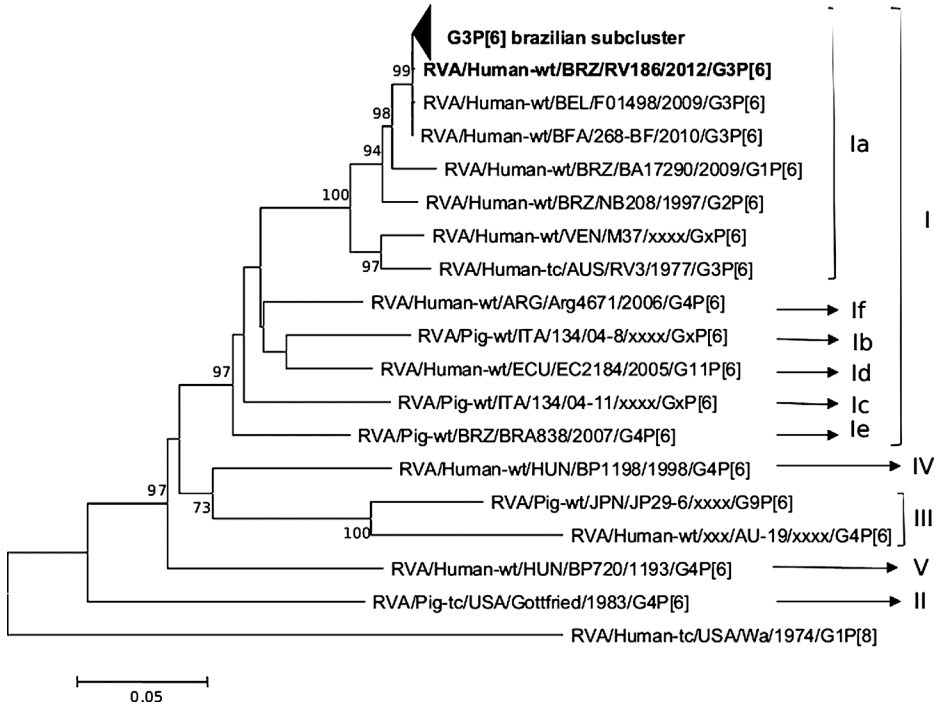

Fig. 4. Phylogenetic analysis of the VP4 protein of circulating G3 Brazilian RVA strains. Neighbor-joining tree was constructed using on the partial nucleotide sequences of VP4 gene (778 bp; nt 51–828; aa 18–276). Bootstrap values above 70% are given at branch nodes. G3 strains analyzed in this study are in bold and condensed into a black triangle.

vaccinated and unvaccinated infants, a finding similar to that reported in another study in Brazil [Soares et al., 2012]. It was detected a trend for a higher frequency of RVA diarrhea among children aged 2–5 years. This is likely to be due to the age for rotavirus vaccination that is for children under 6 months, so unvaccinated children older than 2 years may be more prone to acquire rotavirus infection; however this increase was not statistically significant ($P = 0.32$).

G2P[4] was the predominant genotype (40%) followed by G3P[6] (15%) and G1P[8] (8%). Soares et al. [2012] have reported similar results in a previous study from Northern region of Brazil, between 2008 and 2010, where G2P[4] rotavirus was detected in 46% of patients with acute diarrhea, followed by G1P[8] (22%). Interestingly, it was observed the increase in the rate of G3P[6] genotype, which was the second most frequent genotype circulating in the study population. G3P[6] genotype was detected as from November 2011 and circulated in Amazonas and Acre states only.

G3 rotavirus is a genotype associated with a broad range of hosts. Some studies have shown the increase of G3 frequency associated with P[8] specificity, mostly in Asian countries [Yang et al., 2008; Bányai et al., 2012; Thongprachum et al., 2013]. Recently, G3P[8] was the most frequently detected genotype in Argentina, responsible for about 40% of strains [Stupka et al., 2012]. Human G3 with P[3] and P[9] types have been detected showing higher similarities to feline or canine strains [Grant et al., 2011; Mitui et al., 2011; Maestri et al., 2012]. G3P[6] is a genotype found rarely. In a study conducted in Malawi, G3P[6] strains were responsible for 1% of RVA cases circulating in the nineties [Cunliffe et al., 2010]. In Latin America, G3P[6] strains were detected in 0.2% of RVA genotypes [Linhares et al., 2011].

