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Diversity of Rotavirus Strains Circulating in Botswana before and after introduction of the Monovalent Rotavirus Vaccine

M. Mokomane [a,b,*], M.D. Esona [c], M.D. Bowen [c], J.E. Tate [c], A.P. Steenhoff [d], K. Lechiile [e], S. Gaseitsiwe [f], L.M. Seheri [g], N.B. Magagula [g], G. Weldegebriel [h], J.M. Pernica [i], J.M. Mwenda [j], I. Kasvosve [b], U.D. Parashar [c], D.M. Goldfarb [k]

[a] Department of Medical Laboratory Science, Faculty of Health Sciences, University of Botswana, Gaborone, Botswana
[b] Botswana National Health Laboratory, Gaborone, Botswana
[c] Centers for Disease Control and Prevention, Atlanta, GA, USA
[d] The Children's Hospital of Philadelphia, USA
[e] Botswana-UPenn Partnership, Gaborone, Botswana
[f] Botswana-Harvard AIDS Institute Partnership, Gaborone, Botswana
[g] WHO Regional Rotavirus Reference Laboratory, Diarrhoeal Pathogens Research Unit, Sefako Makgatho Health Sciences University, South Africa
[h] WHO IST East and Southern Africa, Harare, Zimbabwe
[i] McMaster University, Hamilton, Canada
[j] African Rotavirus Surveillance Network, Immunization, Vaccines and Development Cluster, WHO African Regional Office, Brazzaville, Congo
[k] University of British Columbia, Vancouver, Canada

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ABSTRACT

Background: Globally, rotavirus is the leading cause of acute gastroenteritis (AGE) in children aged <5 years. Botswana introduced the monovalent rotavirus vaccine (Rotarix) in July 2012. To study the impact of this vaccine on rotavirus genotypes circulating in Botswana, a comparison of the genotypes pre-vaccination (2011–2012) and post-vaccination (2013–2018) periods was conducted.

Subjects and methods: Residual samples from 284 children <5 years of age that tested positive for rotavirus by enzyme immunoassay were genotyped. One hundred and five samples were from the pre-vaccination period and 179 were from the post-vaccination period. Genotyping was performed using two multiplexed one-step reverse transcription polymerase chain reaction (RT-PCR) assays for the amplification and genotyping of rotavirus VP7 (G) and VP4 (P) genes.

Results: Prior to vaccine introduction, the predominant rotavirus circulating genotypes were G9P[8] (n = 63, 60%) and G1P[8] (n = 22, 21%). During the vaccine period, G2P[4] was the predominant genotype (n = 49, 28%), followed by G9P[8] (n = 40, 22%) and G1P[8] (n = 33, 18.5%). There was a significant decline in the prevalence of G9P[8] (p = 0.001) in the post-vaccination period. There was also a notable decline in G1P[8]. A spike in G2P[4] was observed in 2013, one year post-vaccine introduction. Rotavirus strain G3P[4] (n = 8) was only detected in the post-vaccine introduction period. In 2018 there was a marked increase in genotype G3P[8] (p = 0.0003).

Conclusions: The distribution of circulating rotavirus genotypes in Botswana changed after vaccine implementation. Further studies are needed to examine whether these changes are related to vaccination or simply represent natural secular variation.

1. Introduction

Globally, rotavirus is the leading cause of acute gastroenteritis (AGE) in children aged <5 years, causing 37% of hospitalizations and approximately 215 000 deaths annually [33]. The VP7 and VP4 proteins form the outer layer of the viral capsid and have been used historically to classify rotavirus serotypes and genotypes into respective G and P types [12]. Thirty six G genotypes and 51P genotypes have been identified to date [40] and six strains (G1P[8], G2P[4], G3P[8], G4P[8], G9P[8] and G12P[8]) are associated with 80–90% of the global rotavirus disease burden [8].

Monitoring the vaccine's effects on rotavirus strain diversity following its introduction is recommended [29] as it is critical for

* Corresponding author at: Department of Medical Laboratory Science, Faculty of Health Sciences, University of Botswana, Private Bag UB, 00712 Gaborone, Botswana.
E-mail address: mbafana@gov.bw (M. Mokomane).

