Genomic characterization of uncommon human G3P[6] rotavirus strains that have emerged in Kenya after rotavirus vaccine introduction

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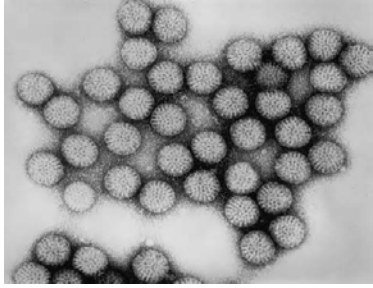
Introduction



- G3 is normally associated with P[8] genotype and a Wa-like genotype constellation

- P[6] is a relatively rare P genotype independent of G genotype combinations and is associated with both Wa-like and DS-1-like genetic backbones

- G3P[6] genotype has been identified in different parts of the world at a low frequency

- Whole genomes of a few G3P[6] strains have been characterized, providing evidence of a DS-1-like genotype constellation of recent G3P[6] strains

[Bwogi et al., 2017; Degiuseppe et al., 2014; Heylen et al., 2013, 2015; Ianiro et al., 2015; Ndze et al., 2014; Nyaga et al., 2018; Utsumi et al., 2018; Matthijnssens and Van Ranst, 2012]

Long-term impact of vaccine pressure on RVA strain distribution, evolution, and selection

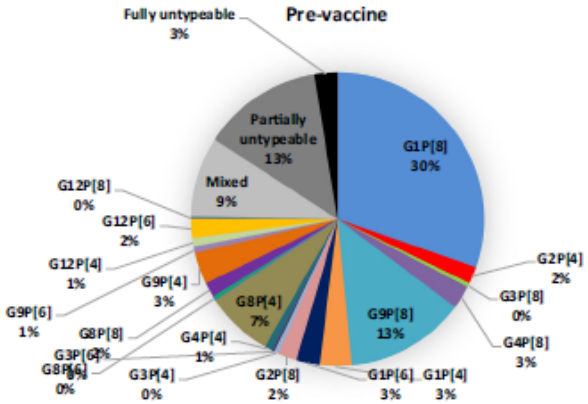
- Although changes in RVA genotype distribution have been observed following mass vaccination with RV1 and/or RV5 in several countries, it remains unclear if these changes are due to the vaccines

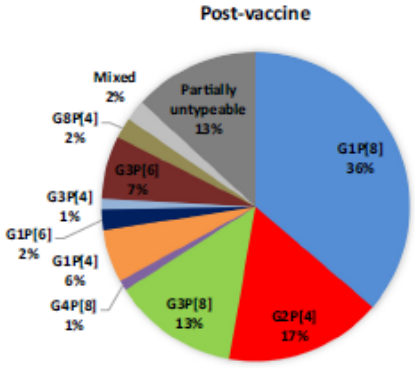
- While G3P[6] genotype has been detected at a considerable rate in some countries, only a few countries have reported a significant increase in the prevalence of this uncommon genotype after vaccine introduction

- However, the prevalence of different G/P genotype combinations often fluctuates from place to place and season to season in the absence of vaccination

[Armahetal.,2016; Bar-Zeev et al., 2015; Carvalho-Costa et al., 2011; Hull et al., 2011; Kirkwoodetal., 2011; ion (Abebe et al., 2018; da Silva Soaresetal.,2014;Heylenetal.,2013;Seherietal.,2017;Bányai et al., 2012; Iturriza-Gómara et al., 2011; Nokes et al., 2010; Zeller et al.,2010]

Study Rationale and Objectives





- Kenya introduced the RV1 rotavirus vaccine in July 2014
- Rotavirus hospitalizations declined by 48% (95% CI: 27-64%)
- There was increased detection of uncommon G3P[6] strains
- We analyzed the whole genomes of two of these post-vaccine G3P[6] strains
- Full genomes of three locally circulating human G8P[4] strains were also sequenced as references

[Wandera et al., 2017]

Materials and Methods

Virus strains

- Identified in stool samples from hospitalized children in Central Kenya, 2009-2016
- Study approved by KEMRI SERU (SSC No. 1323)
- Informed consent obtained from caregivers of participants

