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Rotavirus Strain Surveillance for Three Years Following the Introduction of Rotavirus Vaccine into Belém, Brazil

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The monovalent human rotavirus (RV) vaccine, RIX4414 (Rotarix™, GlaxoSmithKline Biologicals) was introduced into Brazil's Expanded Program on Immunization in March 2006. One year after vaccine introduction, the G2P[4] strain was found to be predominant, with an apparent extinction of many non-G2 strains. This study investigated the diversity of circulating strains in the three years following RIX4414 introduction. Between May 2008 and May 2011, stool samples were collected from children aged ≥12 weeks who were hospitalized for severe lab confirmed RV-gastroenteritis (≥3 liquid or semi-liquid motions over a 24-h period for <14 days, requiring ≥1 overnight hospital stay and intravenous rehydration therapy) in Belém, Brazil. RV-gastroenteritis was detected by ELISA and the G- and P-types were determined by RT-PCR assays. During the first year of surveillance nucleotide sequencing was used for typing those samples not previously typed by RT-PCR. A total of 1,726 of 10,030 severe gastroentertis hospitalizations (17.2%) were due to severe RVGE. G2P[4] was detected in 57.2% of circulating strains over the whole study period, however it predominated during the first 20 months from May 2008 to January 2009. G1P[8] increased in the last part of the study period from May 2010 to May 2011 and represented 36.6% (112/306) of the circulating strains. G2P[4] was the predominant RV strain circulating during the first 20 months of the study, followed by G1P[8]. These findings probably reflect a natural fluctuation in RV strains over time, rather than a vaccine-induced selective pressure. ***J. Med. Virol. 87:1303–1310, 2015.*** © 2015 Wiley Periodicals, Inc.

KEY WORDS: rotavirus; Belém, Brazil; genotypes; post-vaccination; gastroenteritis

INTRODUCTION

Rotavirus (RV) is the leading cause of acute gastroenteritis among children younger than 5 years of age worldwide [Parashar et al., 2006]; it accounts for approximately 40% of all cases of severe infant diarrhea [CDC, 2011]. The World Health Organization (WHO) estimates that in 2008 around 453,000 annual child deaths were due to RV [WHO, 2013a,b].

Abbreviations: CI, confidence interval; ELISA, enzyme linked immunosorbent assay; IV, intravenous; RV, rotavirus; RVGE, rotavirus gastroenteritis; SD, standard deviation; EPI, Expanded Program on Immunization; RT-PCR, reverse transcriptase polymerase chain reaction.

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Recent estimates from Latin America and the Caribbean revealed that, in the absence of vaccination, RV causes up to 229,656 hospitalizations and 6,302 deaths each year among children younger than 5 years of age [Desai et al., 2011]. In Brazil during the pre-vaccine period, RV infections have been estimated to cause 850 annual deaths and 92,453 hospitalizations in children less than five years of age [Sartori et al., 2008].

Two live oral RV vaccines are currently available: a pentavalent, human-bovine reassortant vaccine with RV types G1–G4 and P[8] (RotaTeq®, Merck, NJ, USA) and a monovalent vaccine with an attenuated human G1P[8] RV strain, (RIX4414, [Rotarix™, GSK Biologicals, Rixensart, Belgium]) [Grimwood and Lambert, 2009]. RV strains carrying either G1–G4, or G9, combined with P[4] or P[8] have been found to be the most prevalent causes of RV disease in humans [WHO, 2013a,b; Trojnar et al., 2013]. However, substantial temporal and geographical changes in strain prevalence can lead to the emergence of G- and P-types such as G12 carrying either P[8] or P[6] [Santos and Hoshino, 2005; O'Ryan, 2009] which theoretically could evade immunity provided by the RV vaccines, although P[8], specifically, is included in the composition of both currently available rotavirus vaccines [Matthijnssens et al., 2011].

In early rotavirus vaccine adopter countries the effectiveness of either RotaTeq® or Rotarix® has been demonstrated, as well as the substantial impact on childhood morbidity and mortality due to gastroenteritis [Tate and Parashar, 2014].

