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Effect of Monovalent Rotavirus Vaccine on Rotavirus Disease Burden and Circulating Rotavirus Strains Among Children in Morocco

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Abstract

Rotarix™vaccine was introduced into the National Program of Immunization of Morocco in October 2010, reaching quickly 87% of the target population of children nationally. The incidence of rotavirus gastroenteritis and the prevalence of circulating rotavirus strains has been monitored in three sentinel hospitals since June 2006. The average percentage of rotavirus positive cases among all children under 5 years old hospitalized for gastroenteritis during the pre-vaccine period (2006–2010) was 44%. This percentage dropped to 29%, 15% and 24% in the 3 years post vaccine introduction (2011, 2012 and 2013), which is a decline of 34%, 66%, and 45%, respectively. Declines in prevalence were greatest among children 0–1 years of age (53%) and were most prominent during the winter and autumn rotavirus season. The prevalence of the G2P[4] and G9P[8] genotype sharply increased in the post vaccine period (2011–2013) compared to the previous seasons (2006–2010). Rotavirus vaccines have reduced greatly the number of children hospitalized due to rotavirus infection at the three sentinel hospitals; it is however unclear if the predominance of G2P[4] and G9P[8] genotypes is related to the vaccine introduction, or if this is attributable to normal genotype fluctuations. Continued surveillance will be pivotal to answer this question in the future.

Keywords

rotavirus; genotype; rotavirus vaccine; Morocco

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INTRODUCTION

Rotaviruses are the most common etiologic agents of severe acute gastroenteritis in young children worldwide, accounting for 453,000 deaths in 2008 and over 2 million hospitalizations worldwide among children <5 each year. More than 50% of these 453,000 deaths occur in Africa [Parashar et al., 2006; Tate et al., 2012].

Rotaviruses belong to the *Reoviridae* family and possess a genome containing 11 segments of double stranded RNA. Traditionally, rotaviruses have been classified into groups based on the cross-reactive VP6 capsid antigen. In addition, the two outer capsid proteinsVP7 and VP4 that elicit type-specific neutralizing antibody responses in the host have been used to define rotavirus G and P serotypes originally based on serologic methods. More recently, RT-PCR and sequencing based methods have replaced traditional serologic methods for the dual classification system, defining G and P genotypes, respectively [Estes and Cohen, 1989; Estes, 1996]. Among group A rotavirus, at least 27 G-genotypes and 32 P-genotypes have been described [Matthijnssens et al., 2011]. A total of 12 G and 15 P genotypes have been recovered from humans [Rahman et al., 2005; Martella et al., 2006], which theoretically could reassort to from 180 different G/P combinations. Globally, however, six common G/P combinations among strains infecting humans (G1P[8], G2P[4], G3P[8], G4P[8], G9P[8], and G12P[8]) contribute substantially to the diarrhea burden disease [Matthijnssens et al., 2010; Banyai et al., 2012]. While common overall, large variation in the temporal and geographic distribution of prevalent rotavirus genotypes are well known [Santos and Hoshino, 2005; Banyai et al., 2012]. Regional differences in the diversity and prevalence of genotypes considered uncommon overall, have also been well documented [Gentsch et al., 2005; Santos and Hoshino, 2005]. For example, in Africa, high prevalence of G8 and P[6] strains has been described and linked to possible zoonotic transmission [Cunliffe et al., 1999; Steele and Ivanoff, 2003; Armah et al., 2010].

Recently, two new live-attenuated oral rotavirus vaccines, a monovalent human rotavirus vaccine (Rotarix™; Glaxo-SmithKline Vaccine, Rixensart, Belgium) and a pentavalent human–bovine reassortant rotavirus vaccine (RotaTeq™; Merck & Co, Whitehouse Station, NJ) have been licensed and introduced into routine immunization programs in more than 60 countries worldwide. The monovalent vaccine is based on the attenuated human Wa-like G1P[8] rotavirus strain 89–12, whereas the pentavalent vaccine contains five human–bovine reassortant rotavirus strains containing the human components G1–G4, and P1A[8]. Both vaccines were shown to be highly effective (85%–98%) against severe rotavirus acute gastroenteritis in large clinical trials in developed countries [Ruiz-Palacios et al., 2006; Vesikari et al., 2007a]. The vaccine efficacy was somewhat lower (51%–64%) and more variable in developing countries in Asia and Africa [Armah et al., 2010; Madhi et al., 2010; Zaman et al., 2010], but the public health impact of vaccines was substantial in these settings with greater disease acute gastroenteritis burden. Given these considerations, in 2009, the WHO recommended inclusion of rotavirus vaccination in national immunization programs worldwide [WHO, 2009].

