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REVIEW

Human rotavirus genotypes circulating in Brazil before and after a nationwide rotavirus vaccination program established in 2006

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Abstract: Accounting for an estimated 600,000 deaths worldwide each year, rotaviruses are recognized as the most important etiologic agents causing severe acute gastroenteritis among children under the age of five years. In Brazil, until rotavirus vaccination was established in the public health system in 2006, acute gastroenteritis striking children under five years and caused by these viruses was clearly associated with 3.5 million episodes of diarrhea, 650,000 visits to outpatient health care facilities, 92,000 hospitalizations, and 850 deaths each year. After the introduction of the rotavirus vaccine in Brazil in March 2006, studies all over the country have been comparing rotavirus genotypes circulating in the recent pre- and postvaccination era. Most of these studies have reported a high prevalence of the G2P[4] genotype and also a decrease in rotavirus detection all over Brazil after the introduction of the vaccine. So far, these are preliminary studies, as a longer period of time is necessary to establish if this high prevalence of G2P[4] is due to selective pressure by the vaccine on the circulating viruses or to a normal genotype fluctuation, and if it will have any impact on vaccine efficacy in the future. This review describes results from the most recent studies addressing this issue and on rotavirus genotypic variability in Brazil.

Keywords: human rotavirus, vaccine, genotypes, prevalence, Brazil

Introduction

Accounting for an estimated 527,000–600,000 deaths worldwide each year, rotaviruses are recognized as the most important etiologic agents causing severe acute gastroenteritis in children under 5 years of age. Although most of these deaths (80%–85%) occur in low-income countries like Africa and Asia, rotavirus infection is responsible for significant morbidity and mortality in developing and developed countries.[1–4] The overall median detection rate of these viruses worldwide is 40%, being lowest in the Americas (34%) and highest in the Western Pacific and South-East Asia (45%).[1]

In Brazil, until rotavirus vaccination was established in the public health system in 2006, acute gastroenteritis caused by these viruses striking children younger than five years of age was associated with 3.5 million episodes of diarrhea, 650,000 visits to outpatient healthcare facilities, 92,000 hospitalizations, and 850 deaths each year.[4]

Rotaviruses belong to the genus *Rotavirus* of the Reoviridae family. The nonenveloped virion is 100 nm in diameter, and has a triple-layered capsid surrounding a double-stranded RNA genome of 11 segments. Each double-stranded RNA segment encodes for at least one structural (VP1-4, VP6-7) or nonstructural (NSP1-6) viral protein, except segment 11, in which overlapping open reading frames encode for both NSP5 and NSP6. These viruses can be classified into seven groups (A–G) and most strains related

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to human infection are Group A rotaviruses (RV-A), although some Group B and Group C strains have also been associated with human rotavirus infection.[2,5]

Rotaviruses are classified into G and P genotypes according to two type-specific outer capsid proteins, VP7 (glycoprotein) and VP4 (protease sensitive), respectively. These two proteins play an important role in the body's immune response to rotavirus infection, inducing the production of neutralizing antibodies. Genotype-specific binary classification is based on results from reverse transcription followed by polymerase chain reaction (RT-PCR) and nucleotide sequence analysis. Based on molecular differences, at least 25 different G genotypes and 33 P genotypes have been described so far.[5–12]

The most common G and P genotype combinations identified in human rotavirus strains around the world have been G1P[8], G2P[4], G3P[8], G4P[8], and G9P[8].[1,5,13] However, because VP4 and VP7 genes segregate independently, many G and P combinations can be observed in natural infections, and almost 50 different genotype combinations have been described over the years, varying regionally and temporally.[1,14–20] Especially in developing countries, like Brazil, a diverse range of circulating genotype combinations, including rare genotypes and "mixtures" of more than one genotype G and/or genotype P infecting one patient, have been identified.[13,17,21–25]

The frequent occurrence of unusual G and P combinations is due to the segmented genome of rotaviruses, which can undergo genetic reassortments when at least two different RV-A strains infect a single cell, resulting in (i) new strains, (ii) characterization of more than one G or P-type in one sample, (iii) introduction of animal rotavirus genes in human rotavirus population and vice-versa and (iv) natural RV-A reassortants infecting different animal species.[15,26–29]

