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VP8*P[8] lineages of group A rotaviruses circulating over 20 years in Brazil: Proposal of six different sub-lineages for P[8]-3 clade

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ABSTRACT

Group A rotaviruses (RVA) is the most important cause of severe gastroenteritis among children worldwide. Vaccination is considered the best alternative among public health measures to reduce and prevent the global burden caused by RVA infections. Rotarix™, a monovalent vaccine based on a human strain with a G1P[8]-1 specificity, was introduced in the National Brazilian Immunization Programs (NIP) in March, 2006. RVA P[8] is the most prevalent P genotype worldwide and four distinct phylogenetic lineages: P[8]-1, -2, -3, and -4 have been described. In the current study phylogenetic analysis of the VP8* gene of 135 RVA P[8] Brazilian strains, in combination with G1, G3, G5 or G9 VP7 genotype, collected from 1986 to 2011 were carried out for a better understanding of the evolution of this viral genotype in Brazil. Lineages P[8]-1, P[8]-2, and P[8]-3 were observed circulating in Brazil. In 2001 these three P[8] lineages co-circulated simultaneously and this is the first report in South America to date. Considering the P[8] lineage and the G genotype, all G3 strains were related to lineage P[8]-3, whereas the G9 strains were related to P[8]-2 and P[8]-3 and G1 and G5 were related to P[8]-1, P[8]-2, and P[8]-3. In addition, the phylogenetic analysis based on estimate of genetic distances between P[8]-3 strains and the definition of a 1.5% cutoff value (with relevant statistical support) it was possible to propose a new classification for the P[8]-3 lineage into six different sub-lineages: P[8]-3.1 to P[8]-3.6. These findings reinforce the notion of the existence of constraints within specific RVA strains populations. The results obtained in this study reinforce the importance of a continuous RVA surveillance of circulating strains in order to predict the possible variants that will circulate in a country, assess the effects of vaccination on RVA circulating strains, and ultimately help in the design, challenges, and prospects of RVA vaccines.

1. Introduction

Group A rotaviruses (RVA) are the leading cause of severe gastroenteritis among children, accounting for approximately one third of total diarrhea deaths worldwide (Tate et al., 2010). RVA genome consists of 11 double-stranded RNA (dsRNA) segments, encoding five (or six) nonstructural proteins (NSPs) and six structural proteins (VPs) (Estes and Kapikian, 2007), surrounded by a triple-layered protein capsid. Based on antigenic and genetic differences of two viral surface proteins, VP4 and VP7, RVA can be classified into P (Protease sensitive) and G (Glycolyslated) genotypes, respectively. So far, 27 G and 35 P genotypes have been identified in humans as well as in different animals (Matthijnssens and Van Ranst, 2012).

Mechanisms that promote RVA diversification include point mutations, intersegmental recombination, rearrangements, reassortment, and interspecies transmission (Desselberger, 1996; Iturriza-Gomara et al., 2000; Estes and Kapikian, 2007; Heiman et al., 2008). Point mutations and reassortments are frequently described for RVA and can eventually lead to the emergence of RVA strains that escape neutralization by specific antibodies. Five binary G/P genotype combinations (G1P[8], G3P[8], G4P[8], G9P[8], and G2P[4]) are responsible for more than 90% of human RVA cases detected around the world (Matthijnssens and Van Ranst, 2012). In Brazil, G1-G4 account for 65% of strains but the atypical G5 was also observed (Leite et al., 2008). In order to achieve efficient cell entry, the VP4 protein is cleaved by trypsin into a N-terminal fragment (VP8*) and a C-terminal fragment (VP5*) (Espinola et al., 2008).

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The VP8* region has been described to contain four putative neutralization domains defined by amino acid alignments and mapping of monoclonal antibody escape mutants (Kirkwood et al., 1996). Studies demonstrated antigenic and genomic variations inside the VP8* region, especially among strains bearing G1–G4 and G9 genotypes in association with P[8], which represent the most prevalent genotypes in humans and are the main targets for vaccine studies. So far, studies on the VP8* fragment of P[8] genotype have shown four distinct phylogenetic lineages circulating worldwide: P[8]-1, -2, -3, and -4 (Arista et al., 2006; Cunliffe et al., 2001).

Rotarix™, a monovalent vaccine based on a human strain with G1P[8] (P[8]-1) specificity, was introduced in the National Brazilian Immunization Programs (NIP) in March, 2006. In the present report the genetic variation of P[8] genotype of human RVA strains circulating in Brazil between 1986 and 2011 is described through analysis of 135 RVA strains from vaccinated and non vaccinated children living in 4 out of the 5 Brazilian regions. In addition, a proposal to classify the P[8]-3 lineage into six different sub-lineages: P[8]-3.1 to P[8]-3.6 based on phylogenetic analysis results, is presented.

2. Materials and methods

2.1. Study population

The stool samples were randomly selected from a pool of RVA positive patients, in order to uniformly represent all the study period and all geographic regions involved. Aiming to avoid selection bias, when more than one positive sample was provided by city and year, the same proportion of them were randomly selected from the pool of available samples, reaching thus the final sum of 135 patients (44 G1P[8], 21 G3P[8], 28 G5P[8] and 42 G9P[8]) obtained from children with acute diarrhea living in 4 out of the 5 Brazilian regions: Northern, Northeastern, Southeastern, and Southern between 1986 and 2011. Of the 44 G1P[8] strains, 19 were collected from children vaccinated with one or two doses of Rotarix™. This study was approved by the Institutional Research Ethics Committee (CEP-FIOCRUZ 311/06). The accession numbers of the nucleotide sequences are available in the Supplementary Material.

