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Genetic analysis of HA1 domain of influenza A/H3N2 viruses isolated in Kenya during the 2007–2013 seasons reveal significant divergence from WHO-recommended vaccine strains

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ABSTRACT

Background: Influenza viruses evolve rapidly and cause regular seasonal epidemics in humans challenging effective vaccination. The virus surface HA glycoprotein is the primary target for the host immune response. Here, we investigated the vaccine efficacy and evolution patterns of human influenza A/H3N2 viruses that circulated in Kenyan in the period before and after the 2009 A/H1N1 pandemic, targeting the HA1 domain.

Materials and methods: A hundred and fifteen HA sequences of Kenyan virus viruses were analyzed relative to the corresponding WHO vaccine reference strains using bioinformatics approaches.

Results: Our analyses revealed varied amino acid substitutions at all the five antigenic sites (A–E) of the HA1 domain, with a majority the changes occurring at sites A and B. The Kenyan A/H3N2 viruses isolated during 2007/2008 seasons belonged to A/Brisbane/10/2007-like viruses lineage, while those circulating in 2009–2012 belonged to the lineage of A/Victoria/361/2011-like viruses. The 2013 viruses clustered in clade 3C.3 of the A/Samara/73/2013-like viruses. The mean evolutionary rate of the A/H3N2 viruses analyzed in the study was at 4.17×10^{-3} (95% HPD = 3.09×10^{-3}–5.31×10^{-3}) nucleotide substitutions per site per year, whereas the TMRCA was estimated at 11.18 (95% HPD = 9.00–14.12) years ago from 2013. The prediction of vaccine efficacy revealed modest vaccine efficaciousness during 2008, and 2010 influenza seasons, whilst sub-optimal effectiveness was registered in 2007, 2009, 2012 and 2013. Further, the overall selective pressure acting on the HA1 domain was estimated at 0.56 ($\omega < 1$), suggesting that a majority of codon sites in the HA1 epitopes were evolving under purifying selection.

Conclusions: Generally, our results highlight the genetic plasticity of A/H3N2 viruses and reveal considerable disparity in vaccine efficaciousness against the A/H3N2 viruses that circulated in Kenya, specifically during 2007, 2009, 2012, and 2013 influenza seasons. Our findings underscore the importance and need for consistent surveillance and molecular characterization of influenza viruses, to inform decision making and enhance early of detection of strains with epidemic/pandemic potential as well as benefit in guiding decisions regarding the appropriate annual influenza vaccine formulations.

Introduction

Influenza viruses belong to the *Orthomyxoviridae* family and are grouped into four types: A, B, C, and D (CDC, 2017). Influenza virus type C causes mild respiratory infection and is not associated with epidemics, while type D only infects cattle (CDC, 2017). Influenza virus types A and B cause seasonal/epidemic influenza in humans, but the former is also associated with occasional pandemics (Chen and Holmes, 2006). Among Influenza viruses, type A exhibits the greatest genetic diversity and are further divided into subtypes based on the two virus surface proteins, Hemagglutinin (HA) and Neuraminidase (NA)(Kirkpatrick et al., 2018). To date, there are 18 influenza A subtypes based on HA and 11 subtypes based on NA (CDC, 2017). Before 2009, the influenza virus subtypes A/H1N1 and

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A/H3N2 co-circulated globally in the human population (Zhou et al., 2019). However, in 2009 a novel strain, influenza A/H1N1pdm09, emerged through reassortment of avian, human and swine viruses and eventually spread worldwide establishing itself in humans as a seasonal strain(Dawood et al., 2009; Khan et al., 2009; Majanja et al., 2013). Consequently, the 2009–2011 time period was distinctive in history when, for the first time, three influenza virus A subtypes, A/H1N1, A/H3N2, and A/H1N1pdm09, co-circulated in humans. In Kenya, the influence of more than two influenza A subtypes in the human population on the vaccine efficacy has never been investigated. Incidentally, the current co-circulating seasonal influenza A subtypes are A/H3N2 and A/H1N1pdm09 as the emergence of the latter wholly displaced A/H1N1 (Majanja et al., 2013). Among the two co-circulating influenza A virus subtypes, A/H3N2 has the highest rate of evolution and causes high morbidity and mortality than A/H1N1pdm09 (Westgeest et al., 2014).