Partial sequences were obtained for VP7 genes of G3 strains showing nucleotide similarity higher than 99% to each other over the study period, all of them clustering into lineage I. Trinh et al. [2007] have suggested that two amino acid substitutions (Asp96Asn and Asp213Asn) could be responsible for emergence of G3 rotavirus in China. Similarly these amino acid changes in the VP7 genes were noted in all Brazilian G3 strains identified in the present study. Moreover, Yang et al. [2008] have detected these changes in rotavirus G3 isolated for 1996–2005. Further studies are therefore warranted to explain why there was such a marked emergence of G3 strains in the region.

Brazilian G3 strains showed a high homology with RV3, a human neonatal G3P[6] candidate vaccine, and grouped into lineage I. Previous results during phase II study demonstrated that RV3 protected partially infants against severe diarrhea during successive winter months, even though further studies are needed to better assess the efficacy of this candidate vaccine [Barnes et al., 2002].

Since there was recent introduction of G12P[6] rotavirus in Brazil, the emergence of G3P[6] genotype may be associated possibly with reassortment between G12P[6] and G3P[8] strains. Recently, Heylen et al. [2013] described a full characterization for G3P[6] strains with DS-1-like genotype constellation and proposed a reassortment between different G-genotypes strains. The fact that G3P[6] Brazilian strains exhibited short electropherotypes and some G12P[6] strains possess the I2-R2-C2-M2-A2-N2-T2-E2-H2 genotype constellation supports the hypothesis of genetic exchanging between G3P[8] and DS-1-like strains. Nonetheless, further studies on the molecular characterization of rotavirus genes are needed to better assess a possible origin for these strains. Recently, Maestri et al. [2012] described interspecies transmission of rotavirus detected in Amazon region, including G3P[9] strains that supports a close relationship between human and animal rotavirus genes. Although G3 and P[6] genotypes may be associated with zoonotic transmission this seems unlikely for Brazilian strains, since a high similarity was seen with human strains.

In conclusion, these findings demonstrate the emergence of unusual genotype G3P[6] in Amazon region of Brazil and reinforces the need for continuous long-term monitoring of circulating strains through the national surveillance network, in order to better understand the complex dynamics of RVA molecular epidemiology. Furthermore, the monitoring of unusual genotype emergence that might represent possible challenges to current licensed rotavirus vaccines that do not contain strains with DS-1 genotype constellation.

ACKNOWLEDGMENTS

The authors would like to acknowledge the staff of State Central Laboratories (LACENs) and the technical assistance given by the laboratory personnel at the Virology Section of the Instituto Evandro Chagas, Health Surveillance Secretariat, Brazilian Ministry of Health.

REFERENCES

Assis AS, Valle DA, Antunes GR, Tibiriça SH, Assis RM, Leite JP, Carvalho IP, Rosa E, Silva ML. 2013. Rotavirus epidemiology before and after vaccine introduction. J Pediatr (Rio J) 89:470–476.

Ayres M, Ayres M Jr, Ayres D, Santos A. 2007. BIOESTAT—Aplicações estatísticas nas áreas das ciências bio-médicas. 5th edition. Belém, PA: Ong Mamiraua.

Banerjee I, Ramani S, Primrose B, Iturriza-Gomara M, Gray JJ, Brown DW, Kang G. 2007. Modification of rotavirus multiplex RT-PCR for the detection of G12 strains based on characterization of emerging G12 rotavirus strains from South India. J Med Virol 79:1413–1421.

Bányai K, László B, Duque J, Steele AD, Nelson EAS, Gentsch JR, Parashar UD. 2012. Systematic review of regional and temporal trends in global rotavirus strain diversity in the pre rotavirus vaccine era: Insights for understanding the impact of rotavirus vaccination programs. Vaccine 30:A122–A130.

Barnes GL, Lund JS, Mitchell SV, De Bruyn L, Piggford L, Smith AL, Furmedge J, Masendycz PJ, Bugg HC, Bogdanovic-Sakran

N, Carlin JB, Bishop RF. 2002. Early phase II trial of human rotavirus vaccine candidate RV3. Vaccine 20:2950–2956.

Boom R, Sol CJ, Salimans MM, Jansen CL, Wertheim-van Dillen PM, van der Noordaa J. 1990. Rapid and simple method for purification of nucleic acids. J Clin Microbiol 28:495–503.

Carvalho-Costa FA, Volotão Ede M, de Assis RMS, Fialho AM, de Andrade Jda SR, Rocha LN, Tort LFL, da Silva MFM, Gómez MM, de Souza PM, Leite JPG. 2011. Laboratory-based rotavirus surveillance during the introduction of a vaccination program, Brazil, 2005–2009. Pediatr Infect Dis J 30:S35–S41.

