

## Research Article

# Were the WHO-recommended Human Influenza Vaccine Formulations Appropriate for Kenya During the 2010-2011 Season? Inferences from the HA1 Gene Analysis

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**Background:** The knowledge of evolutionary patterns of the HA gene of the influenza virus is important in vaccine strain selection.

**Objective:** Genetic analysis of HA1 of influenza viruses isolated in Kenya during the 2010-2011 season with reference to WHO vaccine strains.

**Methods:** A total of twenty seven (27) influenza A (H1N1) pdm09, Nineteen (19) influenza A (H3N2) and Sixteen (16) influenza B virus isolates were analyzed. A partial HA1 gene was amplified by RT-PCR and sequenced.

**Results:** Phylogenetic analyses revealed that influenza B viruses were closely related to B/Brisbane/60/2008 vaccine strain while A (H1N1) pdm09 viruses were genetic variants of A/California/07/2009. The Kenyan A (H1N1) pdm09 isolates had P83S, D97N, S185T, I321V and E374K amino acid substitutions. Influenza A/H3N2 isolates showed K62E, T212A and S214I simultaneous amino acid substitutions when compared to A/Perth/10/2009. The K62E change occurred at antigenic site E. Majority of the Kenyan H3N2 isolates further had S45N and K144N amino acid substitutions at sites C and A respectively, which introduced N-glycosylation motifs absent in the vaccine strain.

**Conclusion:** The study showed that although the WHO 2010 vaccine strains recommendations for the southern hemisphere matched with influenza viruses which circulated in Kenya during the 2010-2011 season, the viruses had evolved genetically from the vaccine strains.

**Key words:** Influenza vaccine formulations; HA1 gene; Kenya.

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## 1. Introduction

Influenza is an acute respiratory illness that spreads across the world in annual epidemics and occasional pandemics (WHO, 2003). In annual epidemics, the disease infects about 5 - 15% of the human population

with an estimated 3-5 million severe disease cases and 250,000 - 500,000 deaths (Lin et al, 2004).

Influenza A and B viruses are the main causative agents of the disease (Baras et al, 2008). Type A influenza viruses are further classified into subtypes based on

antigenic properties of surface glycoproteins, hemagglutinin (HA) and neuraminidase (NA). Only a limited number of subtypes have been associated with infections in humans (Baras et al, 2008). Since 1977, subtypes H1N1 and H3N2 viruses have widely co-circulated in the human population (Wang et al, 2009). Recently, a new strain of influenza A (H1N1) virus - the pandemic 2009 H1N1 [A (H1N1) pdm09] - emerged in the human population, causing a global outbreak. This influenza strain has since continued to circulate, and in many parts of the world has displaced the seasonal influenza A (H1N1). Influenza B virus has no subtypes but belongs to two evolutionary lineages that are distinct at the genetic and antigenic levels, and which are represented by B/Yamagata/16/88-like and B/Victoria/2/87-like viruses that have co-circulated in the human population since 1980s (Baras et al, 2008).

Influenza virus surface glycoproteins, HA and NA represent the main immunogenic factors of the virus. However, the virus HA protein is the major primary target against which neutralizing antibodies are directed. It is composed of two subunits, HA1 and HA2 (Wiley and Skehel, 1987; Wright et al, 2007). The HA1 domain is the most rapidly evolving region of the molecule and contains all of the antigenic sites (Webster et al, 1979). These antigenic sites are designated as Sa, Sb, Ca, and Cb for H1, and A, B, C, D and E for H3 HA subtypes (Gerhard et al, 1981; Yewdell and Gerhard, 1981; Caton et al, 1982).

For influenza B virus HA molecule, the antigenic sites occur within 120-Loop, 150-Loop, 160-Loop and 190-Helix (Wang et al, 2008). Mutations occurring in the HA1 globular domain near the receptor binding sites play a significant role in helping the virus evade host immune response (Underwood et al, 1987; Ruigrok et al, 1988).

The primary method for controlling influenza is by vaccination. Consequently, each year WHO convenes technical meetings in February and September to recommend the composition of influenza vaccines for the northern and southern hemispheres respectively. The vaccines for seasonal influenza are trivalent, and contain two influenza A strains (H1N1 and H3N2) and one influenza B strain (WHO, 2005). The recommended vaccines for use in the 2010 influenza season (southern hemisphere winter) contained an A/California/7/2009 (H1N1)-like virus, an A/Perth/16/2009 (H3N2)-like virus and a B/Brisbane/60/2008-like virus. These vaccines are updated annually based on data generated from the global influenza surveillance network to ensure efficacy (WHO, 2008). The Kenya Ministry of Public Health and Sanitation recommends the use of southern hemisphere vaccine formulation for its residents since the influenza season in Kenya coincides with the winter of the southern hemisphere.

