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Research paper

Genomic characterization of uncommon human G3P[6] rotavirus strains that have emerged in Kenya after rotavirus vaccine introduction, and pre-vaccine human G8P[4] rotavirus strains

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ABSTRACT

A monovalent rotavirus vaccine (RV1) was introduced to the national immunization program in Kenya in July 2014. There was increased detection of uncommon G3P[6] strains that coincided temporally with the timing of this vaccine introduction. Here, we sequenced and characterized the full genomes of two post-vaccine G3P[6] strains, RVA/Human-wt/KEN/KDH1951/2014/G3P[6] and RVA/Human-wt/KEN/KDH1968/2014/G3P[6], as representatives of these uncommon strains. On full-genomic analysis, both strains exhibited a DS-1-like genotype constellation: G3-P[6]-I2-R2-C2-M2-A2-N2-T2-E2-H2. Phylogenetic analysis revealed that all 11 genes of strains KDH1951 and KDH1968 were very closely related to those of human G3P[6] strains isolated in Uganda in 2012–2013, indicating the derivation of these G3P[6] strains from a common ancestor. Because the uncommon G3P[6] strains that emerged in Kenya are fully heterotypic as to the introduced vaccine strain regarding the genotype constellation, vaccine effectiveness against these G3P[6] strains needs to be closely monitored.

1. Introduction

Group A rotavirus (RVA) is the primary pathogen of severe diarrhea in young children globally. It is estimated that RVA is associated with approximately 215,000 deaths among children < 5 years annually (Tate et al., 2016). The majority of these deaths occurs in sub-Saharan Africa, where 121,000 children die (Tate et al., 2016). The RVA virion encapsidates an 11-segment genome of double-stranded (ds) RNA (Estes and Greenberg, 2013). Two outer capsid proteins, VP7 and VP4, contain neutralizing epitopes that define the serotypes of RVA. Based on the diversity of the VP7 and VP4 genes, G and P genotypes have been defined for RVA, respectively. To date, at least 36 G and 51 P genotypes have been assigned by the Rotavirus Classification Working Group (RCWG) (https://rega.kuleuven.be/cev/viralmetagenomics/virus-classification/rcwg). Among them, G1-G4, G9, and G12 in association with P[4], P[6], or P[8] are common genotypes associated with human RVA (HuRVA) infections worldwide (Estes and Greenberg, 2013). In addition, studies in Africa have revealed a high prevalence of G8 and P [6] genotypes in various combinations, indicating that both these genotypes should be considered common in Africa (Armah et al., 2010; Cunliffe et al., 1999; Steele and Ivanoff, 2003). Recently, a whole genome-based genotyping system was proposed based on the assignment of all 11 genes, where Gx-P[x]-Ix-Rx-Cx-Mx-Ax-Nx-Tx-Ex-Hx designates the genotypes of the VP7-VP4-VP6-VP1-VP2-VP3-NSP1-NSP2-NSP3-NSP4-NSP5 genes (Matthijnssens et al., 2008). The majority of HuRVAs have genes similar in sequence to those of prototype human strain Wa (genogroup 1 genes) or DS-1 (genogroup 2 genes) (Heiman et al., 2008). The Wa-like strains are characterized by non-G/P genotypes (I1-R1-C1-M1-A1-N1-T1-E1-H1), and tend to possess G/P genotypes G1P[8], G3P[8], G4P[8], G9P[8], and G12P[8]. In contrast, the DS-1-like strains are characterized by non-G/P genotypes (I2-R2-C2-M2-A2-N2-T2-E2-H2), and tend to have G/P genotype G2P[4] (Matthijnssens et al., 2008). G3 is considered one of the most frequent G genotypes worldwide, and is normally associated with the P[8] genotype with a Wa-like genotype constellation (Degiuseppe et al., 2014; Santos and Hoshino, 2005). P[6] is a relatively rare P genotype

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independent of G genotype combinations, but little is known about the genetic composition of P[6] strains (Heylen et al., 2016; Nyaga et al., 2018). In Africa, the P[6] genotype is highly prevalent in combination with G1-G4, G5, G6, G8, G9, and G12, suggesting that P[6] should be considered a common P genotype on the African continent (Armah et al., 2010; Bányai et al., 2012; Ouermi et al., 2017; Seheri et al., 2014; Steele and Ivanoff, 2003). In addition, the P[6] genotype is known to be associated with both Wa-like and DS-1-like genetic backbones (Matthijnssens and Van Ranst, 2012). The unconventional G3P[6] genotype has been identified in different parts of the world at a low frequency (Bourdett-Stanziola et al., 2011; Cunliffe et al., 2010; Ouermi et al., 2017). To date, the whole genomes of a few G3P[6] strains have been fully sequenced and 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), however, little is known about the genetic composition of G3P[6] strains.

Hospital-based HuRVA strain surveillance has been conducted in Kiambu County, in the central region of Kenya, since 2009 (Komoto et al., 2014; Shah et al., 2017; Wandera et al., 2017a and Wandera et al., 2017b), and after the introduction of a monovalent RVA vaccine (RV1 (G1P[8]) (GlaxoSmithKline)) to the national immunization program in Kenya in July 2014, there was increased detection of uncommon G3P[6] strains that coincided temporally with the timing of the vaccine introduction (Wandera et al., 2017b). In brief, G1P[8] was the most prevalent genotype detected in Central Kenya in both the pre-vaccine and post-vaccine periods at 30% and 36%, respectively. Interestingly, the G1P[8] genotype predominated only in the first year of vaccine introduction and this strain was replaced by the G2P[4] genotype during the second year. On the other hand, G9P[8], which had been the second most common genotype in this area in the pre-vaccine period at 13%, was not detected following the vaccine introduction and the strain was replaced by G2P[4], which circulated at 17%. Similarly, G8P[4] was detected at a lower frequency (2%) after vaccine introduction compared to a relatively high frequency of 7% before vaccine introduction. The G3P[8] genotype, which had never been detected in this area before vaccine introduction, became the third most dominant genotype at 13% following the vaccine introduction. Of note is that the G3P[6] genotype, whose prevalence was negligible in the pre-vaccine period (0.2%), was detected at a considerably higher frequency (7%) in the post-vaccine period. Due to their unconventional G/P genotype, these post-vaccine G3P[6] strains might be emerging RVA strains that have escaped the immune protection by the introduced RV1 vaccine. Therefore, in this study, we analyzed the whole genomes of two G3P[6] strains, KDH1951 and KDH1968, that emerged in Kenya just after the vaccine introduction to characterize the post-vaccine G3P[6] strains. Furthermore, the full genomes of three locally circulating human G8P[4] strains possessing a DS-1-like genotype constellation, KDH1111, KDH1255, and KDH1629, isolated just before the vaccine introduction (2011 − 2013), were also sequenced as references to compare the origins of these Kenyan DS-1-like RVA strains.

2. Materials and methods

2.1. Virus strain

Strains KDH1951 and KDH1968 were identified in stool samples from hospitalized children in Kiambu County, Central Kenya during the HuRVA strain surveillance program in this area in 2009–2016, which involved a total of 2204 RVA-positive stool samples (Wandera et al., 2017b). This study was approved by the Kenya Medical Research Institute/National Ethical Review Committee (Ref. no. 1323). Informed written consent was sought from the parents or guardians of all the participating children. Both strains were isolated shortly after the RV1 vaccine was introduced in Kenya in July 2014. Strains KDH1951 and KDH1968 were isolated from a 5-month-old male in July 2014 and a 6-month-old male in August 2014, respectively. Both children had been admitted to Kiambu County Referral Hospital, the main referral hospital in Kiambu County in the central region of Kenya, for severe acute gastroenteritis characterized by watery or loose non-bloody diarrhea, vomiting and high fever. Both children were discharged from the hospital following a good response to rehydration therapy. Neither of the two children had received RVA vaccination.

