Rotavirus group A genotype circulation patterns across Kenya before and after nationwide vaccine introduction, 2010-2018

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Rotavirus group A genotype circulation patterns across Kenya before and after nationwide vaccine introduction, 2010-2018

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Abstract

Background: Kenya introduced the monovalent G1P[8] Rotarix® vaccine into the infant immunization schedule in July 2014. We examined trends in rotavirus group A (RVA) genotype distribution pre- (January 2010 - June 2014) and post- (July 2014-December 2018) RVA vaccine introduction.

Methods: Stool samples were collected from children aged <13 years from four surveillance sites across Kenya: Kilifi County Hospital, Tabitha Clinic Nairobi, Lwak Mission Hospital, and Siaya County Referral Hospital (children aged <5 years only). Samples were screened for RVA using enzyme linked immunosorbent assay (ELISA) and G and P genes sequenced to infer genotypes.

Results: We genotyped 614 samples in pre-vaccine and 261 in post-vaccine introduction periods. During the pre-vaccine introduction period, the most frequent RVA genotypes were G1P[8] (45.8%), G8P[4] (15.8%), G9P[8] (13.2%), G2P[4] (7.0%) and G3P[6] (3.1%). In the post-vaccine introduction period, the most frequent genotypes were G1P[8] (52.1%), G2P[4] (20.7%) and G3P[8] (16.1%). Predominant genotypes varied by year and site in both pre and post-vaccine periods. Temporal genotype patterns showed an increase in prevalence of heterotypic commonly DS-1-like G2P[4] (7.0 to 20.7%, P<.001) and G3P[8] (1.3 to 16.1%, P<.001) genotypes in the post-vaccine introduction period. Additionally, we observed a decline in prevalence of

genotypes G8P[4] (15.8 to 0.4%, P<.001) and G9P[8] (13.2 to 5.4%, P<.001) in the post-vaccine introduction period.

Conclusion: Genotype prevalence varied from before to after vaccine introduction. Such observations emphasize the need for long-term surveillance to monitor vaccine impact. These changes may represent natural secular variation or possible immuno-epidemiological changes arising from the introduction of the vaccine. Full genome sequencing could provide insights into post-vaccine evolutionary pressures and antigenic diversity.

Key words

Rotavirus, genotype, pre-vaccine, post-vaccine, Kenya

1. Background

Childhood diarrhea caused by rotavirus group A (RVA) infection remains a leading cause of morbidity and mortality in young children globally (1). In 2016, RVA infections were estimated to be responsible for 128,500 deaths globally and over 80% of these deaths occurred in developing countries (1). Upon infection by the virus, immune response to RVA by the host is directed to genes of the VP4 and VP7 surface proteins which are encoded by the highly variable P (VP4) and G (VP7) genes, respectively, found on two separate segments of the double-stranded RNA genome (2). RVA P and G genotypes exist as multiple variants in nature, few of which have been found to infect humans (2). Up to 36 G and 51 P genotypes have been detected globally in both humans and animals, with multiple G-P combinations (3). Molecular studies have characterized circulating genotypes worldwide with predominance of genotype

G1P[8], G2P[4], G3P[8], G4P[8], G9P[8] and G12P[8] (in decreasing order of prevalence) (4,5). Although the distribution of these genotypes varies from region to region and from one season to another, genotype G1P[8] has remained the most dominant genotype globally (4).

In 2009, the World Health Organization (WHO) recommended the inclusion of RotaTeq® (Merck Vaccines, Whitehouse Station, New Jersey) or Rotarix® (GlaxoSmithKline Biologicals, Rixensart, Belgium) vaccines in the national immunization programs of countries that experience high diarrhea morbidity and mortality burden due to RVA disease (6). Kenya incorporated the monovalent G1P[8] Rotarix® (RV1) vaccine into the national immunization program in July 2014, administered in two oral doses offered at weeks 6 and 10 of age. The Rotavirus Immunization Programme Evaluation in Kenya (RIPEK) was established as a collaboration among existing rotavirus surveillance platforms across Kenya to monitor the impact of RV1 introduction against rotavirus disease and circulating RVA genotypes. Substantial effectiveness of the vaccine in Kenya and the entire sub-Saharan region (where disease burden is high) has been recorded, and the decline in incidence of all-cause and rotavirus associated diarrhea admissions has been attributed to vaccine implementation (7–11). However, there are limited data on RVA diversity in post-vaccine introduction periods in this region. The current report describes the distribution and temporal patterns of RVA genotypes observed before and after RV1 vaccine introduction in Kenya.

2. Methods

2.1 Rotavirus surveillance

RVA surveillance was carried out in four health facilities in coastal, western and central regions of Kenya. These surveillance sites were: Kilifi County Hospital (KCH) in Kilifi County, Tabitha Clinic (TC), in Kibera, Nairobi County, Saint Elizabeth Lwak Mission Hospital (LMH) and Siaya County Referral Hospital (SCRH) in Siaya County (Supplementary Figure 1). Surveillance was conducted during January 2010 –December 2018 for all the sites except for SCRH where surveillance ended in December 2016. Stool samples were collected from children aged <13 years (in KCH, TC, LMH) and <5 years (in SCRH) of age presenting with acute gastroenteritis (AGE). AGE was defined as $\geq$ 3 watery stools passed within a 24-hour period during the illness for KCH, TC and LMH, while for SCRH, AGE was defined as ≥ 3 loose stools and/or ≥ 1 episode of unexplained vomiting followed by loose stool within a 24-hour period beginning no more than 7 days before the visit for Siaya County Referral Hospital.

