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Distribution of Rotavirus Strains before and after Introduction of the Rotarix Vaccine in the Expanded Program on Immunization (EPI) in Senegal

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Abstract

Senegal introduced the monovalent Rotavirus vaccine into its Expanded Program on Immunization in November 2014. The main objective of this study was to monitor the distribution of Rotavirus strains following vaccine introduction in Senegal. Materials and Methods Stool specimens were collected from children under 5 years of age who were hospitalized or under observation at the Albert Royer National Children's Hospital in Dakar from January 1, 2011 to December 31, 2020. Rotavirus antigen detection was performed using an enzyme-linked immunosorbent assay (ELISA), and molecular characterization of ELISA-positive samples was conducted at the West African Regional Rotavirus Reference Laboratory in Accra, Ghana. Results: A total of 313 ELISA-positive samples underwent molecular characterization: 235 samples (75.07%) were collected before vaccine introduction and 78 samples (24.93%) after. During the pre-vaccine period, VP7 genotypes G1 and G12 and VP4 genotypes P[6] and P[8] accounted for more than 90% of circulating G and P genotypes. In the post-vaccine period, VP7 genotypes G3 and G1 represented over 47% of strains, while VP4 genotypes P[6] and P[8] comprised nearly 90%. The predominant strain combinations were G12P[8] and G1P[6] before vac-

cine introduction, shifting to G3P[8] and G1P[8] afterward. Conclusion G12P[8] and G1P[6] genotypes predominated in the pre-vaccine era, whereas G3P[8] and G1P[8] were the most frequent after vaccine introduction. Continuous surveillance in the post-vaccine period is essential to monitor circulating Rotavirus strains and detect unusual or emerging genotypes.

Keywords

Genotypes, Rotavirus, Monovalent Vaccine, Senegal

1. Introduction

Acute gastroenteritis (AGE) remains a major public health concern and is one of the leading causes of pediatric hospitalization. In low- and middle-income countries, morbidity and mortality rates remain high [1] [2]. Rotaviruses are the principal etiological agents of infantile gastroenteritis. Antigenic diversity within the viral outer capsid proteins VP7 and VP4, as well as genetic variability of their encoding genes, has led to a classification into G and P genotypes [3]. At least 32 G genotypes and 47 P genotypes have been described, producing numerous G/P combinations [3]. Molecular epidemiology studies have significantly advanced Rotavirus vaccine development. In November 2014, the Senegalese Ministry of Health and Social Action, with Gavi support, introduced the two-dose monovalent live-attenuated human Rotavirus vaccine RV1 (RIX4414 strain, G1P[8] specificity; Rotarix, GlaxoSmithKline Biologicals) into the national Expanded Program on Immunization (EPI). RV1 is administered at 6 and 10 weeks of age. Although genotypes G1 to G4 are globally prevalent, emergent genotypes such as G9 and G12 currently not included in vaccines are being increasingly reported worldwide [4] [5]. The emergence of novel genotypes highlights the need for ongoing Rotavirus surveillance both before and after vaccine introduction. The primary objective of this study was to monitor the molecular evolution of circulating rotavirus strains in children under 5 years of age in Senegal, comparing the periods before and after implementation of the national Rotavirus vaccination program.

2. Methods

2.1. Study Population

It was a prospective study covering the period from January 1st, 2011 to December 31, 2020, conducted at the Rotavirus sentinel surveillance site at the Albert Royer National Children's Hospital in Dakar. This surveillance has been established since 2005 by the World Health Organization (WHO), under the authority of the Ministry of Health and Social Action, therefore with the agreement of the national epidemiological surveillance officials and the hospital. Surveillance years were defined as March of one year through February of the following year. Given that vaccine introduction occurred in November 2014, March 2014-February 2015 was

considered a transitional period.

Fecal samples were collected from children less than 5 years of age admitted with a primary diagnosis of AGE within 48 h of hospitalization. AGE is defined as three or more watery stools per 24-hour period, lasting for a period of 7 days or less. Upon enrolment, informed consent was obtained from the child's parent or guardian, a questionnaire was administered to obtain demographic and clinical information, and 10 grams of stool was collected and transported to the CHNEAR laboratory for analysis. Detection of group A Rotavirus antigen was performed by using enzyme immunoassay (EIA) (ProSpecTTM, Oxoid Cambridge, United Kingdom).

Rotavirus-positive stools were subsequently stored at −80˚C before their molecular characterization at the West African Regional Rotavirus Reference Laboratory (RRL) located at the Noguchi Memorial Institute for Medical Research, University of Ghana.

2.2. Polyacrylamide gel Electrophoresis (PAGE)

All RV EIA-positive stool specimens were subjected to Polyacrylamide Gel Electrophoresis (PAGE) to ascertain the integrity of the RNA genome. Simarly, all EIA-negative samples were also subjected to PAGE to screen for any non-group A Rotavirus. Briefly, viral RNA was extracted from 10% faecal suspensions by the Bender method [6] with slight modification for PAGE analysis [7]. The extracted double-stranded RNA (dsRNA) was electrophoresed on a 10% polyacrylamide slab gel for 18 - 20 h at 100 V using the discontinuous buffer system as described by Laemmli [8]. A 3% stacking gel was employed to enhance the resolution of the segmented genes. Bands were visualized by silver-staining technique [9].