Illumina MiSeq sequencing

1 Library Preparation

RNA extraction using QiAamp Viral RNA Mini Kit

200 bp fragment library built using NEBNext Ultra Library Prep Kit

2 Cluster Generation

cDNA library purified using Agencourt AMPure XP magnetic beads

Quality and quantity of the purified cDNA library assessed

3 Sequencing

Sequencing performed on an Illumina MiSeq sequencer

4 Data Analysis

Data analysis performed using a CLC Genomics Workbench v8.0.1

RVA nucleotide sequences translated into amino acid sequences using GENETYX v11

Determination of RVA genotypes

- The genotype of each gene of the study strains was determined with the RotaC v2.0 automated genotyping tool

Phylogenetic analysis

- Multiple alignment of each gene performed using ClustalW
- Maximum-likelihood phylogenetic trees constructed using the Jukes-Cantor substitution model with MEGA7.0.26

Nucleotide sequence accession numbers

- The nucleotide sequence data for the study strains have been deposited in the DDBJ and EMBL/GenBank data libraries.

[Maes et al., 2009; Matthijnssens et al., 2011; Kumar et al., 2016]

Results and Discussion

- Complete or nearly complete nucleotide sequences of all 11 genes of the study strains could be determined

- The 11 gene segments of study strains were both assigned as
  G3-P[6]-I2-R2-C2-M2-A2-N2-T2-E2-H2

- Both G3P[6] strains possessed a complete DS-1-like genomic backbone

- The two study strains showed very high nucleotide sequence identities (99.4–99.9%) for all the 11 genes

- The three locally circulating G8P[4] strains were also shown to possess a complete DS-1-like genomic backbone

[Bwogi et al., 2017; Degiuseppe et al., 2014; Heylen et al., 2013, 2015; Ianiro et al., 2015; Ndze et al., 2014; Nyaga et al., 2018]

Genotype constellations of the 11 gene segments of Kenyan G3P[6] strains compared with other human rotavirus strains