The influenza virus A virus consists of a multipartite negative-sense RNA genome segmented into eight genes (Otieno et al., 2016; Samji, 2008). Among the encoded proteins, the virus surface glycoproteins HA and NA harbour major antigenic epitopes (Wiley and Skehel, 2003). NA is essential for the release of virus particles to infect other cells in the same host, while HA surface glycoprotein contains defined antigenic and receptor-binding sites (Wiley and Skehel, 2003; Matrosovich et al., 2004; Tewawong et al., 2015). During viral entry, conformational and posttranslational changes, including enzymatic cleavage of the full HA protein (denoted HA0) into two sub-units; HA1 and HA2 occur (Cross et al., 2001). HA1 forms the globular domain exposed on the surface of the virus and is visible to the host immune system, while HA2 forms the stem portion of the protein that is inserted in the lipid bilayer (Kurosawa et al., 2014). Specific antibodies to the HA1 can block the attachment of the virus to host cells, conferring immunity against the infecting virus (Kurosawa et al., 2014). Mutations occurring in the HA1 globular domain can lead to virus immune escape (Kurosawa et al., 2014). These mutations are mostly thought to drive antigenic drift among influenza viruses (Koelle, 2007; Lamb et al., 1981).

The recommended approach to reduce morbidity and mortality due to influenza is through annual vaccination (Deem and Pan, 2009). Presently, the WHO-recommended human influenza vaccines contain HA components of A(H1N1)pdm09, A/H3N2, and one lineage of type B (trivalent vaccine) or both lineages of type B viruses (quadrivalent vaccine) that match to a great extent antigenic properties of circulating influenza virus strains (Tewawong et al., 2015). However, high mutation rates associated with these viruses necessitate not only annual vaccine re-formulation but also limit influenza virus vaccine efficaciousness to about 50–60% (Tewawong et al., 2015). Influenza A/H3N2 virus HA1 domain contains five major antigenic sites (epitopes) designated A, B, C, D, and E against which host neutralizing antibodies are directed (Deem and Pan, 2009).

There are several methods for estimating the antigenic distance between influenza vaccine strains and the circulating viruses (Anderson et al., 2018; Cai et al., 2011, 2012; Fouchier and Smith, 2010; Mary, 2009; Wang et al., 2016). The $P\text{-}_{epitope}$ model, which quantifies the number of amino acid changes in dominant epitopes of the vaccine strain and the circulating virus strain is reliable in estimating antigenic distance (Tewawong et al., 2015). The calculated antigenic distance linearly correlates well with the vaccine efficacy (Deem and Pan, 2009; Gupta et al., 2006). Given the simplicity and the amenability of the $P\text{-}_{epitope}$ model, we sought to interrogate the vaccine efficacies of A/H3N2 components of the WHO recommended vaccines for the southern hemisphere against respective virus strains that circulated in Kenya during the study period. We further examined the evolutionary dynamics and antigenic properties of these viruses, targeting the HA1 domain of the hemagglutinin gene.

Materials and methods

Study design

This study was carried out using HA sequences of human influenza A/H3N2 viruses previously generated at the National Influenza (NIC) laboratories within the Kenya Medical Research Institute (KEMRI) and deposited in Genbank and GISAID databases between 2007 and 2013.

Ethical considerations

The study did not involve human or animal subjects. Permission to use the HA sequences of human influenza A/H3N2 viruses isolated in Kenya and previously deposited in Gen Bank was sought and granted by the Principal Investigator of the USAMRD-A Influenza surveillance program protocol (KEMRI SERU#981 WRAIR31267).

Sequence data

A total of one hundred and fifteen ($n = 115$) HA sequences of human influenza A/H3N2 viruses isolated in Kenya and previously deposited in GenBank (http://www.ncbi.nlm.nih.gov/genbank) and GISAID (http://platform.gisaid.org/epi3/frontend) databases were retrieved alongside those of corresponding WHO-recommended vaccine strains for the respective years. Noteworthy, HA sequences of the local A/H3N2 strains had previously been sequenced from viruses obtained from clinical specimens collected from hospitals constituting the human respiratory virus surveillance program, within the Kenya Medical Research Institute (KEMRI). HA sequences of less than 987 nucleotides in size (HA1 subunit) including those not well-curated were left out. Fifty-six ($n = 56$) HA sequences of other relevant global reference strains were also included, bringing the whole sequence dataset analyzed to one hundred and seventy-one ($N = 171$). The hospitals are well distributed across the country. The accession numbers of HA sequences for the study viruses are:

CY022909-12, EU204965-68, EU221476, FJ662951-54, FJ662956, FJ662959-61, FJ662963-69, FJ6622971-72, FJ662975-76, FJ662978, FJ662980, FJ662986, FJ662988-90, FJ662992-99, J663000-02, FJ663004-05, FJ663007-08, HM347425-29, HQ185194, HQ185197-203, HQ185205-208, HQ185210, HQ229610–HQ229617, HQ229619–HQ229640, JQ396182-83, KF451873, KF451875-78, KF451880-85, KX431186-88, KX431190-92, KX431194-95; while those of reference strains are: EPI342104, EPI556816, EPI352716, EPI346601, EPI356601, EPI305337, EPI607276, EPI606448, EPI684771, EPI684747, EPI335734, EPI316191, EU103823, CY062334, FJ445770, CY031827, CY087311, CY087468, CY032199, CY087652, CY034499, CY032557, KP456778, CY062347, GQ385918, CY031829, JX239588, KM978061, KM978061, KP457611, EU100720, EU659851, KP456824, KP456369, CY087747, CY062335, CY062331, KF598718, JX978453, KT889256, KT734915, KC535027, CY099722, KC999475, EPI335854, EPI460558, KM821347, HM628694, GQ293081, KM821334, KC882777, KP458092, KF790147, EPI405878, KF789535.