2.2 Laboratory processing

RVA was tested by use of commercially available enzyme immunoassays. The ProSpecT™ Rotavirus Kit (Oxoid, Basingstoke UK) was used to test samples collected from KCH while the Rotaclone® kit (Premier^{TM} Meridian Bioscience, Cincinnati, Ohio, USA) was used to test samples collected from LMH, SCRH, and TC. For positive samples collected from SCRH, sample processing and genotyping was performed as previously described (12). For samples collected from LMH, KCH and TC, partial fragments of the segments encoding the outer capsid proteins, VP4 (660 bp) and VP7 (881 bp), were amplified in a One-step Reverse Transcriptase Polymerase Chain Reaction(RT-PCR) using previously described primer pairs (13,14). Successful

amplification was visualized by electrophoresis of the PCR product in a 2% agarose gel. PCR products of confirmed positives were purified using GFX DNA purification kit (GFX-Amersham, Amersham, UK), according to the manufacturer's instructions. Confirmed positives were then sequenced using Big Dye Terminator 3.1 (Applied Biosystems, Foster City, California, USA) with the same primers as in PCR amplification on an ABI Prism 3130xl Genetic Analyzer (Applied Biosystems, Foster City, California, USA).

2.3 RVA genotyping

Reads from the sequencer were trimmed (removing regions including primer sequences) and assembled into contigs (consensus sequence formed from aligning the forward and reverse reads) using Sequencher version 5.4.6 (Gene Codes Corp Inc., Ann Arbor, MI, USA). G and P genotypes were determined using the online automated Virus Pathogen Resource online genotyping tool(15). Using this method only a single genotype per specimen was identified. However, 8.6% (n=113) of the samples were typed for only one of the genes due to failure in sequencing and/or contig assembly, supplementary table 1, and were excluded from the main analysis. Genotyping of RVA positive samples collected from SCRH was performed by use of type-specific primers as described(12). In this instance, untypable G (8.9%, n=15/168) and P (22.6%, n=38/168) genotypes and mixed infections (18.5%, n=31/168) were observed and sub-sequently excluded from analysis.

2.4 Data Analysis

Data collating and analyses were performed in Microsoft Excel and R version 3.5. Genotype data collected from LMH and SCRH were merged to constitute data from Siaya County. The summary of proportions was conducted for each genotype by site and season of detection. Frequency distribution and temporal pattern graphs were generated. Two-sided proportional tests of the most common genotypes during the pre-vaccine (January 2010 thru June 2014) and post-vaccine (July 2014 thru December 2018) eras were performed in R, and P-values < 0.05 were considered statistically significant.

2.5 Ethics statement

The study protocol was approved by institutional review boards (IRBs) of Kenya Medical Research Institute (Scientific Ethics Review Unit protocol no. #3049) and United States Centers for Disease Control and Prevention (CDC protocol no. #6968). CDC's human subjects research office relied on KEMRI for IRB oversight. Written informed consent for enrolment into the study was obtained from parents or guardians of children admitted with diarrhea symptoms.

3. Results

A total of 6,562 stool samples were collected from health facilities in LMH, SCRH, TC and KCH. Of the 1,312 (20.0%) RVA positive samples, 875 (66.7%) were successfully genotyped for both G and P genes [LMH (n=38), SCRH (n=161), TC (n=84), and KCH (n=592)]. Of these, 614 (70.2%) were observed in the pre-vaccine (January 2010 – June 2014) and 261 (29.8%) in the post-vaccine (July-2014 – December 2018) introduction periods, respectively.

3.1 G and P genotypes circulating in Kenya

Overall, nine different G types (G1-G4, G8-G10, G12, G29) and four P types (P[4], P[6], P[8] and P[14]) were observed in Kenya. The most common G type was G1 (49.5%) followed by G9 (12.7%), G8 (12.0%), G2 (11.1%) and G3 (10.1%), Table 1. Genotypes G4, G10, G12 and G29 were detected at low frequencies (<4%). The most common P types were P[8] (68.4%) and P[4] (23.6%), while P[6] (7.7%) and the less common P[14] (0.3%) were also observed, Table 1.