Both rotavirus vaccines showed good protection against severe acute gastroenteritis caused by a range of rotavirus strains that circulated during pre-licensure trials, including strains

that do not share either of vaccine's two surface proteins. While the efficacy of the monovalent rotavirus strain in the pivotal trial in Latin America appeared to be lower against the fully heterotypic G2P[4] strains, a subsequent trial in Europe showed good efficacy against this strain [Ruiz-Palcios et al., 2006; Vesikari et al., 2007b]. Also, while a great diversity of rotavirus strains circulated during the clinical trial of monovalent rotavirus vaccine in South Africa and Malawi, the vaccine appeared to provide similar protection against acute gastroenteritis caused by vaccine type and non-vaccine type strains [Steele et al., 2012]. Nevertheless, concerns have been raised about the relatively unusual dominance of G2P[4] strains observed in some Latin American countries following national implementation of rotavirus vaccination [Gurgel et al., 2007; Nakagomi et al., 2008]. The concurrent G2P[4] dominance in other Latin American countries without rotavirus vaccine programs and case-control studies documenting good effectiveness of monovalent rotavirus vaccine against this strain as well as other non-vaccine type strains provide some reassurance that these changes in prevalent strains may not be vaccine related but due to natural temporal variation [Patel et al., 2008, 2013; Yen et al., 2011]. However, the impact of vaccination on strain diversity requires further monitoring, especially in low income settings where vaccine efficacy is lower [Snelling et al., 2011].

To control the burden of severe and fatal diarrheal disease, the Ministry of Health of Morocco introduced the single strain rotavirus vaccine, Rotarix™, into the national immunization program in October 2010, making Morocco the first African and Eastern Mediterranean country to introduce rotavirus vaccine into the routine infant immunization schedule. Quickly, after its launch the rotavirus vaccine coverage in Morocco reached 86%, 86.5% and 88% in 2011, 2012 and 2013 respectively [Ministry of Health of Morocco data source].

In Morocco, rotavirus is the most common cause of severe diarrhea in children <5 years of age, accounting for 40% of children hospitalized with acute gastroenteritis in the pre-vaccine era (May 2006–June 2010) [Benhafid et al., 2012]. Previous data indicated that G1P[8] was the most prevalent rotavirus strain in Morocco during the pre-vaccine period accounting for 55% of all samples. However, great diversity and rapid changes in the prevalence of rotavirus strains was observed in Morocco over a relatively short time before rotavirus vaccine introduction (June 2006–May 2009) [Benhafid et al., 2013].

In this study, the impact of vaccination in reducing the prevalence of rotavirus among Moroccan children hospitalized with diarrhea was assessed. Rotavirus genotypes were also described in three sentinel hospitals during 3 years (January 2011 through December 2013) after Rotarix™ vaccine introduction into the national program of immunization in Morocco and compared to prevalent strains prior to vaccine introduction.

MATERIALS AND METHODS

Rotavirus Surveillance

Stool samples were collected from children under 5 years admitted to hospital for acute gastroenteritis between December 1, 2011 and January 31, 2013, at one of three hospitals in geographically distinct regions of Morocco: Mohamed V Hospital in Tanger (Northern

Region), Al Farabi Hospital in Oujda (Eastern Region) and Prefectoral Hospital in Benimellal (Center Region). These three sentinel hospital located in three different strategic regions of Morocco were selected as they are the main hospitals that children present for diarrhea in these major cities, with about 400 diarrhea cases annually in each hospital. The prevalence of rotavirus disease from the surveillance sites provides a reasonably geographically diverse representation from cities where a large portion of the Moroccan population resides, but the data may not be might fully generalizable to all Moroccan children.

A case of acute gastroenteritis was defined as the acute onset of three or more loose stools or two or more episodes of vomiting in a 24 hr period, which were not explained by another diagnosis. Acute onset was defined as symptom(s) onset of <7 days before presentation.

At the three sentinel sites, stool samples were tested routinely at the local hospital laboratory for rotavirus using a commercial enzyme immunoassay (ProSpecT Rotavirus ELISA kit (Oxoid, Cambridge-shire, UK). The Central Virology Laboratory at the National Institute of Hygiene in Rabat was responsible for genotyping and quality control. All tested locally positive samples and 20% of negative samples from the three sentinel sites were sent to the National Institute of Hygiene and positive samples were stored at −70 °C for future molecular characterization.

Viral RNA Extraction and RT-PCR Genotyping

Rotaviral double-stranded RNA was extracted from fecal suspensions using the QIAamp® Viral RNA Mini kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The VP7 gene was amplified the Beg9 and End9 primers under conditions described previously [Gouvea et al., 1990]. G typing was performed by using a primer set which incorporated primer RVG9, and primers aBT1, aCT2, aET3, aDT4, aAT8, aFT9, G10, and G12B, which are specific to G types 1, 2, 3, 4, 8, 9, 10, and 12, respectively [Gouvea et al., 1990; Iturriza-Gómara et al., 2004].

For the RT-PCR of VP4, the consensus primers Con3 and Con2 were employed under the conditions described previously [Gentsch et al., 1992]. For P typing, a seminested PCR was used to incorporate primer Con3 and primers 1T-1, 2T-1, 3T-1, 4T-1, and 5T-1, which are specific to types P[8], P[4], P[6], P[9], and P[10], respectively [Gentsch et al., 1992]. All PCR products were examined by gel electrophoresis in 2% agarose gel containing 4 mg of EtBr.