Strain	Genotype										
	VP7	VP4	VP6	VP1	VP2	VP3	NSP1	NSP2	NSP3	NSP4	NSP5
RVA/Human-wt/KEN/KDH1951/2014/G3P[6]	G3	P[6]	I2	R2	C2	M2	A2	N2	T2	E2	H2
RVA/Human-wt/KEN/KDH1968/2014/G3P[6]	G3	P[6]	I2	R2	C2	M2	A2	N2	T2	E2	H2
RVA/Human-tc/USA/Wa/1974/G1P[8]	G1	P[8]	I1	R1	C1	M1	A1	N1	T1	E1	H1
RVA/Human-tc/USA/DS-1/1976/G2P[4]	G2	P[4]	I2	R2	C2	M2	A2	N2	T2	E2	H2
RVA/Human-tc/AUS/RV3/1993/G3P[2]	G3	P[2]	I1	R1	C1	M1	A1	N1	T1	E1	H1
RVA/Human-wt/HUN/ERN5523/2012/G3P[4]	G3	P[4]	I2	R2	C2	M2	A2	N2	T2	E2	H2
RVA/Human-tc/AUS/RV3/1977/G3P[6]	G3	P[6]	I1	R1	C1	M1	A1	N1	T1	E1	H1
RVA/Human-wt/ZAF/MRC-DPRU4992/1997/G3P[6]	G3	P[6]	I2	R2	C2	M2	A2	N2	T2	E2	H2
RVA/Human-wt/CHN/R946/2006/G3P[6]	G3	P[6]	I1	R1	C1	M1	A1	N1	T1	E1	H1
RVA/Human-tc/VNM/NT0001/2007/G3P[6]	G3	P[6]	I5	R1	C1	M1	A8	N1	T1	E1	H1
RVA/Human-wt/ETH/MRC-DPRU1873/2008/G3P[6]	G3	P[6]	I2	R2	C2	M2	A2	N2	T2	E2	H2
RVA/Human-wt/BEL/BE1322/2009/G3P[6]	G3	P[6]	I2	R2	C2	M2	A2	N2	T2	E2	H2
RVA/Human-wt/GHA/Ghan-007/2009/G3P[6]	G3	P[6]	I2	R2	C2	M2	A2	N2	T2	E2	H2
RVA/Human-wt/ITA/NA06/2009/G3P[6]	G3	P[6]	I2	R2	C2	M2	A2	N2	T2	E2	H2
RVA/Human-wt/TGO/MRC-DPRU5138/2010/G3P[6]	G3	P[6]	I2	R2	C2	M2	A2	N2	T2	E2	H2
RVA/Human-wt/ARG/Arg9448/2011/G3P[6]	G3	P[6]	I2	R2	C2	M2	A2	N2	T2	E2	H2
RVA/Human-wt/CMR/ES276/2011/G3P[6]	G3	P[6]	I2	R2	C2	M2	A2	N2	T2	E2	H2
RVA/Human-wt/CMR/MA155/2011/G3P[6]	G3	P[6]	I2	R2	C2	M2	A2	N2	T2	E2	H2
RVA/Human-wt/UGA/MUL-12-104/2012/G3P[6]	G3	P[6]^{a,b}	I2	R2^{a,b}	C2	M2	A2	N2^{a,b}	T2	E2	H2^{a,b}
RVA/Human-wt/UGA/MUL-12-117/2012/G3P[6]	G3	P[6]	I2^{a,b}	R2	C2	M2	A2^{a,b}	N2	T2^{a,b}	E2^{a,b}	H2^{a,b}
RVA/Human-wt/UGA/MUL-13-166/2013/G3P[6]	G3^{a,b}	P[6]	I2^{a,b}	R2	C2	M2^{a,b}	A2	N2	T2	E2	H2
RVA/Human-wt/IDN/SOEP128/2016/G3P[6]	G3	P[6]	I2	R2	C2	M2	A2	N2	T2	E2	H2
RVA/Human-tc/USA/P/1974/G3P[8]	G3	P[8]	I1	R1	C1	M1	A1	N1	T1	E1	H1
RVA/Human-wt/USA/DC23/1976/G3P[8]	G3	P[8]	I1	R1	C1	M1	A1	N1	T1	E1	H1
RVA/Human-tc/JPN/YO/1977/G3P[8]	G3	P[8]	I1	R1	C1	M1	A1	N1	T1	E1	H1
RVA/Human-wt/CHN/E2421/2010/G3P[8]	G3	P[8]	I1	R1	C1	M1	A1	N1	T1	E1	H1
RVA/Human-tc/JPN/AU-1/1982/G3P[9]	G3	P[9]	I3	R3	C3	M3	A3	N3	T3	E3	H3
RVA/Human-wt/KEN/KDH1111/2011/G8P[4]*	G8	P[4]	I2	R2	C2	M2	A2	N2	T2	E2	H2
RVA/Human-wt/KEN/KDH1255/2012/G8P[4]*	G8	P[4]	I2	R2	C2	M2	A2	N2	T2	E2	H2
RVA/Human-wt/KEN/KDH1629/2013/G8P[4]*	G8	P[4]	I2	R2	C2	M2	A2	N2	T2	E2	H2
RVA/Human-wt/UGA/MUL-13-427/2013/G8P[4]	G8	P[4]	I2	R2^{a,b}	C2^{a,b}	M2	A2	N2	T2	E2	H2
RVA/Human-wt/UGA/MUL-13-308/2013/G8P[6]	G8	P[6]	I2	R2	C2	M2	A2	N2	T2^{a,b}	E2	H2

Nucleotide sequence identity between Kenyan G3P[6] strains and close strain(s) in each gene segment