2.2. Viral dsRNA extraction, cDNA library construction, and Illumina MiSeq sequencing

RVA dsRNAs were extracted from fecal suspensions using a QIAamp Viral RNA Mini Kit (Qiagen). The extracted dsRNAs were subjected to Illumina MiSeq sequencing as described previously (Dennis et al., 2014; Komoto et al., 2016a). Briefly, a 200 bp fragment library ligated with bar-coded adapters was built using an NEBNext Ultra RNA Library Prep Kit for Illumina v1.2 (New England Biolabs) according to the manufacturer's instructions. The cDNA library was purified using Agencourt AMPure XP magnetic beads (Beckman Coulter). After assessing the quality and quantity of the purified cDNA library, nucleotide sequencing was performed on an Illumina MiSeq sequencer (Illumina) using a MiSeq Reagent Kit v2 (Illumina) to generate 151 paired-end reads. Sequence reads were trimmed to remove the adapter, primer, and low-quality sequences, using a CLC Genomics Workbench v8.0.1 (CLC Bio). The parameter settings for the quality trimming were as follows: trim using quality scores, limit = 0.08; trim ambiguous nucleotides, maximum number of ambiguities = 4; and filter on length, discard reads below length = 15. Data analysis was performed using a CLC Genomics Workbench v8.0.1. Contigs were assembled from the obtained sequence reads (trimmed) by *de novo* assembly. Using the assembled contigs as query sequences, the Basic Local Alignment Search Tool (BLAST) non-redundant nucleotide database was searched to determine which contig represents the full-length nucleotide sequence for each segment of the study strains. To further improve the contigs, the sequence reads of each segment were mapped back to the assembled contigs. The RVA nucleotide sequences were translated into amino acid sequences using GENETYX v11 (GENETYX).

2.3. Determination of RVA genotypes

The genotype of each gene of the study strains was determined with the RotaC v2.0 automated genotyping tool (http://rotac.regatools.be/) (Maes et al., 2009) according to the guidelines proposed by the RCWG (Matthijnssens et al., 2011).

2.4. Phylogenetic analysis

Multiple alignment of each gene was performed using ClustalW. Maximum-likelihood phylogenetic trees were constructed using the Jukes-Cantor substitution model with MEGA7.0.26 (Kumar et al., 2016). The best substitution models for the 11 genes were chosen based on the corrected Akaike information criterion value as implemented in MEGA7.0.26. The reliability of the branching order was estimated from 1000 bootstrap replicates.

2.5. Nucleotide sequence accession numbers

The nucleotide sequence data for the study strains have been deposited in the DDBJ and EMBL/GenBank data libraries. The accession numbers for the nucleotide sequences of the VP1-VP4, VP6, VP7, and NSP1-NSP5 genes of strains KDH1951, KDH1968, KDH1111, KDH1255, and KDH1629 are LC406786-LC406796, LC406797-LC406807, LC406808-LC406818, LC406819-LC406829, and LC406830-LC406840, respectively.

Table 1
Genotype natures of the 11 gene segments of two Kenyan G3P[6] strains, KDH1951 and KDH1968, compared with those of selected human strains with known genomic constellations.

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-wt/AUS/D388/2013/G3P[8]	G3	P[8]	I2	R2	C2	M2	A2	N2	T2	E2	H2
RVA/Human-wt/THA/SKT-281/2013/G3P[8]	G3	P[8]	I2	R2	C2	M2	A2	N2	T2	E2	H2
RVA/Human-wt/HUN/ERN8263/2015/G3P[8]	G3	P[8]	I2	R2	C2	M2	A2	N2	T2	E2	H2
RVA/Human-wt/JPN/IS1078/2015/G3P[8]	G3	P[8]	I2	R2	C2	M2	A2	N2	T2	E2	H2
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

3. Results and Discussion

3.1. Nucleotide sequencing and whole genotype constellation

Illumina MiSeq sequencing yielded 7.6×10^5 reads (~142 bp average length), 6.4×10^5 reads (~143 bp average length), 9.7×10^5 reads (~141 bp average length), 18.9×10^5 reads (~144 bp average length), and 9.8×10^5 reads (~144 bp average length) for strains KDH1951, KDH1968, KDH1111, KDH1255, and KDH1629, respectively. Seventy% of sequences were ≥ Q30 (99.9% accuracy of base calling at a particular sequence position). Complete or nearly complete nucleotide sequences of all 11 genes of the study strains could be determined. The lengths of the nucleotide and amino acid sequences of the 11 segments of the study strains, with related sequence read data, are summarized in Supplementary Table S1.

The 11 gene segments of strains KDH1951 and KDH1968 were both assigned as G3-P[6]-I2-R2-C2-M2-A2-N2-T2-E2-H2 (Table 1). The two strains were ascertained to possess the G3P[6] genotype, as suggested on RT-PCR-based G/P genotyping (Wandera et al., 2017b). Strains KDH1951 and KDH1968 were named RVA/Human-wt/KEN/KDH1951/2014/G3P[6] and RVA/Human-wt/KEN/KDH1968/2014/G3P[6], respectively, according to the guidelines for the uniformity of RVAs nomenclature proposed by the RCWG (Matthijnssens et al., 2011). Comparison of the complete genotype constellations of these two strains with those of other G3 and non-G3 HuRVAs is shown in Table 1. Both G3P[6] strains possessed a complete DS-1-like genomic backbone, which is commonly found in African and African-like 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). Furthermore, the two study strains showed very high nucleotide sequence identities (99.4–99.9%) for all the 11 genes (data not shown), indicating the derivation of these G3P[6] strains from a common origin. In contrast, the 11 genes of three locally circulating strains, KDH1111, KDH1255, and KDH1629, were all assigned as G8-P[4]-I2-R2-C2-M2-A2-N2-T2-E2-H2 (Table 1), and named RVA/Human-wt/KEN/KDH1111/2011/G8P[4], RVA/Human-wt/KEN/KDH1255/2012/G8P[4], and RVA/Human-wt/KEN/KDH1629/2013/G8P[4], respectively. These three G8P[4] strains were also shown to possess a complete DS-1-like genomic backbone.

3.2. Phylogenetic analysis

Since phylogenetic analysis of RVA nucleotide sequences offers valuable information to elucidate possible genetic linkages among RVA segments (Ghosh and Kobayashi, 2011; Matthijnssens et al., 2008), strains KDH1951 and KDH1968 were further characterized by constructing phylogenetic trees using the full-length gene sequences for each of the 11 segments, (Fig. 1a-k). The nucleotide sequence identities between the two study strains and close strain(s) in each segment are shown in Table 3. Phylogenetically, each of the 11 segments of strains KDH1951 and KDH1968 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, which had also not introduced an RVA vaccine at that time (Fig. 1a-k). Moreover, all 11 genes of strains KDH1951 and KDH1968 were very closely related to those of Ugandan G3P[6] strains, indicating the derivation of these East African G3P[6] strains from a common ancestor. Notably, the VP7 genes of strains KDH1951 and KDH1968 formed a cluster with those of African and African-like G3P[6] strains in G3 lineage-1, away from the clusters comprising the non-P[6] G3 strains in G3 lineages-1/2/3 (Fig. 1a). On the other hand, non-G/P-defining genes of the two study strains were scarcely related to those of locally circulating G8P[4] strains (Fig. 1c-k), indicating the distinct evolution of Kenyan DS-1-like strains having the G3P[6] and G8P[4] genotypes.

3.3. Antigenic epitope comparison of the VP7 proteins of Kenyan G3P[6] strains and those of vaccine strains

We performed amino acid sequence alignments to compare protein composition between the VP7 proteins of the study strains, KDH1951 and KDH1968, and those of currently licensed RVA vaccine strains (RV1 and RV5 (Merck)). We identified seven residues in the 326-amino acid VP7 protein that differ between both strains KDH1951 and KDH1968, and the VP7 components of both RV1 and RV5 (data not shown). A total of 58 and 8 residues differ between the VP7 proteins of Kenyan G3P[6] strains, and the VP7 protein(s) of RV1 and RV5, respectively (data not shown). To gain an insight into the antigenic properties of Kenyan G3P[6] strains, we identified VP7 amino acids that reside in predicted antigenic epitopes of RV1 and RV5 (Table 2). VP7 antigenic epitopes have been predicted by mapping antibody neutralization-escape mutants, from structural information from VP7-antibody complexes, and by identifying surface-exposed amino acids that vary among G genotypes (McDonald et al., 2009; Aoki et al., 2009; Taniguchi et al., 1988). Amino acid changes were observed in the 7-1a, 7-1b, and 7–2 regions, and the 7-1b and 7–2 regions in comparison with the RV1 and RV5 vaccine strain, respectively (Table 2). Thus, it was found that Kenyan G3P[6] strains showed specific amino acid changes in the 7-1a, 7-1b, and 7–2 regions compared with the introduced RV1 strain, and potentially alter the antigenic properties of the study viruses.