Surveillance of influenza in Kenya is conducted by the National Influenza Center (NIC) at the Kenya Medical Research Institute (KEMRI), Nairobi, in collaboration with the US Army Medical Research Unit-Kenya (USAMRU-K), Centers for Disease Control and Prevention-Kenya (CDC) and the Ministry of Public Health and Sanitation (MOPHS). In this study we present genetic data of HA1 domain of representative influenza A (H3N2), pandemic H1N1 and influenza B

viruses isolated in the country during 2010 to 2011 season and analyze their antigenic properties with reference to vaccine strains using this genetic data.

## 2. Materials and Methods

### 2.1 Sample collection

Sentinel surveillance network comprised the following district hospitals: Mbagathi, New Nyanza, Malindi, Isiolo, Mombasa, Port Reitz, and Kericho. The study sites were selected based on geographical regions and population demographics. Nasopharyngeal swabs were collected from patients over 2 months of age showing acute respiratory illness (ARI) symptoms and transported in viral transport media (VTM) to the laboratory on a weekly basis for processing.

### 2.2 Virus Isolation and Characterization

All specimens testing positive for influenza by real-time PCR (RT-PCR) were inoculated onto Mardin-Darby canine kidney (MDCK) cells prepared in culture tubes. The tubes were incubated at 37 °C with 5% CO<sub>2</sub>, and virus growth monitored at 33 °C with reference to cytopathic effects (CPEs) for up to 2 weeks. All isolates were thereafter typed and subtyped by hemagglutination inhibition assay (HAI) in accordance with Centers for Disease Control and Prevention (CDC) protocols (WHO, 2011b).

### 2.3 RNA Extraction and PCR

Viral RNA was extracted from 100 µL of virus-infected MDCK culture fluid with a QIAmp Viral RNA Mini Kit (Qiagen, Inc., Valencia, CA, USA) according to the manufacturer's protocols.

The HA sequences for A/H1N1pdm09, A/H3N2 and B influenza viruses were amplified by RT-PCR using specific primer sets tagged with M13. These included:

H3\_HA\_M13\_F: 5'-TGT AAA ACG ACG GCC AGT AAA GCA GGG GAT AAT TCT A -3' & H3\_HA\_1778\_M13\_R: 5'-CAGGAA ACA GCT ATG ACC AGT AGA AAC AAG GGT GTT TT -3' for amplification of seasonal H3;

pH1\_HA\_F\_4: 5'-TGT AAA ACG ACG GCC AGT ATA CGA CTA GCA AAA GCA GGG G -3' & pH1\_HA\_F\_1778: 5'-CAG GAA ACA GCT ATG ACC GTG TCA GTA GAA ACA AGG GTG TTT -3' for amplification of pandemic H1; and

B\_HA\_F\_7: 5'-TGT AAA ACG ACG GCC AGT GCA GAG CAT TTT CTA A -3' & B\_HA\_R\_1869: 5'-CAG GAA ACA GCT ATG ACC AGT AGT AAC AAG AGC AT -3' for amplification of influenza B H.

Cycling parameters included 30 cycles of 94 °C for 2 minutes, 50 °C for 30 seconds, and 72 °C for 1 minute, with a final extension cycle at 72 °C for 7 minutes. Amplicons were analyzed by 1% gel electrophoresis and consisted of 1.8 kb, 1.8 kb and 1.87 kb for H3, pH1 and B H respectively. These were purified using a QIAquick Gel extraction Kit (Qiagen, Valencia, CA).

### 2.4 Nucleotide sequencing and Analysis

Sequencing reactions were performed using Big Dye Terminator v3.1 cycle sequencing kit and extension products resolved using an ABI 3500 series Genetic Analyzer (Applied Biosystems) according to the manufacturers' specifications. Sequencing primers included one forward (M13F) and one reverse (M13R) M13 primers. Nucleotide contigs were assembled using DNA baser version 3.5 (SRL, 2011). Sequences were aligned using Muscle 3.6 (Edgar, 2004a; Edgar, 2004b). Phylogenetic analyses were performed with Mr Bayes 3.1.2 (Huelsenbeck et al, 2001; Ronquist and Huelsenbeck, 2003). Sequences used in this study have been submitted to GenBank and GISAID databases under accession numbers JQ396182-JQ396184, JQ396198-JQ396212, JQ396224-JQ396243, EPI307143-EPI307151, EPI307154-EPI307156 and EPI356816-EPI356915.