Over the past 20 years, many studies have established that naturally acquired RV-A infections may confer protection against reinfections and subsequent severe diarrheic episodes.[2,17,30] Primary rotavirus infections stimulate serotype-specific immune response (homotypic immune response) and reinfections are usually caused by a rotavirus with different genotypes than those responsible for the primary infection. However, subsequent infections may stimulate heterotypic immune responses.[31] The cross-immunity protective potential is not completely elucidated and becoming infected by one rotavirus serotype does not necessarily mean that the patient is protected against infection caused by other serotypes, but only against more severe acute gastroenteritis.[31,32]

An important study conducted in Mexico showed that protection against moderate and severe diarrheic episodes caused by rotavirus infection was established after two primary infections, even when those were asymptomatic, as a result of establishment of immunity against the virus. Almost all children enrolled in this survey (96%) became infected before two years of age, 69% were infected twice, 42% were infected three times and some experienced four or five rotavirus infections. Natural infection was found to offer low protection against moderate diarrheic episodes and even lower protection against asymptomatic infections.[33]

These findings were responsible for encouraging scientists to develop a vaccine against rotaviruses over the years. As rotaviruses can affect children in both developed and developing countries with distinct economical and social environments, improvements in sanitary measures are not sufficient to mitigate the spread of rotavirus among children.[1–3,34] Therefore, vaccination against rotavirus constitutes a more effective means of preventing and controlling the transmission of the virus in all populations.[1,10,35–37] It is estimated that vaccination of small children against rotaviruses would be able to prevent 352,000 to 592,000 deaths each year, especially in regions like Africa, Asia, India and China, where 90% of the deaths occur.[2]

There have been several attempts at RV-A vaccine strategies over the last two decades.[37,38] The first candidate ever authorized for administration in children was the RotaShield® vaccine (Wyeth Lederle Vaccines, Philadelphia, PA), a tetravalent vaccine, composed of one attenuated virus (Simian/MMU18006 with G3 specificity) and three recombinant viruses (corresponding to G1, G2, and G4 human serotypes). This vaccine displayed a 90% efficiency rate against RV-A severe diarrheic episodes and 60% against all other cases of RV-A infection, and it was licensed in October, 1998 in the United States, to be administrated in three doses (2, 4, and 6 months of age). However, nine months after licensing and vaccination of more than 600,000 children, the use of RotaShield was suspended by the Food and Drug Administration (FDA) as 15 cases of intussusceptions were detected in healthy children, two weeks after vaccination. After investigation, it was concluded that the risk of intussusception in infants younger than 12 months of age, within two weeks after receiving the first dose of Rotashield, increased 20 to 30 times than would be expected for children at that age. The risk also tripled or was seven times greater than expected when the second dose of the vaccine was considered, after two weeks of its administration. For these reasons, RotaShield was withdrawn from the market in October, 1999.[37,38]

Nowadays, two vaccines against rotavirus are licensed and in use in many countries around the world, including

Brazil. RotaTeq (Merck & Co, Inc, Whitehouse Station, NJ) vaccine was licensed in the United States in 2006 and in April, 2008 in Brazil and consists of a live-attenuated oral pentavalent vaccine that contains five live reassortant rotaviruses: four reassortant rotaviruses expressing VP7 (genotypes G1, G2, G3, or G4) from a human rotavirus strain and VP4, from the bovine rotavirus strain WC3 (G6P[7]). The fifth reassortant virus expresses VP4, genotype P1[8], from a human rotavirus strain and VP7, genotype G6, from the same bovine rotavirus strain, WC3. It must be administrated in three doses to infants between 6 and 32 weeks of age. The first dose of RotaTeq should be administered between 6 and 12 weeks of age.[39] This vaccine gives protection against rotavirus displaying genotypes G1, G2, G3, G4 and P[8], which represents 75% of the genotypes that infect humans worldwide. Also, efficacy and safety trials through the first rotavirus season after vaccination showed that RotaTeq has a 74% primary efficacy against any grade of severity of rotavirus gastroenteritis caused by naturally occurring serotypes G1, G2, G3, or G4 and primary efficacy against severe rotavirus gastroenteritis caused by the same serotypes of 98% and no risk of intussusception was detected.[39]