In March 2006, Brazil was one of the first countries to introduce the monovalent human rotavirus vaccine into their Expanded Program on Immunization (EPI), which covers a birth cohort of around 2.9 million [DATASUS, 2013]. By December 2014, 73 countries had introduced RV vaccines into their EPI's, therefore increasing the need for conducting post-licensure surveillance studies [PATH, 2014]. Although such studies have provided reassuring evidence for the monovalent human rotavirus vaccine impact and effectiveness, whether vaccine-induced selective pressure might impact circulating RV strains is still debated [Gentsch et al., 2009; Tate et al., 2010; Patel et al., 2011; Matthijnssens et al., 2012]. The implementation of the monovalent human rotavirus vaccine into the Brazilian EPI in 2006 coincided with a dramatic increase in circulating G2P[4], leading some investigators to suggest that a serotype replacement had occurred as a result of vaccine-induced selective pressure mechanisms [Gurgel et al., 2008; Leite et al., 2008; Nakagomi et al., 2008; van Doorn et al., 2009; Carvalho-Costa et al., 2011; Linhares et al., 2011; Dulgheroff et al., 2012; Oliveira et al., 2012]. However, as most of these studies covered just a short surveillance period following vaccine introduction, the data obtained could reflect a natural fluctuation of G2P[4] over time, rather than a consequence of vaccination.

In this study results from a long-term (2008–2011) hospital-based surveillance study of RV strains among children with severe RV gastroenteritis in Belém, Northern Brazil is reported.

MATERIALS AND METHODS

Study Setting and Design

This hospital-based study was conducted in Belém, Brazil between May 2008 and May 2011. Belém has a population of 2.08 million and an annual birth cohort of 24,054 [Justino et al., 2011]. Strain surveillance was performed in two stages: May 2008–May 2009 in parallel with a case-control study to estimate the effectiveness of RIX4414 at four large urban hospitals [Justino et al., 2011]; and for an additional two years (May 2009–May 2011) at two of these hospitals, which received 50% of all gastroentertis-related pediatric hospitalizations in this area, the covered population was still considered to be representative of Belém as a whole.

The protocol was approved by the Independent Ethics Committee of the Brazilian Ministry of Health's National Rotavirus Reference Laboratory, Instituto Evandro Chagas (IEC) and the Brazilian Ministry of Health. The study was carried out in accordance with the Declaration of Helsinki and Good Clinical Practice. Written informed consent was obtained from the parents/legal guardians of children before enrolment.

Case Definition

Cases were defined as children at least 12 weeks of age, who had been born after March 6, 2006, and were hospitalized for laboratory-confirmed severe RVGE ($\geq$3 liquid or semi-liquid motions over a 24-h period for $<$14 days, requiring $\geq$1 overnight hospital stay and intravenous rehydration therapy) [Justino et al., 2011]. This ensured that children were eligible to have received at least one vaccine dose at enrolment; each child was included only once in the study.

Assessments

Parents/guardians were interviewed to collect demographic data and relevant medical history. Individual vaccination history was not collected since evaluating vaccine effectiveness was not the purpose of the study.

As part of routine practice, stool samples were collected within 48 hr of admission and transported to the IEC, for RV testing using enzyme-linked immunosorbent assay (ELISA) (RIDASCREEN® Rotavirus; R-Biopharm, Darmstadt, Germany). The tests were performed according to the manufacturer's instructions and included positive and negative controls.

Exclusion criteria included logistical reasons, late screening or collection, insufficient sample. However, genotyping was completed for 1,076 samples as two samples had insufficient quantities. Genotyping was

done using reverse transcriptase-polymerase chain reaction (RT-PCR), to determine G- and P-types. RT-PCR was performed using a two-step amplification process as previously described [Boom et al., 1990; Gouvea et al., 1990; Gentsch et al., 1992; Das et al., 1994; Leite et al., 1996]. During the case-control study only (first year of monitoring), nucleotide sequencing was performed with strains not typed previously by RT-PCR. Briefly, amplified first round products of the VP7 and VP4 genes were sequenced using the Big Dye Terminator Cycle Sequencing kit (Applied Byosystems, Foster City, CA), following the manufacturer's instructions. As per protocol nucleotide sequencing was not performed during the second and third years of surveillance.

