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Review Article

Rotavirus serotype distribution in northern Brazil trends over a 27 year period pre and post national vaccine introduction

Alessilva Oliveira [a], Joana D'Arc P. Mascarenhas [a], Luana S. Soares [a], Sylvia F.S. Guerra [a], Yvone B. Gabbay [a], Nervo Sánchez [b], Romulo E. Colindres [b], Maria Cleonice A. Justino [a], Alexandre C. Linhares [a,*]

[a] Instituto Evandro Chagas, Secretaria de Vigilância em Saúde, Virology Section, Avenida Almirante Barroso, 492, 66.090-000 Belém, Pará, Brazil
[b] GlaxoSmithKline Biologicals, estrada dos Bandeirantes 8464, 22.783-110 Jacarepaguá, Rio de Janeiro, RJ, Brazil

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ABSTRACT

In Brazil, a rotavirus vaccine was included in the public sector in March 2006. In order to identify a possible effect of vaccination on rotavirus strains we evaluated the distribution of serotypes/genotypes in northern Brazil during pre (1981–2005) and early post (2006–2008) national rotavirus vaccine introduction periods. Of 1286 rotavirus strains, 993 (77.2%) were successfully G typed. G1 strains were detected throughout the years, accounting for the majority of typed isolates ($n = 426$; 42.9%). G2 rotaviruses displayed a cyclic pattern of occurrence over time, re-emerging recently in early 2006, with detection rates as high as 91%, and remained the predominant circulating strain through 2008. G9 rotaviruses appeared during 1990–1992, re-emerged from 1998 to 2000 and rose to 43% in a gastroenteritis outbreak in northwestern Brazil in 2005. The most common combinations overall were G2P[4] (55.1%; 136/247), and G1P[8] (24.7%; 61/247). Although our data show the predominance of G2P[4] early after vaccine introduction, there is a need for continuous, long-term surveillance of circulating strains to better assess a possible effect of rotavirus vaccination on the strain ecology.

Contents

1. Introduction

Rotavirus remains the most common cause of severe acute gastroenteritis in infants and young children worldwide, being responsible for an estimated 527,000 deaths annually. Parashar et al. [1] have estimated that 82% of these deaths occur in children in the less developed regions of the world, where a rotavirus vaccine is most needed.

Currently, there are two licensed oral live attenuated rotavirus vaccines, *Rotarix*™ (GlaxoSmithKline, Rixensart, Belgium) and *Rotateq*™ (Merck Research Laboratories, USA). In large, phase III

Abbreviations: UMV, Universal Mass Vaccination; MoH, Ministry of Health; PCR, polymerase chain reaction; RT-PCR, reverse transcription polymerase chain reaction; PAGE, polyacrylamide gel electrophoresis; ST, G serotyping.

* Corresponding author. Address: Instituto Evandro Chagas, Secretaria de Vigilância em Saúde, Ministério da Saúde, Avenida Almirante Barroso, 492, 66.090-000 Belém, Pará, Brazil. Tel.: +55 91 3214 2007x2002; fax: +55 91 3214 2005.

E-mail addresses: alessilvaoliveira@iec.pa.gov.br (A. Oliveira), joanamascarenhas@iec.pa.gov.br (J.D'.P. Mascarenhas), luanasoares@iec.pa.gov.br (L.S. Soares), sylviaguerra@iec.pa.gov.br (S.F.S. Guerra), yvonegabbay@iec.pa.gov.br (Y.B. Gabbay), nervo.o.sanchez@gsk.com (N. Sánchez), romulo.e.colindres@gskbio.com (R.E. Colindres), mariajustino@iec.pa.gov.br (M.C.A. Justino), alexandrelinhares@iec.pa.gov.br (A.C. Linhares).

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trials in Latin America, Europe and the United States these two vaccines proved to be safe and have demonstrated excellent protection (>85%) against severe rotavirus disease [2,3].

Rotarix™ and *Rotateq*™ have been pre-qualified by WHO and are increasingly being introduced in national immunization programs of many countries worldwide, mainly in those regions where successful phase III efficacy trials had been conducted [4]. In this context, Brazil was one of the first countries to introduce universal immunization in March 2006 with *Rotarix*™. Brazil is also the largest country to introduce *Rotarix*™ into Universal Mass Vaccination (UMV), covering a birth cohort of 3.3 million. According to the Brazilian Ministry of Health [5], since the introduction of nation-wide rotavirus vaccination it has been observed an increase in the uptake of vaccine over time, with 81% of children having had two doses of the vaccine by 2008. However, the coverage rates of the second dose remain suboptimal in the Northern region, ranging from 27% to 65% in 2006 and 2008, respectively.

Of importance, trials recently completed in Africa and Asia have provided evidence that both vaccines are quite beneficial in poor settings and this warranted a further WHO global recommendation to include rotavirus vaccines in every nation's immunization program [6–9].

While *Rotarix*™ (monovalent; G1P[8]) and *Rotateq*™ (pentavalent; G1, G2, G3, G4 and P[8]) differ in strain composition, both vaccines appear to provide significant protection against a variety of rotavirus strains [10,11]. Nevertheless, a continuous monitoring of circulating rotavirus strains is needed to detect the possible emergence of uncommon or novel types in the community that may pose a challenge to the efficacy of the available vaccines [4].