2.3. Molecular Characterization by RT-PCR

RVA dsRNA was extracted from 10% fecal suspensions of EIA-positive and EIA negative PAGE-positive samples by the phenol/chloroform method as described by Steele and Alexander and purified with an RNaid Kit (Bio 101, Carlsbad, USA) [9]. RT-PCR was carried out using consensus primers Beg9/End9 and Con2/Con3 to amplify the VP7 and VP4 genes respectively [10] [11]. Semi-nested multiplex PCR was done for G- and P-typing by using genotype-specific primers as described previously [12] [13]. The amplified product was electrophoresed on a 2% agarose gel, and the genotypes determined by the sizes of the amplicons. All demographic, clinical and laboratory data were entered into a database and analyzed using Stata version 14. We used the chi-square test to calculate the value of probability p to determine. P-values < 0.05 were considered statistically significant. Data were entered and analyzed using Epi Info 3.5 and Microsoft Excel.

3. Results

During the pre-vaccine period (2010-2014), a total of 683 stool specimens were collected, of which 333 tested positive for Rotavirus, corresponding to a positivity

rate of **48.76%**. This rate declined to **15.29%** in the post-vaccine period (2015-2020), when 89 of 582 samples were positive. The p value indicates a statistically significant difference ($p < 0.05$) (Table 1).

Table 1. Distribution of rotavirus cases according to vaccination periods.

	Number of stools collected	Number of positive stools	Percentage of Rotavirus positives	p-value
Pre-vaccine period	683	333	48.76%	$p < 0.01$
Post-vaccine period	582	89	15.29%	

A total of 313 Rotavirus-positive stools underwent genotyping: 235 from the pre-vaccine period and 78 from the post-vaccine period.

Pre-vaccine genotypes

VP7 genotypes

Among the 235 strains, 177 (75.31%) were successfully typed for VP7. The VP7 genotyping revealed five different G genotypes: **G1, G2, G8, G9, and G12**, with **G1 and G12** predominating (Table 2).

Table 2. Distribution of rotavirus genotypes during the pre-vaccine period (2011-2014).

		VP4			Total
		P[6]	P[8]	P[Mix]	
VP7	G1	38	26	4	68
	G2	8	2	1	11
	G8	7	1	0	8
	G9	1	28	0	29
	G12	9	51	1	61
	GNT	29	28	1	58
	Total	92	136	7	235

VP4 genotypes

Two P genotypes were identified **P[6] and P[8]** with **P[8]** predominating at approximately 57.87% (Table 2).

VP7/VP4 combinations

Simultaneous typing of VP7 and VP4 was achieved for 170 samples (72.34%), revealing distinct G/P associations (Table 2). The most frequent combination was **G12P[8] (28.81%)**, followed by **G1P[6] (21.46%)**, **G9P[8] (15.81%)**, and **G1P[8] (14.68%)**.

Post-vaccine genotypes

VP7 genotypes

Four VP7 genotypes were detected: **G1, G2, G3, G9, and G12** with **G3** being the most common, followed by **G1** (Table 3).

Table 3. Distribution of Rotavirus genotypes during the post-vaccine period (2015-2020).

		VP4				Total
		P[4]	P[6]	P[8]	P[Mix]	
VP7	G1	1	6	8	0	15
	G2	1	4	1	1	7
	G3	2	6	12	2	22
	G9	0	1	4	0	5
	G12	0	4	1	1	7
	G mix	0	5	4	1	8
	Total	4	22	48	4	78

VP4 genotypes

VP4 genotyping identified three primary genotypes: **P[4], P[6], and P[8]**, along with mixed types **P[8] P[6]** and **P[4] P[6]**. **P[8]** remained the dominant VP4 genotype (Table 3).

VP7/VP4 combinations

Concurrent VP7/VP4 genotyping was achieved for 66 strains (84.61%). The most frequent G/P association was **G3P[8] (18.18%)**, followed by **G1P[8] (12.12%)** (Table 3).

4. Discussion

Acute gastroenteritis remains one of the leading causes of morbidity and mortality among children under five years of age in developing countries, with Rotavirus as the most common infectious etiology. Laboratory-diagnosed Rotavirus AGE, particularly among younger children, substantially declined following Rotavirus vaccine introduction in Senegal. Rotavirus vaccine was introduced in November 2014, and rapidly reached 89% two-dose coverage by the end of 2015, and 96% by 2020 [14]. Infants were the first to benefit from vaccine. Our findings reveal a substantial decline in the number of circulating Rotavirus strains in the country after vaccine introduction. Genotyping at the Rotavirus Reference Laboratory (collaborating laboratory of the World Health Organization) was carried out according to the available budget. That's why all positive Rotavirus were not genotyped.