The second step was a nested PCR using G or P specific oligonucleotide primers targeted at G (G1–G4 and G9) and P (P[4], P[6], P[8], and P[9]) RV types. Genotyped RV strains were categorized according to their possible origin, as reported before by Iturriza-Gómara et al. [Iturriza-Gómara et al., 2011].

Statistical Analyses

Data analyses were performed using SAS version 9.1 (SAS Institute, Inc., Cary, NC).

The distribution of RV G- and P-types was tabulated and compared with respect to age (3–5 months, 12–23 months and >24 months), origin (common human strains, reassortants among common human strains, possible zoonotic strains and possible animal human hybrids) and time of the year using chi-square and Fischer exact tests. All tests were two-tailed and differences between variables were considered statistically significant at P-values ≤ 0.05.

RESULTS

Of 10,030 severe gastroenteritis hospitalizations that were screened for RV (between May 2008 and May 2011), RV was identified in 1,726 (17.2%) cases by ELISA-525 (out of 538 samples collected from May 2008 to April 2009, during the case-control study), 260 from May 2009 to April 2010, and 293 from May 2010 to May 2011. Among 1,726 ELISA-positive stool samples, a subset consisting of 1,078 samples was further analyzed (62%).

The mean age of the subjects was 18.5 ($\pm$9.4) months and 52.8% were male (Table I). The highest percentage of hospitalizations for severe RV gastroenteritis was seen in children $\geq$12 months of age (76.1%; 820/1,078).

RV genotyping by RT-PCR was done on 1,076 samples and enabled G- and P-types to be successfully determined in 88.7% (954/1,076) of cases. Strains that could not be fully G- and/or P-typed represented 11.3% (122/1,076) of the total samples. Single G and P strains were present in 88.6% (845/954) of samples and 11.4% (109/954) had mixed RV strains. G2P[4] was the most commonly observed RV strain (57.2% [615/1,076]) followed by G1P[8] (14.9% [160/1,076]). The most common mixed RV strains were G2P[4]-+P[6] (2.9%; 31/1,076) and G1+G2P[4] (2.2%; 24/1,076) (Fig. 1).

RV strains were classified according to their possible origins as: common human strains (74.1%; 797/1,076); reassortant among common human strains (0.6%; 6/1,076); potential zoonotic strains (0.2%; 2/1,076) and possible human-animal hybrids (2.7%; 29/1,076) (Table II).

G2P[4] was the most common strain in all age groups: 3–5 months (46.9% [95% CI: 29.1-65.3]; 6–11 months (56.0% [95% CI: 49.3–62.6]); 12–23 months (60.9% [95% CI: 56.6–65.0]); $\geq$24 months (52.0% [95% CI: 46.0–58.0]). G1P[8] was the second most prevalent RV strain across the four age groups ranging between 6.3% and 18.6% (data not shown).

Between May 2008 and April 2009, the most commonly found multiple combinations were G2P[-Mixed] (79.5%; 31/39). GMixedP[4] (47.2%; 17/36) was frequently seen between May 2009 and April 2010. The majority (70.5%; 86/122) of either partially typed or fully untypeable RV strains were detected from May 2010 to May 2011 (Table II).

G2P[4] strains were identified throughout the study period, but predominated from May 2008 until December 2009. Frequency rates ranged from 100% (May and June 2008) to 29.4% (June 2009). An increase in G1P[8] strains was observed from

TABLE I. Baseline Characteristics of Children <5 Years of Age (N = 1,078)

Characteristics	Categories	n	Value	%
Age (months)	Mean	1,078	18.5	–
	SD		9.4	
Gender	Female	509	–	47.2
	Male	569	–	52.8
Race	African heritage	35	–	3.3
	Asian heritage	1	–	0.1
	White Caucasian	11	–	1.0
	Other*	1,030	–	95.6
	Missing	1	–	0.1
Currently live in Belem	Yes	971	–	90.1
	No	107	–	9.9

N, number of severe RVGE hospitalizations; n, number of subjects in a given category; value, value of the considered parameter; % = n/N × 100; Other*, mixed race; SD, standard deviation.