Phylogenetic analyses

The HA nucleotide sequences were aligned using the Multiple Sequence Comparison by Log-Expectation (MUSCLE) V3.8 (Edgar, 2004). The alignment was imported into Bioedit Sequence Alignment Editor software and trimmed, leaving only the region encoding the HA1 domain. Translation of the HA1 domain

nucleotide code into amino acid code was also carried out using Bioedit software (Hall et al., 2011). Phylogenetic clustering was estimated using MrBayes, a Bayesian inference that uses Markov chain Monte Carlo (MCMC) methods to estimate the posterior distribution of model parameters (Huelsenbeck and Ronquist, 2001). The best-fit nucleotide substitution model for the tree reconstruction was predicted by the jModel Test (Posada, 2008; Ronquist et al., 2011) implemented in MEGA V.6.0 (Kumar et al., 2018). The best-fit nucleotide substitution model was found to be K2 + Gamma.

Analyses of natural selective pressure and prediction of N-glycosylation

The overall selection pressure driving evolution of the HA1 domain of the Kenyan A/H3N2 viruses was determined by the single likelihood ancestor counting (SLAC) alongside the fixed effects likelihood (FEL) methods implemented in the online Data Monkey online tool (http://www.datamonkey.org). The prediction of potential N-linked glycosylation sites within the HA1 domain was performed using the online NetNGlyc 1.0 server (http://www.cbs.dtu.dk/services/NetNGlyc/). A threshold value of >0.5 was considered indicative of glycosylation.

Prediction of vaccine efficacy using the $P_{\text{-epitope}}$ model

The $P_{\text{-epitope}}$ model method was used to estimate vaccine efficacy (VE) of A/H3N2 components of WHO vaccine strains against the viruses that circulated in Kenya during 2007–2013 seasons. The $P_{\text{-epitope}}$ was calculated based on amino acid substitutions in the dominant HA1 epitope of the viruses relative to the corresponding vaccine strain for each year. The association between the VE and the $P_{\text{-epitope}}$ for A/H3N2 is given by the equation $VE = -2.47 \times P_{\text{epitope}} + 0.47$, where VE is 47% when $P_{\text{-epitope}} = 0$ (Gupta et al., 2006).

Evolutionary analyses

Estimation of molecular evolutionary rates, time of the most recent common ancestor (TMRCA), and time-scaled phylogeny of the local strains were performed using the Bayesian statistical inference approach implemented in the Bayesian Evolutionary Analysis Sampling Trees (BEAST) package v1.7.5 (Drummond et al., 2012). The BEAST was run for 100 million MCMC chains with sampling at every 100,000th generation. A relaxed molecular clock (uncorrelated lognormal) and an HKY + Gamma model of nucleotide substitution were used. The successful convergence of the runs was assessed by the interpretation of generated .log txt files in Tracer v1.6 software (Drummond et al., 2012). Convergence was established at Effective Sample Size (ESS) values >200 on various statistical parameters estimated by the completed BEAST run. Subsequently, a maximum credibility tree (MCC) was generated using the Tree Annotator v1.8.2 software, incorporated in the BEAST package, and visualized and annotated appropriately using the Fig tree v1.40 software (Ober and Heider, 2010).

Results

The HA1 domain sequences of the hemagglutinin gene of Kenyan A/H3N2 viruses obtained in 2007–2013 influenza seasons were compared to those of WHO southern hemisphere vaccine strains, alongside other relevant global strains. The A/H3N2 viruses obtained in 2007 contained nine amino acid differences (G50E, D122N, S138A, V186G, I223V, and K140I) relative to the vaccine strain A/Wisconsin/67/2005 (Table 1). Amino acid changes S138A and K140I affected antigenic site A while V186G was at antigenic site B. Those obtained in 2008 influenza season differed from A/Brisbane/10/2007 at three amino acid residue positions V112I, N144S/K and K173E/Q, with substitutions N144S/K and K173E/Q affecting antigenic sites A and D, respectively (Table 1). Furthermore, the 2009 viruses contained five amino acid variations: K158N, K173Q, N189K, T212A, and I214S/T relative to A/Brisbane/10/2007. The amino acid changes K158N and N189K affected antigenic site B, and K173Q was at antigenic site D. The Kenyan 2010 viruses displayed five amino acid differences: S45N, K62E, K144N, T212A, and S214I relative to A/Perth/16/2009 (Table 1). Accordingly, amino acid changes S45N affected antigenic site C, K62E antigenic site E, while K144N was at antigenic site A. For the 2011 influenza season, no Kenyan A(H3N2) sequences were found in the genetic databases. However, five viruses obtained in 2012 contained six amino acid changes relative to A/Perth/16/2009 (Table 1). The S45N mutation affected antigenic site C, K62E antigenic site E, while K144N was at antigenic site A. Furthermore, the Kenyan 2013 viruses harboured eleven amino acid differences relative to A/Victoria/361/2011 vaccine virus. These included Q33R, G78D, T128A, I140R, R142G, N145S, R156H, V186G, E190D, Y219S, and N278K (Table 1). The G78D change occurred at antigenic site E;