Correia JB, Patel MM, Nakagomi O, Montenegro FMU, Germano EM, Correia NB, Cuevas LE, Parashar UD, Cunliffe NA, Nakagomi T. 2010. Effectiveness of monovalent rotavirus vaccine (Rotarix) against severe diarrhea caused by serotypically unrelated G2P[4] strains in Brazil. J Infect Dis 201: 363–369.

Cortes J, Arvelo W, Lopez B, Reyes L, Kerin T, Gautam R, Patel M, Parashar U, Lindblade KA. 2012. Rotavirus disease burden among children <5 years of age—Santa Rosa, Guatemala, 2007–2009. Trop Med Int Health 17:254–259.

Cunliffe NA, Ngwira BM, Dove W, Thindwa BDM, Turner AM, Broadhead RL, Molyneux ME, Hart CA. 2010. Epidemiology of rotavirus infection in children in Blantyre, Malawi, 1997–2007. J Infect Dis 202:S168–S174.

Dennehy PH. 2012. Effects of vaccine on rotavirus disease in the pediatric population. Curr Opin Pediatr 24:76–84.

Estes MK, Kapikian AZ. 2007. Rotaviruses. In: Knipe DM, Howley PM, editors. Fields virology. 5th edition. Philadelphia, PA: Lippincott William and Wilkins, pp 1917–1974.

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 30:1365–1373.

González R, Rivero L. 2013. Genetic diversity of rotavirus group a: Correlation between G3 type and severity of the infection. Valencia, Venezuela. Invest Clin 54:34–46.

González GG, Liprandi F, Ludert JE. 2011. Molecular epidemiology of enteric viruses in children with sporadic gastroenteritis in Valencia, Venezuela. J Med Virol 83:1972–1982.

Gouvea V, Glass RI, Woods P, Taniguchi K, Clark HF, Forrester B, Fang ZY. 1990. Polymerase chain reaction amplification and typing of rotavirus nucleic acid from stool specimens. J Clin Microbiol 28:276–282.

Grant L, Esona M, Gentsch J, Watt J, Reid R, Weatherholtz R, Santosham M, Parashar U, O'Brien K. 2011. Detection of G3P [3] and G3P[9] rotavirus strains in American Indian children with evidence of gene reassortment between human and animal rotaviruses. J Med Virol 83:1288–1299.

Gurgel RQ, Cuevas LE, Vieira SCF, Barros VCF, Fontes PB, Salustino EF, Nakagomi O, Nakagomi T, Dove W, Cunliffe N, Hart CA. 2007. Predominance of rotavirus P[4]G2 in a vaccinated population, Brazil. Emerg Infect Dis 13:1571–1573.

Heylen E, Zeller M, Ciarlet M, De Coster S, Van Ranst M, Matthijnssens J. 2013. Complete genetic characterization of human G2P[6] and G3P[6] rotavirus strains. Infect Genet Evol 13:27–35.

Justino MCA, Linhares AC, Lanzieri TM, Miranda Y, Mascarenhas JDP, Abreu E, Guerra SFS, Oliveira ASL, da Silva VB, Sanchez N, Meyer N, Shafi F, Ortega-Barria E, Soriano-Gabarró M, Colindres RE. 2011. Effectiveness of the monovalent G1P[8] human rotavirus vaccine against hospitalization for severe G2P [4] rotavirus gastroenteritis in Belém, Brazil. Pediatr. Infect Dis J 30:396–401.

Kimura M. 1980. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. J Mol Evol 16:111–120.

Lanzieri TM, Linhares AC, Costa I, Kolhe DA, Cunha MH, Ortega-Barria E, Colindres RE. 2011. Impact of rotavirus vaccination on childhood deaths from diarrhea in Brazil. Int J Infect Dis 15: e206–e210.

Leite JPG, Carvalho-Costa FA, Linhares AC. 2008. Group A rotavirus genotypes and the ongoing Brazilian experience: A review. Mem Inst Oswaldo Cruz 103:745–753.

Linhares AC, Stupka JA, Ciapponi A, Bardach AE, Glujovsky D, Aruj PK, Mazzoni A, Rodriguez JAB, Rearte A, Lanzieri TM, Ortega-Barria E, Colindres R. 2011. Burden and typing of rotavirus group A in Latin America and the Caribbean: Systematic review and meta-analysis. Rev Med Virol doi: 10.1002/rmv.682

Lorenzetti E, da Silva Medeiros TN, Alfieri AF, Alfieri AA. 2011. Genetic heterogeneity of wild-type G4P[6] porcine rotavirus strains detected in a diarrhea outbreak in a regularly vaccinated pig herd. Vet Microbiol 154:191–196.