3.4. Antigenic epitope comparison of the VP4 proteins of Kenyan G3P[6] strains and those of vaccine strains

Through amino acid sequence alignments, we identified 104 residues in the 775/776-amino acid VP4 protein that differ between both KDH1951 and KDH1968, and the VP4 components of both RV1 and RV5 (data not shown). A total of 160 and 119 residues differ between the VP4 protein of Kenyan G3P[6] strains, and the VP4 protein(s) of RV1 and RV5, respectively (data not shown). VP4 antigenic epitopes have been predicted by mapping neutralizing escape mutants and identifying surface-exposed amino acids in VP4 trypsin cleavage products, VP8* and VP5*, that vary among P genotypes (Dormitzer et al., 2002, 2004; McDonald et al., 2009; Taniguchi et al., 1987). Kenyan G3P[6] strains carried 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 (Table 2). It was observed that Kenyan G3P[6] strains exhibited amino acid changes in the 8–1, 8–2, 8–3, 8–4, 5–1, and 5–3 regions compared with the introduced RV1 strain, suggesting altered antigenic properties of the study viruses. Thus, the high number of consistent amino acid differences in the antigenic epitopes of VP7 and VP4 proteins between the Kenyan G3P[6] strains and those of the RV1 and RV5 strains could result in reduced antibody binding and thus reduced neutralization of these Kenyan G3P[6] strains.

The long-term impact of vaccine pressure on RVA strain distribution, evolution, and selection remains unclear. Although changes in RVA genotype distribution have been observed following mass vaccination with RV1 and/or RV5 in several countries (Armah et al., 2016; Bar-Zeev et al., 2015; Carvalho-Costa et al., 2011; Hull et al., 2011; Kirkwood et al., 2011), it remains unclear if these changes are due to the vaccines. In this study area, usual global G genotypes G2, G3, and G4 circulated at remarkably low frequencies during the pre-vaccine period (Wandera et al., 2017b). However, following the RV1 vaccine introduction, we observed a sudden increase in the prevalence of G3P[6] strains (Wandera et al., 2017a). While the G3P[6] genotype has been detected at a considerable rate in some countries (Abebe et al., 2014; Lartey et al., 2018; Ndombo et al., 2017; Seheri et al., 2017), only a few countries such as Belgium, Brazil, and Ethiopia have reported a significant increase in the prevalence of this uncommon genotype after nationwide RVA vaccine introduction (Abebe et al., 2018; da Silva Soares et al., 2014; Heylen et al., 2013; Seheri et al., 2017). However, the prevalence of different G/P genotype combinations often fluctuates

from place to place and season to season in the absence of vaccination (Bányai et al., 2012; Iturriza-Gómara et al., 2011; Nokes et al., 2010; Zeller et al., 2010), making it difficult to determine if these changes in G3P[6] prevalence are due to the vaccines.

Nonetheless, due to the unconventional G/P genotype combination of G3P[6] strains, we analyzed the whole genomes of two of these post-vaccine G3P[6] strains, KDH1951 and KDH1968. Both strains were found to possess a complete DS-1-like genomic backbone and to be very closely related to Ugandan G3P[6] strains phylogenetically. However, it should be noted that several G3P[6] strains having a complete DS-1-like genotype constellation have also been reported recently from different locations on the African continent other than Kenya (Cameroon,

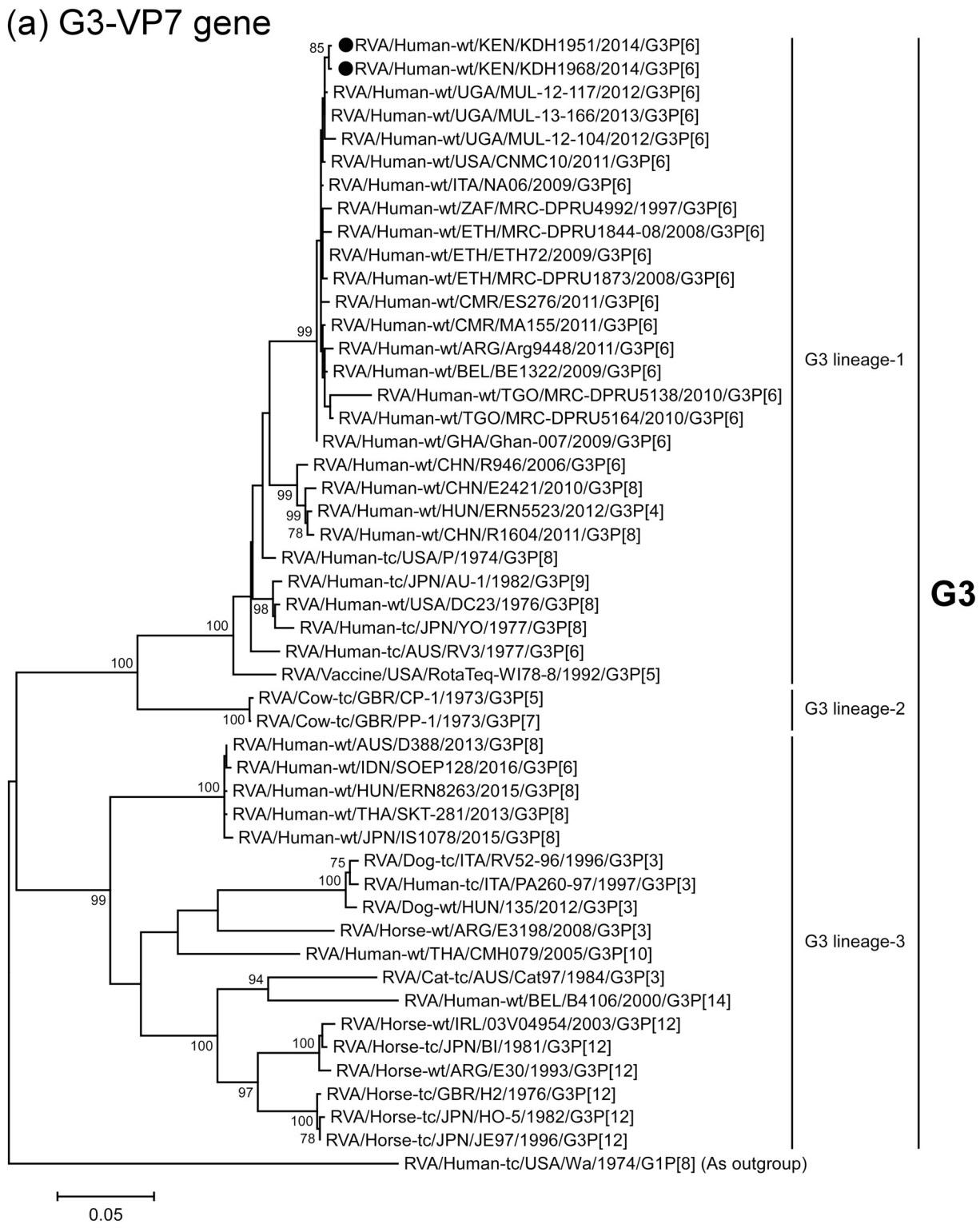


Fig. 1. Phylogenetic trees constructed from the nucleotide sequences of the VP7 (a), VP4 (b), VP6 (c), VP1 (d), VP2 (e), VP3 (f), NSP1 (g), NSP2 (h), NSP3 (i), NSP4 (j), and NSP5 (k) genes of strains KDH1951 and KDH1968, and representative RVA strains. In all the trees, the positions of strains KDH1951 and KDH1968 are shown by filled circles. Asterisks indicate locally circulating strains KDH1111, KDH1255, and KDH1629. Bootstrap values of < 75% are not shown. Scale bars, 0.02 (c, e, and h–k) or 0.05 (a, b, d, f, and g) substitutions per nucleotide.