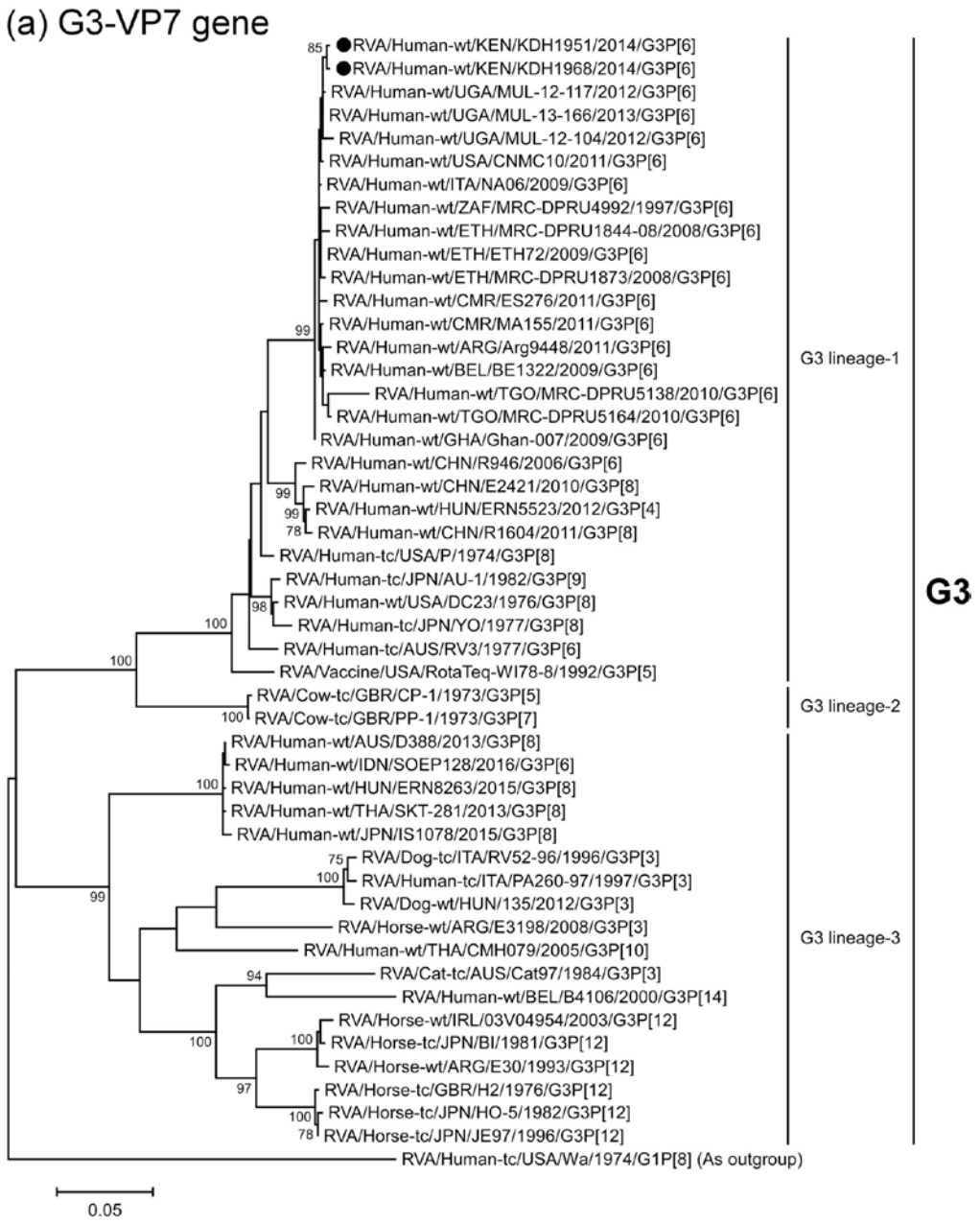
Gene	Strains which exhibit close nucleotide sequence identities in the BLAST database	% identity	Reference
VP7	RVA/Human-wt/UGA/MUL-13-166/2013/G3P[6]	99.5–99.6	Bwogi et al., 2017
VP4	RVA/Human-wt/UGA/MUL-12-104/2012/G3P[6]	99.5–99.6	Bwogi et al., 2017
VP6	RVA/Human-wt/UGA/MUL-12-117/2012/G3P[6]	99.6–99.9	Bwogi et al., 2017
	RVA/Human-wt/UGA/MUL-13-166/2013/G3P[6]	99.6–99.9	Bwogi et al., 2017
VP1	RVA/Human-wt/UGA/MUL-12-104/2012/G3P[6]	99.7	Bwogi et al., 2017
	RVA/Human-wt/UGA/MUL-13-427/2013/G8P[4]	99.7	Bwogi et al., 2017
VP2	RVA/Human-wt/UGA/MUL-13-427/2013/G8P[4]	99.7–99.8	Bwogi et al., 2017
VP3	RVA/Human-wt/UGA/MUL-13-166/2013/G3P[6]	99.7	Bwogi et al., 2017
NSP1	RVA/Human-wt/UGA/MUL-12-117/2012/G3P[6]	99.5–99.6	Bwogi et al., 2017
NSP2	RVA/Human-wt/UGA/MUL-12-104/2012/G3P[6]	99.7–99.8	Bwogi et al., 2017
NSP3	RVA/Human-wt/UGA/MUL-12-117/2012/G3P[6]	99.5–99.7	Bwogi et al., 2017
	RVA/Human-wt/UGA/MUL-13-308/2013/G8P[6]	99.5–99.7	Bwogi et al., 2017
NSP4	RVA/Human-wt/UGA/MUL-12-117/2012/G3P[6]	99.3–99.6	Bwogi et al., 2017
NSP5	RVA/Human-wt/UGA/MUL-12-104/2012/G3P[6]	99.4	Bwogi et al., 2017
	RVA/Human-wt/UGA/MUL-12-117/2012/G3P[6]	99.4	Bwogi et al., 2017