3.2 Rotavirus Genotype Distribution in pre- and post- vaccination eras

In the entire period, 22 different GP combinations were identified, Table 2. G1P[8] (47.7%) was the most common genotype followed by G8P[4] (11.2%), G2P[4] (11.1%), G9P[8] (10.9%), G3P[8] (5.7%) and G3P[6] (2.9%). In addition to these common genotypes, multiple other rare genotypes were observed in low frequency (<2%), Table 2. Notably, only one (n = 1/417, 0.2%) of the G1P[8] isolates was phylogenetically identified as a Rotarix vaccine strain. This was included in subsequent analysis

Figures 1 a, b, c and d show the temporal distribution of RVA genotypes in Kilifi, Siaya (i.e, pooled SCRH and LMH data) and Nairobi counties, and Kenya (pooled countrywide), respectively. Genotypes G1P[8], G2P[4], G8P[4], G3P[6], G3P[8] and G9P[8] were observed across all three sites. G1P[8] was observed in all seasons, in all sites, except in 2011 and 2016 in Nairobi. In contrast, genotypes G2P[4], G3P[8], G3P[6], G8P[4] and G9P[8] showed a fluctuating pattern in all three sites. Notably, genotype G2P[4] was observed in pre- and post-vaccine periods in Kilifi and Nairobi, and only in the post- vaccine period in Siaya. Furthermore,

genotype G3P[8] was observed in Kilifi and Nairobi counties only, while G3P[6] was detected in all three sites, although in moderate proportions. Additionally, genotype G9P[8] was observed in pre- and post- vaccine periods in Kilifi, unlike in Nairobi and Siaya where it was only observed in the pre-vaccine period. Similarly, genotype G8P[4] occurred in pre- vaccine and post-vaccine periods in Nairobi county and only in the pre-vaccine period in Kilifi and Siaya counties.

During the period before vaccine introduction in Kenya, G1P[8] (45.8%) was the predominant genotype observed in this population, followed by G8P[4] (15.8%), G9P[8](13.2%), G2P[4] (7.0%) and G3P[6] (3.1%). After vaccine introduction, G1P[8] remained the dominant genotype (52.1%), followed by G2P[4] (20.7%), G3P[8] (16.1%), G9P[8] (5.4%) and G3P[6] (2.7%). Multiple other genotypes were also observed in pre- and post-vaccine periods, although in low proportions (<2%), Tables 2, 3. Overall, at these surveillance sites, the first four RVA seasons, saw an alternating pattern of dominance between G8P[4] and G1P[8] (Figure 1d). G8P[4] was the predominant genotype in 2010 (54/153; 35.3%) and in 2012 (42/150;28.0%), while G1P[8] predominated in 2011 (117/160;73.1%) and 2013 (60/104;57.7%) through to 2014 (vaccine introduction season) breaking the cyclic pattern. During this season, G2P[4] and G9P[8] circulated in moderate proportions (1-20%) and (2-14%), respectively (Table 2). After vaccine introduction, G1P[8] dominated immediately in the first year of vaccine introduction (2015) (91/101, 90.1%). This phenomena was however, short lived and there was a re-emergence of G2P[4] (35/44; 79.5%) in the second year of vaccine period (2016), which was among the common genotypes in 2017 together with genotype G1P[8]. G3P[8] (41/66;62.1%) dominated in the fourth year post-vaccine introduction period (2018), whereas G1P[8]

193 (17/66;25.8%) and G2P[4] (4/66;6.1%) continuously circulated, although in reduced
194 proportions.
195 In the post-vaccine introduction period, relative to the pre-vaccine period, we observed a
196 significant increase in prevalence of genotypes G2P[4] (7.0% vs. 20.7%, P<0.001) and G3P[8]
197 (1.3% vs. 16.1%, P <0.001) and significant decline in prevalence of genotypes G8P[4] (15.8% vs.
198 0.4%, P<0.001) and G9P[8] (13.2% vs. 5.4%, P<0.001), Figure 2. No significant difference in the
199 prevalence of the vaccine genotype G1P[8] (45.8 vs. 52.1%,P=0.35) was measured before and
200 after vaccine introduction. A decline in genotype diversity was observed after vaccine
201 introduction (11 genotypes) as compared to pre-vaccine period (21 genotypes).
202
203 **4. Discussion**
204 Data generated for a period of nine years, 2010 to 2018, showed that G1P[8], G2P[4],
205 G8P[4], G9P[8] and G3P[8] were the common genotypes, accounting for over 90% of all RVA-
206 associated diarrhea in children. Prior to vaccine introduction, G1P[8] was the predominant
207 genotype. However, this genotype's prevalence varied substantially from year to year
208 (dominant in 2011, 2013, 2014 and 2015; less so in 2010 and 2012), emphasizing the limits of
209 short-term surveillance and the potential for misrepresentation of patterns. During 2010 and
210 2012, a large proportion of children were infected with genotype G8P[4], which was not
211 detected in any years following vaccination (except during 2017). Genotypes G1P[8], G2P[4]
212 and G9P[8] have also been identified as dominant genotypes in a study involving six countries
213 from Eastern and Southern Africa(5).