Sequence Analysis of Untypeables Strains

Samples with adequate stool in which the P or G types could not be determined by RT-PCR were subjected to nucleotide sequence analysis. The PCR products of VP7 gene (1062 base pairs) were amplified using Beg9 and End9 primers [Gouvea et al., 1990]. Fragments of VP4 gene (876 base pairs) were amplified using con2 and con3 primers [Gentsch et al., 1992]. The nucleotide sequence of each PCR product was determined using the Big-Dye terminator cycle sequencing kit (Applied Bio-systems, Foster City, CA) and an automated DNA sequencer (ABI 3130XL; Perkin Elmer-Applied Biosystem). The sequences were compared

with rotavirus sequences in the GenBank database with use of the Blast program, and genotypes were assigned by relatedness to reference VP7 or VP4 genes.

Ethical Considerations

Informed consent was obtained from the parents or legal guardians of minors. The Ethics Office from the WHO and the Moroccan Ministry of Health deemed this study as a public health practice.

RESULTS

Prevalence of Rotavirus in Children Hospitalized With Diarrhea Before and After Vaccine Introduction

In pre-vaccine years from 2006–2010, a total of 1861 children were hospitalized with acute gastroenteritis at the three surveillance hospitals, of which 766 (41%) tested positive for rotavirus. In contrast, in post-vaccine years from 2011–2013, 533 children were hospitalized with acute gastroenteritis, of which 128 (24%) were positive for rotavirus. This represented an overall decline in prevalence of rotavirus of 41.5%. Although there were fluctuations in the number of hospitalized acute gastroenteritis cases and in the number of rotavirus positive cases over the years before the introduction of vaccination (2006–2010), the percentage of rotavirus positive cases was relatively stable and ranged between 41% and 47%. However, in the rotavirus seasons following the introduction of vaccination, this percentage dropped to 29% (2011), 15% (2012) and 24% (2013) (Fig. 1). Compared to the average percentage of RV positive cases (43.7%) in 4 years before vaccine introduction (2006–2010), the decline in the percentage of RV positive cases was 33%, 66% and 44% in 2011, 2012 and 2013, respectively. Declines in the prevalence of RV positives cases were seen at each of the three sentinel hospitals and were 36%, 56% and 51% in Oujda, Tanger and Benimellal, respectively.

Age Distribution of Rotavirus Infection Before and After Rotavirus Vaccination

The analysis of distribution of positive RV cases, according to age, in the pre- and post-vaccination periods demonstrated a significant decline in the number and prevalence of cases of rotavirus disease in all age groups <2 years of age, and were greatest in children between 0 and 1 year (535 (43%) vs. 72 (19.8%), $P < 0.001$). Significant reduction in the prevalence of cases of rotavirus disease was also observed in children aged between 1 and 2 years (168 (42.2%) vs. 35 (23.3%), $P < 0.05$). In contrast, no significant difference in the prevalence of rotavirus disease was observed in children aged between 2 and 5 years (63 (28.3%) vs. 21 (21%), $P = 0.63$) (Table I).

Seasonal Pattern of Rotavirus Infection Before and After Rotavirus Vaccination

The monthly analysis of rotavirus infection during pre-vaccine and post-vaccine periods are presented in Figure 1 and show clearly a decrease in acute gastroenteritis rotavirus cases. Rotavirus infections occurred year-round, and activity peaked in autumn (September, October, and November) and winter (January and February), including a defined peak in

November during the pre-vaccine period. In contrast, two defined peaks in November and February were observed in the post-vaccine period. (Fig. 2).

Distribution of G and P Genotypes After Rotavirus Vaccine Introduction

Of the 128 rotavirus-positive samples in post-vaccine years from 2011–2013, 126 (98.4%) were subjected to G and P genotyping. Four G genotypes were found: G1 was the predominant G-type (45.2%), followed by G2 (31.7%), G9 (15.1%) and G4 (0.8%). Mixed G infection represented 7.1% of all strains. Three P genotypes were found: P[8] (65%) was the most prevalent, followed by P[4] (21.4%) and P[6] (1.6%). Mixed P infections represented 11.8% of total strains (Table II). Among genotype combinations, G1P[8] was the most prevalent accounting for 40.5%, followed by G2P[4] (21.4%), G9P[8] (15%) and G4P[8] (0.8%). Two uncommon strains were identified at low prevalence G2P[8] (4%) and G1P[6] (1,6%). A high frequency of mixed infections was found (16.4% of all samples) of which G2P[4]P[8] (6.3%) accounted for the majority (Table II)

Comparison of Genotype Distribution Before and After Rotavirus Vaccine Introduction

Data for four rotavirus seasons pre-vaccine introduction (June 2006–December 2010) showed large fluctuation of rotavirus genotype distribution with multiple genotypes circulating each year (Fig. 3). G1P[8] was the dominant genotype for the 4 years pre-vaccine introduction (57%) followed by G2P[4] (15%), G9P[8] (7.8%), G2P[6] (2.4%), G4P[8] (0.6%) and G3P[8] (0.3%). Mixed infections were found in 11% of samples. Several other strains were identified including G1P[4], G1P[6], G2P[8], G3P[6], G3P[4], and G9P[6] which together accounted for 6% of all samples. Interestingly, the globally emergent G9P[8] genotype that was the second most prevalent strain in 2006–2007 (30.5% of all samples) decreased sharply to 6% in 2007–2008, 4% in 2008–2009 and 1% in 2009–2010. G2P[4] strains were detected at low prevalence during 2006–2007 and 2007–2008 (1.5% and 1.3% of all samples, respectively), increased sharply in 2008–2009 and 2009–2010 (25.6% and 20,5% of all samples, respectively) and became the second most prevalent genotype in the 2 years before vaccine introduction (Fig. 2). G4P[8] and G3P[8] strains were detected at low frequency during the pre-vaccine introduction accounting for 0,7% and 0,2% respectively. The detection rate for mixed rotavirus infection was relatively high accounting for 11% of all samples.