- Each of the 11 segments of Kenyan G3P[6] strains was found to be very closely related to that of human G3P[6], G8P[4], and/or G8P[6] strains isolated in 2012–2013 in the neighboring Uganda

Phylogenetic analysis of Kenyan G3P[6] Strains

- All 11 genes of Kenyan G3P[6] were very closely related to those of Ugandan G3P[6]

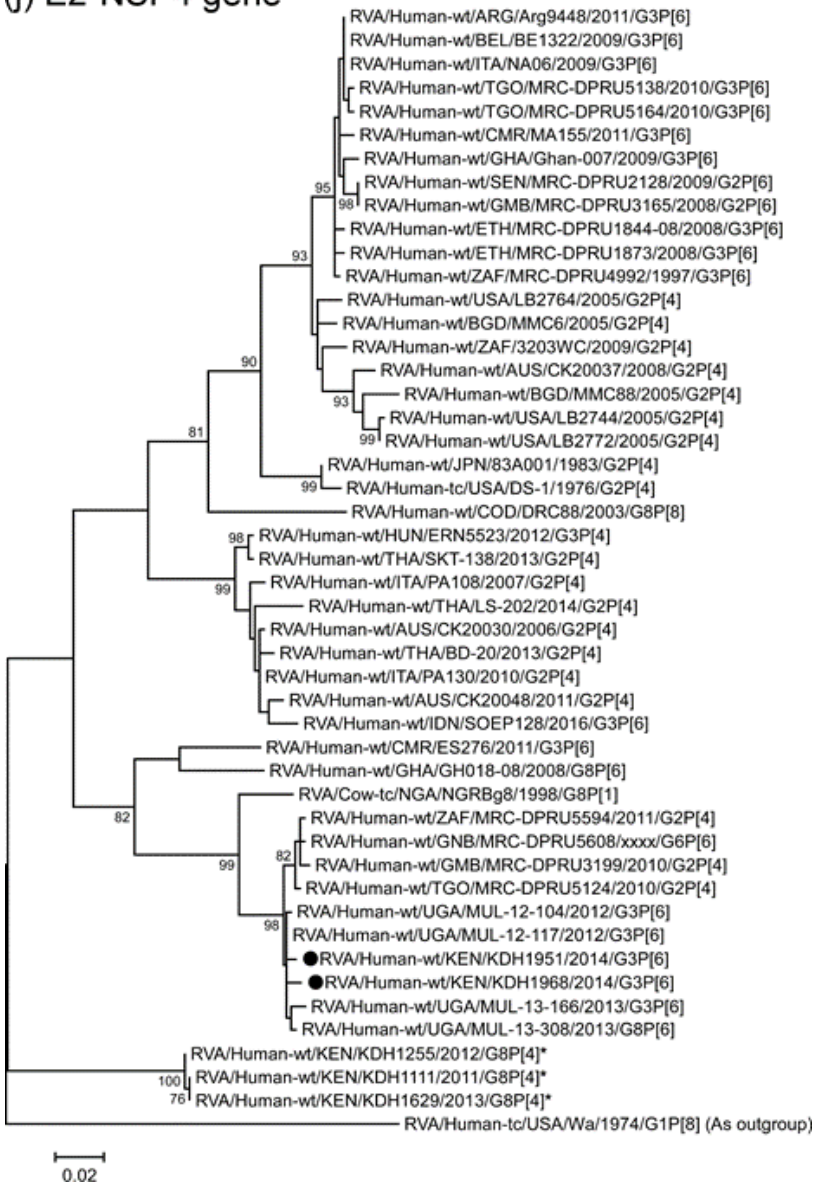
- VP7 genes of Kenyan G3P[6] strains formed a cluster with those of African and African-like G3P[6] strains in G3 lineage-1