Rotarix (GlaxoSmithLine Biologicals, Rixensart, Belgium) is the other vaccine against rotavirus infection being used worldwide. It was first licensed in Mexico and Dominican Republic in 2004, followed by Brazil in 2006 and as of May 2007, Rotarix has been approved in 50 countries in Latin America, Europe, Asia, and Africa, with more than 11 million doses distributed.[38] In April, 2008 it was approved by the FDA and licensed to be used in the United States. It is a live-attenuated oral monovalent vaccine derived from the human 89-12 rotavirus strain, which belongs to the most common genotype that infects humans: G1P[8].[38] Rotarix administration is recommended in a two-dose vaccination schedule beginning at 6 weeks of age, with an interval of at least four weeks between the first and second dose. The two-dose series should be completed by 24 weeks of age.[40]

After a large trial conducted in eleven Latin American countries and in Finland with more than 63,000 infants enrolled, results showed that Rotarix does not increase the risk of intussusception among it is users and also shows protection rates of 85% against severe rotavirus gastroenteritis, 100% against the most severe dehydrating rotavirus gastroenteritis, 92% efficacy against any grade of severity of rotavirus gastroenteritis caused by G1 genotype and 88%, against genotypes G3, G4 and G9. Nevertheless, it is important to mention that during this large trial, efficacy against genotype G2 was very low (41%) and thus was not significant.[40]

However, even displaying a relatively low efficacy rate against genotype G2, Rotarix showed 81% cross-protection against G2P[4] strain, when using the meta-analysis of efficacy trials against non-G1 and non-P[8] strains.[11] Constant monitoring of circulating genotypes in a population where Rotarix has been implemented is crucial for understanding its cross-protection potential and how it will affect emerging rotavirus genotypes and rotavirus-caused gastroenteritis all over the world.

Brazil was the first country to include vaccination against rotavirus in its public health system by introducing Rotarix vaccine into the Brazilian Expanded Immunization Program in 2006. Therefore, the purpose of this paper was to describe rotavirus genotypes circulating in the Brazilian population more recently and to investigate a possible genotype shift as a consequence of vaccination. This analysis was based on data available for this period (data published in 2009, 2010, and early 2011).

As a brief retrospective, it is important to mention genotypes circulating in Brazil during the 1980s, before reverse transcription followed by polymerase chain reaction and nucleotide sequence analysis were implemented for rotavirus genotyping, until 2006. Serotypes G1 and G2 circulated in Brazil from 1983 to 1985, and serotype G3 also emerged in 1988, playing an important role in many rotavirus gastroenteritis cases.[30] In the early 1990s, when the reverse transcription followed by polymerase chain reaction technique was established for rotavirus genotyping,[41–44] the most frequent genotypes described around the world (G1, G2, G3, and G4) were circulating in Brazil. An uncommon genotype also emerged (G5P[8], G5 is a typical porcine genotype), quickly spreading among children with diarrhea in this country (through nine Brazilian states and in the Federal District) and in South America.[13] It is worth mentioning that, during this period, the detection rate of G2P[4] genotype decreased from 26% during the 1982–1995 period to 2% during the 1996–2005 period.[17] Linhares[30] also mentioned that in 1995–1999, the G2 genotype had a prevalence of 80% in Brazil and of 35% in 1998–2000. During the latter period, the G1 genotype accounted for 42% of severe episodes of infectious diarrhea.