Although recent recommendations have been proposed for the classification of group A rotaviruses using all 11 genomic RNA segments, currently a system exists for the dual classification of serotype specificities are based mainly on the segregation of VP4 (protease-sensitive; P types) and VP7 (glycoproteins; G types) genes [12]. Based on the diversity of VP4 and VP7 proteins present on the outer shell, rotaviruses are classified into 23 G and 31P types [13]. Currently, the most common strains in human disease belong to G1, G2, G3, G4, and G9 types in combination with either P[4], P[6] or P[8] types [14]. Several studies have reported that approximately 90% of the human rotavirus strains include G1P[8], G2P[4], G3P[8], G4P[8], and G9P[8] combinations.

Several serotyping/genotyping studies have been conducted across Brazil during the past two decades, underscoring the broad diversity of circulating strains over time, including the common human rotavirus strains as well as uncommon strains (G5) or globally emerging (G9) types [15]. A number of surveys conducted beginning in early 2006, coinciding with the year that the vaccine was introduced, indicate an apparent predominance of G2P[4] strains in northern, northeastern and southeastern regions of Brazil [16–19] and led to speculation that the vaccine was causing strain replacement [20].

This review assesses the diversity of rotavirus strains in northern region of Brazil between 1981 and 2008, highlighting the distribution profiles of circulating serotypes/genotypes before and early after introduction of *Rotarix*™ into the national immunization program. The patterns of genotype distribution over time provided us with the opportunity of an early insight on the issue of whether changes following introduction of universal rotavirus vaccination in Brazil might be due to natural fluctuation or possible vaccine pressure.

2. Material and methods

We reviewed data from 11 studies assessing diarrhoea burden in children and adults, conducted between 1981 and 2006 in northern Brazil [21–28]. In addition, a review was made using specifically data from the Brazilian Ministry of Health's (MoH) surveillance network, established in February 2006. This national, hospital-based surveillance was part of a program implemented by Pan American Health Organization in Latin American and Caribbean countries to assess the burden of rotavirus disease and monitoring circulating strains in the pre-vaccine era. While samples from the 11 studies were obtained from the states of Pará ($n = 10$) and Maranhão ($n = 1$), the official MoH's network included seven states located in the Amazonian region of Brazil-Acre, Amazonas, Roraima, Pará, Amapá, Maranhão and Tocantins (Table 1). Overall, there were nine hospital-based studies, one community-based study and two vaccine trials comprising variable age groups: 0–3 years (3 studies), 0–5 years (2), 0–2 years (1), 0–28 days (1), 1 month–2 years (1), 2 months–1 year (2), 1 month–29 years (1), and 0–55 years (1). The surveillance network implemented by the MoH included diarrhoeic children of $\leqslant$5 years.

Stool specimens were obtained as soon as possible after an episode of diarrhoea was detected. An aliquot of each sample was stored at 2–8 °C for a maximum of 24 h until being transported on ice to Instituto Evandro Chagas, a MoH's Rotavirus National Reference Centre. All samples were screened for the presence of group A rotaviruses by commercially available enzyme-linked immunosorbent assays (Dakopatts, Denmark or Rotaclone, USA). Serotyping/genotyping was performed using monoclonal antibodies, solid phase immuno-electron microscopy or reverse-transcription polymerase chain reaction (RT-PCR), depending on the techniques available during the conduct of each study over time. In order to assess the RNA electrophoretic profiles, polyacrylamide gel electrophoresis (PAGE) was carried out on selected faecal suspensions using a standard method which includes extraction of nucleic acid by using glass powder. G serotyping (ST) using monoclonal antibodies was performed in five studies; ST and solid-phase immune electron microscope in one, ST and RT-PCR genotyping (GT) in 2, and GT only in 4. Samples from the MoH's surveillance network were genotyped only by PCR.

3. Results

Of the 1286 stool specimens that yielded group A rotavirus antigen positive result by ELISA, a G serotype could be assigned to 993 (77.2%). These isolates (either single or mixed) comprised the five globally relevant G serotypes, that is, G1, G2, G3, G4 and G9, which make up the majority of strains associated with gastroenteritis in humans. The predominant G serotypes detected from 1981 to 2008 were G1 (426; 43.0%), followed by G2 (337; 33.9%), G9 (142; 14.3%), G4 (45; 4.5%) and G3 (15; 1.5%). In addition, 28 (2.8%) rotavirus strains had mixed serotype-specificities (Fig. 1).

G1 strains were identified throughout the years, being the predominant serotype detected in 6 out of the 11 periods of observation of each study, at prevalence rates that ranged from 24.6% to 66.7%. Rotavirus strains bearing G2 type-specificity occurred at rates that varied from 14.8% to 26.3% during studies conducted during 1981–1990, 1982–1986, and 1990–1992 and became predominant in 1992–1994 (76.7%). In the 1998–2000 period G2 types were recognized at a rate comparable to that of G1 (34.1% and 30.3%, respectively) and this was followed by an abrupt decrease in prevalence rates (0–2.3%) in studies conducted during the following 5 years. G2 then re-emerged as the leading serotype beginning early in 2006 (Study K; just before and a few months after introduction of rotavirus vaccine in Brazil), as well as during the official national surveillance (2006–2008), at rates that ranged from 60.6% to 91.0%.

With the exception of one isolate during a 1990–1992 vaccine trial in Belém, Brazil, G9 could not be recognized in six studies