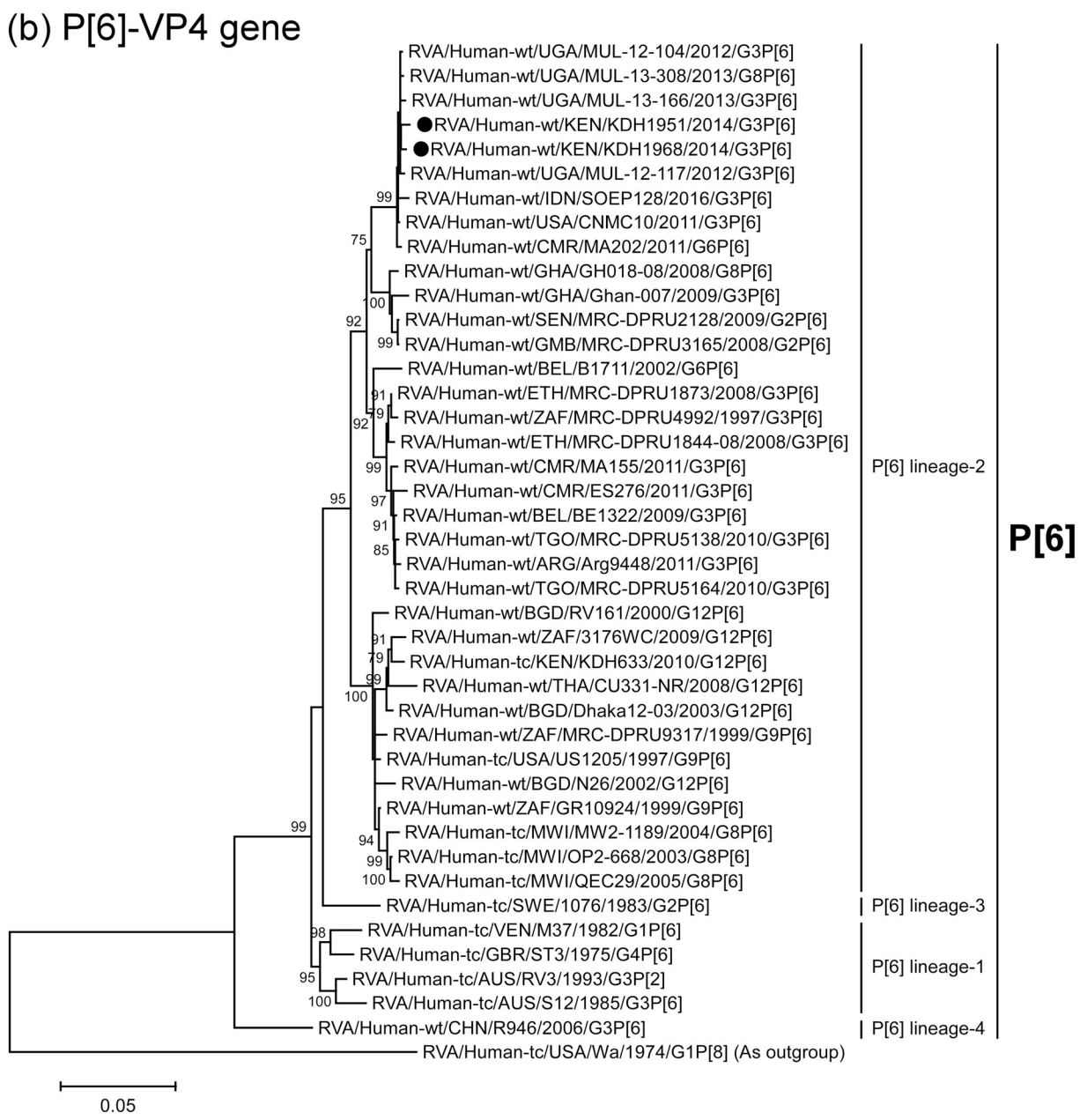
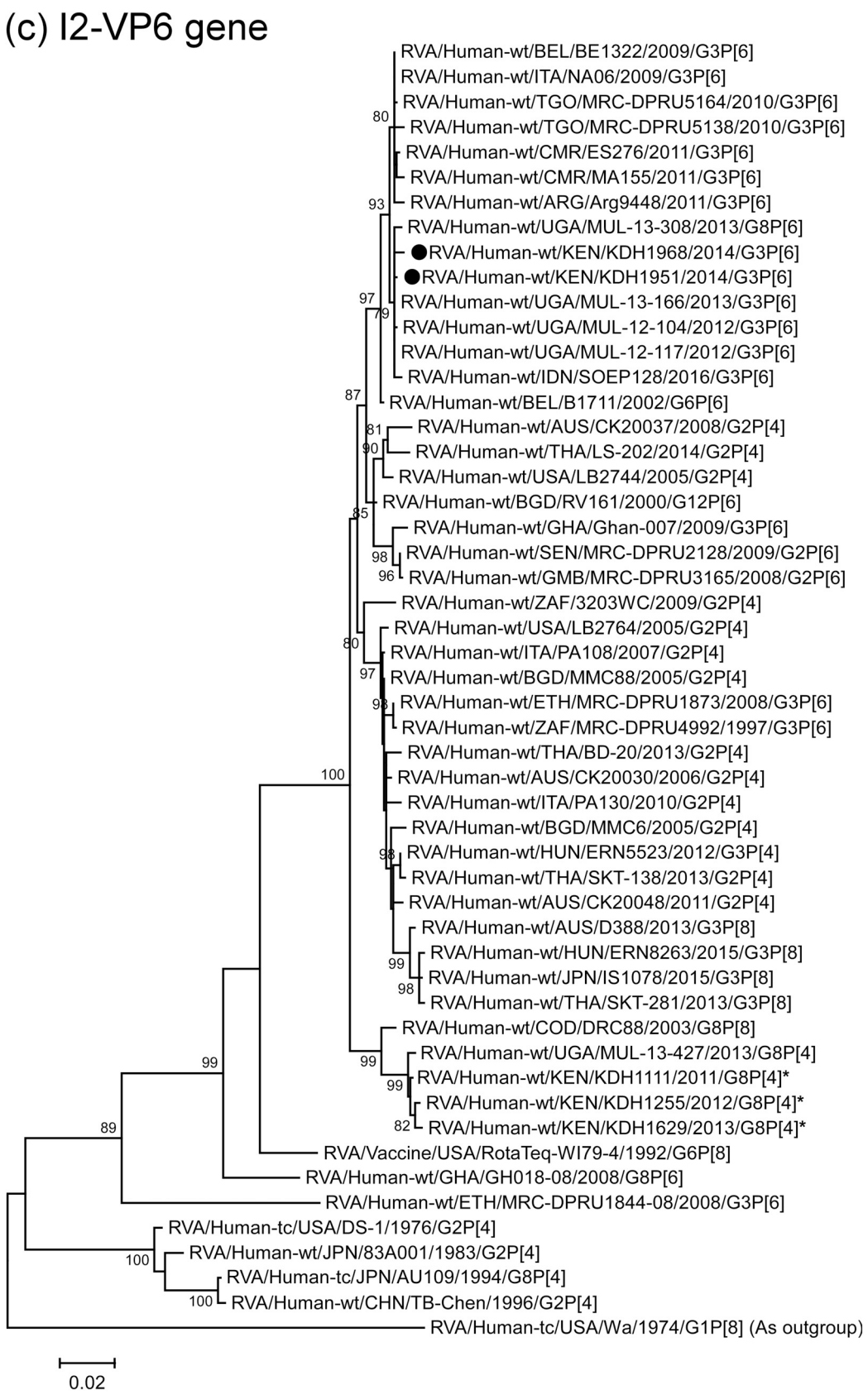


Fig. 1. (*continued*)

Ethiopia, Ghana, South Africa, Togo, and Uganda) (Abebe et al., 2014; Bwogi et al., 2017; Heylen et al., 2015; Ndze et al., 2014; Nyaga et al., 2018; DDBJ and EMBL/GenBank data libraries) and Europe (Italy) (Ianiro et al., 2015) before RVA vaccine introduction. Thus, these G3P [6] strains associated with a DS-1-like genomic backbone might be under ongoing expansion, especially on the African continent. A few studies have been carried out for complete genomic characterization of G3P[6] strains following vaccine introduction. For example, in Belgium, full-genomic analysis of the G3P[6] strains detected in the post-vaccine period exhibited a full DS-1-like genotype constellation (Heylen et al., 2013). Of note is that the Kenyan G3P[6] strains were most closely related to the Ugandan G3P[6] strains that were isolated in 2012–2013, a time when the neighboring country, Uganda, had not yet introduced RVA vaccine into her nationwide immunization program (Bwogi et al., 2017).