Genotype prevalence varied year to year and from before to after vaccine introduction. Interestingly, while genotype G1P[8] showed no overall change in prevalence compared to the pre-vaccine period, it predominated only in the first year after vaccine introduction (2015), and declined in prevalence thereafter, in particular to be replaced by G2P[4] in 2016, and by G3P[8] in 2018. Our phylogenetic analysis found only a single case of a child who had received the first dose of the Rotarix vaccine, shedding the vaccine strain while the remainder of the identified G1P[8] strains were wild-type. Genotypes G8P[4] and G9P[8], which were the second and third common genotypes in pre-vaccine period, significantly declined in the post-vaccine period. Predominance of G2P[4] and G3P[8] has been reported worldwide following universal RV1 vaccine introduction. For instance, G2P[4] was observed in Australia(16) (exclusively in states using RV1) and Belgium(17) after vaccine introduction, while data from Ethiopia(18) and Madagascar(19) depicted an increase in prevalence of G3P[8] in post-vaccine introduction season. G2P[4] is one of the genotypes of concern since this fully heterotypic genotype has a different genomic constellation (DS-1-like) to the genotype in the monovalent RV1 vaccine (Wa-like) (20). There is no conclusive evidence associating the increase in prevalence of these genotypes to selective vaccine pressure created by implementation of RV1 monovalent vaccine. Whilst the vaccine offers both homotypic and heterotypic protection (21,22), the emergence of the heterotypic G2P[4] and G3P[8] genotypes and persistence in circulation of the homotypic G1P[8] genotype after vaccine introduction emphasizes the need for continued monitoring of impact of vaccine on genotypes.

We observed multiple uncommon GP combinations including G1P[4], G1P[6], G2P[8],G4P[4], G8P[8],G8P[14], G10P[8], G12P[4] and G12P[6] at frequency of <2%. Genotype

P[14] has been described sporadically in humans and is believed to have originated from animal rotavirus and introduced into humans through interspecies transmission and/or reassortment events(23). The circulation of these uncommon genotypes demonstrates the high diversity of RVA genotypes in Kenya and concurs with findings from other African countries(5). However, genotype diversity was seen to decline in post-vaccine period (only 11 GP combinations, compared to 21 in pre-vaccine period), mirroring the experience of other countries, including Brazil and Zimbabwe, which also indicated a decline in genotype variation after vaccine introduction(24,25) Although diversity seems to decrease following vaccine introduction, it is unknown whether the observed trends will be sustained in the long-term, especially in African settings where pre-vaccine genotype diversity is high.

This study provides substantial epidemiological information on changes in distribution of RVA genotypes in Kenya. Some limitations of this study included fewer surveillance sites and unequal distribution of the number of samples among the sites, potentially underestimating the type of genotypes circulating in the post-vaccine period. However, it is important to consider that there was an overall decline in rotavirus circulation in the post-vaccine era, hence the fewer specimens and less diversity could be a reflection of less rotavirus circulation (7,8). Additionally, since this is an ecological study, the changes in distribution and diversity of genotypes in the post-vaccine era cannot directly be attributed to vaccine introduction. Furthermore, due to unsuccessful sequencing and/or contig assembly only two thirds of the total RVA positive samples were fully genotyped. Lastly these findings may not be generalized to the whole country because analysis was based on rotavirus cases observed in health facilities in three counties only.

In conclusion, we highlight the importance of monitoring the transition in the prevalence of genotypes for a better understanding of the performance of the currently available vaccines. The emergence of the fully heterotypic G2P[4] and partially heterotypic G3P[8] genotypes after vaccine introduction raises questions about the epidemiological dynamics following vaccine introduction. Previous analysis in Kenya showed that the vaccine had a significant impact on G1P[8] and non-significant G2P[4] (although with limited statistical power)(7), hence, continuous monitoring of the circulating genotypes in the post-vaccine era is necessary. Additionally, continued surveillance of the genetic characteristics of RVs circulating is recommended to obtain a full view of the long-term effects of vaccine introduction. Since immunity to RVA involves immune responses conferred by genes other than the commonly studied P and G genes, vaccine effectiveness might be challenged by changes occurring on non-capsid genes. It is therefore recommended that full genome analysis of genotypes collected in different time or geographic regions be conducted to improve understanding of their evolutionary profile during the post-vaccine introduction period.

5. Acknowledgment

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6. Data Availability

Partial sequences for the VP7 and VP4 genes reported in this work have been deposited to GenBank database. Sequence accession numbers for the VP7 and VP4 genes are indicated in Supplementary table 2 and 3, respectively. The datasets used and analyzed during this study are available from the Harvard Dataverse: https://doi.org/10.7910/DVN/0VQ2OK. Users who wish to use the data should send a request to the KEMRI Wellcome Trust Research Programme data governance committee, which can be contacted by emailing: dgc@kemri-wellcome.org.

7. Conflict of Interest

The authors declare no conflict of interest.

8. Funding

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9. Author Contributions

Mwanga J. Mike: Data curation and collation, formal analysis, investigation, methodology, project administration and coordination, manuscript writing. Owor E. Betty conceptualization, investigation, methodology. Ochieng B. John: formal analysis, investigation. Mwanajuma J. Ngama: Patient recruitment and consenting, Sample collection. Ogwel Billy: Data curation and collation. Onyango Clayton: Sample processing and project administration. Juma Jane: Sample processing. Njeru Regina: Sample processing. Gicheru Elijah: Sample processing. Otieno P. Grieven: Data collation and curation. Khagayi Sammy: Data collation and curation. Agoti N. Charles: Conceptualization, methodology, validation, formal analysis, Data curation. Omore Richard: Project administration, validation. Bigogo M. Godfrey: Project administration,

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10. Abbreviations

RVA – Rotavirus group A

WHO – World Health Organization

AGE – Acute Gastroenteritis

VP – Viral protein

RV1 – Rotarix monovalent Vaccine

RIPEK - Rotavirus Immunization Programme Evaluation in Kenya

11. Disclaimer

The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the Centers for Disease Control and Prevention.