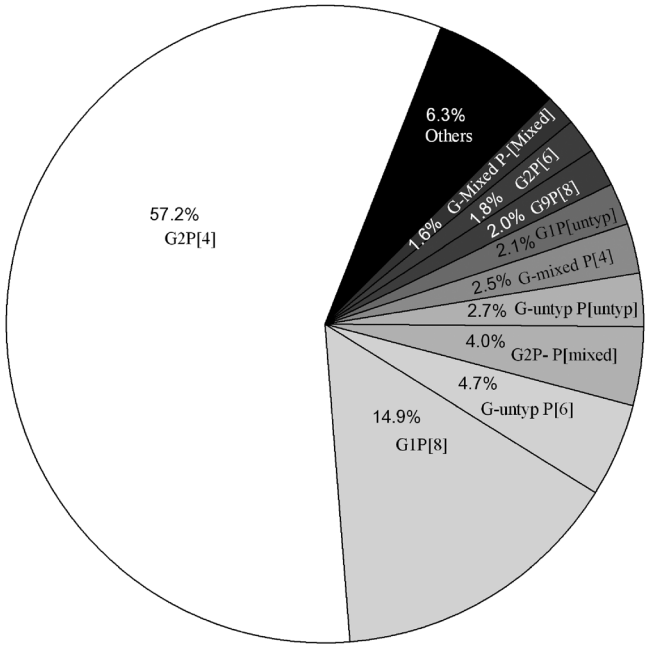

Fig. 1. Strain distribution (N = 1,076). Others = G12P[6], G2UNTYP, G1P-Mixed, G1P[6], G-Mixed P[8], G-Mixed, UNTYP P, G1P[4], G9P[4], G-Mixed P[6], G12P-Mixed, G9P[6], G9UNTYP, GUNTYPP-Mixed, G3P[8] and G4P-Mixed.

May 2010 to May 2011, representing 38.2% (112/293) of the circulating strains (Fig. 2). G1P[8] RV strains were not detected during the first 9 months of the study but were found at monthly low frequencies ranging from 2.5% (1/40) to 21.6% (6/37) from February 2009 until January 2010.

DISCUSSION

The present analysis is an extension of a previously published 12-month case-control study, which assessed the effectiveness of a full 2-dose series of RIX4414 vaccine in preventing severe RV gastroenteritis hospitalization in Belém [Justino et al., 2011]. The total duration of our RV strain surveillance was 36 months during which time it was essentially assessed if any significant changes in the temporal distribution of RV strains were evident following the introduction of the monovalent human rotavirus vaccine in the Brazilian EPI.

Overall, RV was identified in 17.2% of children who received treatment for severe gastroenteritis between May 2008 and May 2011. Although this follow up study was not designed to assess effectiveness or impact of the vaccine over the 3 years of study, this proportion of RV-positive cases among all GE cases was lower than that previously reported (46%) for Belém in 2002–2003 [Linhares et al., 2012], as well as other regions around Brazil (>30%) before the introduction of the vaccine [Carvalho-Costa et al., 2011; O'Ryan et al., 2011; Munford et al., 2009]. This is consistent with recent findings demonstrating a marked decline (59%) in hospitalizations of RV gastroenteritis among infants in the immediate post-vaccine era as compared with the pre-vaccine era [Sáfadi et al., 2010].

Although not individual rotavirus vaccination history was collected from participants, the majority of hospitalizations for severe RV gastroenteritis were seen in children age at least 12 months. These observations may warrant further investigation to assess the extension of long-term protection after 12 months of age, as demonstrated in pre-licensure efficacy studies. Indeed, phase III trials in Latin America and Europe have reported an efficacy for the first 2 years of life of 83% (73.1–89.7) and 96% (83.8–99.5), respectively, against hospital admission for rotavirus gastroenteritis [Vesikari et al., 2007; Linhares et al., 2008;]. Furthermore, in developed countries within Asia, vaccine efficacy against severe RV gastroenteritis was 96.9% (95% CI: 88.3–99.6%) during the first three years of life [Phua et al., 2012].

