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SHORT REPORT

CIRCULATING PROFILE OF ROTAVIRUS A, G AND P GENOTYPES BEFORE AND AFTER VACCINE INTRODUCTION IN THE BRAZILIAN MID-WEST 1986-2015

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ABSTRACT

The purpose of this study was to perform a comparative analysis of Rotavirus A (RVA) G and P genotypes circulating in the Brazilian Mid-West in the period 1986-2015. Seven studies conducted from 1986 to 2009 were included, as well as fecal samples obtained in the period 2014-2015. RVA was screened by ELISA and/or PAGE; genotyping by conventional RT-PCR and/or genomic sequencing. A temporal variation in the predominance of G genotypes mainly G1 and G2 with G9 and G12 emergence was observed. Even with vaccination, RVA continues to circulate in the population, requiring continuous virus monitoring.

KEY WORDS: Rotavirus infections; genotype; vaccination.

Rotavirus A (RVA) is an important causative agent of acute gastroenteritis (AGE) mainly affecting infants. The viral particle is non-enveloped and formed by three concentric protein layers that surround 11 segments of double-stranded RNA (dsRNA) (Estes & Greenberg, 2013).

Each RVA genomic segment has extensive variability, which may reflect in antigenic diversity of the respective proteins, mainly VP7 and VP4 proteins that comprise the outer layer. RVA has been classified in a binary system represented by the combination of G genotypes (VP7) and P (VP4) genotypes (Estes & Greenberg, 2013). There are, so far, 32G (G1-G32) and 47P (P[1]-P[47]) genotypes described (RCWG, 2016). In humans, the most common combinations are G1P[8], G2P[4], G3P[8], G4P[8], G9P[8], and G12P[8] (Santos & Hoshino, 2005, Dóró et al., 2014).

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Worldwide G1 and P[8] have been the predominant genotypes (Santos & Hoshino, 2005), being replaced at regular intervals, especially by G2 and P[4] (Dóró et al., 2014), as well as other emerging genotypes such as G5 and G9 (Santos & Hoshino, 2005). Many studies of the genotypic profile of RVA, in the Brazilian Mid-West, have been conducted since 1986, detecting the occurrence of distinct G and P genotypes with changes over time, notably G1P[8], G2P[4] and G9P[8] (Cardoso et al. 2000, Souza et al. 2003, Costa et al. 2004, Andreasi et al. 2007, Munford et al. 2009, Borges et al. 2011, Almeida et al. 2015).

Because of the severity of acute gastroenteritis caused by RVA, especially in children under five years of age, there have been two licensed vaccines available for use in Brazil since 2006: Rotarix™ (Glaxo Smith Kline) and RotaTeq™ (Merck Sharp & Dohme). Rotarix™ has also been included in the Brazilian National Immunization Program (Dóró et al., 2014).

Studies conducted in the Brazilian Mid-West have shown a temporal variation in the predominance of certain G and P genotypes, characterizing the occurrence of an antigenic shift (Cardoso et al., 2000, Souza et al., 2003, Costa et al., 2004, Andreasi et al., 2007, Munford et al., 2009, Borges et al., 2011, Almeida et al., 2015).

Considering that the pediatric population in Brazil has access to both RVA vaccines, this study presents the genotypic variability of RVA in the region, in the pre- and post-vaccine periods.

In order to analyze the circulation of G and P genotypes in the Brazilian Mid-West, data was compared from studies conducted in the Mid-West in the pre-vaccine period (Cardoso et al., 2000, Souza et al., 2003, Costa et al., 2004, Andreasi et al., 2007, Munford et al., 2009), and post-vaccine period (Munford et al., 2009, Borges et al., 2011, Almeida et al., 2015), with data from samples obtained in the period from 2014 to 2015.

In previous studies, fecal samples were collected from children with AGE (Souza et al., 2003, Costa et al., 2004, Andreasi et al., 2007, Munford et al., 2009, Almeida et al., 2015), without AGE (Borges et al., 2011), and from children with or without AGE (Cardoso et al., 2000). These studies included hospitalized children (Souza et al., 2003, Costa et al., 2004, Andreasi et al., 2007, Munford et al., 2009, Almeida et al., 2015), hospitalized and seen in outpatient clinics (Cardoso et al., 2000) and children that attended day-care centers (Borges et al., 2011).

The study conducted during 2014-2015 included samples obtained from children under five years of age, with or without AGE symptoms hospitalized in a child-care referral hospital in the state of Goiás (*Hospital Materno Infantil de Goiânia*). One fecal sample was obtained from each child after the parent or legal guardian signed the written consent form.