Phylogenetic analysis of Kenya G3P[6] Strains

Fig. 1 (*continued*)

(j) E2-NSP4 gene



- Non-G/P-defining genes of the G3P[6] were scarcely related to those of locally circulating G8P[4] strains, indicating the distinct evolution of Kenyan DS-1-like strains

Antigenic epitope variation between the VP7 proteins of Kenyan G3P[6] strains and those of vaccine strains

VP7 epitope	7-1a														7-1b						7-2								
	87	91	94	96	97	98	99	100	104	123	125	129	130	291	201	211	212	213	238	242	143	145	146	147	148	190	217	221	264
G1 RV1	T	T	N	G	E	W	K	D	Q	S	V	V	D	K	Q	N	V	D	N	T	K	D	Q	N	L	S	M	N	G
G1 RV5	T	T	N	G	D	W	K	D	Q	S	V	V	D	K	Q	N	V	D	N	T	K	D	Q	S	L	S	M	N	G
G2 RV5	A	N	S	D	E	W	E	N	Q	D	T	M	N	K	Q	D	V	S	N	S	R	D	N	T	S	D	I	S	G
G3 RV5	T	T	N	N	S	W	K	D	Q	D	A	V	D	K	Q	D	A	N	K	D	K	D	A	T	L	S	E	A	G
G4 RV5	S	T	S	T	E	W	K	D	Q	N	L	I	D	K	Q	D	T	A	D	T	R	A	S	G	E	S	T	S	G
G6 RV5	V	N	A	T	E	W	K	D	Q	D	A	V	E	K	Q	N	P	D	N	A	K	D	S	T	Q	S	T	T	G
G3 KDH1951	T	T	N	N	S	W	K	N	Q	D	A	V	D	K	Q	D	T	N	N	N	K	D	V	T	L	S	E	D	G
G3 KDH1968	T	T	N	N	S	W	K	D	Q	D	A	V	D	K	Q	D	T	N	N	N	K	D	V	T	L	S	E	D	G
	*	*	*	*	*	*	*	*	*						*	*		*	*			*		*	*	*	*		*

Amino acid changes were observed in the 7-1a, 7-1b, and 7–2 regions, in comparison with the RV1 and the 7-1b and 7–2 regions in comparison with the RV5 vaccine strain

Antigenic epitope variation between the VP4 proteins of Kenyan G3P[6] strains and those of vaccine strains