Lucero Y, Mamani N, Cortés H, Peña A, Vergara R, O'Ryan M. 2012. Rotavirus genotypes in children with gastroenteritis assisted in two public hospitals from Chile: Viral strains circulating in a country without a universal vaccination against rotavirus. Rev Chilena Infectol 29:142–148.

Maestri RP, Kaiano JHL, Neri DL, Soares Lda S, Guerra Sde FDS, Oliveira Dde S, Farias YN, Gabbay YB, Leite JPG, Linhares Ada C, Mascarenhas JDP. 2012. Phylogenetic analysis of probable non-human genes of group A rotaviruses isolated from children with acute gastroenteritis in Belém, Brazil. J Med Virol 84:1993–2002.

Martella V, Bányai K, Ciarlet M, Iturriza-Gómara M, Lorusso E, De Grazia S, Arista S, Decaro N, Elia G, Cavalli A, Corrente M, Lavazza A, Baselga R, Buonavoglia C. 2006. Relationships among porcine and human P[6] rotaviruses: Evidence that the different human P[6] lineages have originated from multiple interspecies transmission events. Virology 344:509–519.

Martella V, Bányai K, Matthijnssens J, Buonavoglia C, Ciarlet M. 2010. Zoonotic aspects of rotaviruses. Vet Microbiol 140: 246–255.

Martínez-Laso J, Román A, Rodriguez M, Cervera I, Head J, Rodríguez-Avial I, Picazo JJ. 2009. Diversity of the G3 genes of human rotaviruses in isolates from Spain from 2004 to 2006: Cross-species transmission and inter-genotype recombination generates alleles. J Gen Virol 90:935–943.

Mascarenhas JDP, Leite JPG, Lima JC, Heinemann MB, Oliveira DS, Araújo IT, Soares LS, Gusmão RHP, Gabbay YB, Linhares AC. 2007. Detection of a neonatal human rotavirus strain with VP4 and NSP4 genes of porcine origin. J Med Microbiol 56:524–532.

Matthijnssens J, Van Ranst M. 2012. Genotype constellation and evolution of group A rotaviruses infecting humans. Curr Opin Virol 2:426–433.

Matthijnssens J, Heylen E, Zeller M, Rahman M, Lemey P, Van Ranst M. 2010. Phylodynamic analyses of rotavirus genotypes G9 and G12 underscore their potential for swift global spread. Mol Biol Evol 27:2431–2436.

Matthijnssens J, Ciarlet M, McDonald SM, Attoui H, Bányai K, Brister JR, Buesa J, Esona MD, Estes MK, Gentsch JR, Iturriza-Gómara M, Johne R, Kirkwood CD, Martella V, Mertens PPC, Nakagomi O, Parreño V, Rahman M, Ruggeri FM, Saif LJ, Santos N, Steyer A, Taniguchi K, Patton JT, Desselberger U, Van Ranst M. 2011. Uniformity of rotavirus strain nomenclature proposed by the Rotavirus Classification Working Group (RCWG). Arch Virol 156:1397–1413.

Matthijnssens J, Otto PH, Ciarlet M, Desselberger U, Van Ranst M, Johne R. 2012. VP6-sequence-based cutoff values as a criterion for rotavirus species demarcation. Arch Virol 157:1177–1182.

Mitui MT, Chan PKS, Nelson EAS, Leung TF, Nishizono A, Ahmed K. 2011. Co-dominance of G1 and emerging G3 rotaviruses in Hong Kong: A three-year surveillance in three major hospitals. J Clin Virol 50:325–333.

Munos MK, Walker CLF, Black RE. 2010. The effect of rotavirus vaccine on diarrhoea mortality. Int J Epidemiol 39:i56–i62.

Nordgren J, Nitiema LW, Sharma S, Ouermi D, Traore AS, Simpore J, Svensson L. 2012. Emergence of unusual G6P[6] rotaviruses in children, Burkina Faso, 2009–2010. Emerg Infect Dis 18:589–597.

O'Ryan M, Lucero Y, Linhares AC. 2011. Rotarix: Vaccine performance 6 years postlicensure. Expert Rev Vaccines 10:1645–1659.