As discussed by Leite et al,[17] many studies from the 1996–2005 period showed that G9P[8] genotype detection in Brazil increased, following a worldwide pattern.[13] According to these authors, this genotype was detected in 27% of rotavirus samples and showed a wide geographic distribution. A wide variety of G and P genotype combinations (including uncommon ones and combinations with

typical animal rotavirus genotypes) and samples displaying multiple mixtures of G and/or P genotypes were also described by these authors. A study of rotavirus samples from the northeast region of Brazil, collected in 1994–1996 also reported a great diversity of genotypes, including a prevalence of G type mixtures of 41.5%, P genotypes of 27.6%, and unusual G/P combinations containing mixtures of human and animal rotavirus genotypes, suggesting genetic reassortment and interspecies transmission.[21] In a recently published article, Caruzo et al[5] described unusual G/P combinations, occurrence of human P genotypes and 51.6% of P genotype mixtures for bovine rotavirus samples from the central region of Brazil, reinforcing the occurrence of genetic reassortment and interspecies transmission in nature, as referred to by many other authors.[12,18,19,27,31,45,46]

It is also important to mention that another uncommon G genotype, G12, appears to be the new G genotype emerging in the human population, which has been detected in many countries since 1986, predominantly associated with either P[8] or P[6].[47,48] As mentioned by Matthijnssens et al,[10] this genotype has shown an almost exponential increase in detection around the world, especially after 1999. In Brazil, this genotype has been reported in 2002–2004.[10,24] These emerging G12 strains also appear to have a rather variable overall genetic constellation, although displaying highly conserved VP7 genes, which suggests, once more, the frequent occurrence of genetic reassortments among rotavirus strains.[10,16,46]

All data reviewed by Linhares,[30] Leite et al,[17] and Matthijnssens et al[10] show the great variety of genotypes circulating in Brazil over the years and the importance of constant epidemiologic vigilance for introduction of efficient vaccines against rotavirus[49] and possible strain replacement in the vaccines, as recommended by the World Health Organization.[50]

Following the introduction of rotavirus vaccine in Brazil in March 2006, Leite et al[17] also reviewed eight scientific papers regarding genotype distribution in different Brazilian states. The reviewers found a predominance of G2P[4] and G2PNT (NT = nontypable) in those studies as G2 accounted for 74% (148) of 199 positive rotavirus samples tested. Genotypes G1 (3%), G3 (3%), G9 (11%), and mixed/atypical genotypes (8%) were also detected. In 2006–2007, the G2 genotype was detected in different regions in Brazil as the southeast region (Rio de Janeiro and Minas Gerais) and the northeast region (Sergipe, Pernambuco, and Piauí). Also, in Northern Brazil, a study detected the same G2 genotype in 90% of all rotavirus isolates collected in early 2006.[17]

For the present review, seven scientific papers were analyzed in order to add more data on the rotavirus genotypes circulating in Brazil immediately before and after the introduction of the rotavirus vaccine in this country. Two articles were published in 2009, and analyzed samples collected in 2005–2007.[51,52] The other five articles (four published in 2010[53–56] and one in 2011[57]) reported rotavirus genotyping data for positive samples collected in 2004–2009. These studies included samples from different regions of Brazil.

Munford et al[52] analyzed 221 rotavirus-positive samples from four different regions in Brazil: southeast, south, northeast, and central regions. They were collected from children under five years of age in 2005–2006. Results showed that genotype G9 alone was the most prevalent (51.6%), followed by G2 (34.8%), and G1 (15.4%). However, the G/P genotype combination G2P[4] was the most frequent one (28.7%), followed closely by G9P[8] (28.2%), G9P[4] (14.9%), and G1P[8] (10.1%). Interestingly, when the data are analyzed by region, it can be concluded that genotype G2P[4] was predominant in the central (58.5%) and northeast (48.9%) regions, and genotypes G9P[8] and G1P[8] were predominant in the southeast (48.9%) and south (75%) regions.

Carvalho-Costa et al[51] studied 133 rotavirus-positive samples collected from February 2005 to December 2007 in Rio de Janeiro (southeast region). Among patients ineligible for full vaccination, genotype G9P[8] was the most frequent one (45%) in 2005, followed by G3P[8] (30%), G1P[8] (14%), G2P[4] (1.4%), and other genotypes, mixed or nontypable (9.5%). In 2006, genotype G2P[4] was the most frequent one (41%), followed by other genotypes, mixed or nontypable (23%), G3P[8] (8%), G9P[8] (5%), and G1P[8] (2.9%). Positive samples from 2007 revealed only two genotypes: G2P[4] (92%) and G1P[8] (8%). These authors also genotyped positive samples from vaccinated and nonvaccinated patients from 2007, and all samples displayed genotype G2P[4].