A marked increase in the relative prevalence of G2P[4] was observed during 2008 and 2009. This is consistent with findings from Brazil and Latin America, where a sharp increase in the fully heterotypic G2P[4] RV strain was seen during this time period. This trend was seen in countries with nationwide introduction of the monovalent human rotavirus vaccine [Munford et al., 2009; Carvalho-Costa et al., 2011; Dulgheroff et al., 2012; Oliveira et al., 2012] and more notably, also in countries where RV vaccination had not been implemented. Some South-American countries, such as Argentina and Paraguay had predominant G2P[4] strains even before introduction of rotavirus vaccine [Patel et al., 2011; Oliveira et al., 2012]. Furthermore, in Nicaragua, where a pentavalent rotavirus vaccine was introduced in 2006, one year later, G2P[4] was also identified in 88% of the rotavirus cases that required hospitalization [Patel et al., 2009]. During the first year of surveillance in a case-control study in Belém, G2P[4] accounted for 82.0% of RV gastroenteritis hospitalizations [Justino et al., 2011]. In contrast to the results of this study which showed an increase in the prevalence of G1P[8] starting in 2010, a recent 4-year follow-up study in Triângulo Mineiro, Brazil, showed that G2P[4] largely predominated over the other circulating strains in 2010, possibly reflecting a continuation of an "epidemic cycle" in this particular region [Dulgheroff et al., 2012]. These contrasting findings highlight the well-known temporal and geographical patterns in RV strains circulation [Santos and Hoshino, 2005; O'Ryan et al., 2011].

While it has been hypothesized that the 'emergence' of the G2P[4] strain may reflect a true shift in the RV strain distribution due to vaccine-induced selective pressure [Gurgel et al., 2008; Leite et al., 2008; Nakagomi et al., 2008; van Doorn et al., 2009; Linhares et al., 2011], it could possibly be due to natural strain fluctuation [Munford et al., 2009; van Doorn et al., 2009; Esteban et al., 2010; Kirkwood

TABLE II. Distribution of Rotavirus Strains Between 2008 and 2011 in Belém, Brazil (N = 1076)[a]

	May 2008–April 2009[b]		May 2009–April 2010		May 2010–May 2011		Total	
Genotype	N	%	n	%	n	%	n	%
Common human strains								
G1P[8]	11	2.1	37	14.5	112	38.0	160	14.9
G2P[4]	434	82.7	123	48.0	57	19.3	614	57.1
G3P[8]	0	0.0	0	0.0	1	0.3	1	0.1
G9P[8]	2	0.4	18	7.0	1	0.3	21	2.0
Reassortants among common human strains								
G1P[4]	0	0.0	3	1.2	0	0.0	3	0.3
G9P[4]	2	0.4	1	0.4	0	0.0	3	0.3
Potential zoonotic strains								
G9P[6]	1	0.2	1	0.4	0	0.0	2	0.2
Possible human-animal hybrid strains								
G1P[6]	1	0.2	3	1.2	6	2.0	10	0.9
G2P[6]	14	2.7	4	1.6	1	0.3	19	1.8
G12P[6]	11	2.1	0	0.0	0	0.0	11	1.0
Mixed infections (single G-genotype with multiple P-genotypes								
G1P[4] + P[6]	0	0.0	1	0.4	0	0.0	1	0.1
G1P[4] + P[8]	2	0.4	1	0.4	3	1.0	6	0.6
G1P[6] + P[8]	1	0.2	1	0.4	1	0.3	3	0.3
G2P[4] + P[6]	25	4.8	4	1.6	2	0.7	31	2.9
G2P[4] + P[8]	1	0.2	2	0.8	5	1.7	8	0.7
G4P[4] + P[6] + P[8]	1	0.2	0	0.0	0	0.0	1	0.1
G12P[4] + P[6]	2	0.4	0	0.0	0	0.0	2	0.2
G2P[4] + P[6] + P[8]	5	1.0	0	0.0	0	0.0	5	0.5
Mixed infections (multiple G-genotypes with a single P-genotype)								
G1 + G2P[4]	2	0.4	14	5.5	8	2.7	24	2.2
G1 + G2P[6]	0	0.0	0	0.0	2	0.7	2	0.2
G1 + G2P[8]	1	0.2	1	0.4	0	0.0	2	0.2
G1 + G9P[4]	0	0.0	1	0.4	0	0.0	1	0.1
G1 + G9P[8]	0	0.0	2	0.8	3	1.0	5	0.5
G1 + G2 + G9P[4]	0	0.0	2	0.8	0	0.0	2	0.2
Mixed infections (multiple G- and P-genotypes)								
G1 + G2P[4] + P[6]	1	0.2	3	1.2	0	0.0	4	0.4
G1 + G2P[4] + P[8]	0	0.0	1	0.4	6	2,0	7	0.7
G1 + G2P[6] + P[8]	1	0.2	0	0.0	0	0.0	1	0.1
G2 + G9P[4] + P[8]	0	0.0	2	0.8	0	0.0	2	0.2
G1 + G2P[4] + P[6] + P[8]	2	0.4	1	0.4	0	0.0	3	0.3
Partially genotyped (G-genotyped and P-untypeable)								
G1P[NT]	0	0.0	8	3.1	15	5.1	23	2.1
G2P[NT]	1	0.2	4	1.6	5	1.7	10	0.9
G9P[NT]	0	0.0	1	0.4	0	0.0	1	0.1
G1 + G2P[NT]	0	0.0	1	0.4	1	0.3	2	0.2
G1 + G9P[NT]	0	0.0	4	1.6	1	0.3	5	0.5
Partially genotyped (G-untypeable and P-genotyped)								
GNTP[6]	2	0.4	8	3.1	41	13.9	51	4.7
GNTP[4] + P[6]	1	0.2	0	0.0	0	0.0	1	0.1
G and P-untypeable								
GNTP[NT]	1	0.2	4	1.6	24	8.1	29	2.7
Total	525	100	256	100	295	100	1076	100