In the first year (2011) after the introduction of Rotraix™, G1P[8] (54.7%) remained the most prevalent strain followed by G2P[4] (9.5%) and G4P[8] (1.2%). In the second year after vaccine introduction, G2P[4] strains were predominant accounting for 54% of all hospitalizations followed by G1P[8] (20.8%) and G9P[8] (16.6%). The third year after vaccine introduction was characterized by a major change in the rotavirus genotypes profile. Genotype G1P[8], was not detected and the only two genotypes were G9P[8] (67%) and G2P[4] (33%) (Fig. 2). In the post-vaccine period G4P[8] was detected only in one sample during 2011 while G3P[8] was not detected. The frequency of G/P mixed infection declined progressively after vaccine introduction, from 23.4% in 2011 to 4% in 2012 to 0% in 2013.

Geographical Distribution of Strains at the Three Sentinels Hospitals Before and After Vaccine Introduction

To further understand the genotype distribution pre-(2006–2010) and post-(2011–2013) vaccine introduction at a local level, the distribution of rotavirus genotypes circulating in the 3 sentinels hospitals was compared (Fig. 4). In Oujda, G1P[8] was the dominant type identified for the 4 years pre-vaccine introduction representing 58% of sample followed by G2P[4] (17.5%) and G9P[8] (6%). In the post-vaccine introduction period (2011–2013), G1P[8] (49%) remained the most observed genotype followed by G2P[4] (14.4%) and G9P[8] (9%) (Fig. 3A). In Benimellal hospital, G1P[8] accounted for 60.6% of strains in the pre-vaccine period, whereas in the post-vaccine period, G1P[8] accounted for only 9% of strains, while G9P[8] (47.6%) was the most predominant strain followed by G2P[4] (38%) (Fig. 3B). In Tanger hospital, G1P[8] (43.5%) was the most prevalent strain in pre-vaccine period followed by G2P[4] (11.4%) and G9P[8] (2%). Conversely, in the post-vaccine period G2P[4] (40%) emerged as the most observed genotype followed G1P[8] (33.3%) and G9P[8] (6%) (Fig. 3C). The uncommon strain G2P[6], which was detected at low frequency during the pre-vaccine period in each of the 3 sentinels hospitals was not found in post-vaccine years. Mixed infections were detected at each of the 3 sentinels hospital during the pre-vaccine period at variable frequency (24% in Tanger, 8.4% in Oujda and 5% in Benimellal). In contrast, mixed genotypes were detected only in Oujda during the post-vaccine period, accounting for 23.3% of samples (Fig. 3 A–C).

DISCUSSION

This study is among the first to evaluate the effect of Rotarix™ vaccine on prevalence of rotavirus in children hospitalized with diarrhea and the distribution of circulating rotavirus strains in African countries introducing national Rotarix™ vaccination. Vaccination was started on October 2010, reaching quickly 87% of the target population of children nationally. The comparative analysis between the pre- and post-vaccination periods demonstrated a signification reduction in the prevalence of rotavirus among children hospitalized with diarrhea at the sentinel hospitals from 41% to 24%, representing a 41.5% reduction. The reduction of prevalence was greatest among children 0–1 years of age and was more prominent during the winter rotavirus season. In each of the three sites, the reduction in rotavirus prevalence were observed post-vaccine introduction. These findings convincingly demonstrate the impact of vaccination in reducing the burden of severe rotavirus disease in Moroccan children, consistent with data from other studies worldwide studies [Zeller et al., 2010; Assis et al., 2013; Pendleton et al., 2013]. During the pre-vaccine period, the prevalence of rotavirus hospitalizations peaked during the cooler months of September–December with a defined peak in November. In contrast, the rotavirus peaked in November and February during the post-vaccine period. Changes in rotavirus activity was also observed in countries with rotavirus vaccine programs such as the US and Belgium, the timing and onset of the annual rotavirus epidemic has been delayed by 4–6 weeks after the introduction of rotavirus vaccine [Zeller et al., 2010; Tate et al., 2011]. In addition, a recent model has suggested that, following rotavirus vaccine implementation larger seasonal peaks could occur in countries with year-round disease [Pitzer et al., 2011].