Oliveira A, Mascarenhas JDP, Soares LS, Guerra SFS, Gabbay YB, Sánchez N, Colindres RE, Justino MCA, Linhares AC. 2012. Rotavirus serotype distribution in northern Brazil trends over a 27 year period pre and post national vaccine introduction. Trials Vaccinol 1:4–9.

Patel MM, Patzi M, Pastor D, Nina A, Roca Y, Alvarez L, Iniguez V, Rivera R, Tam KI, Quaye O, Bowen M, Parashar U, De Oliveira LH. 2013. Effectiveness of monovalent rotavirus vaccine in Bolivia: Case–control study. BMJ 346:f3726.

Patton JT. 2012. Rotavirus diversity and evolution in the post-vaccine world. Discov Med 13:85–97.

Pereira HG, Azeredo RS, Leite JP, Barth OM, Sutmoller F, de Farias V, Vidal MN. 1983. 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 78:483–490.

Rahman M, Matthijnssens J, Yang X, Delbeke T, Arijs I, Taniguchi K, Iturriza-Gómara M, Iftekharuddin N, Azim T, Van Ranst M. 2007. Evolutionary history and global spread of the emerging g12 human rotaviruses. J Virol 81:2382–2390.

Rippinger CM, Patton JT, McDonald SM. 2010. Complete genome sequence analysis of candidate human rotavirus vaccine strains RV3 and 116E. Virology 405:201–213.

Santos N, Hoshino Y. 2005. Global distribution of rotavirus serotypes/genotypes and its implication for the development and implementation of an effective rotavirus vaccine. Rev Med Virol 15:29–56.

Sartori AMC, Valentim J, de Soárez PC, Novaes HMD. 2008. Rotavirus morbidity and mortality in children in Brazil. Rev Panam Salud Publica 23:92–100.

Soares Lda S, Lobo Pdos S, Mascarenhas JDP, Neri DL, Guerra Sde F dos S, de Oliveira A do SL, Maestri RP, Oliveira Dde S, de Menezes EM de FC, Linhares Ada C. 2012. Identification of lineage III of G12 rotavirus strains in diarrheic children in the Northern Region of Brazil between 2008 and 2010. Arch Virol 157:135–139.

Stupka JA, Carvalho P, Amarilla AA, Massana M, Parra GI. 2009. National Rotavirus Surveillance in Argentina: High incidence of G9P[8] strains and detection of G4P[6] strains with porcine characteristics. Infect Genet Evol 9:1225–1231.

Stupka JA, Degiuseppe JI, Parra GI. 2012. Increased frequency of rotavirus G3P[8] and G12P[8] in Argentina during 2008–2009 : Whole-genome characterization of emerging G12P[8] strains. J Clin Virol 54:162–167.

Tate JE, Burton AH, Boschi-Pinto C, Steele AD, Duque J, Parashar UD. 2012. 2008 estimate of worldwide rotavirus-associated mortality in children younger than 5 years before the introduction of universal rotavirus vaccination programmes: A systematic review and meta-analysis. Lancet Infect Dis 12:136–141.

Thongprachum A, Chan-it W, Khamrin P, Okitsu S, Nishimura S, Kikuta H, Yamamoto A, Sugita K, Baba T, Mizuguchi M, Maneekarn N, Hayakawa S, Ushijima H. 2013. Reemergence of new variant G3 rotavirus in Japanese pediatric patients, 2009–2011. Infect Genet Evol 13:168–174.

Trinh QD, Pham NTK, Nguyen TA, Phan TG, Khamrin P, Yan H, Hoang PL, Maneekarn N, Li Y, Kozlov V, Kozlov A, Okitsu S, Ushijima H. 2007. Amino acid substitutions in the VP7 protein of human rotavirus G3 isolated in China, Russia, Thailand, and Vietnam during 2001–2004. J Med Virol 79:1611–1616.

Trojnar E, Sachsenröder J, Twardziok S, Reetz J, Otto PH, Johne R. 2013. Identification of an avian group A rotavirus containing a novel VP4 gene with a close relationship to those of mammalian rotaviruses. J Gen Virol 94:136–142.

WHO. 2009. Rotavirus vaccines: An update. Wkly Epidemiol Rec 84:533–540.

WHO. 2013. Rotavirus vaccines. WHO position paper—January 2013, 2013. Wkly Epidemiol Rec 88:49–64.

Yang X-L, Matthijnssens J, Sun H, Muhamaiti J, Zhang B, Nahar S, Van Ranst M, Rahman M. 2008. Temporal changes of rotavirus strain distribution in a city in the northwest of China, 1996–2005. Int J Infect Dis 12:e11–e17.