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13. Figure and Table Legends

Figure 1; Temporal rotavirus genotype distribution in the three surveillance sites across Kenya; a –Kilifi County Hospital in Kilifi County, b – Tabitha Clinic in Kibera, Nairobi County, c – Siaya County (combined genotype data from Lwak Mission Hospital and Siaya Referral Hospital) and d - combination of the three Counties in Kenya between 2010 to 2018.

Figure 2; Comparison of prevalence of the dominant genotypes (G1P[8], G2P[4], G3P[8], G8P[4] and G9P[8]) at 95% confidence interval (CI) during the pre- (Jan 2010-Jun 2014) and post-(July 2014 – Dec 2018) vaccine introduction periods in Kenya. The predominant genotypes were selected based on their frequency as indicated in Table 3.

Supplementary Figure 1; Geographical boundaries and location of the health facilities participating in the rotavirus genotype surveillance program represented by the diamond shapes. Green – Siaya County Referral Hospital, blue – Lwak Mission Hospital, red – Tabitha Clinic and orange – Kilifi County Hospital.

Supplementary Table 1; Frequency of partially typed G and P genotypes. Gx and P[x] were un-successfully typed due to failure in sequencing and/or contig assembly.

Supplementary Table 2; GenBank accession numbers of all VP7 G gene sequences.

Supplementary Table 3; GenBank accession numbers of all VP4 P gene sequences.

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Table 1: Frequency of RVA G and P genotype circulation in Kenya between 2010-2018

G Genotypes	n	%	P Genotypes	n	%
G1	459	49.5	P[4]	213	23.6
G2	103	11.1	P[6]	70	7.7
G3	94	10.1	P[8]	618	68.4
G4	1	0.1	P[14]	3	0.3
G8	111	12.0			
G9	118	12.7			
G10	5	0.5			
G12	36	3.9			
G29	1	0.1			
Total	928		Total	904	

Table 2: Temporal distribution of RVA GP genotypes observed in Kenya between 2010–2018.

	2010		2011		2012		2013		2014		2015		2016		2017		2018		Total	
	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
G1P[8]	40	26.1	117	73.1	39	26	60	57.7	38	46.9	91	90.1	8	18.2	7	43.8	17	25.8	417	47.7
G8P[4]	54	35.3	0	0	42	28	1	1	0	0	0	0	0	0	1	6.3	0	0	98	11.2
G2P[4]	11	7.2	1	0.6	19	12.7	5	4.8	14	17.3	3	3	35	79.5	5	31.3	4	6.1	97	11.1
G9P[8]	18	11.8	20	12.5	30	20	8	7.7	17	21	1	1	0	0	0	0	1	1.5	95	10.9
G3P[8]	0	0	0	0	0	0	4	3.8	4	4.9	0	0	0	0	1	6.3	41	62.1	50	5.7
G3P[6]	2	1.3	0	0	2	1.3	13	12.5	2	2.5	4	4	0	0	2	12.5	0	0	25	2.9
G1P[6]	1	0.7	1	0.6	2	1.3	9	8.7	0	0	0	0	0	0	0	0	0	0	13	1.5
G9P[6]	8	5.2	1	0.6	1	0.7	0	0	1	1.2	0	0	0	0	0	0	0	0	11	1.3
G12P[6]	2	1.3	5	3.1	4	2.7	1	1	1	1.2	0	0	0	0	0	0	1	1.5	14	1.6
G12P[8]	2	1.3	6	3.8	1	0.7	0	0	0	0	1	1	0	0	0	0	1	1.5	11	1.3
G1P[4]	5	3.3	1	0.6	2	1.3	0	0	0	0	1	1	0	0	0	0	0	0	9	1
G9P[4]	4	2.6	2	1.3	1	0.7	0	0	0	0	0	0	0	0	0	0	0	0	7	0.8
G10P[8]	0	0	4	2.5	0	0	1	1	1	1.2	0	0	0	0	0	0	0	0	6	0.7
G2P[8]	1	0.7	0	0	0	0	1	1	1	1.2	0	0	0	0	0	0	1	1.5	4	0.5
G8P[6]	1	0.7	0	0	3	2	0	0	0	0	0	0	0	0	0	0	0	0	4	0.5
G8P[8]	1	0.7	1	0.6	3	2	0	0	0	0	0	0	0	0	0	0	0	0	5	0.6
G12P[4]	2	1.3	1	0.6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0.3
G3P[4]	0	0	0	0	0	0	0	0	2	2.5	0	0	0	0	0	0	0	0	2	0.2
G8P[14]	1	0.7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0.1
G9P[14]	0	0	0	0	1	0.7	0	0	0	0	0	0	0	0	0	0	0	0	1	0.1
G1P[14]	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	1	0.1
G4P[4]	0	0	0	0	0	0	0	0	0	0	0	0	1	2.3	0	0	0	0	1	0.1
Total	153		160		150		104		81		101		44		16		66		875	

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460 **Table 3:** Frequency of RVA genotypes detected in Kenya in pre-vaccine (2010 - June 2014) and

461 post-vaccine (July 2014-2018).