Table 1
Mutation at the antigenic sites of influenza A/H3N2 circulating in Kenya compared to the contemporaneous vaccine strain from 2007 to 2013.

Influenza subtype	Year	Vaccine strain	Mutations in circulating strains	Mutation at the antigenic sites	
				Site	Mutation
A/H3N2	2007	A/Wisconsin/67/2005	G50E, D122N, S138A, V186G, I223V, K140I	A	S138A, K140I
				B	V186G
	2008	A/Brisbane/10/2007	V112I, N144S/K K173E/Q	A	N144S/K
				D	K173E/Q
				B	K158N, N189K
				D	K173Q
	2010	A/Perth/16/2009	S45N, K62E, K144N, T212A, S214I	A	K144N
				C	S45N
				E	K62E
	2011	A/Perth/16/2009	NO Kenyan viruses retrieved		
	2012	A/Perth/16/2009	S45N, K62E, K144N, V224I, T212A, S214I	A	K144N
				C	S45N
				E	K62E
	2013	A/Victoria/361/2011	Q33R, G78D, T128A, I140R, R142G, N145S, R156H, V186G, E190D, Y219S, 278K	A	I140R, R140G, N145S
				B	T128A, R156H, V186G
				C	N278K
				D	G78D

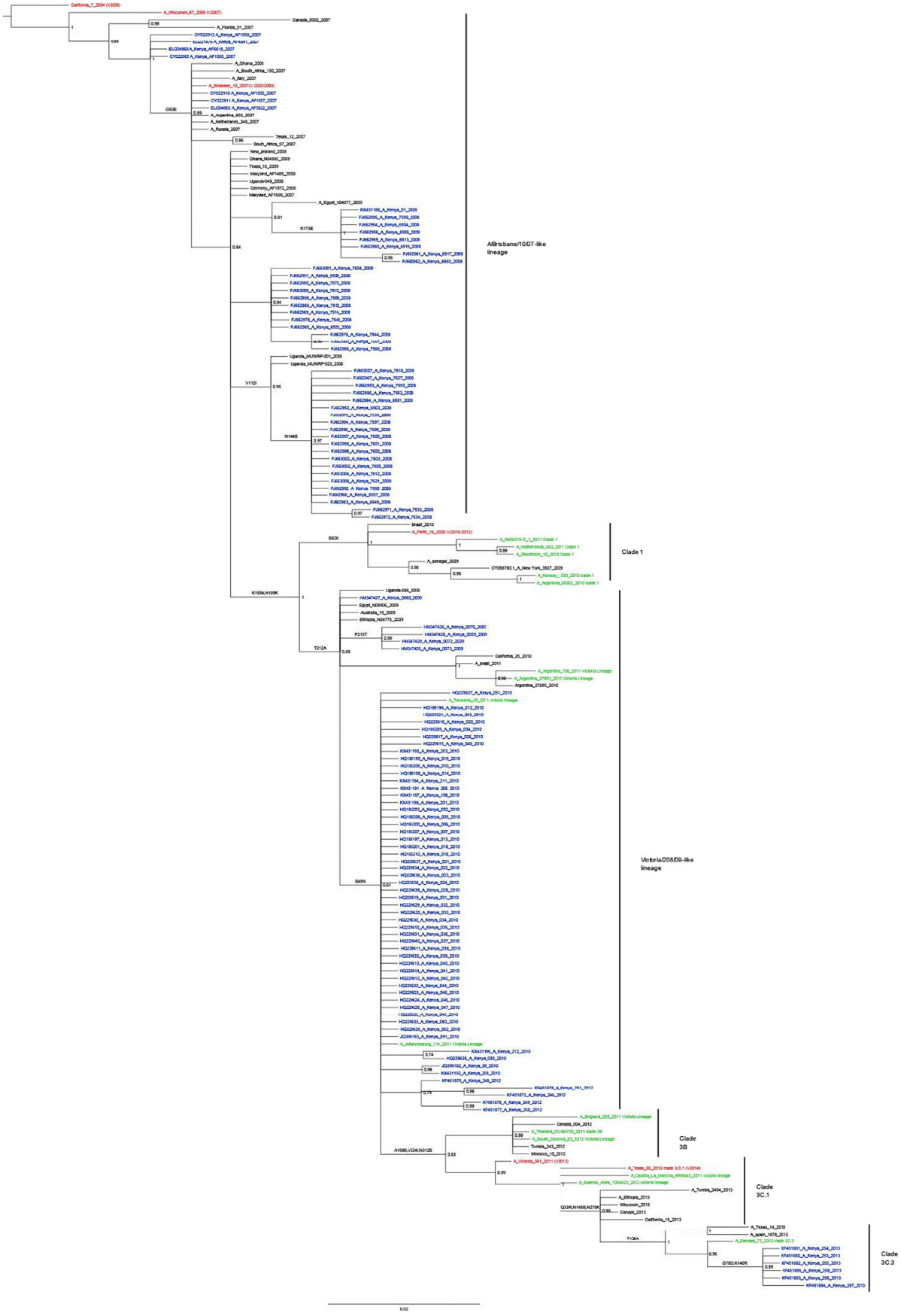

Figure 1. Phylogenetic analysis of influenza A/H3N2 virus HA1 amino acid sequences. The Kenyan viruses are indicated in blue colour. Those in green represent reference strains of known clades. Those in red represent WHO vaccine reference strains for the southern hemisphere. Those in black represent global strains.

Table 2
Efficacy estimation among A/H3N2 vaccine strains and mutations found on the dominant epitope of Influenza A(H3N2) viruses that circulated in Kenya (2007–2013).

Year	Vaccine strain	No. of strains	Dominant epitope	No. of mutations	Residue variations	$P_{-epitope}$	Efficacy (47%)
2007 $N=7$	A/Wisconsin/67/2005	7	A	3	D122N, S138A, K140I,	**0.1579**	8
2008 ($N=41$)	A/Brisbane/10/2007	21	A	1	N144S	**0.0526**	34.01
		6	B	1	K158R	0.0476	35.24
		9	C	1	T48A	0.03707	37.84
		2	D	1	K173E	0.0488	34.95
		3	E	1	C57R	0.0455	35.76
2009 $N=5$		4	B	2	K158R, N189K	**0.0952**	23.49
		1	D	3	K173C, T212A, V214T	0.0976	22.89
2010 $N=51$	A/Perth/16/2009	45	**A**	1	K144N	**0.0526**	34.01
		2	C	2	S45N, G275N	0.0741	28.7
		4	D	4	D175E, T212A, S214I	0.07322	28.91
2012 $N=5$		2	**A**	2	I140R, R142G, K144N, N145S, G186S	**0.2105**	−5
		2	C	2	S45N, D53N	0.0741	29
		1	D	1	D175E	0.073	29
2013 $N=6$	A/Victoria/361/2011	6	**A**	3	I140R, R142G, N145S	**0.1579**	8

I140R, R140G, and N145S at antigenic site A; T128A, R156H, and V186G at antigenic site B; and N278K antigenic site C.