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evaluating vaccine-induced changes in genotype distribution [32], as well as detecting strains which might not be covered by the vaccine [15]. Due to the segmented nature of the rotavirus genome, reassortment events involving different strains are common and recognized as one of the mechanisms governing the evolution of novel viruses, therefore leading to the emergence of rotavirus variants that may potentially challenge current rotavirus vaccination strategies [19]. Conducting surveillance of circulating rotavirus strains is important to identify reassortants and emergence of latest genotypes.

The Botswana Ministry of Health and Wellness introduced universal rotavirus vaccination (RV1 - Rotarix®) into Botswana's national immunization programme in July 2012 following three diarrhoea outbreaks that occured in 2005, 2006 and 2011 which resulted in a cumulative total of 57,879 cases and 777 deaths [6,38]). Rotavirus vaccine introduction was associated with significant reductions in gastroenteritis admissions and mortalities [9] and the measured vaccine effectiveness was found to be similar to those seen elsewhere in sub-Saharan Africa [13]. In this study, we report on the distribution of rotavirus G and P genotypes circulating in Botswana before and after introduction of rotavirus vaccination.

2. Materials and methods

2.1. Study samples

Two hundred and eighty-four (284) rotavirus positive stool samples from children <5 years of age admitted to hospitals with acute gastroenteritis defined as either ≥3 episodes of diarrhea (looser than normal stools) or ≥2 episodes of emesis and any episodes of diarrhea within a 24-h period, and lasting <7 days. Children were enrolled through active surveillance at four different clinical sites across Botswana. These were residual samples that had already been tested for rotavirus using a commercial Enzyme Immunoassay (EIA) or with Polymerase Chain Reaction (PCR) in previous studies [35,16,28]. One hundred and five samples were collected prior to vaccine introduction (May 2011 - December 2012) and 179 samples were from the post-vaccination period (January 2013 – November 2018). Stool samples were collected within 48 h of hospital admission, transported to the laboratory in chilled cooler boxes and were stored at −80°C until analysis. Ethical approval was obtained from the Ministry of Health Research and Development Committee (HRDC) and institutional review boards of Princess Marina Hospital, University of Pennsylvania and McMaster University. Consent was obtained from a parent or guardian of each child.

3. Laboratory investigations

3.1. Nucleic acid extraction

The laboratory investigations for the 235 samples were conducted at the Botswana National Health Laboratory. Stool samples were pre-treated as previously described [16]. Total nucleic acid extraction was performed on the NucliSENS easy MAG instrument (bioMérieux, Marcy l'Étoile, France) as previously described [27]. Furthermore, 49 additional samples were shipped in cold packs to the WHO Regional Rotavirus Reference Laboratory in South Africa for genotyping.

3.2. Genotyping

Two hundred and thirty-five (235) samples were genotyped in Botswana using previously described multiplexed one-step reverse transcription polymerase chain reaction (RT-PCR) assays for the amplification and genotyping of rotavirus VP7 (G) and VP4 (P) genes on all EIA rotavirus positive samples [11].

The 49 samples from the 2018 season were sent to the WHO Regional Rotavirus Reference Laboratory in South Africa for genotyping. The genotyping method used was previously described [31,41]. Rotavirus RNA from these samples was extracted using QIAamp viral RNA extraction kit according to manufacturer's instructions. Rotavirus reverse transcription polymerase chain reaction and genotyping was performed according to the methods described by Gentsch et al. [14], Gouvea et al. [18], Aladin et al. [1].

3.3. Visualization of genotypes

The amplified RT-PCR products were visualized by gel electrophoresis using 3% UltraPure™Agarose gel (Invitrogen by Life Technologies, Carlsbad, CA), in 1X TBE buffer at 100 V for 3 h. A 100 bp DNA ladder (New England Biolabs, Inc., UK) was used as the molecular size marker and the bands were visualized by UV translumination using a Molecular Imager® Gel Doc™ XR+ System with Image Lab™ Software (Bio-Rad Laboratories, Inc. California, United States).