This study was approved by the Ethics Committee of Research in the Hospital das Clínicas of the Universidade Federal de Goiás, (Protocol: 19948113.6.0000.5078).

All the samples obtained in the period 1986-2009 were screened by Enzyme-Linked Immunosorbent Assay (ELISA), according to the manufacturer's instructions and/or Polyacrylamide Gel Electrophoresis (PAGE) using protocols previously described (Pereira et al., 1983).

RVA positive samples obtained in this period underwent G and P genomic amplification by conventional RT-PCR. The dsRNA was extracted using Trizol reagent (Invitrogen™/Life Technologies, Foster City, USA), following the manufacturer's instructions. The reverse transcription of dsRNA and the amplification reaction were performed using specific primer pairs for the VP7 and VP4 encoding genes, previously described (Gouvea et al., 1990, Gentsch et al., 1992). Negative (sterile water) and positive (RVA positive samples) controls were also included in each run.

The amplification products were analyzed in 1.5% agarose gel, 0.5X TBE buffer (Tris-HCl-Borate-EDTA) with ethidium bromide staining, compared to the 100 bp ladder (Invitrogen™/Life Technologies, Foster City, USA), and visualized under UV light (Vilbert Loumart). Fragments with an expected size of 876 and 1.062 bp (VP4 and VP7, respectively) were considered positive. All procedures were conducted in the proper environment in order to avoid contamination.

For the samples obtained in 2014-2015, RVA screening was performed using PAGE following a previously described protocol (Pereira et al., 1983). RVA positive samples underwent genomic sequencing of genes encoding VP7 (G) and VP4 (P) proteins using the same primers and conditions of conventional RT-PCR (Gouvea et al., 1990, Gentsch et al., 1992).

The genomic sequencing was performed using the BigDye Terminator kit (Applied Biosystems™, USA) in automatic sequencer (ABIPrism 3130, Applied Biosystems™, USA). The genotyping was performed by comparison with sequences deposited in the National Center for Biotechnology Information (NCBI) (https://www.ncbi.nlm.nih.gov).

RVA G and P genotypes were detected in previous studies conducted in the Brazilian Mid-West, in 1986-2009, when G1 and P[8] genotypes proved predominant among 474 RVA positive samples with positivity rate ranging from 3.6 to 41.8% (Table 1).

Analyses regarding the collection period showed that in the pre-vaccine period, G2 predominated in the years 1986-1995 and after that, G1 emerged. G5 was only detected in 1986-1995, and G9 emerged in 1998. Also P[8] proved predominant in all studies and two P[9] samples were detected in 2005. In the post-vaccine period, G2 and P[4] were predominated until 2009.

In 146 samples with G and P combination, G1P[8] proved the most frequent in the pre-vaccine period and after that G2P[4] samples emerged in the post-vaccine period (Table 2).

Table 1. Studies of Rotavirus A conducted in the Brazilian Mid-West, considering G and P genotypes from 1986 to 2009

	Sampling period	N	%	Genotype G (VP7)									Genotype P (VP4)						
				G1	G2	G3	G4	G5	G8	G9	MIX	GNT	P[4]	P[6]	P[8]	P[9]	MIX	PNT	Ref.
Pre-vaccine period	1986-1995	132	11.8	26	37	13	2	3	-	-	-	51	*	*	*	*	*	*	Cardoso et al. 2000
	1998-2000	120	23.3	92	6	1	-	-	-	6	-	15	-	20	33	-	-	67	Souza et al. 2003
	2000-2002	77	37.2	38	-	-	2	-	-	21	-	16	-	3	23	-	16	35	Costa et al. 2004
	2000-2004	85	23.2	49	-	1	5	-	-	1	-	29	-	-	37	-	-	48	Andreasi et al. 2007
	2005-2006[a]	13	13.3	11	-	-	-	-	-	1	1	-	-	-	6	2	7	-	Munford et al. 2009
Post-vaccine period	2005-2006[b]	28	28.5	1	27	-	-	-	-	-	-	-	24	-	2	-	-	-	Munford et al. 2009
	2008-2008	08	3.6	-	7	-	-	-	1	-	-	-	6	-	-	-	-	2	Borges et al. 2011
	2008-2009	11	16.9	-	10	-	-	-	-	-	-	1	4	-	-	-	1	6	Almeida et al. 2015
Total		474		217	87	15	9	3	1	29	1	112	34	23	101	2	24	158	

a =data from 2005; b =data from 2006; N =total number of RVA positive samples; % =percentage of positive samples; MIX =co-detection of genotypes; NT =non-typeable; * =not tested.