Phylogenetic inference showed that Kenyan A/H3N2 viruses clustered into three different clades/lineages, consistent with year of isolation (Figure 1). Those isolated in 2007/2008 fell into the A/Brisbane/10/2007-like virus cluster, while those obtained in 2009/2012 clustered with A/Perth/16/2009-like viruses (Figure 1). The Kenyan 2013 A/H3N2 viruses belonged to clade 3C.3 of the A/Samara/73/2013-like viruses, divergent from clade 3C.1 of the A/Victoria/361/2011-like viruses (vaccine strain for 2013 in the Southern Hemisphere) (Figure 1). All the Kenyan viruses contained potential N-linked glycosylation motifs at HA1 residue positions 8, 22, 63, 133, 165, and 285. However, these were all present in the vaccine virus strains.

The estimated vaccine efficacies of the 2007–2013 vaccine strains against the Kenyan A/H3N2 viruses are summarized in Table 2. The $P_{-epitope}$ between A/Wisconsin/67/2005 vaccine strain and Kenyan 2007 viruses was 0.1579 (dominant epitope A; amino acid changes D122N, S138A, and K140I), suggesting a vaccine efficacy (VE) of 17% ($E=8\%$ of 47%). The $P_{-epitope}$ between A/Brisbane/10/2007 and Kenyan 2008/2009 viruses was 0.0526 (dominant epitope A; mutation N144S/K) and 0.0952 (dominant epitope B; mutation K158N and N189K) suggesting a VE of 72.36% and 49.98%, respectively. A/Perth/16/2009 was the recommended WHO vaccine strain for 2010, 2011, and 2012 influenza seasons. The $P_{-epitope}$ value between A/Perth/16/2009 and Kenyan 2010 A/H3N2 viruses was 0.0526 (dominant epitope A; mutation N144K), suggesting vaccine efficacy of 72.36%. No sequences for A/H3N2 viruses that circulated in Kenya during the 2011 influenza season were available in GenBank/GSAID databases; thus, no analysis was carried out for this period. For the 2012 influenza season, 40% (2/5) of the analysed A/H3N2 viruses possessed I140R, R142G, K144N, N145S, and G186S amino acid changes at site A of the HA1 domain relative to A/Perth/16/2009. These resulted in negative vaccine efficacy (Table 2). All the Kenyan 2013 viruses contained three amino acid substitutions (T128A, R156H, and V186G) affecting site A of the HA1domain. The estimated $P_{-epitope}$ value between these viruses and the vaccine strain A/Victoria/361/2011 was 0.1579 (Table 2), signifying a low vaccine efficacy.