For samples tested in Botswana, VP4 (P) genotypes were determined by direct observation of one of more of the following RT-PCR products, 199 bp for P[6], 339 bp for P[8], 391 bp for P[9], 497 bp for P[4] and 583 bp for P[10]. For VP7 (G), genotypes were determined by direct observation of one of more of the following RT-PCR products: 849 bp for G1, 146 bp for G2, 732 bp for G3, 198 bp for G4, 353 bp for G9 and 283 bp for G12. While in the WHO Regional Rotavirus Reference Laboratory in South Africa, VP4 genotypes were determined by direct observation of one of more of the following amplicon sizes: 267 bp for P[6], 345 bp for P[8], 483 bp for P[4], 543 bp for P[14]. For VP7, genotypes were determined by direct observation of one of more of the following amplicon sizes: 749 bp for G1, 652 bp for G2, 813 bp for G3, 583 bp for G4, 306 bp for G9, 885 bp for G8 and 559 bp for G12. In the absence of RT-PCR amplicons or the presence of bands with amplicon sizes that do not correspond to a reference P or G type, a sample was designated as non-typeable.

3.4. Statistical analysis

All rotavirus positive samples were stratified according to whether the genotypes appeared prior to or after vaccine introduction. Genotypes were reported as a proportion and expressed as percentage. A z-test was used to compare the proportions of genotypes prior to and post rotavirus vaccination and p-value of <0.05 was considered statistically significant.

4. Results

A total of 284 rotavirus positive samples were genotyped, 105 samples from the pre-vaccination period and 179 from the post-vaccination period. Demographic data were available for 91 samples in the pre-vaccine period, and age ranged from 3 days to 27 months with a median of 8 months. In the post-vaccine period, demographic data was available for 127 samples, and the age ranged from 5 days to 51 months with a median of 9 months. The proportion of females was comparable between the groups, 44% versus 41%, respectively. Prior to introduction of the monovalent rotavirus vaccine, the predominant circulating rotavirus genotypes were G9P[8] (60%, n = 63) and G1P[8] (21%, n = 22). In the post vaccination period, G2P[4] was the predominant genotype (28%,

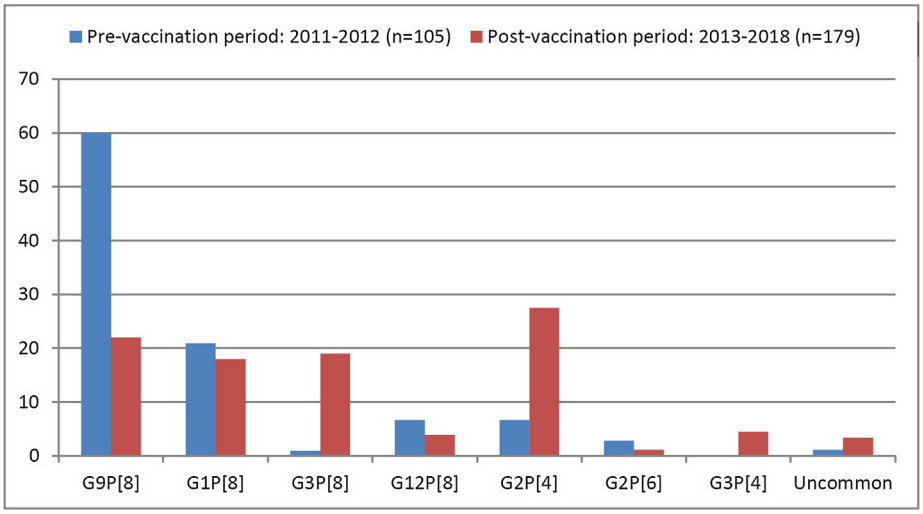
n = 49), followed by G9P[8] (22%, n = 40), G3P[8] (19.1%, n = 34) and G1P[8] (18.5%, n = 33), Fig. 1.

When comparing the two periods, there was a significant decline in the prevalence of G9P[8] (60% versus 22%, p = 0.001, (Table 1). Of note is that most G1P[8] genotypes in the post vaccination era were reported in 2014, with a few cases reported in 2018. A spike in G2P[4] (n = 37, 20%; p < 0.0001) was noted in 2013, one year post-vaccine introduction. In the fourth and fifth years post-vaccine introduction, that is in 2016 and 2017, G3P[4] (n = 8) genotype, which was not detected in the pre-vaccination period, emerged. In addition, in 2018, there was a significant increase of G3[P8] (1% vs. 19%; p = 0.0003), seven years after the last case was detected.