Table 2. Combinations of G and P genotypes of Rotavirus A samples obtained from different studies conducted in the Brazilian Mid-West from 1998 to 2009

	Sampling period		G (VP7) and P (VP4) combinations										
		N	G1P[6]	G1P[8]	G2P[4]	G2P[6]	G2P[8]	G3P[8]	G4P[8]	G8P[4]	G9P[6]	G9P[8]	Ref.
Pre-vaccine period	1998-2000	46	13	25	-	1	2	-	-	-	5	-	Souza et al. 2003
	2000-2002	18	1	14	-	-	-	-	1	-	-	2	Costa et al. 2004
	2000-2004	37	-	33	-	-	-	1	2	-	-	1	Andreasi et al. 2007
	2005-2006[a]	9	-	8	-	-	-	-	-	-	-	1	Munford et al. 2009
Post-vaccine period	2005-2006[b]	26	-	1	24	-	1	-	-	-	-	-	Munford et al. 2009
	2008-2008	6	-	-	5	-	-	-	-	1	-	-	Borges et al. 2011
	2008-2009	4	-	-	4	-	-	-	-	-	-	-	Almeida et al. 2015
Total		146	14	81	33	1	3	1	3	1	5	4	

a = data from 2005; b = data from 2006; N = total number of RVA positive samples.

In the study conducted in 2014-2015, in which 335 fecal samples were analyzed, 134 were obtained from children with AGE and 201 from children without AGE symptoms. Nine samples (2.6%) were RVA positive; eight were from children with AGE and one sample from a child without AGE symptoms. From the eight samples from children with AGE, four were G12P[8], one was P[8], one G12 and the other two were non-typeable for G or P. The positive sample from the child without AGE symptoms was P[8] and nontypeable G.

RVA vaccination has resulted in an important reduction in the number of AGE cases and disease severity, and consequent hospitalization, around the world (Kollaritsch et al., 2015). This situation has also been documented in Brazil, including the Mid-West Region, where studies during the pre-vaccine period showed detection rates of up to 37.2% (Cardoso et al., 2000, Souza et al., 2003, Costa et al., 2004, Andreasi et al., 2007). In studies conducted after the vaccine implementation, lower positive rates were noted (Borges et al., 2011, Almeida et al., 2015).

It is noteworthy that lower positivity rates were observed in the study performed in 1986-1995 (Cardoso et al., 2000) with children with and without AGE and in the study performed in 2008 (Borges et al., 2011) which included children without AGE, performed in the pre- and post-vaccine periods, respectively.

In the 2009-2010 study (Munford et al., 2009) conducted with hospitalized children with AGE, including samples from both periods, a higher detection rate (41.8%) was observed. This data contrasts with the literature and also the results from the study conducted in the period 2014-2015 when 2.6% positivity for RVA was observed, even considering only the AGE population (6.0%).

It was supposed that this study (2014-1015) would be limited due to the use of a single technique (PAGE) to screen RVA. Although this methodology presents good specificity, its sensitivity is lower than other techniques like ELISA. Even so, the reduction of RVA detection may be related to vaccine benefits in the Mid-West Region.

Regarding G and P combinations, a predominance of G1P[8] samples from the 1998-2004, pre-vaccine period was observed (Souza et al., 2003, Costa et al., 2004, Andreasi et al., 2007), and the emergence of G2P[4] samples between 2006 and 2009 in the post-vaccine period (Munford et al., 2009, Borges et al., 2011, Almeida et al., 2015). Additionally, in spite of the time lapse, the 2014-2015 sampling, reveals the G12P[8] combination. These data corroborated previous studies in reinforcing the fluctuation of RVA genotypes, as well as the emergence of G12P[8] (Santos & Hoshino, 2005, Dóró et al., 2014).

Additionally, considering samples collected between 2006 and 2009, post-vaccine perid, it was observed the emergence of G2P[4] samples (Munford et al., 2009, Borges et al., 2011, Almeida et al., 2015). In spite of the time lapse,

the 2014-2015 sampling, reveals the G12P[8] combination, which corroborates previous studies reinforcing the fluctuation of RVA genotypes, as well as the emergence of these genotypes (Dóró et al., 2014).

These data indicate a regular tendency to fluctuation in RVA G and P genotypes in the human population. This situation cannot be solely attributed to vaccination since RVA may mutate over relatively short period of time resulting in shifts in the circulation of predominant genotypes over time.