The overall selection pressure underlying the evolution of the HA1 domain of the Kenyan A/H3N2 viruses was estimated at 0.56 (d_N/d_S) using the SLAC method. Conversely, no individual codon sites within the HA1 domain of the Kenyan viruses were found to be under negative or positive selection by both the SLAC or FEL methods, with a strong significance statistical support. Time-scaled phylogenetic analyses estimated TMRCA) for the whole sequence dataset at 11.18 (95% HPD = 9.00–14.12) years ago from 2013, which translates to September 2001 (95% HPD = September 1998 to October 2003) (Figure 2). The mean evolutionary rate of Kenyan viruses viruses was 4.17×10^{-3} (95% HPD = 3.09×10^{-3}–5.31×10^{-3}) nucleotide substitutions per site per year.

Discussion

In this report, we examined the antigenic and evolutionary aspects of human influenza A/H3N2 virus strains isolated in Kenya from 2007 to 2013 influenza seasons relative WHO-recommended vaccine strains and other relevant global strains, focusing on the HA1 domain of the hemagglutinin gene. Genetic variations observed among influenza viruses are often attributed to the virus-encoded non-proofreading, error-prone RNA-dependent RNA polymerase (RdRP), an enzyme involved in transcription and virus genome replication mechanisms (Samji, 2008; Manley, 2013). The overall effect of the low-fidelity RdRP is the emergence of influenza virus strains with the potential to evade host immunity due to accumulated point mutations at the antigenic sites that are also the protective immunological epitopes (Deem and Pan, 2009; Gupta et al., 2006; Manley, 2013). In the present work, different genetic variations were observed among the Kenyan viruses, not common in the WHO-recommended vaccine strains for the respective years. Some of these changes were antigenically significant as they occurred within the HA1 antigenic receptor binding sites. Amino acid variations, specifically those affecting antigenic sites A and B of the HA1 protein, have been shown to cause major antigenic alteration of influenza A/H3N2 viruses leading to vaccine failures (Korsun et al., 2017).

Phylogenetic inference revealed that the Kenyan A/H3N2 virus strains clustered into three genetic clades clustering together according to the three vaccine strains. Those isolated in 2007–2008 influenza seasons clustered within the A/Brisbane/10/2007-like virus clade; the 2009–2012 viruses clustered within the A/Victoria/361/2011-like virus clade, whereas those obtained in 2013 belonged to clade 3C.3 of the of A/Samara/73/2013(H3N2)-like virus. Overall, these results show that the Kenyan viruses evolved along the same trajectories as global strains. Findings in this study are compatible with those of other studies that reported circulation of A/Brisbane/10/2007, A/Victoria/361/2011 and clade 3C.3 of the A/Samara/73/2013(H3N2)-like viruses during 2008–2009 and 2013 seasons, respectively (Tewawong et al., 2015; Dawood et al., 2014). Consistent with findings of a separate study (Agustiningsih et al., 2010), the majority of the virus strains clustered based on the isolation year. Conversely, as indicated by Suwannakarn et al. (Eick-Cost et al., 2012), the Kenyan 2007 A/H3N2 viruses were drift variants of the vaccine strain. This was due to additional amino acid changes in the HA1 absent in A/Wisconsin/67/2005 (Southern Hemisphere 2007 and Northern