5. Discussion

The common genotypes observed in Botswana, G9P[8], G1P[8], G3P[8] and G2P[4], correlate well with what has been reported worldwide as predominant genotypes[8]. In addition, the predominance of G2P[4] immediately after introduction of the vaccine has also been reported in many other countries including Africa, following introduction of the monovalent rotavirus vaccine. A large proportion of children were infected with G2P[4] rotavirus strain in 2012 and there was also a steady annual increase in four African countries namely; Ethiopia, Mauritius, Tanzania and Zambia) [31]. The same observation was made in developed countries as well. In Japan for instance, the detection rate of G2P[4] increased soon after vaccine introduction [22] and the same was observed in other countries like Brazil [20], Australia [7] and Belgium [34]. In fact, in Australia, a G2P[4] rotavirus outbreak occurred 28 months after the introduction of the Rotarix® vaccine [7]. However, it appears different vaccines exert different immunologic pressure, as reported in Australia where both RotaTeq and Rotarix were implemented and differences in genotype dominance were observed [30].

Following introduction of the rotavirus vaccine, there was a significant reduction in the prevalence of G9P[8], and significant increases in G2P[4] and G3P[8]. Genotype G1P[8] was one of the most prevalent genotypes detected during the pre-vaccination period, however post-vaccination, it was not detected a year after introduction of the vaccine, but reappeared in the second year post-vaccine introduction with a slight but non-significant increase in genotype distribution between the two periods (9% vs. 18%). However, subsequently it was undetected for three years thereafter, except for three cases in 2018. These findings support results from other studies that show that Rotarix rotavirus vaccine, which is composed of the G1P[8] strain, confers significant protection against this homologous genotype [24]. Protection against G9 strains by Rotarix has also been documented, possibly effected via the P[8] component of most G9 strains [10,23], hence the reason



[i] Uncommon genotypes: G9P[6], G9P[4], G3P[6], G4P[8], G8P[4]

Fig. 1. Comparison of prevalence of circulating rotavirus genotypes detected prior to and post vaccination periods.

Table 1
Annual distribution of rotavirus genotypes during the two vaccination periods under comparison.

Genotype	Pre-vaccine period n = 105		Post-vaccine period n = 179					
	2011	2012	2013	2014	2015	2016	2017	2018
G9 P[8]	59	4	2	9	26	0	0	3
G1P [8]	1	21	0	30	0	0	0	3
G3 P[8]	1	0	0	0	0	0	0	34
G12 P[8]	6	1	0	0	1	1	5	0
G2 P[4]	0	7	37	2	1	2	4	3
G2 P[6]	3	0	0	2	0	0	0	0
G3P [4]	0	0	0	0	0	7	1	0
Uncommon	0	2	0	0	0	0	0	6

Uncommon genotypes: G9P[6], G9P[4], G3P[6], G4P[8], G8P[4].

for the decline in detection of G9P[8] during the post-vaccination rotavirus seasons. The other genotypes showed no significant differences between the two periods. Previous studies have shown that rotavirus vaccines are effective in preventing severe rotavirus disease due to homotypic and heterotypic rotavirus vaccine strains [4]. In one study, a systematic review of published work to assess the strain-specific effectiveness of these vaccine was performed and it was found that for Rotarix, vaccine effectiveness (VE) was 94% (95% CI 80–98) against homotypic strains, 71% (39–86) against partly heterotypic strains, and 87% (76–93) against fully heterotypic strains, in high income countries [24].

In 2018, Botswana experienced another rotavirus diarrhea outbreak seven years after the last outbreak, and rotavirus G3P[8] was the predominant genotype detected [37]. What is notable is that G3[P8] had not been detected in the previous six years (except for one case that was detected in 2011 – before introduction of rotavirus vaccine). Therefore, the re-emergence of G3[P8], with serious consequences resulting in an outbreak, emphasizes the need to strengthen rotavirus surveillance to monitor trends for any emerging and re-emerging genotypes and advise policy makers accordingly. After the diarrhea outbreak, rotavirus vaccination coverage was hypothesized as a contributing factor to this outbreak. Reports from the Expanded Programme on Immunization (EPI) Unit have indicated that the vaccine coverage had been ranging from 82% in 2013 to 72.6% in 2018 (with an average of 78%) [39].