Therefore, despite the benefits of vaccines, considering the reduction of RVA positivity and hospitalization cases of AGE, the virus continues to circulate, with the same vaccine genotypes or in a different one, such as G12P[8]. Therefore, continuous monitoring of RVA samples circulating among the human population remains important.

Finally, the continuity of studies that focus on RVA dynamics in the human population may help to predict changes in the genotypic profile of the circulating samples and in determining preventive measures such as the development of vaccines from the predominant genotypes over time.

REFERENCES

1. Almeida TN, Fiaccadori FS, Souza M, Borges AM, Cardoso Dd. Molecular characterization of group A rotavirus before and after the introduction of vaccines in Brazil. *Rev Soc Bras Med Trop 48:* 599-602, 2015.
2. Andreasi MS, Batista SM, Tozetti IA, Ozaki CO, Nogueira MM, Fiaccadori FS, Borges AM, Santos RA, Cardoso Dd. Rotavirus A among hospitalized infants, up to three years of age, with acute gastroenteritis in Campo Grande, State of Mato Grosso do Sul. *Rev Soc Bras Med Trop 40*: 411-414, 2007.
3. Borges AM, Dias e Souza M, Fiaccadori FS, Cardoso Dd. Monitoring the circulation of rotavirus among children after the introduction of the Rotarix™ vaccine in Goiânia, Brazil. *Mem Inst Oswaldo Cruz 106*: 499-501, 2011.
4. Cardoso DDP, Soares CMA, Azevedo MSP, Leite JPG, Munford V, Rácz ML. Serotypes and subgroups of rotavirus isolated from children in central Brazil. *J Health Popul Nutr 18*: 39-43, 2000.
5. Costa PS, Cardoso DD, Grisi SJ, Silva PA, Fiaccadori F, Souza MB, Santos RA. Rotavirus A infections and reinfections: genotyping and vaccine implications. *J Pediatr 80:* 119-122, 2004.
6. Dóró R, László B, Martella V, Leshem E, Gentsch J, Parashar U, Bányai K. Review of global rotavirus strain prevalence data from six years post vaccine licensure surveillance: is there evidence of strain selection from vaccine pressure? *Infect Genet Evol 28*: 446-461, 2014.
7. Estes MK, Greenberg HB. Rotaviruses. *In*: Knipe DM, Howley PM, eds. *Fields Virology*. 6th ed. Lippincott Williams and Wilkins, Philadelphia, 2013.
8. Gentsch JR, Glass RI, Woods P, Gouvea V, Gorziglia M, Flores J, Das BK, Bhan MK. Identification of group A rotavirus gene 4 types by polymerase chain reaction. *J Clin Microbiol 30*: 1365-1373, 1992.
9. Gouvea V, Glass RI, Woods P, Taniguchi K, Clark FH, Forrester B, Fang ZY. Polymerase chain reaction amplification and typing of rotavirus nucleic acid from stool specimens. *J Clin Microbiol 28*: 276-282, 1990.

10. Kollaritsch H, Kundi M, Giaquinto C, Paulke-Korinek M. Rotavirus vaccines: a story of success. *Clin Microbiol Infect 21*: 735-743, 2015.

11. Munford V, Gilio AE, de Souza EC, Cardoso DM, Cardoso Dd, Borges AM, Costa PS, Melgaço IA, Rosa H, Carvalho PR, Goldani MZ, Moreira ED Jr, Santana C, El Khoury A, Ikedo F, Rácz ML. Rotavirus gastroenteritis in children in 4 regions in Brazil: a hospital-based surveillance study. *J Infect Dis 200 (Suppl 1)*: S106-113, 2009.

12. Pereira HG, Azeredo RS, Leite JP, Barth OM, Sutmoller F, de Farias V, Vidal MN. 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, 1983.

13. RCWG (Rotavirus Classification Working Group). Newly assigned genotypes [Internet]. Leuvan (Belgium). 2016 - [cited 2016 Jun 10]. Available from: https://rega.kuleuven.be/cev/viralmetagenomics/virus-classification/rcwg.

14. Santos N, Hoshino Y. 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, 2005.

15. Souza MB, Rácz ML, Leite JP, Soares CM, Martins RM, Munford V, Cardoso DD. Molecular and serological characterization of group A rotavirus isolates obtained from hospitalized children in Goiânia, Brazil, 1998-2000. *Eur J Clin Microbiol Infect Dis 22*: 441-443, 2003.