n, number of subjects in a given category; N, number of severe RVGE hospitalizations.
[a]Two samples were excluded from RV-testing by PCR.
[b]Nucleotide sequencing was performed with strains untyped by RT-PCR during this period only.

et al., 2011; Matthijnssens and Van Ranst, 2012]. This 3-year RV strain distribution surveillance study in Belém provides additional evidence to support the latter hypothesis, as the sharp decline in the relative prevalence rates of G2P[4] was followed by an increase in the detection of G1P[8] strains. Another recent study from Northern Brazil found similar patterns: G2 strains displayed a typical cyclical pattern of occurrence and re-emergence during the 2006–2008 period [Oliveira et al., 2012].

However, these findings remain potentially inconclusive for two reasons: firstly, the monovalent human rotavirus vaccine is composed of a G1P[8] species A, an RV strain related to the Wa-like genotype constellation, that fully differs from G2P[4], which possesses the DS-1-like genotype constellation [Matthijnssens et al., 2012]. Secondly, the decline in prevalence rates of G2P[4] in this study might also be influenced by an increasing proportion of children aged below 5 years who might had previously been

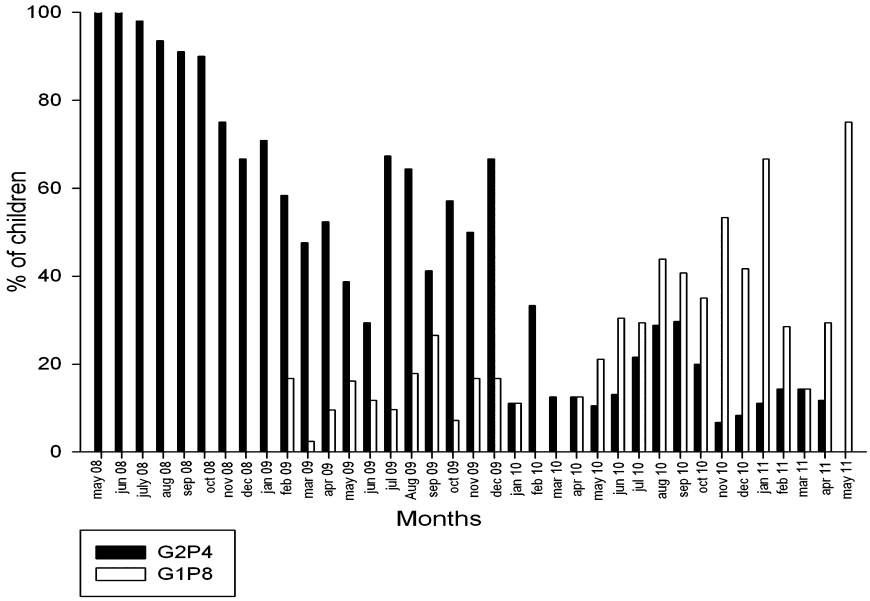

Fig. 2. Annual distribution of G2P[4] and G1P[8]. % = n/N × 100 N, number of severe RVGE hospitalizations; n, number of subjects in a given category.

infected with circulating G2P[4] strains and had developed homotypic immunity.