VP8* epitope	8-1											8-2		8-3									8-4		
	100	146	148	150	188	190	192	193	194	195	196	180	183	113	114	115	116	125	131	132	133	135	87	88	89
P[8] RV1	D	S	S	N	S	S	A	N	L	N	N	E	R	N	P	V	D	S	S	N	D	N	N	T	N
P[8] RV5	D	S	S	N	S	N	A	N	L	N	D	E	R	N	P	V	D	N	R	N	D	D	N	T	N
P[5] RV5	G	T	I	G	R	I	T	N/K	Y	A	S	E	N	T	S	E	T	S	S	N	A	D	T	G	P
P[6] KDH1951	D	G	V	A	Y	S	S	N	L	S	E	E	H	T	N	Q	S	T	E	N	N	N	T	N	Q
P[6] KDH1968	D	G	V	A	Y	S	S	N	L	S	E	E	H	T	N	Q	S	T	E	N	N	N	T	N	Q
	*	*	*	*	*	*			*			*	*		*		*			*	*	*	*	*	*

VP5* epitope	5-1								5-2	5-3	5-4	5-5
	384	386	388	393	394	398	440	441	434	459	429	306
P[8] RV1	S	Y	S	A	W	N	L	R	E	N	S	L
P[8] RV5	R	H	S	A	W	N	L	R	E	N	S	L
P[5] RV5	D	S	A	Q	W	K	T	R	E	R	R	M
P[6] KDH1951	N	N	Q	A	W	S	L	R	E	H	S	L
P[6] KDH1968	N	N	Q	A	W	S	L	R	E	H	S	L
	*	*	*	*	*	*	*	*	*	*	*	*

Kenyan G3P[6] strains exhibited numerous amino acids changes when compared to the RV1 and RV5 strains in the 8–1, 8–2, 8–3, 8–4, 5–1, and 5–3 regions

Summary

- Kenyan G3P[6] strains possessed a complete DS-1-like genomic backbone, with very high nucleotide sequence identities
  - derivation of Kenyan G3P[6] strains from a common origin

- Phylogenetically, the Kenyan G3P[6] strains were most closely related to the Ugandan G3P[6] strains isolated in 2012-2013
  - derivation of East African G3P[6] strains from a common ancestor
  - limits attempts to attribute the changing prevalence of these strains to vaccine-induced selective pressure

- Kenyan G3P[6] strains were scarcely related to the locally circulating DS-1-like G8P[4] strains
  - distinct evolution of Kenyan DS-1-like strains

- Kenyan G3P[6] strains showed specific amino acid changes in the VP7 and VP4 antigenic epitopes compared with the introduced RV1 vaccine strain
  - potentially alter the antigenic properties of the study viruses

Conclusions

- The low genomic correlation between Kenyan DS-1-like G3P[6] strains and the emerging DS-1-like G8P[4] strains indicates the distinct evolution of these DS-1-like G3 strain

- The high number of amino acid differences in the antigenic epitopes of VP7 and VP4 proteins between the Kenyan G3P[6] and the vaccine strains could result in reduced antibody binding and thus reduced neutralization of these Kenyan G3P[6] strains

- Since these uncommon G3P[6] strains are fully heterotypic to the introduced vaccine strain regarding the genotype constellation, vaccine effectiveness against these G3P[6] strains needs to be closely monitored

Acknowledgements

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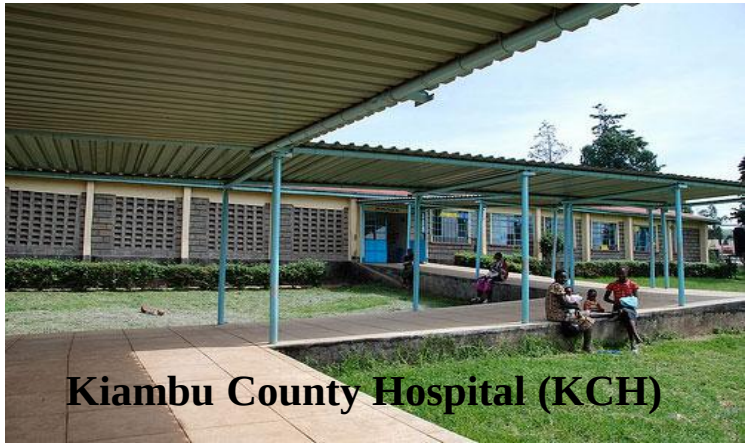

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