DS-1-like G3P[8] strains with an equine-like G3 genotype (G3-P[8]-I2-R2-C2-M2-A2-N2-T2-E2-H2) deserve mention because these unusual strains have emerged on different continents in a very short time period (Arana et al., 2015; Cowley et al., 2016; Dóró et al., 2016; Guerra et al., 2016; Kikuchi et al., 2018; Komoto et al., 2016b, 2017; Perkins et al., 2017; Utsumi et al., 2018). Moreover, DS-1-like G3P[8] strains have emerged as major strains in some countries on them (Arana et al., 2015; Cowley et al., 2016; Utsumi et al., 2018). However, the low genomic correlation between Kenyan DS-1-like G3P[6] strains and the emerging DS-1-like G3[8] strains indicates the distinct evolution of these DS-1-like G3 strains (Fig. 1a–k).

Thus, the above observations put together, and in the absence of data on strain-specific vaccine effectiveness in this setting and the limited post-vaccine introduction period that did not allow for monitoring for any sustained predominance of the G3P[6] strains, it is difficult to conclusively attribute the changing prevalence of these strains to vaccine-induced selective pressure. It is likely that the rising G3P[6] genotype prevalence following vaccine introduction in Kenya is due to temporal fluctuations. Nevertheless, further whole genome-based studies are needed to better understand the evolutionary dynamics of these G3P[6] strains.



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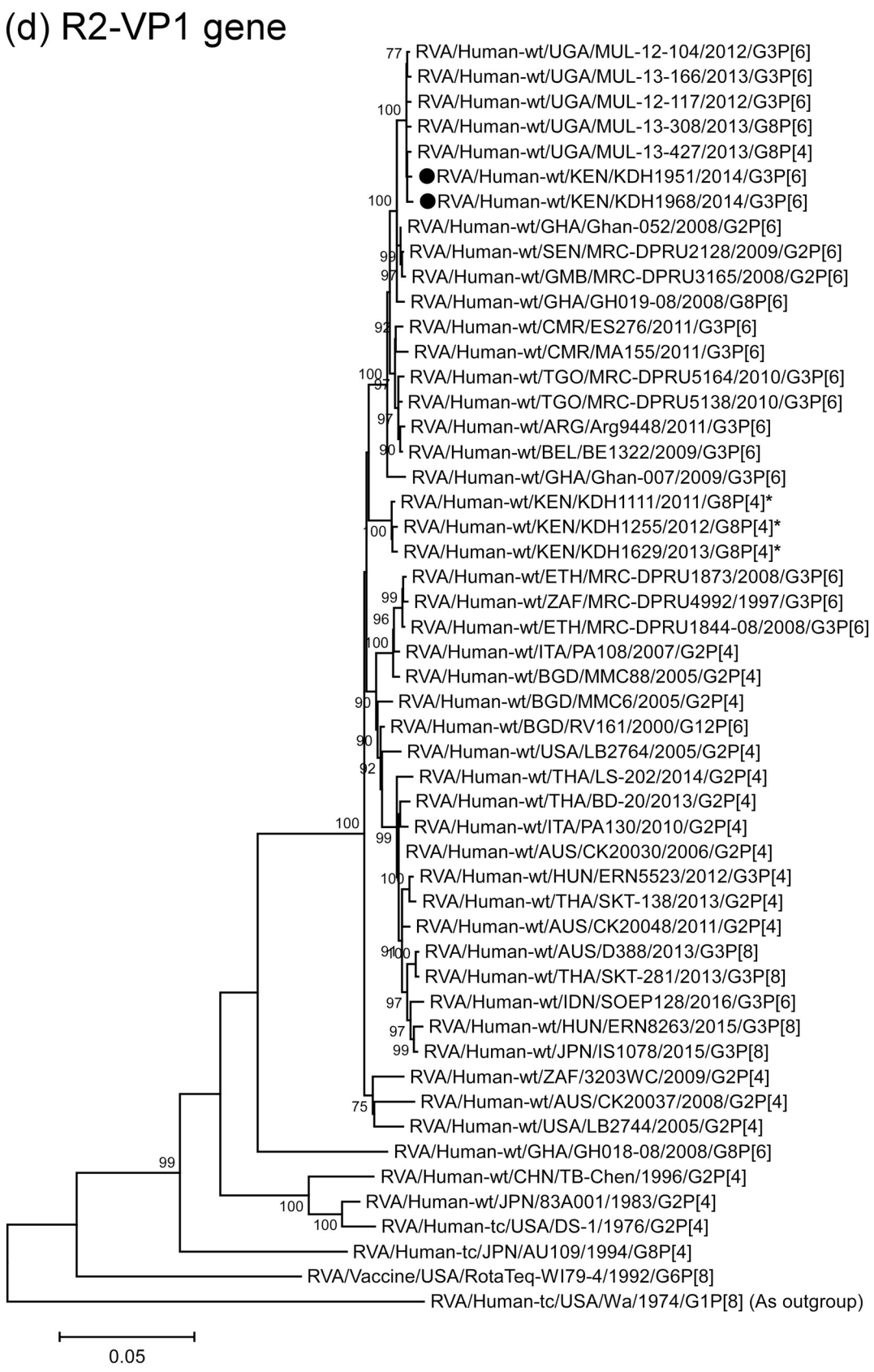

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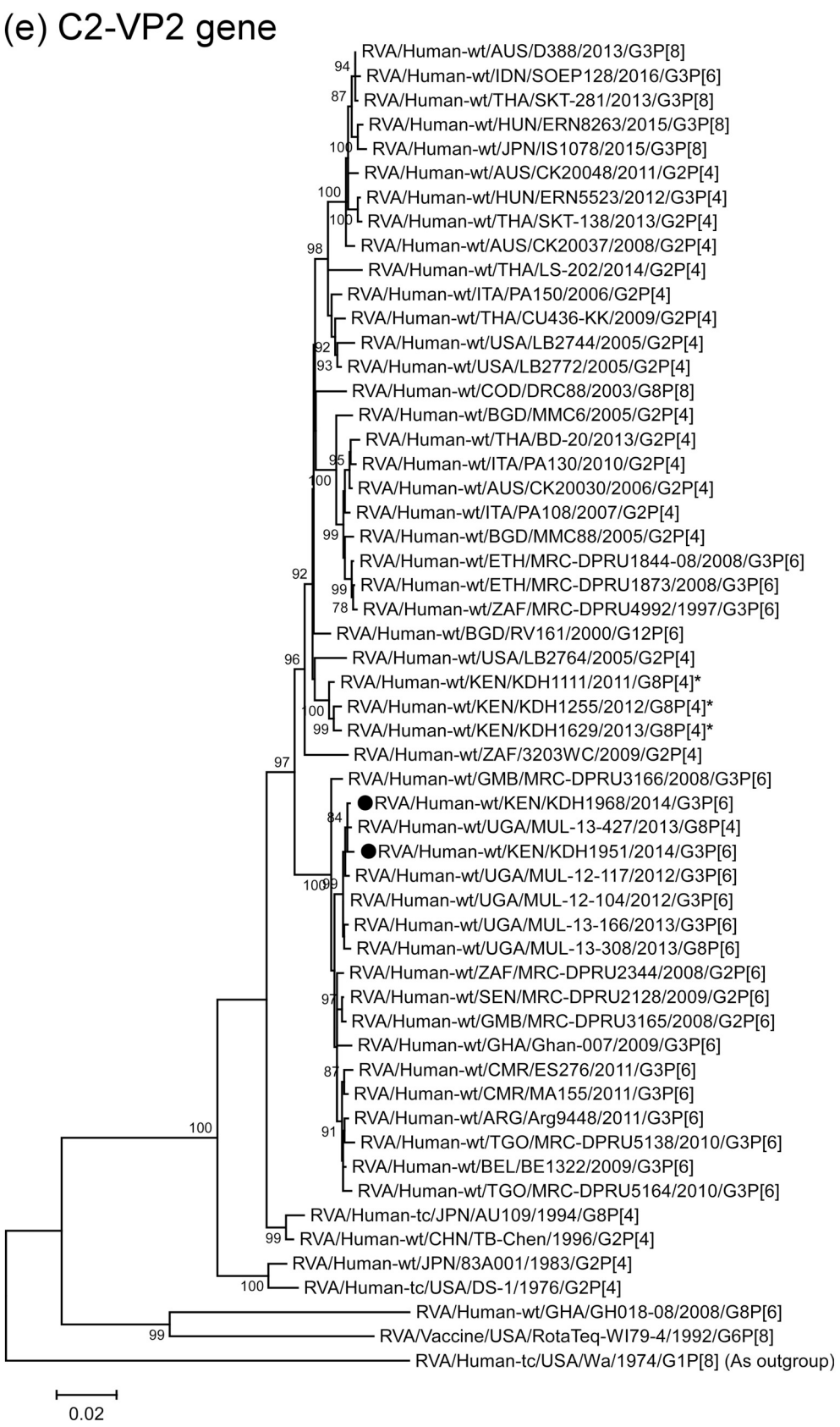