Questions have been raised about how well the monovalent Rotarix vaccine will protect against non-vaccine type strains, particularly against G2P[4] strains that do not share either of vaccine's two surface proteins. In one report from Brazil [Gurgel et al., 2007] after introduction of Rotarix™ in 2006 showed all strains identified belonged to G2P[4] genotype. In addition, a similar association has been observed in Australia where G2P[4] strains appeared to be more common in locations after introduction of Rotarix™ [Kirkwood et al., 2011]. In our study, while the prevalence of G2P[4] was low during the 2 first years of the pre-vaccine period, it emerged to become the second most prevalent strain in the next two years immediately before rotavirus vaccination introduction, accounting for 22,5% of all isolates. The post-vaccine-period was characterized by a continuous high prevalence of G2P[4], which was the most prevalent genotype in the second year after rotavirus vaccination. The emergence of G2P[4] strains prior to vaccine implementation in Morocco suggests that this phenomenon may be related to natural temporal variation rather than vaccine pressure. Similar cyclic peaks in G2P[4] prevalence have been described in several countries including Venezuela, Thailand, Bangladesh, and several African countries during the last decade, in the absence of vaccine programs [Page and Steele, 2004; Khamrin et al., 2007; Rahman et al., 2007; Vizziet al., 2011; Ferreira et al., 2012]. In addition, G2P[4] have also been reported as the dominant circulating genotype in several non vaccinated population in Central America and Europe [Patel et al., 2008; Iturriza-Gómara et al., 2009]. Furthermore, post-licensure studies conducted in Brazil, Belgium, and Bolivia have shown good effectiveness of Rotarix against G2P[4] strains [Matthijnssens et al., 2014]. Thus, continuous surveillance is required to determine whether the current trend toward increase of G2P[4] after introduction of Rotarix™, is long lasting, and affects vaccine effectiveness.

Interestingly and unexpectedly, the globally emergent G9P[8] genotype that almost disappeared in the year before vaccine introduction (1% of samples in 2010) emerged as the most prevalent strain in the third year after vaccination accounted for 67% of samples followed by G2P[4] (33%). This increased prevalence of G2P[4] and G9P[8] was also reported in states using Rotarix™ in Australia [Kirkwood et al., 2011]. This is probably due to natural genotype fluctuations, as strong yearly and geographical fluctuations in the prevalence of different genotype have been previously reported in pre-vaccine period in Morocco [Benhafid et al., 2013] and Australia [Kirkwood et al., 2009]. Despite a relatively small sample size collected during the third year following vaccine introduction, it might be tempting to speculate that the circulation of only two genotypes (G2P[4] and G9[8]) in this year may be due of vaccine driven selection. Nevertheless, the observed increasing of prevalence of two genotype G2P[4] and G9P[8] in the post-vaccine period is rather unusual and warrants further monitoring.

Fluctuations in genotype prevalence were also analyzed before and after rotavirus introduction at each of the three sites. It is possible that these regional genotype differences could simply represent a natural fluctuation unrelated to vaccination. However, in order to assess the impact of vaccination on the fluctuation of different rotavirus genotypes, it is necessary to perform continuous epidemiological surveillance after vaccine introduction.

The prevalence of the globally common strain G1P[8] decreased from 54.7% in 2011 to 20.5% in 2012. Interestingly, G1P[8] was undetected in the third year following vaccine

introduction (2013). The absence of acute gastroenteritis cases exclusively related to genotype G1P[8] strain in 2013 seems to indicate vaccine protection against homotypic G1P[8] rotavirus. In fact, clinical trials have demonstrated high (>87%) efficacy of rotavirus vaccine against rotavirus of the most prevalent G1P[8] strain [Salinas et al., 2005; Vesikari et al., 2007; Linhares et al., 2008]. At first sight, the G1P[8] genotype seems to be affected particularly by routine vaccination. Nonetheless, it will be important to monitor G1P[8] prevalence carefully in the Moroccan Rotarix™ vaccination program in the forthcoming years to discriminate whether this is due to vaccine driven immunity or normal fluctuation of rotavirus genotypes.

The selective pressures from the vaccine may be subtle; many years may be required before making a conclusion concerning this phenomenon [Patton, 2012]. Likewise, to further investigate the possibility of impact of vaccine pressure on changes of rotavirus strains in Morocco, more detailed analysis of the complete genomes of rotavirus strains collected before and after rotavirus vaccination will likely be necessary to detect such changes [Matthijnssens et al., 2008].

A major limitation of this analysis was the limited number of hospitals participating in the post-vaccine surveillance study and a lack of information of child's vaccination status. Nevertheless, the current study represents the longest-term study of rotavirus strain prevalence in Morocco and provides baseline data on which to subsequently assess the impact of the new rotavirus vaccination program on circulating strains.

In summary, the findings of this study highlight the need for continuing and extending surveillance at other sites in Morocco to monitor changes in the epidemiology of rotavirus disease and to identify dynamic shifts in circulating rotavirus strains. These data will be critical for better understanding of rotavirus evolution and assessment of the vaccine program.