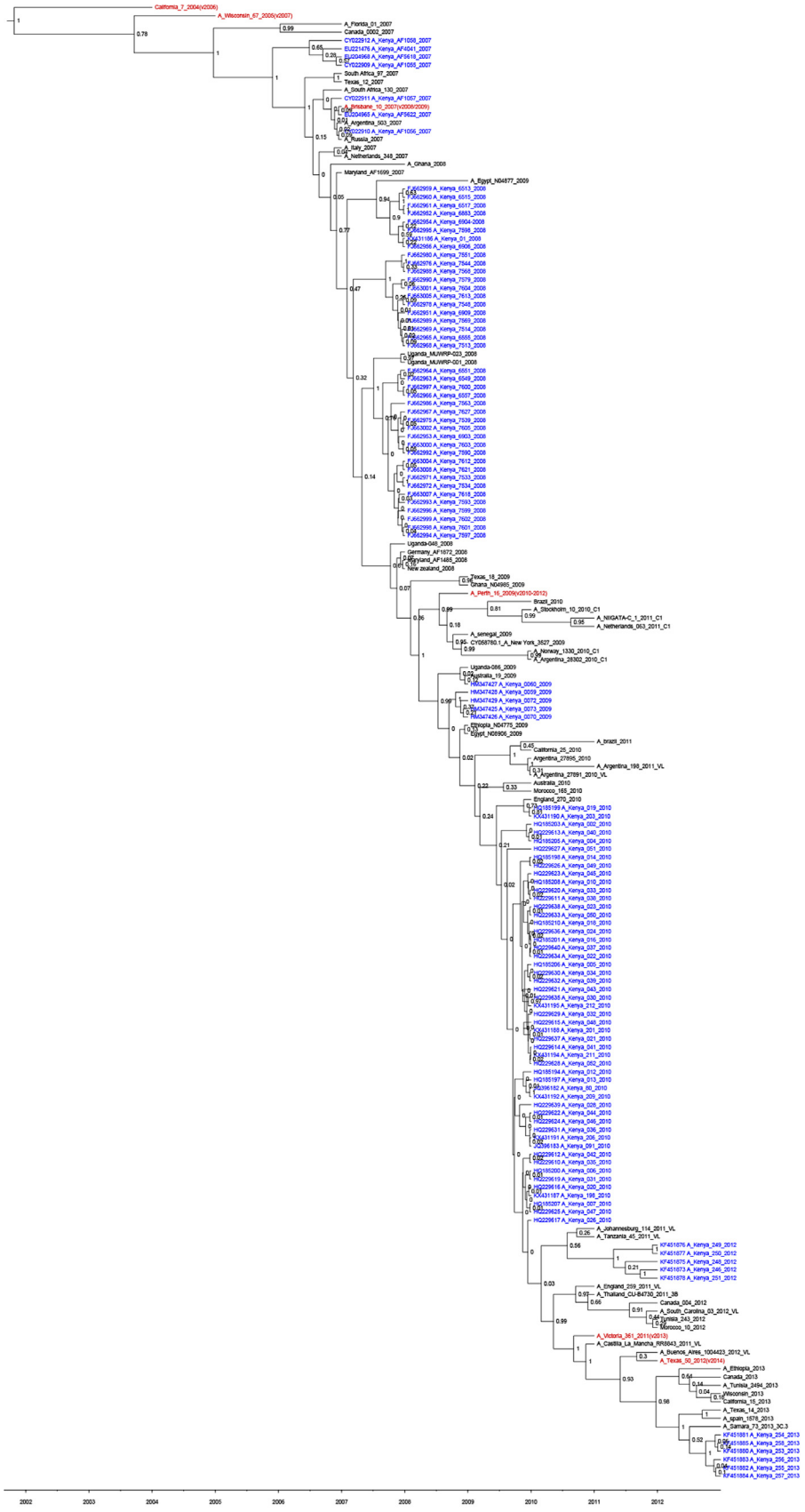

Figure 2. Maximum clade credibility tree from Bayesian analysis of Kenyan human influenza A/H3N2 virus isolate strains. The numbers at the nodes represent heights. The Kenyan viruses are indicated in blue colour. Those in black represent global strains. Those in red represent WHO vaccine reference strains for the southern hemisphere.

Hemisphere 2007–2008 vaccine strain) but present in A/Brisbane/10/2007 (Southern Hemisphere 2008 and Northern Hemisphere and 2008–2010 vaccine strain) and 2008 A/H3N2 viruses strains. Contrary to findings by Falchi et al. (2011), the A/H3N2 viruses circulating in Corsica highlands in 2007 were closely related to A/Wisconsin/67/2005.virusesviruses The Kenyan 2008 and 2009 viruses shared T212A and N144S & K158N amino acid substitutions respectively, with those that circulated in a neighbouring country in the same period (Dawood et al., 2014). Consistent with findings by Tewawong et al. (2015), all the Kenyan viruses obtained in 2013 belonged to clade 3C.3 and were characterized by T128A and R142G amino acid substitutions among other mutations, relative to the vaccine strain A/Victoria/361/2011.

In support of previous findings (Falchi et al., 2011; Skowronski et al., 2014), all potential N-glycosylation sites detected among the Kenyan A/H3N2 strains were conserved among the vaccine strains. The gain or loss of N-linked glycosylation can affect the antigenicity of the virus HA glycoprotein (Falchi et al., 2011). Regarding natural selection, the estimated mean d_N/d_S value of 0.56 revealed that the evolution of the HA1 domain of Kenyan A/H3N2 virus strains was largely driven by purifying selection, signifying that a majority of the amino acid changes in the HA1 domain did not favor virus adaptability to the host. Although this value is slightly higher, these findings are in agreement with those of previous studies (Chen and Holmes, 2006, 2008; Tewawong et al., 2015) whose estimated d_N/d_S values range from 0.130 to 0.37 ($\omega < 1$). The mean evolutionary rate for the Kenyan A/H3N2 viruses was estimated as 4.17×10^{-3} (95% HPD = 3.09×10^{-3}–5.31×10^{-3}) nucleotide substitutions per site per year. This estimate is similar to the global nucleotide substitution rate for A/H3N2 viruses in the HA1 [4.84×10^{-3} (95% HPD = 4.32×10^{-3}–5.38×10^{-3})] for the period between 1968 and 2011 (Westgeest et al., 2014). Besides, coalescent analyses showed that the time of circulation of the most recent common ancestor (tMRCA) for A/H3N2 virus strains analyzed in this study was September 2001 (95% HPD = September 1998 to October 2003).