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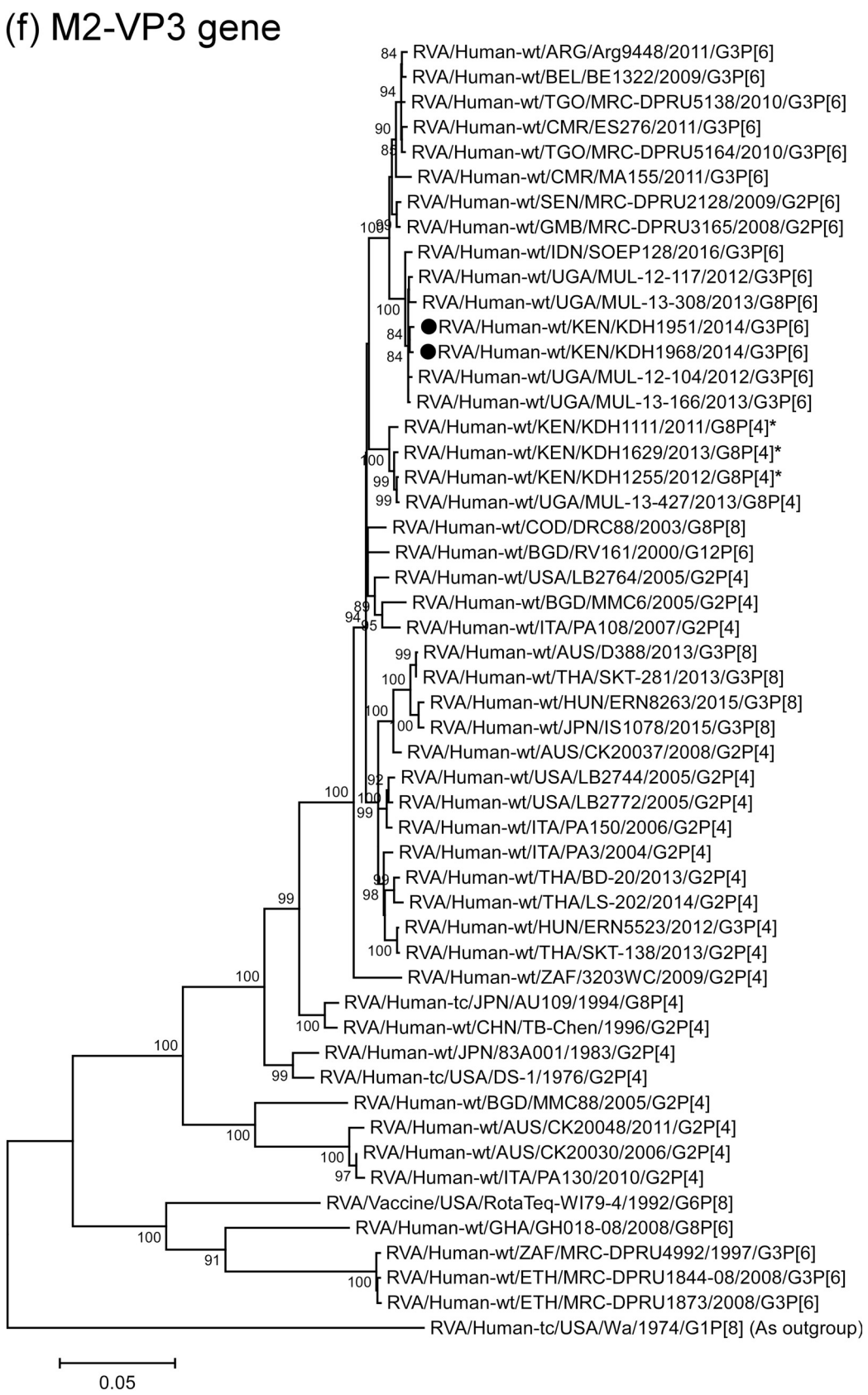

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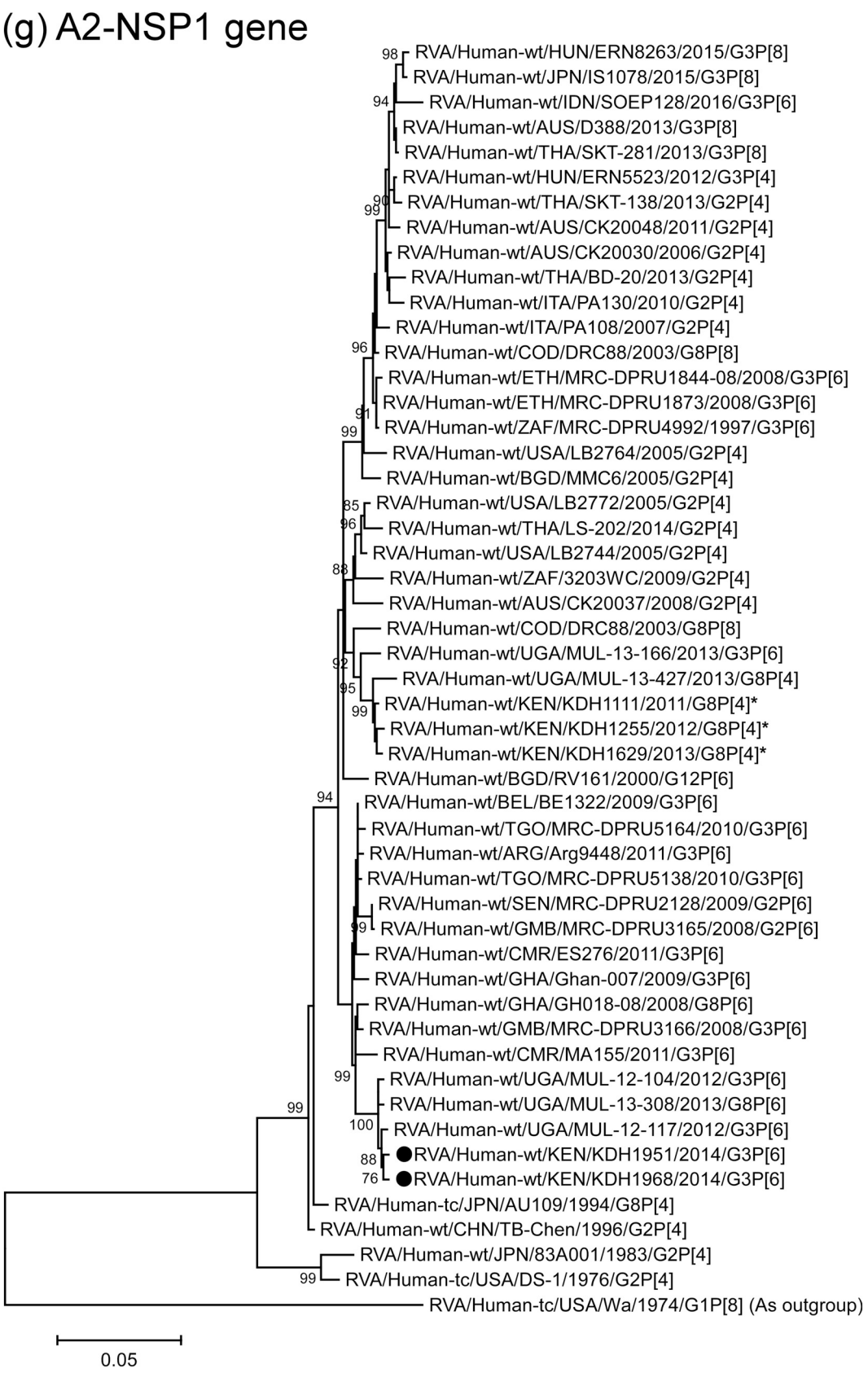