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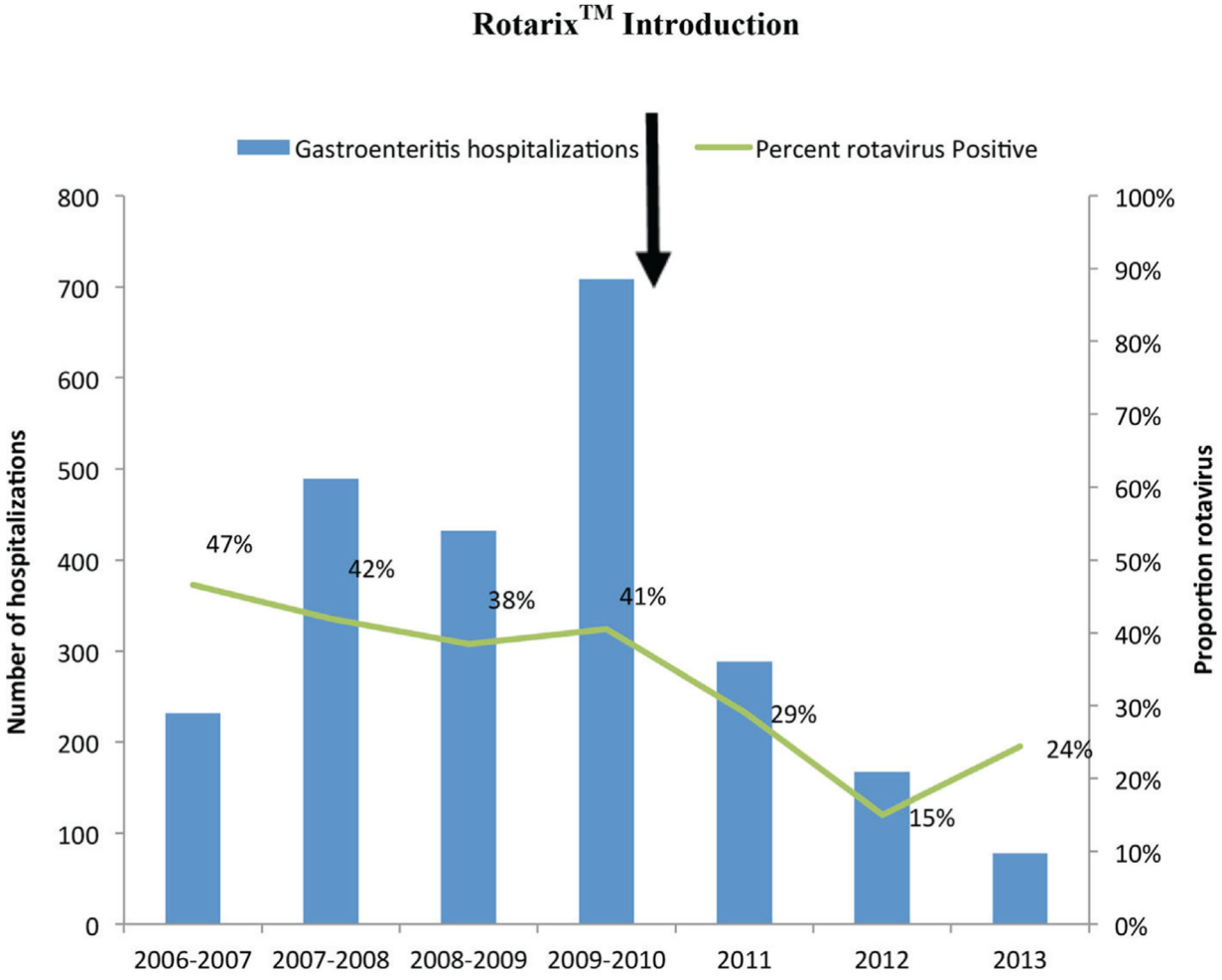

Fig. 1.

Evolution of the number of hospitalized AGE cases and prevalence of Rotavirus tested positive before and after rotavirus vaccination, Morocco, 2006–2013.

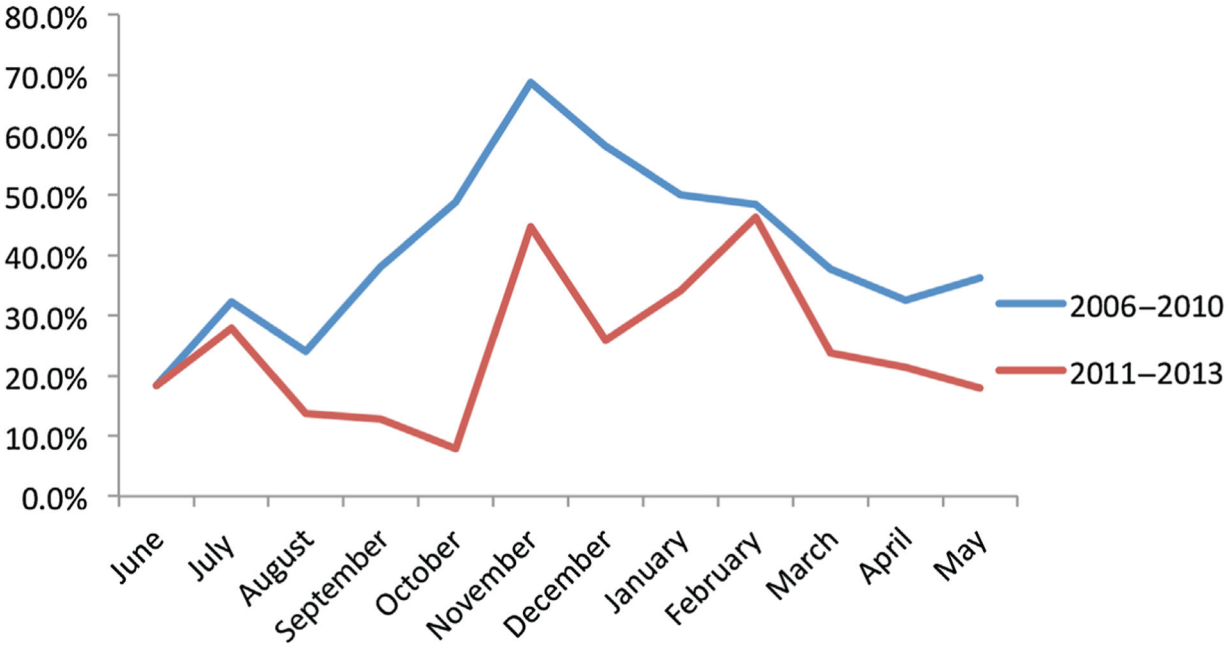

Fig. 2.