Among the G genotypes identified, G1 was most frequent during the pre-vaccine period of our study, followed by G12 and G9, whereas after vaccination G3 became predominant, followed by G12, G1, and untyped genotypes. The genotyping protocol used makes it possible to detect more than 95% of the main genotypes responsible for human Rotavirus infections. So the untyped strains will not have an impact on the distribution of genotypes, especially during the pre-vaccination period. These results confirm that the globally common strains (G1, G2, G3, G4, and G9) are not detected in the same proportions in Senegal compared with developed countries [15]-[17] and even some other African nations. For example, in

Zimbabwe the predominant strains were G9 (34%), G2 (26%), G1 (13%), G8 (5%), and G12 (4%) [4]; similarly, in Thailand G1 accounted for 50%, G2 for 26.9%, G3 for 10.1%, and G9 for 4.3% [18].

Since vaccine introduction, genotype G3 has dominated in Senegal, similar to findings in Malaysia [19], whereas G12 predominates in Ivory Coast [20]. Although G3 was never a predominant genotype in Senegal studies. The dominant genotypes in Zimbabwe (G9, G2) differ from those in Senegal [4]. Our results show that P[8] and P[6] genotypes were the most common both before and after vaccination, mirroring the relatively stable P-type distribution observed in Zimbabwe, despite a marked quantitative decline [4].

Before vaccination, the predominant strains in our study were G12P[8] (28.81%), G1P[6] (21.46%), G9P[8] (15.81%), and G1P[8] (14.68%). Globally, prior to vaccine introduction, five G/P genotype combinations were most often associated with rotavirus diarrhea: G1P[8], G2P[4], G3P[8], G4P[8], and G9P[8] [17] [21] [22]. Other studies have shown that globally rare strains were more frequently detected in Africa (27%), Asia (14%), and South America (11%) compared with North America (5%) and Europe (4%) [23] [24]. Among these African strains, some (G12P[8], G3P[6], G1P[6], and G9P[6]) were present and predominant in Senegal before vaccination [24].

However, after introduction of the G1P[8] Rotarix vaccine, G12P[8] was replaced by G3P[8] (18.18%), followed by G1P[8] (12.12%), G3P[6] (9.09%), and G1P[6] (9.09%) in our study. This aligns with the work of Bilbera and al., who reported that the most important circulating genotypes during the post-vaccination period included G2P[4] and five other G/P combinations (G1P[8], G3P[8], G9P[8], G4P[8], G12P[8]) [25]. These findings highlight the strong effectiveness of the vaccine against genotypes such as G12P[8] and G1P[6].

Although G3P[8] has been detected in several countries, the detection rate varies. Few G3P[8] strains have been detected in Germany, Hungary, Japan and the USA. In Australia and Spain, the detection rate was moderate, between 14.4 and 37.4%. G3P[8] was predominant in Brazil, Indonesia and Thailand during the 2016-2017 seasons [19]. This emergence of G3P[8] could be due to a high coverage rate of the Rotarix vaccine. In Australia, the dominance G3P[8] is attributed to high Rotarix vaccine coverage-related vaccine-induced selective pressure. The dominance of G3P[8] in Thailand and Hungary is also attributed to vaccine-induced selective pressure, although Rotarix is only available on the private market and has lower national coverage in these two countries. Furthermore, the G3P[8] dominance in Spain is also attributed to vaccine-induced selective pressure, although RotaTeq has been primarily used there [19].

Notably, 90% of circulating strains worldwide share epitopes on VP4 and 30% on VP7, which enables cross-recognition by neutralizing antibodies [26]. The Rotarix® vaccine (GSK) contains an attenuated strain belonging to the same G1P[8] group as the Wa strain, conferring protection against both homotypic and heterotypic strains [22]. Regardless of the changes in genotype distributions, the overall

prevalence of rotavirus has decreased substantially in all countries following rotavirus vaccine introduction [27].

Because of its segmented genome, the virus can undergo point mutations, gene rearrangements, or gene reassortment, generating extensive genetic diversity. Mixed-genotype infections may also allow reassortment of RNA segments. Numerous reports have described natural reassortment events between animal and human rotavirus strains [28].

5. Conclusions

Unusual genotype combinations were observed during the study period. From 2010 to 2014, the dominant G and P genotypes were **G1, G12, P[8] and P[6],** whereas between 2015 and 2020 the predominant types shifted to **G3, G12, P[8] and P[6]**.

Before vaccine introduction, the leading genotype associations were **G12P[8]**, **G1P[6]**, **G9P[8]**, and **G1P[8]**. After vaccine rollout, **G3P[8]** became the most prevalent combination, followed by **G1P[8]**, **G1P[6]**, and **G3P[6]**.

The emergence of the G3 genotype could pose a challenge to vaccine effectiveness. Enhanced surveillance of these newly circulating genotypes is therefore essential to ensure early detection and to guide vaccine policy and effectiveness assessments.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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