A significant proportion of amino acid substitutions detected among the Kenyan A/H3N2 viruses affected the antigenic sites. Accordingly, vaccine efficacies (antigenic distance) against the A/H3N2 strains assessed for respective years, utilizing the in-silico P-$_{epitope}$ model (Gupta et al., 2006) revealed sub-optimal vaccine efficaciousness against the A/H3N2 strains that circulated in Kenya during 2007, 2009, 2012, and 2013 influenza seasons. The amino acid variations S138A, K140I (2007 viruses relative to A/Wisconsin/67/2005); K158R, N189K (2009 viruses relative to A/Brisbane/10/2007); K144N (2012 viruses relative to A/Perth/16/2009) and K140R, R140G, N145S (2013 viruses relative to A/Victoria/361/2011) on A epitopes of the HA1 domain of the hemagglutinin gene were plausibly attributable for the decline in vaccine efficacy against the virus strains. Conversely, the A/Brisbane/10/2007 and A/Perth/16/2009 2008 vaccine strains exhibited modest vaccine efficacy against the Kenyan A/H3N2 strains that circulated in 2008 and 2010 influenza seasons, respectively. Overall, these results are compatible with findings by Tewawong et al. (2015), Dawood et al. (2014), Eick-Cost et al. (2012) and Kittikraisak et al. (2015) who in their separate works reported modest effectiveness of A/H3N2 vaccine component against the circulating strains and influenza-associated illnesses in the same period in different parts of the world. Moreover, as reported by Tewawong et al. (2015), the change in influenza virus vaccine component for A/H3N2 to A/Victoria/361/2011-like strain for Southern hemisphere in 2013 season did not offer sufficient protection against the circulating A/H3N2 strains as the latter had evolved away from A/Victoria/361/2011-like viruses belonging to genetic sub-clade 3C.1 to 3C.3 of A/Samara/73/2013-like viruses.

This study had a few shortcomings. First, most of the HA sequences available were of partial lengths, necessitating the use of only the HA1 region of the HA gene for the analyses. Some of the sequence data lacked full information regarding collection dates, limiting temporal analysis. There were no HA sequences available in GenBank for A/H3N2 viruses that circulated in Kenya in 2011 to include in the analysis. Furthermore, the study did not incorporate hemagglutination inhibition assay (HAI) for serologic antigenic analyses. These data would have served to confirm the net effect of genetic variations noticed in the HA1 domain to vaccine efficacy calculated by the P-$_{epitope}$ method. Cognisant of the fact that the antigenic properties of A/H3N2 strains and vaccine efficacy data reported in the present study were generated based solely on genetic and in silico P-$_{epitope}$ method, we submit that our findings should be interpreted with caution since vaccine efficacy studies in the context of the antigenic distance between the vaccine and circulating strains can be inaccurate due to mutations introduced to the vaccinating virus during propagation in eggs (Skowronski et al., 2014). Despite the limitations, the present study has outlined the genetic and evolutionary aspects of human influenza A/H3N2 viruses that circulated in Kenya from 2007 to 2013 influenza seasons and highlighted the discrepancy in vaccine efficaciousness.

Our findings underscore the importance of and need for consistent surveillance and advanced molecular characterization of influenza viruses globally. Information from surveillance programs such as the one instituted in Kenya by DoD GEIS inform decision making across the world and enhance global health security by promoting detection of novel/drifted influenza viruses, enhancing detection of those with epidemic and pandemic potential and has benefit in guiding decision regarding the appropriate annual influenza vaccine formulations.

Authors contributions

Mr. Edward Mairura was responsible for data analysis and manuscript preparation; W.D. Bulimo was responsible for experimental design, data analysis, and manuscript preparation; E. Magiri was responsible for study design and manuscript preparation; S. Opanda and V. Mobegi were involved in data analysis and manuscript writing.

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These materials have been reviewed by the Walter Reed Army Institute of Research (WAIR). There is no objection to its presentation and/or publication. The opinions or assertions contained herein are the private views of the authors and are not to be construed as official, or as reflecting correct views of the Department of the Army or the Department of Defence. The investigators have adhered to the policies for the protection of human subjects as prescribed in AR 70–25, and declare no conflict of interest.

Conflict of interest

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