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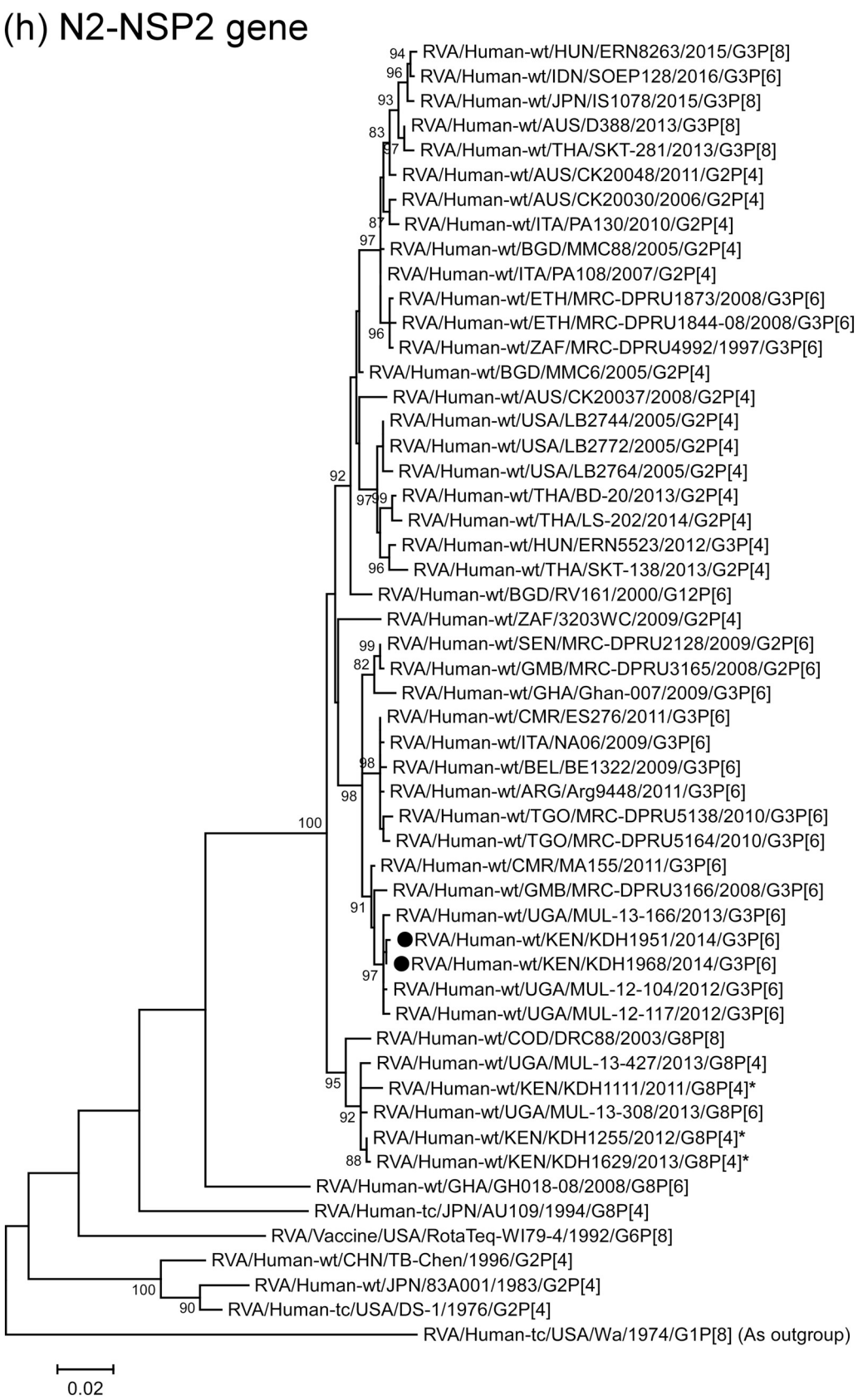

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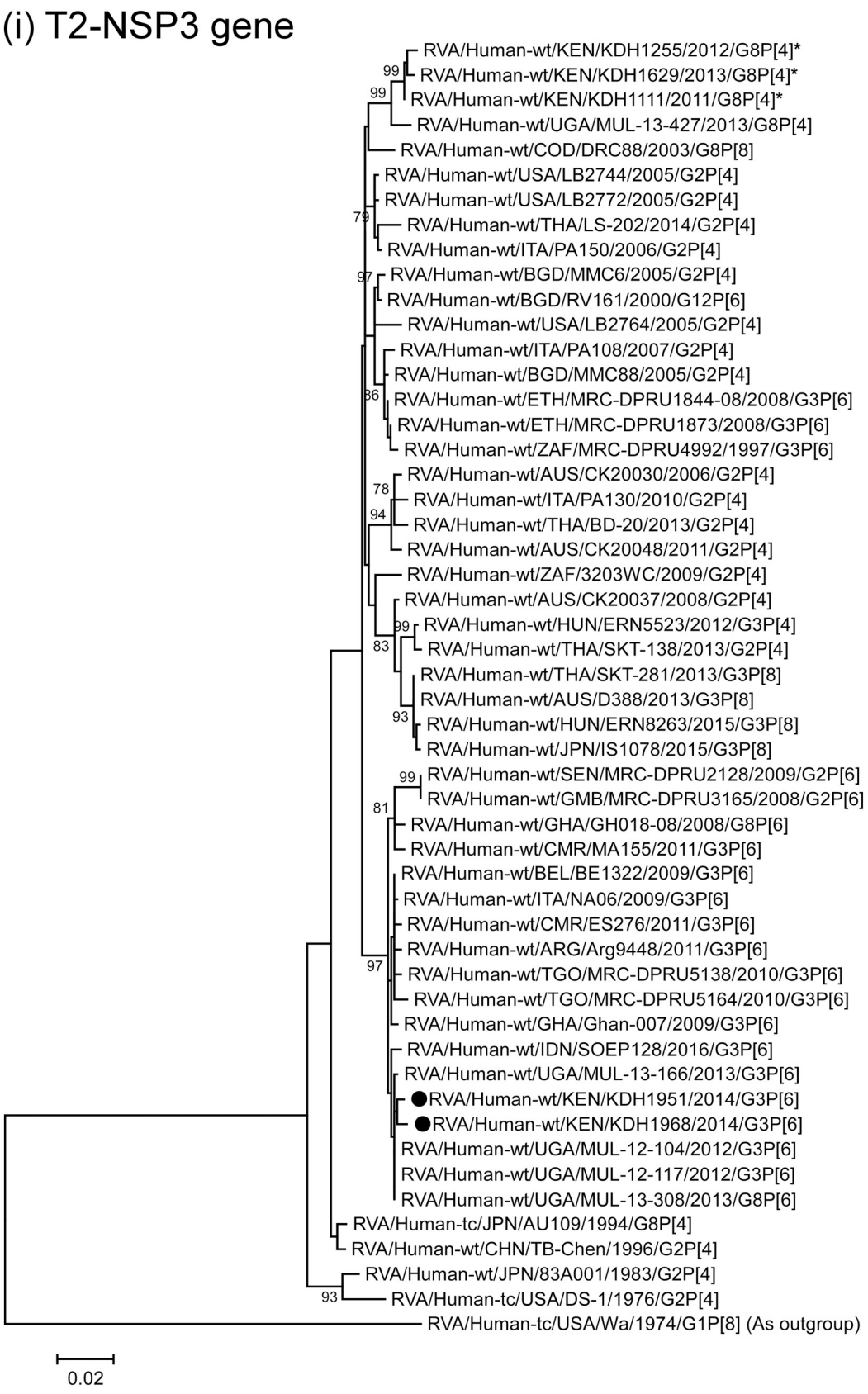