Seasonality of hospitalization for rotavirus infection in Morocco before (2006–2010) and after Rotavirus vaccination (2011–2013).

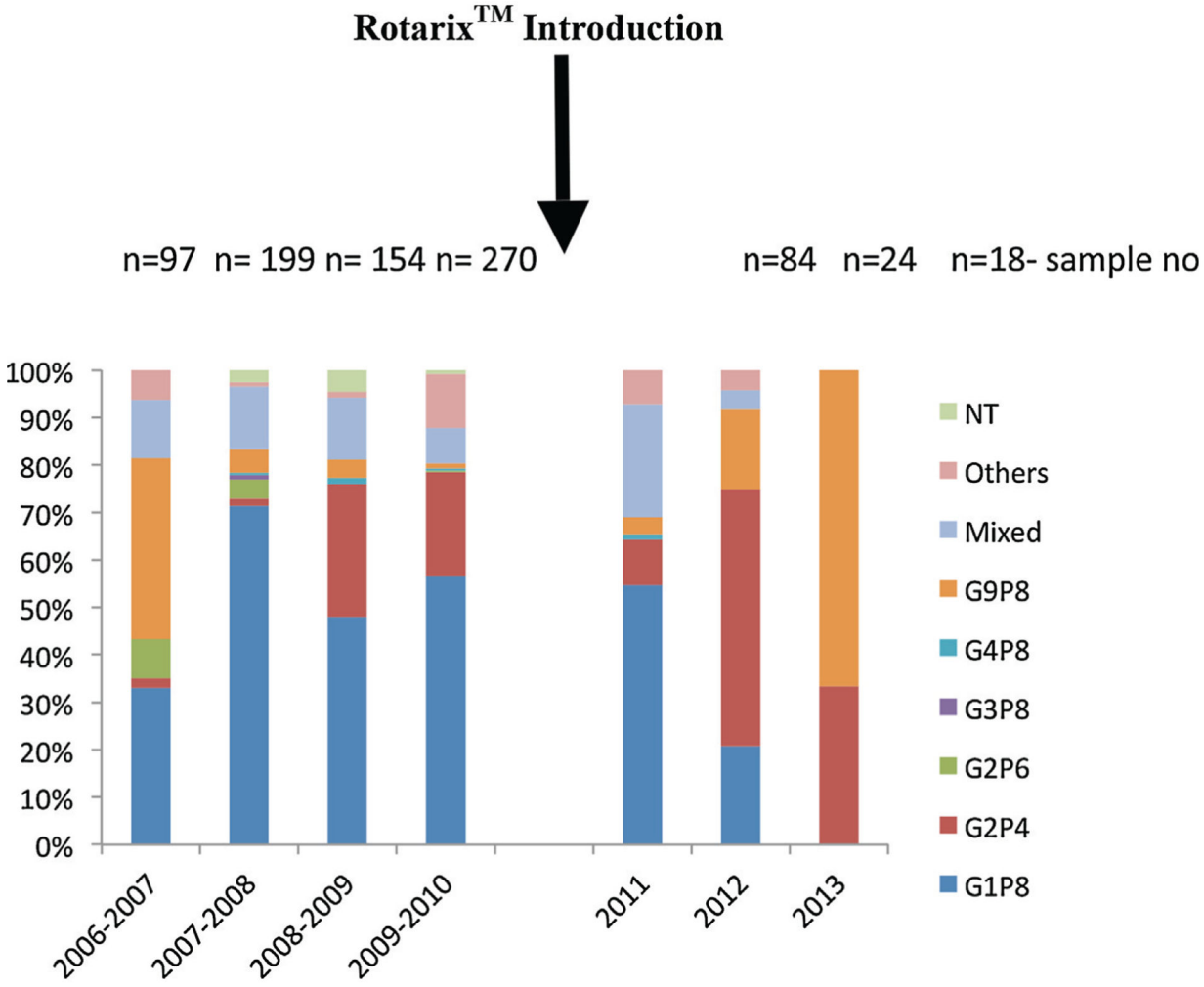

Fig. 3.

Genotype distribution of rotavirus G and P type before and after rotavirus vaccine introduction in Morocco. The arrow indicates when vaccine was introduced in Morocco (October, 2010).