However, emergence of G3[P8] causing diarrhea outbreak does not seem to be unique to Botswana. It has previously been observed that following introduction of rotavirus vaccines, G3 strains were detected worldwide in humans in combination with P[4], P[6], P[9] and P[8] [19]. G3P[8] together with G3P[4], G3P [6] were detected in other African countries like Angola, Ethiopia, Madagascar, Mauritius, Uganda, Seychelles, South Africa, Zambia and Zimbabwe in 2016 and 2017 [36]. These strains, particularly G3[P8] gradually increased in 2018 and became more predominant in Botswana resulting in the diarrhea outbreak [37]. In developed countries, G3P[8] rotavirus emerged as the dominant strain in children with severe rotavirus gastroenteritis in Australia and Italy [5,26]. Although it has been established that the Australian strain had an equine-like VP7 gene [5], further studies like whole genome characterization of the strain observed in Botswana still need to be conducted to establish its lineage.

A previous study conducted in 2001, more than 10 years before the introduction of rotavirus vaccine, found that rotavirus strains G1P[8] and G1P[6 + 8] were the most common genotypes in Botswana [21]. In the current study, the findings indicate that G9P[8] was the predominant genotype detected followed by G1P[8]. However, it has been observed that the predominant strains causing severe rotavirus disease change from year to year and this genetic diversity is generated by several mechanisms, among them being natural evolution due to accumulation of point mutations, reassortment between human and animal strains [23]. Thus, changes in circulating genotypes pre- and post-vaccine introduction may also be due to natural secular variation and not related to vaccine introduction.

When comparing these circulating rotavirus strains in Botswana post vaccine introduction, to those found in other African countries using the same monovalent rotavirus vaccine (Rotarix), the most common genotypes in Malawi were G2P[4] (25%), G1P [8] (21%), G12P[6] (10%) and G2P[6] (10%) [2]. In South Africa, the common genotypes were G12P[8] (43%), G2P[4] (14%), G1P [8] (12%), G9P[8] (8%), G8P[4] (7%) and G2P[6] (5%) [17]. This suggests the diversity of rotavirus strains in different countries and regions, hence the need for countries to establish rotavirus strain surveillance programs to monitor rotavirus strains and to understand these changes in relation to vaccine effectiveness. G3P[4], which is a less common genotype of equine origin has been observed in Ghana, [3] China and Japan [25].

This study had several limitations. The short time frame of surveillance prior to vaccine introduction makes it difficult to attribute changes in genotype diversity to natural fluctuation or vaccine pressure. Genotyping data from other countries in the region both with and without rotavirus vaccine showed similar fluctuations in circulating strains [31]. We did not assess the effectiveness of the vaccine against any of the detected strains in this analysis although this vaccine has been shown to provide protection against homologous and heterologous strains [24]. Finally, there was also a relatively small number of samples analyzed, particularly in 2016 and 2017.

6. Conclusion

The predominant circulating rotavirus genotypes in Botswana prior to introduction of the monovalent rotavirus vaccine were G9P[8] and G1P[8] but during the vaccine period, G2P[4] became the most common genotype, followed by G9P[8] and G1P[8]. The G3P[4] strain emerged in the post vaccination period, as well as the re-emergence of G3P[8] during the 2018 diarrhea outbreak. This observation reinforces the need to continue with the rotavirus surveillance program in Botswana to ensure monitoring of and detection of changes in rotavirus strains in the country. Predominance of these new strains may lead to potential rotavirus gastroenteritis epidemics, once the traditional common strains decline in prevalence potentially due to the effects of vaccine pressure, as seen in the Botswana 2018 rotavirus diarrhea outbreak due to G3[P8].

7. Disclaimer

The findings and conclusions in this report are those of the author(s) and do not necessarily represent the official position of the Centers for Disease Control and Prevention and World Health Organization (WHO). Names of specific vendors, manufacturers, or products are included for public health and informational purposes; inclusion does not imply endorsement of the vendors, manufacturers, or products by the Centers for Disease Control and Prevention or the US Department of Health and Human Services and World Health Organization (WHO).

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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