	Pre-vaccine (2010-June 2014)					Post-vaccine (July 2014-2018)			
	P[4]	P[6]	P[8]	P[14]	Total	P[4]	P[6]	P[8]	Total
G1	8	13	281	1	303	1	0	136	137
G2	43	0	2	0	45	54	0	2	56
G3	2	19	8	0	29	0	7	42	49
G4	0	0	0	0	0	1	0	0	1
G8	97	4	5	1	107	1	0	0	1
G9	7	11	81	1	100	0	0	14	14
G10	0	0	6	0	6	0	0	0	0
G12	3	12	9	0	24	0	2	1	3
Total	160	59	392	3	614	57	9	195	261

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Figure 1

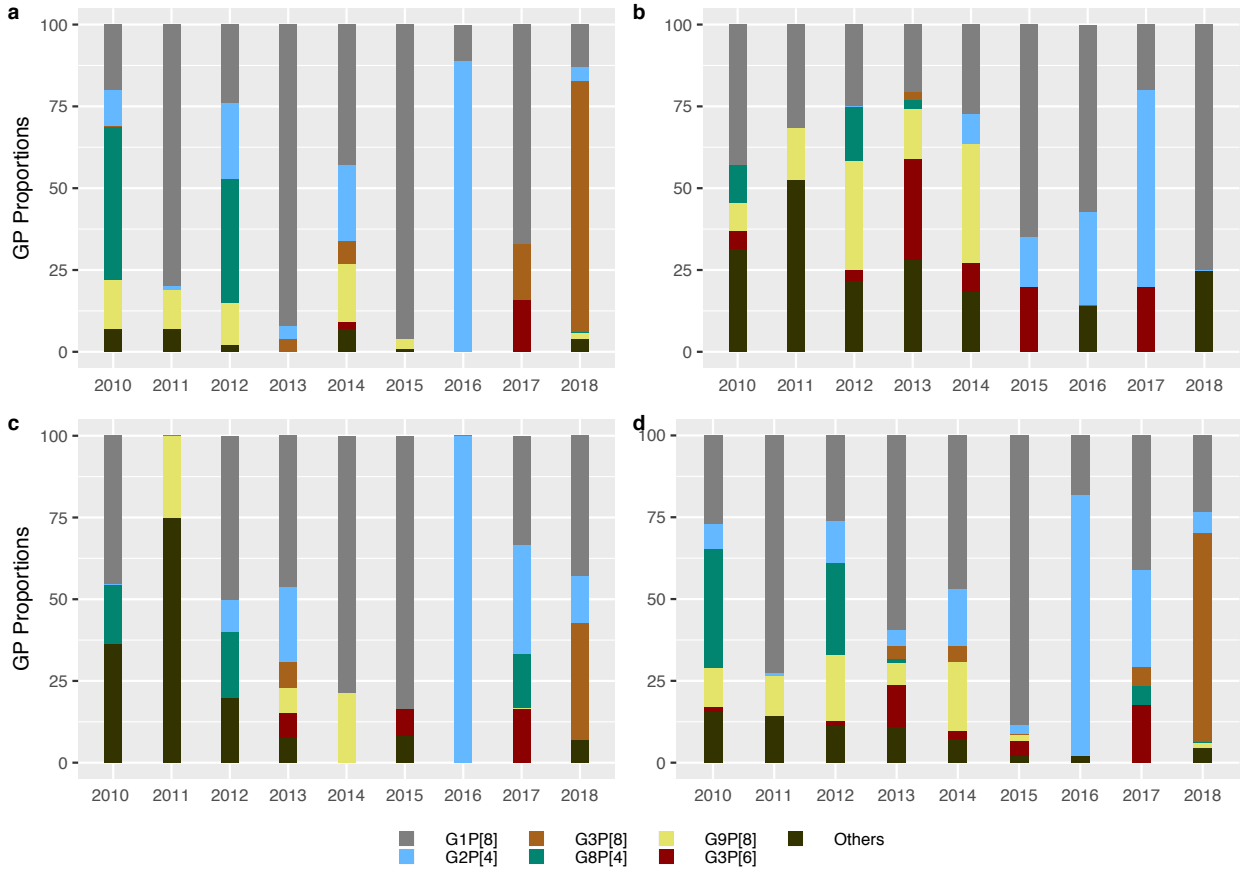
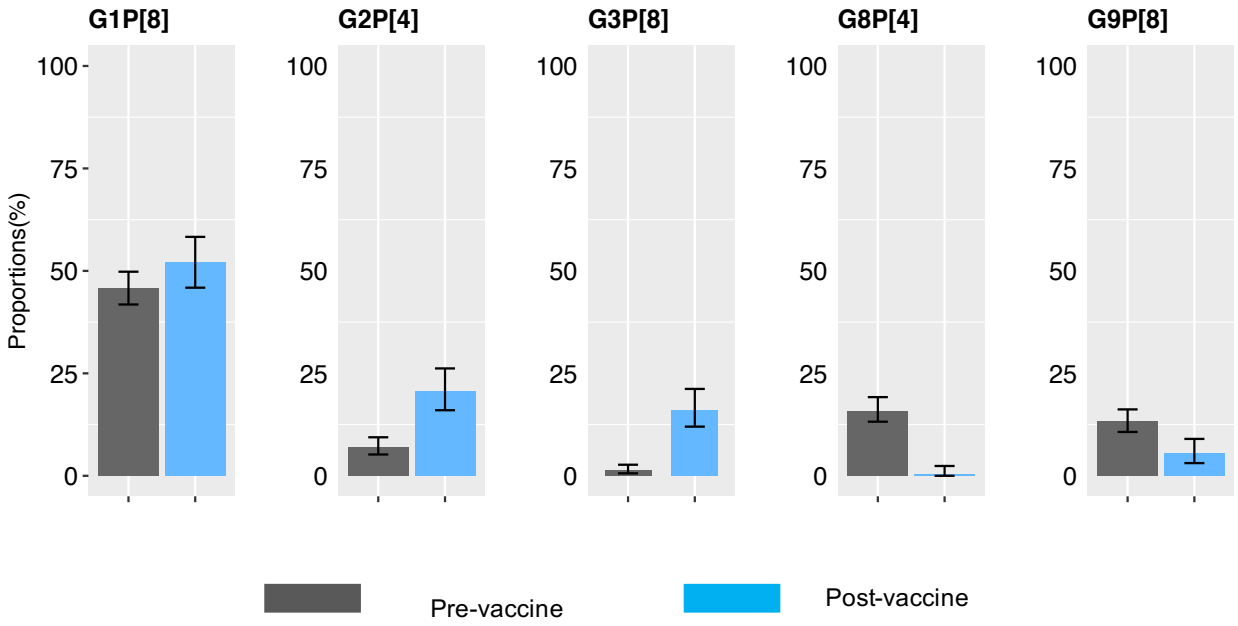