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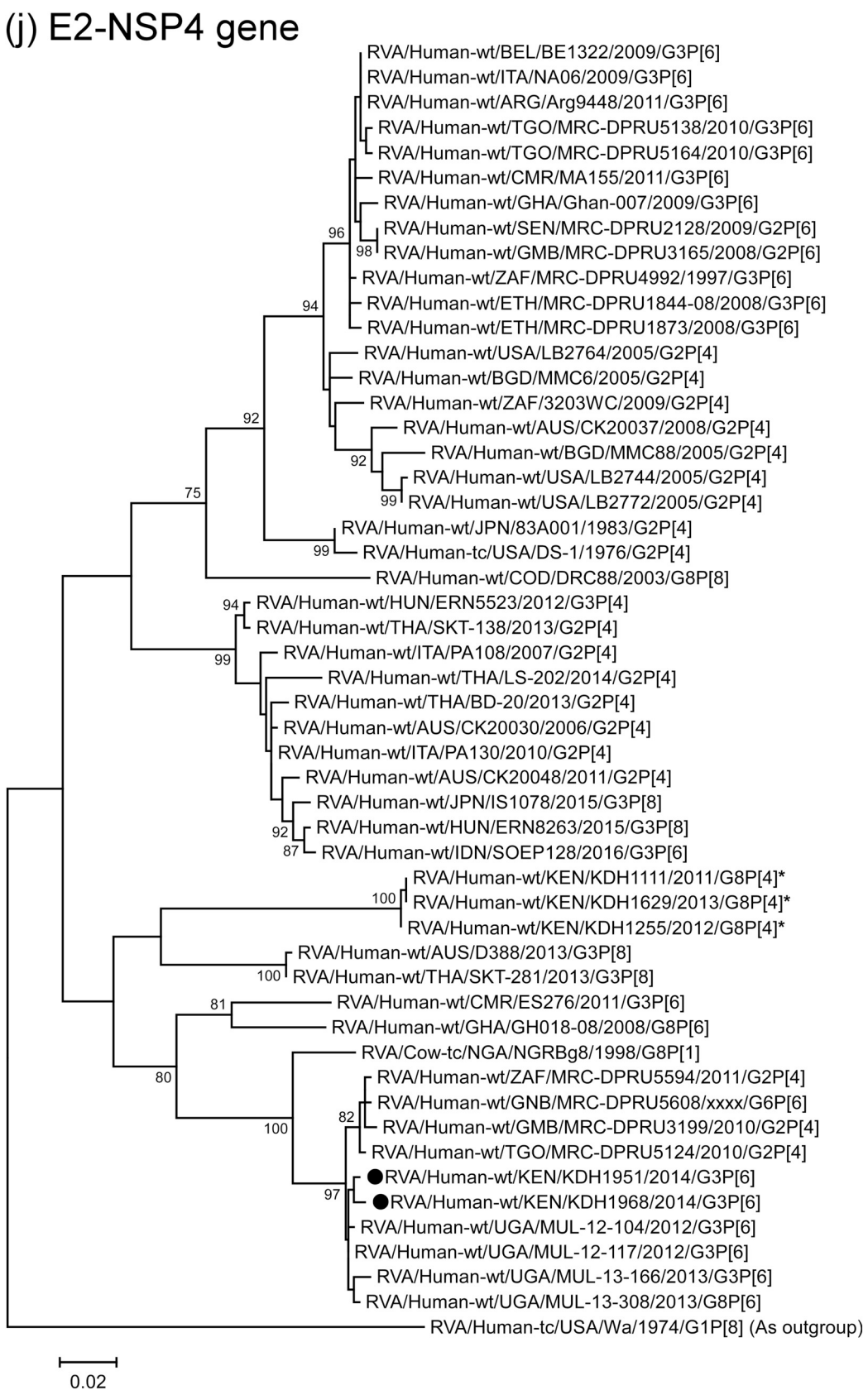

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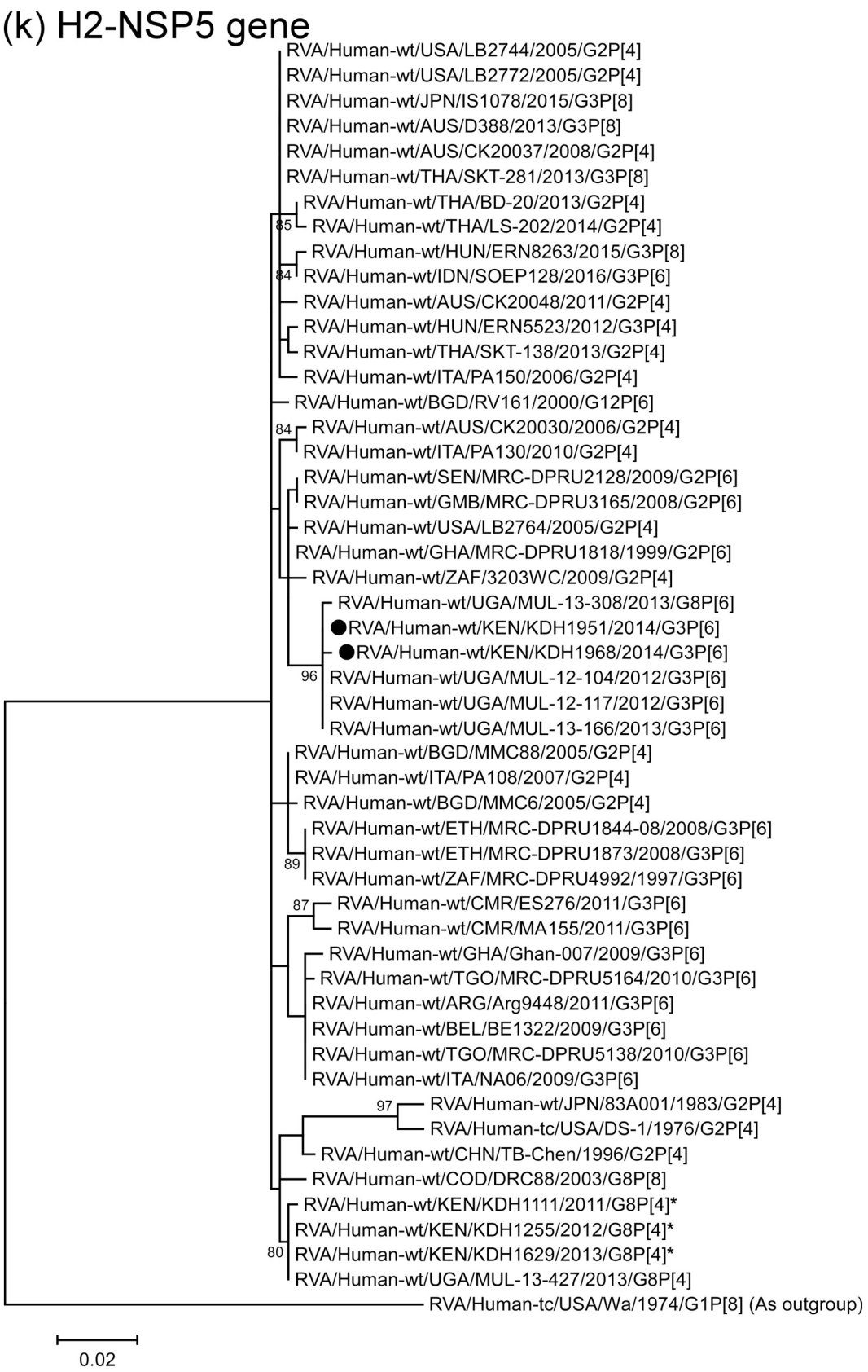

Fig. 1. (*continued*)

Table 2
Antigenic epitope variation between the VP7 and VP4 proteins of two 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
	*	*	*	*	*	*	*	*	*						*	*		*	*			*		*	*	*	*		*

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
	*	*	*	*	*	*	*	*	*	*	*	*

Table 3
Nucleotide sequence identity between two Kenyan G3P[6] strains (KDH1951 and KDH1968) 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

4. Conclusion

To our knowledge, this is the first description of full genome-based characterization of G3P[6] strains from Kenya. Because it has not been examined whether or not the currently licensed RVA vaccines (RV1 and RV5) are effective for prevention against uncommon strains, such as G3P[6] strains that share neither the P[6] genotype nor a full DS-1-like genotype constellation with strains in these vaccines, continuing RVA surveillance of G3P[6] strains is essential.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.meegid.2018.12.004.

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