2.2. Nucleic acid purification, amplification and sequencing

Nucleic acids were extracted by the glass powder method (Boom et al., 1990) with modifications described by Leite et al. (1996). The viral dsRNA was reverse transcribed (RT) and the primers used for amplification of VP8* gene were designed to generate an amplicon of 887 bp from the 5′ terminus of the gene (Gentsch et al., 1992). The VP8* primers, used to obtain the RT-PCR amplicon, were employed individually for gene sequencing. Forward and reverse strand amplification reaction was carried out for each sequence obtained at least twice. DNA sequencing was performed with an ABI Prism Big Dye Terminator Cycle Sequencing Ready Reaction Kit® and an ABI Prism 3730 Genetic Analyzer® (both from Applied Biosystems, Foster City, CA, USA) by Genomic Platform of DNA sequencing PDTIS/FIOCRUZ. All procedures to avoid cross-contamination were performed, including negative controls (DNase/RNase free water – Gibco, Grand Island, USA – with RNase-Out – Invitrogen, Carlsbad, USA), during all steps.

2.3. Phylogenetic and genetic distance analyses

Mean nucleotide distances within and among different P[8] lineages and sub-lineages were estimated using the Tamura-Nei model (Tamura and Nei, 1993) as implemented in MEGA 4 v.4.0 software package (Tamura et al., 2007). Mean nucleotide distances between P[8]-3 sub-clusters were $\geqslant$.017, while mean nucleotide distances within P[8]-3 sub-clusters were $\leqslant$0.012. Thus, based on genetic distance results, it was proposed a cutoff value of 1.5% to define distinct P[8]-3 sub-lineages.

3. Results and discussion

To evaluate the genetic diversity of RVA strains circulating in a country considering evolutionary forces involved are important issues in studies of anti-RVA vaccines. In order to reach a better understanding of how these forces act, numerous studies have investigated the genetic variation of the P[8] genotype worldwide (Arista et al., 2006, Espinola et al., 2008; Iturriza-Gomara et al., 2000; Stupka et al., 2012).

A total of 135 RVA P[8] strains belonging to VP7 genotypes: G1, G3, G5, and G9 were sequenced in order to evaluate the evolutionary relationships between the P[8] lineages of human RVA detected in Brazil before and after Rotarix™ introduction in the NIP in comparison with human and animal RVA strains detected worldwide. The results presented here demonstrate that P[8]-1, P[8]-2, and P[8]-3 lineages were detected in Brazil in the last 25 years. Fig. 1 shows the chronology of the three P[8] lineages detected in Brazil over the studied period. To our knowledgment, these lineages are preserved overtime and in different countries (Espinola et al.,

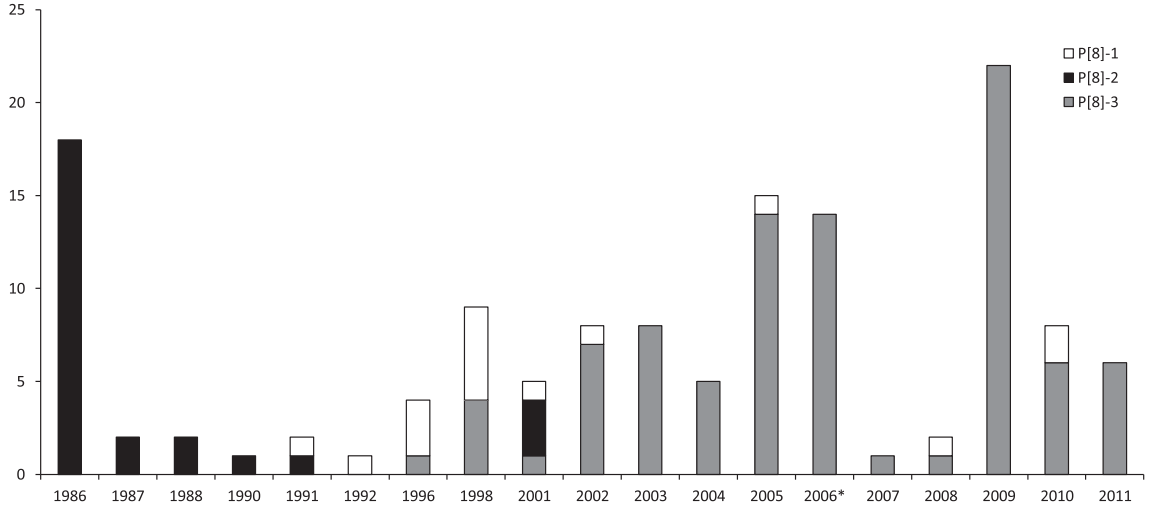

Fig. 1. P[8] lineages chronology in Brazil from 1986 to 2011. The products of the cleavage of the protein VP4 (VP5 * and VP8 *) are actually represented with the symbol *.