It is important to mention that Carvalho-Costa et al[51] also reported that rotavirus detection rate decreased from 38% in 2005 to 24% in 2007, suggesting that vaccination may be reducing the risk of severe diarrhea among children under five years of age. Sáfadi et al[56] also observed a reduction in the rate of rotavirus detection among young children (42.2%), especially in infants (69.2%), associated with a 59% reduction in hospitalization events due to rotavirus gastroenteritis in the postvaccination period. The authors conducted a prospective surveillance at a sentinel hospital in São Paulo (southeast region) in 2004–2008. This reduction in the number of

rotavirus-caused gastroenteritis cases, and rotavirus detection due to introduction of one or both rotavirus vaccines, Rotarix and RotaTeq, has also been observed elsewhere, as discussed by Matthijnssens et al.[10]

Sáfadi et al[56] genotyped 70 (86.4%) of 81 rotavirus-positive samples collected in 2006–2008 and observed an increase in genotype G2P[4]. In 2006, G9P[8] accounted for 41.2% of all samples tested, followed by G9P[4] (23.5%), G2P[4] (8.8%), G2P[8] (5.9%), and other genotypes, mixed or nontypable (20.6%). However, in 2007, G2P[4] was detected in 58.8% of all samples tested, and genotypes G9P[8], G2P[8], and GNTP[8] were present in lower percentages of 29.4%, 5.9%, and 5.9%, respectively. Finally, in 2008, the authors observed the highest occurrence of genotype G2P[4] at 73.7%, followed by only 10.5% and 15.8% of genotypes G2P[6] and G2P[NT], respectively.

Another study selected for this review showed that, in the northern region of Brazil, the emergence of genotype G2P[4] occurred in 2006–2008.[54] A total of 30 rotavirus-positive samples (12.45%) were genotyped, and 90% of them were characterized as G2P[4], followed by G1P[8] (6.67%) and G9P[8] (3.33%). These authors also performed nucleotide sequencing of the VP7 gene and its phylogenetic analysis in 15 of the G2 strains. They identified two possible new distinct sublineages of this genotype, and reinforced the need for surveillance of rotavirus in order to detect the possible appearance of mutant escape strains. The occurrence of these mutant escape strains may eventually pose a challenge to vaccine strategies, reflecting selective pressure induced by the vaccine. However, the authors were cautious to say that the predominance of G2P[4] detected in their study was more likely to reflect the cyclic pattern of occurrence of this serotype in northern Brazil and also elsewhere in Latin America, but that constant surveillance of RV-A in the post-vaccination era is crucial for understanding the cyclic circulation of genotype G2 in Brazil.[54]

Morrillo et al[55] also reported the reemergence of G2P[4] genotype in 2007 when they analyzed rotavirus-positive samples from day care centers in São Paulo, and characterized 28.5% of them as G9P[8], 14.2% as G1P[8], and 20% as GNTP[NT] in 2004; 28.5% as G9P[8] and 5.4% as G1P[8] in 2005; 9% as GNTP[NT] in 2006; and 11.1% as G2P[4] and as 38.8% GNTP[NT] in 2007. They also detected a reduction in rotavirus detection rate from 65.7% in 2004 to 50% in 2007, with no cases in 2008. These authors also suggested that the re-emergence of the G2P[4] genotype may be due to a natural genotype fluctuation, but that "implementation of post marketing surveillance is recommended, especially because diverse RVA strains cocirculate 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". Furthermore, they emphasize that a better understanding of rotavirus infection ecology will confirm that the observed reduction in the detection rate is due to vaccine implementation or to occurrence of genotypic patterns of the virus in Brazil.