The trend for higher prevalence rates of G1P[8] could be interpreted as resulting from an apparent decline in protection after 1 year of age, as suggested by post-licensure studies conducted in Brazil and Latin America, even though further investigation on this particular issue is required [Gentsch et al., 2005; Correia et al., 2010; de Palma et al., 2010; Justino et al., 2011; O'Ryan et al., 2011; Patel et al., 2012].

A remarkable variability was observed in co-circulating strains from January 2010 onwards; the majority was either partially typed or fully untypeable strains, or mixed infections. Mixed infections, which may have occurred due to exposure of children to a heavily contaminated environment, were predominantly represented by G2P[4] + P[6] and G1 + G2P[4] strains which were common throughout the study period and could possibly challenge the RV vaccine effectiveness [Gentsch et al., 1996; Fischer et al., 2005; Santos and Hoshino, 2005].

A finding of particular interest was the detection of a high proportion of untypeable RV strains during 2010–2011, in comparison with the previous follow-up period. This occurrence may reflect the circulation of common RV strains that underwent genetic variation, and is supported by studies showing that standard RT-PCR methods may fail to determine genotype-specificities, due to possible silent mutations in the primer-binding site [Iturriza-Gómara et al., 2000; Soares et al., 2012]. We were unable to detect RVs bearing G12 type-specificity during the second and third years of follow-up, which, according to a study in Northern Brazil, is a recently emerging strain [Matthijnssens et al., 2010; Soares et al., 2012].

Another plausible explanation for the emergence of new RV strains is that in this study, potential zoonotic strains (G9P[6]) and strains, which are likely to originate from reassortment between human and animal RV strains (G1P[6] and G2P[6]) were detected at very low frequencies, suggesting that they do not spread efficiently among humans. Nonetheless, one cannot rule out the possibility that such unusual strains were generated through reassortant events involving common circulating human strains and the emerging G12P[6] strain.

The main limitation in this study was that vaccine protection was assessed only during the first year of surveillance where effectiveness against G2P[4] was75%. This study was not designed to evaluate vaccine protection during the remaining two years of follow-up. A possible limitation of this study is the difference in sample size over the four year study period: in the first two years we covered 80% of severe gastroenteritis cases in Belem compared with only 50% of cases in the remaining two years. In addition, the set of primers that were used did not target either the G12 or G5 type-specificities at least for the second and third years of surveillance, where nucleotide sequencing was not performed, we may have missed detecting G12 RV strains bearing either P[6] or P[8] types. These are known to have emerged worldwide and may possibly have arrived in the Northern region of Brazil [Matthijnssens et al., 2010; Soares et al., 2012]. An additional limitation of the current study was the lack of complete analysis of the entire RV genotype constellations for a long-term

assessment of vaccine effect on strain type, as based on the currently adopted classification of rotaviruses [Matthijnssens et al., 2012]. In this regard, molecular analyses to identify lineages from G1P[8] and G2P[4] genotypes are worth to be done for a better understanding of strain fluctuation over time.

Finally, although the monitoring of RV strains in our study was conducted over a relatively extended period, continued surveillance would be useful in detecting trends in the occurrence of the prevailing and potentially emerging new strains that may pose a challenge to the currently licensed RV vaccines. In conclusion, G2P [4] was predominantly observed during the first 20 months of our study, followed thereafter by G1P[8], which is suggestive of natural RV strain fluctuation over time, rather than vaccine-induced selective pressure on circulating RV strains. Future strain surveillance activities will be beneficial to further clarify the overall impact of RV vaccines.

TRADEMARK

Rotarix is a registered trademark of the Glaxo SmithKline group of companies.

Rotateq is a registered trademark of Merck & Co. Inc.

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