a
b
c
d
GP Proportions
GP Proportions
100
75
50
25
0
2010
2011
2012
2013
2014
2015
2016
2017
2018
G1P[8]
G2P[4]
G3P[8]
G8P[4]
G9P[8]
G3P[6]
Others

Figure 2


G1P[8]
G2P[4]
G3P[8]
G8P[4]
G9P[8]
Proportions(%)
100
75
50
25
0
Pre-vaccine
Post-vaccine

Figures

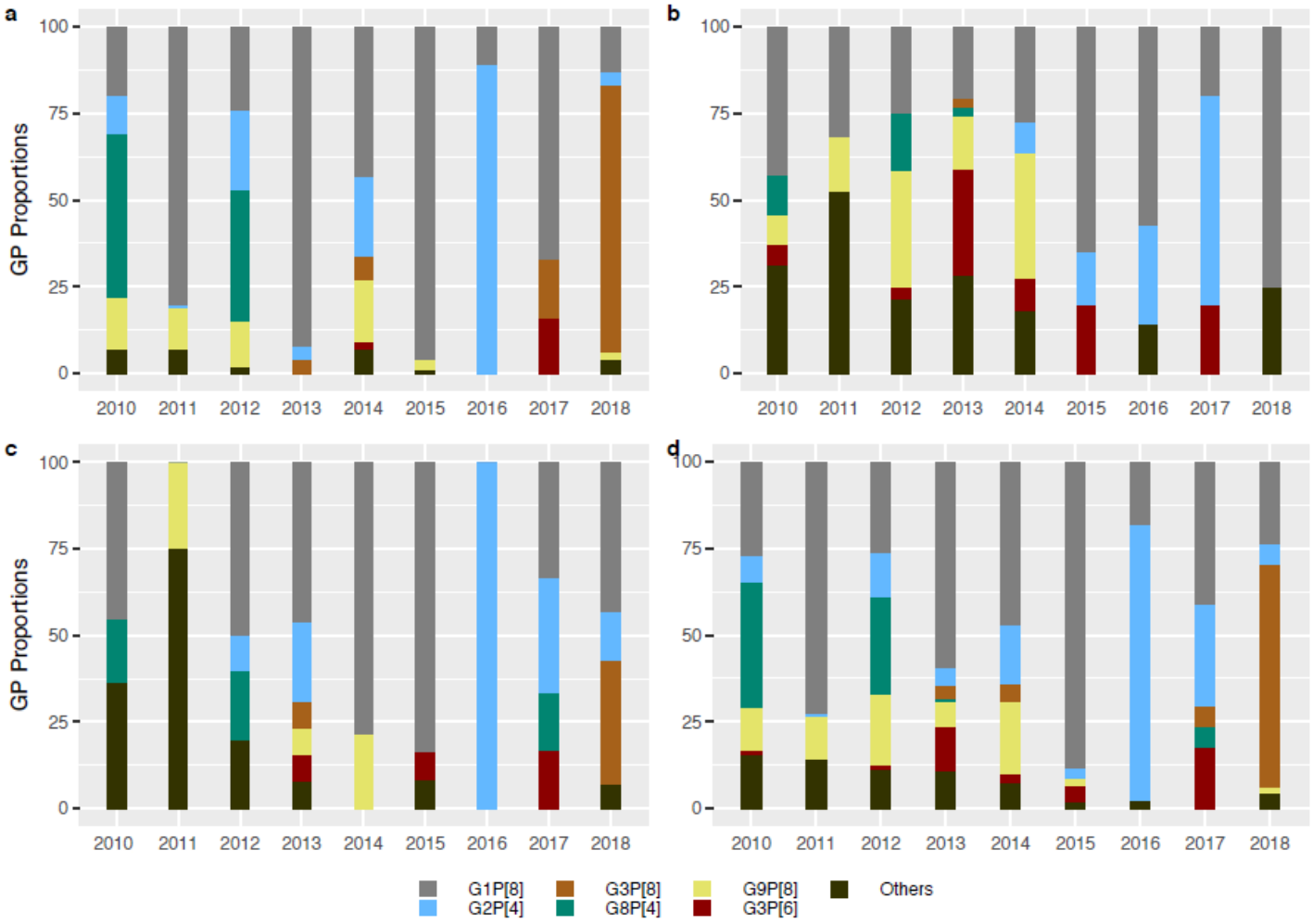


Figure 1

Temporal rotavirus genotype distribution in the three surveillance sites across Kenya; a –Kilifi County Hospital in Kilifi County, b – Tabitha Clinic in Kibera, Nairobi County, c – Siaya County (combined genotype data from Lwak Mission Hospital and Siaya Referral Hospital) and d - combination of the three Counties in Kenya between 2010 to 2018.