In one of the largest studies regarding circulation of rotavirus genotypes in Brazil,[57] 6109 stool samples, collected from January 2005 to December 2009 in 18 Brazilian states, were tested and 1242 (20.3%) were positive for rotavirus. These samples were molecularly characterized by RT-PCR into genotypes G and P. Briefly, the most prevalent genotype in 2005 was G9P[8]/G9P[NT], accounting for 52% of the samples; in 2006–2008 there was an important increase in prevalence of genotype G2P[4]/G2P[NT], accounting for 42%, 66%, and 85%, respectively and in 2009, this genotype was still the most frequent one, but its prevalence reduced to 37.5%. During those five years, other genotypes were also characterized, but less frequently: G1P[8]/G1P[NT], G3P[8]/G3P[NT], G4P[8]/G4P[NT], GNTP[4], GNTP[6], GNTP[8], mixed genotypes and atypical genotypes or atypical G/P combinations, such as G5P[6], G5P[8], G12P[9], G2P[6], G4P[9], G2P[8], and G9P[6]. The authors also noticed a decrease in rotavirus detection during the five years of study, among samples from children under two years of age, regardless vaccination: 33.8% in 2005, 27.7% in 2006, 16.8% in 2007, 22.9% in 2008, and 18.3% in 2009.

Carvalho-Costa et al[57] emphasize the importance of knowing the distribution of rotavirus genotypes in a country/geographic area which intends to implement a rotavirus vaccine and, the need for constant surveillance in order to understand rotavirus genotypic diversity and to investigate possible strain replacements after introduction of massive rotavirus vaccination. However, at this time, they consider the re-emergence of genotype G2P[4] in 2005–2008 and its decrease in 2009 as a natural fluctuation of this genotype, and a distinguishing mark of rotavirus genotype distribution.

To underscore the great variability of rotavirus genotypes in Brazil (Figure 1), it is worth mentioning a study from Gómez et al[53] in which two strains from Rio de Janeiro, collected in 2002, displayed unusual P/G combinations, ie, G8P[8] and G8P[4], which could be a result of genetic reassortment between rotavirus G8P[4] and G9P[8] strains circulating in Rio de Janeiro (southeast region) in that same year. The authors also did a phylogenetic analysis on those strains, and showed a close genetic relationship with strains

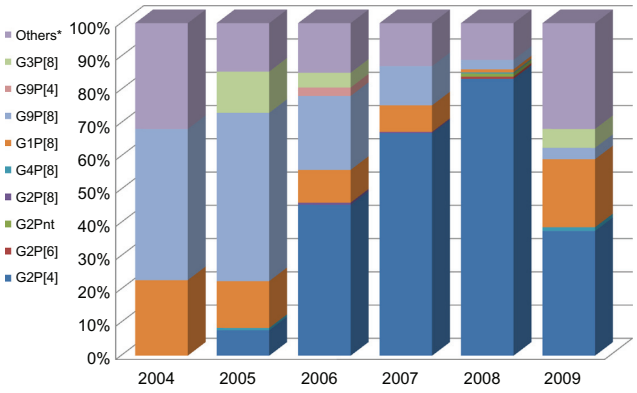

Figure 1 Human rotavirus genotypes distribution in Brazil during 2004–2009, based only on new data analyzed for this review. Data from Munford et al[42] were not included in the graph as the authors did not specify the year of sample collection, only the period (August 2005 to August 2006).
Notes: Data from Gomez et al[21] were not included, as this study selected only two samples for phylogenetic analysis. *Includes other genotypes, mixed infections, and nontypable samples.

from Africa, where it had become prevalent over the years. Gómez et al[53] findings regarding G8 strain were also similar to those reported by Montenegro et al[58] for strains from Recife, located at the northeast region of Brazil.