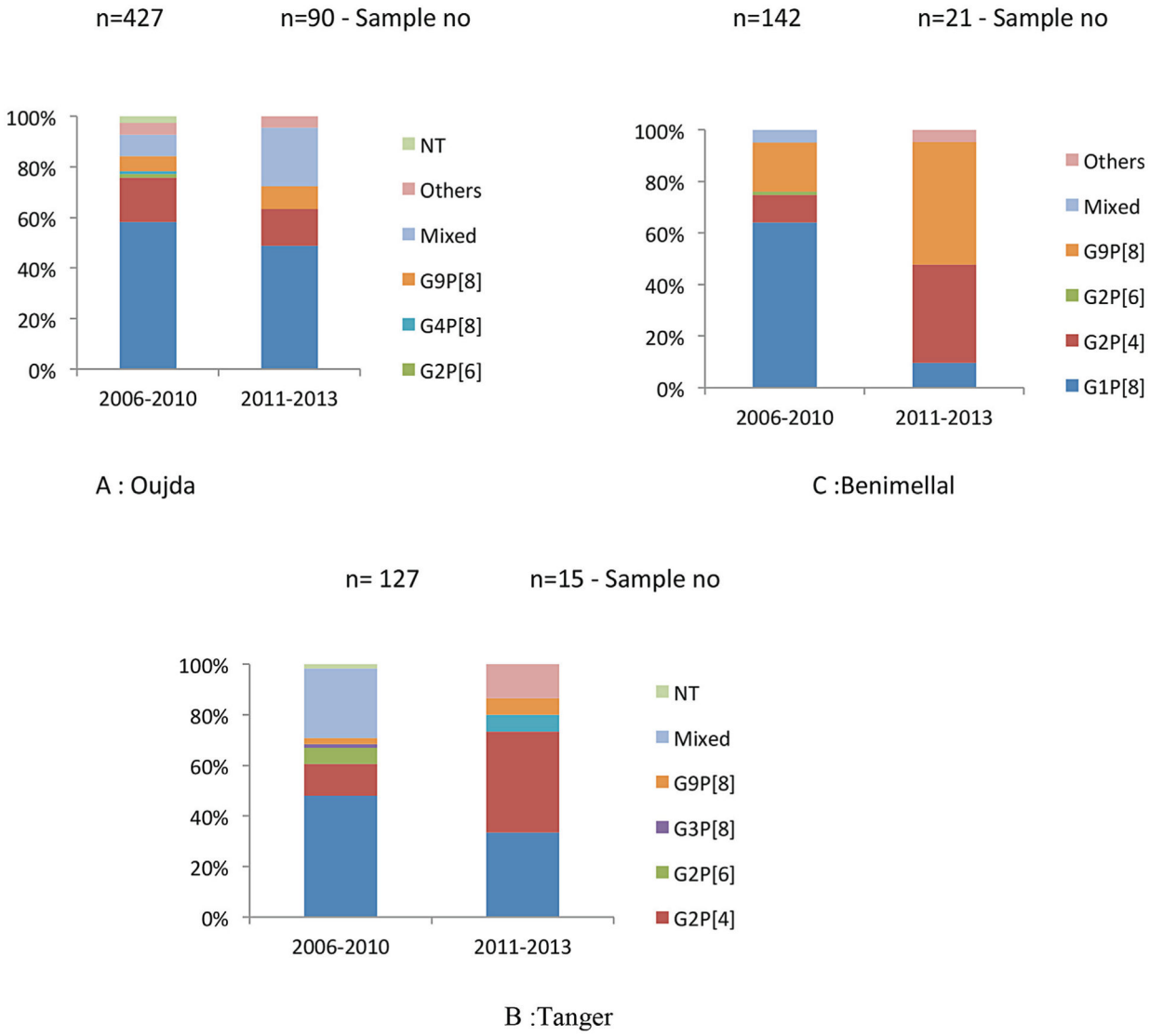

Fig. 4.

Geographical distribution of rotavirus G and P genotypes before and after rotavirus vaccination at the three sentinel hospitals. **A:** Oujda; **B:** Tanger and **C:** Benimellal.

TABLE I.

Number of Rotavirus Positive Cases Broken Down Per Age Group During Pre (2006–2010) Post (2011–2013) Vaccine Periods

	RV positives cases/total age (percentage)		
Age range (years)	**Pre-vaccine (2006–2010)**	**Post-vaccine (2011–2013)**	***P*-value**
[0–1]	535/1241 (43.1%)	72/363 (19.8%)	<0.001
[1–2]	168/398 (42.2%)	35/150 (23.3%)	<0.05
[2–5]	63/222 (28.3%)	21/100 (21%)	0.63
Total	766/1861 (41.1%)	128/613 (20.8%)	<0.001

TABLE II.

Distribution of Rotavirus G and P Types Among Children Under 5 Years of Age After Rotarix™ Introduction, Morocco, 2011–2013

	P type, no. (%) strains				
Gtype	**P[8]**	**P[4]**	**P[6]**	**Mixed**	**Total no. (%)**
G1	51(40,5)	0	2(1,6)	4(3,1)	57(45,2)
G2	5(4)	27(21,4)	0	8(6,3)	40(31,7)
G4	1(0,8)	0	0	0	1(0,8)
G9	19(15,1)	0	0	0	19(15,1)
Mixed	6(4,7)	0	0	3(2,4)	9(7,1)
Total no. (%)	82(65,1)	27(21,4)	2(1,6)	15(11,8)	126(100)

The mixed G type found in this study were: G1G2 (0,8%, n = 1); G1G9 (0,8%, n = 1); G2G9 (3,2%, n = 4); and G1G2G9 (1,6%, n = 2).
The mixed P type found in this study were: P[4]P[8].