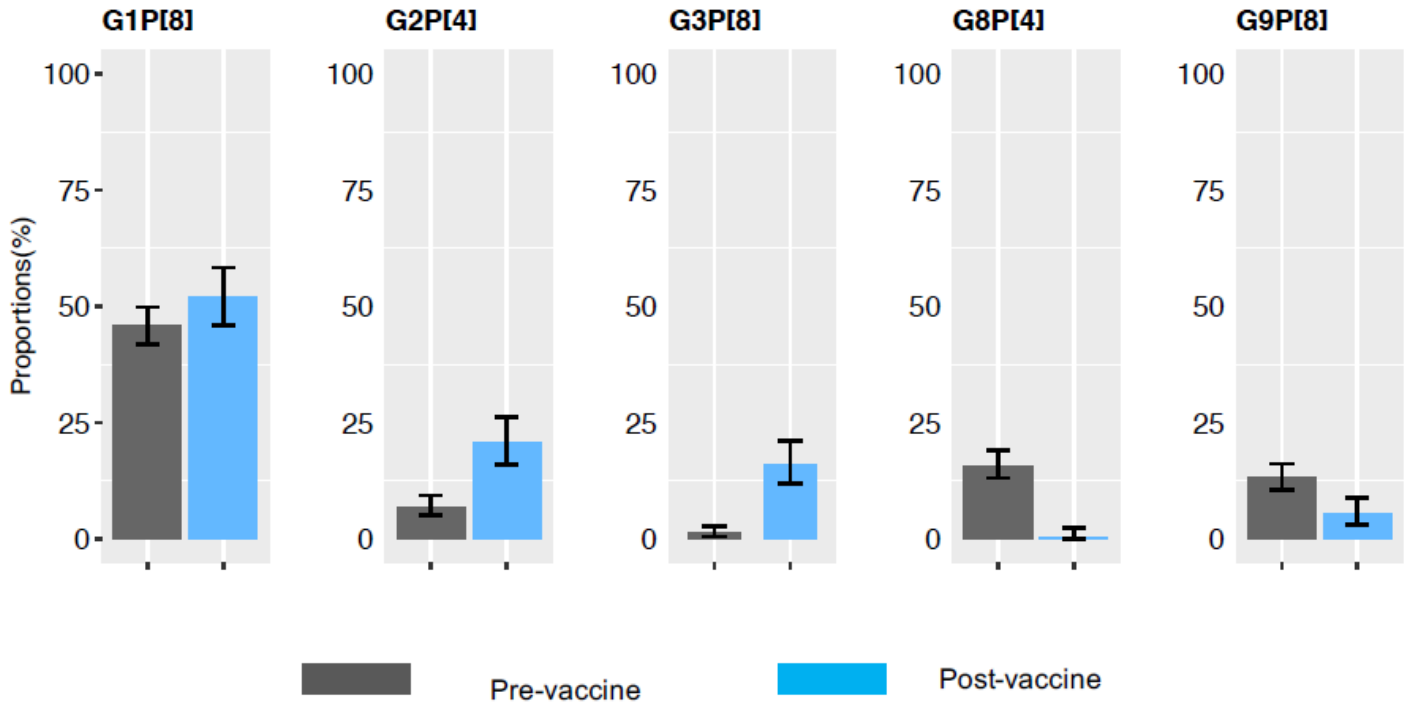


Figure 2

Comparison of prevalence of the dominant genotypes (G1P[8], G2P[4], G3P[8], G8P[4] and G9P[8]) at 95% confidence interval (CI) during the pre- (Jan 2010-Jun 2014) and post-(July 2014 – Dec 2018) vaccine introduction periods in Kenya. The predominant genotypes were selected based on their frequency as indicated in Table 3.

Supplementary Files

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- SupplementaryFigure1.pdf
- Supplementarytable3.pdf
- Supplementarytable2.pdf
- Supplementarytable1.pdf