Rotavirus genotype variability in Brazil and throughout the world has been reported over the years, as reviewed by Linhares,[30] Santos and Hoshino,[13] Leite et al,[17] Greenberg and Estes,[5] Matthijnssens et al,[10] and elsewhere. Data collected for over more than 20 years showed the capability of rotavirus to infect humans and other animals, crossing the species-specific barrier, resulting in a constellation of genes circulating in nature. These interspecies infections, together with genetic reassortment events, point mutations and, nowadays, the possibility of selective pressure on emerging rotavirus genotypes due to mass vaccination, only reinforce the need for constant surveillance all over the world and a better understanding of ecology and epidemiology of rotaviruses and their implication on the future of rotavirus vaccination and public health care.[10,59] In addition, the decrease in rotavirus detection rates reported by the authors mentioned in this review must be investigated and better understood by comparing data from pre- and post-vaccination eras in order to confirm whether it may be due to the introduction of the vaccine in the population,[10,51,55,56,60] or not, as stated by Carvalho-Costa et al,[57] who noticed this reduction in 2005–2009 regardless of vaccination, and by Gouvea et al[61] who also believe that rotavirus "… vaccination impacted marginally, if at all, on the incidence of childhood gastroenteritis" when comparing data from pre- and post-vaccination periods, after a 4.5-year study in Rio de Janeiro.

Regarding the re-emergence of genotype G2P[4] after introduction of the rotavirus vaccination in Brazil, it is important to say that, based on all studies reviewed for this article, it is not yet possible to assume that this reemergence is due to selective pressure created by the vaccine. Data are still needed, especially in the next season, to determine if the appearance of genotype G2P[4] is a result of natural fluctuation of rotavirus genotypes, which appears to last ten years for G2P[4], or if the selective pressure caused by the vaccine is acting on the rotavirus circulating population.[55] An interesting study conducted in Australia has shown that regions in that country which only introduced the Rotarix vaccine reported a higher prevalence of serotypes G2 and G9, and regions that introduced only the RotaTeq vaccine reported an increased prevalence of G3.[62] The authors of this study concluded that it was too soon to affirm that these patterns were not natural fluctuations of those genotypes, but it gives more data on understanding the impact of the vaccine in the genotype variability.

Another study in Belgium,[60] which introduced Rotarix to the market in 2006 and RotaTeq in 2007, also reported a significant increase in G2 prevalence in postvaccination seasons (30%–40%). The authors were still cautious on stating that G2 prevalence increase may be due to vaccination and suggest that constant surveillance must be carried on to elucidate this question in the future.

It is noteworthy that Carvalho-Costa et al[57] discussed this, although an increase in the prevalence of G2P[4] was clearly observed in their study for 2006–2008: "… the early circulation of G2P[4] rotavirus in Central-Western Brazil in August 2005, prior to Rotarix vaccination introduction, in states that border other South American countries; this includes i) the state of MatoGrosso do Sul, which borders Bolivia and Paraguay, a country where rotavirus genotype G2 circulation was reported during 2004–2005 and rotavirus vaccination is not performed; ii) the state of Acre, which borders Peru and Bolivia, countries where universal rotavirus vaccination was still not implemented. The analysis of the geographic localization of the G2 rotavirus detection from 2005 to 2009 suggests that this genotype could have been introduced in Western Brazil and have spread progressively and, in 2008, was present in most surveyed localities". Another example of this close relationship among rotavirus genotypes spreading and South American countries is the reemergence of the G2P[4] genotype in Argentina since 2004.[63] In that study, the authors reported 21.4% of 140 rotavirus-positive stool samples collected in 2004–2007, as G2P[4]. Therefore, the

explanation may be found in this situation for such an expressive and unusual re-emergence of genotype G2P[4] in Brazil and it may be taken into account for future analysis.

The need for constant rotavirus genotype monitoring has always been reinforced over more than 20 years of studies on rotavirus epidemiology and was crucial for the development of efficient vaccines.[8,30,49,50] Now that those vaccines are finally being implemented around the world, these studies will be even more indispensable.[1,10,55,57] Understanding natural genotype fluctuations, selective pressure caused by the vaccine, vaccine efficacy against different genotypes, genetic reassortments, mutations, and rotavirus interspecies transmission will give us more opportunities to improve health care with vaccination, reducing rotavirus detection rates, disease severity, hospitalizations and human and economic losses worldwide.

Disclosure

The author reports no conflict of interest in this work.

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