### 2.5 Ethical considerations

The Kenya Medical Research Institute (KEMRI) and the Walter Reed Army Institute of Research (WRAIR) institutional review boards reviewed and approved the study protocol with approval numbers SSC#981 and WRAIR#1267, respectively. Informed consent was obtained from patients prior to study participation.

### 3. Results

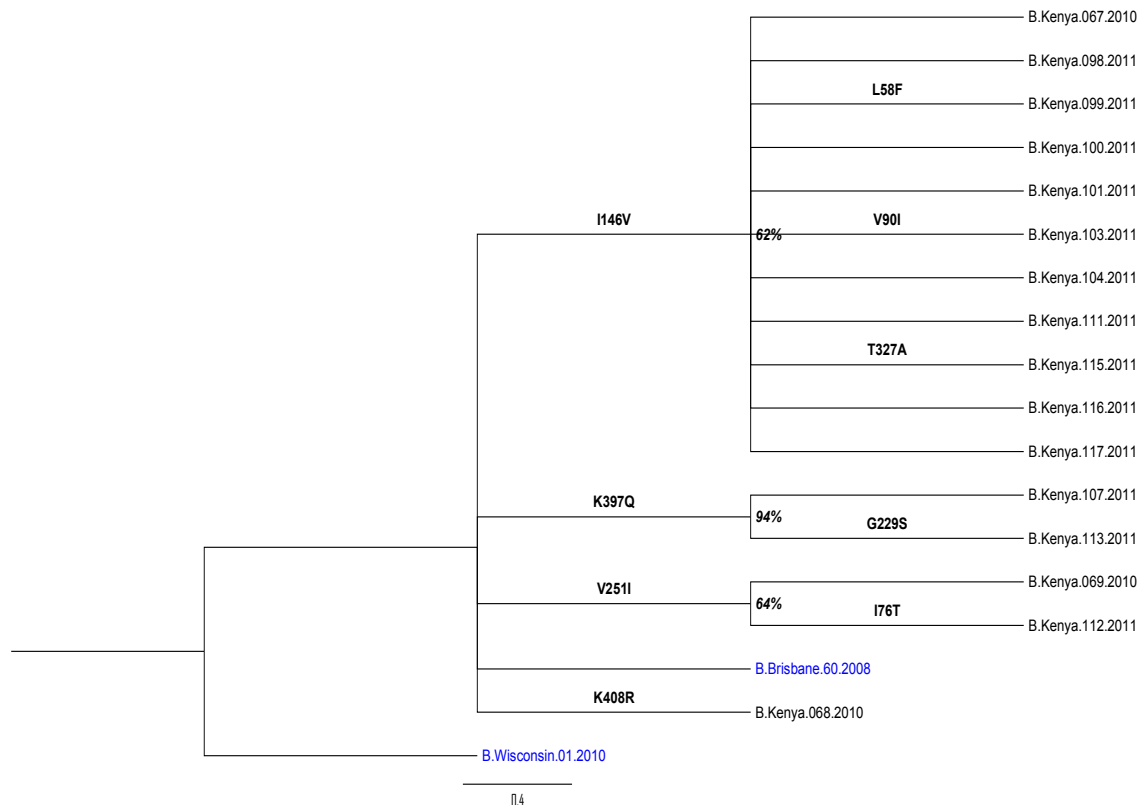
**Table 1:** Amino acid substitution in the HA1 hemagglutinin of Influenza B viruses isolated in Kenya during 2010 to 2011 season.

|                    | Amino acid substitution in the HA1 |    |    |     |     |     |     |     |     |     |
|--------------------|------------------------------------|----|----|-----|-----|-----|-----|-----|-----|-----|
|                    | 58                                 | 76 | 90 | 146 | 225 | 229 | 256 | 327 | 397 | 408 |
| B/Brisbane/60/2008 | L                                  | I  | V  | I   | V   | G   | G   | T   | K   | K   |
| B/Kenya/067/2010   | .                                  | .  | .  | V   | .   | .   | .   | .   | .   | .   |
| B/Kenya/068/2010   | .                                  | .  | .  | .   | .   | .   | .   | .   | .   | R   |
| B/Kenya/069/2010   | .                                  | .  | .  | .   | I   | .   | .   | .   | .   | .   |
| B/Kenya/098/2011   | .                                  | .  | .  | V   | .   | .   | .   | .   | .   | .   |
| B/Kenya/099/2011   | F                                  | .  | .  | V   | .   | .   | .   | .   | .   | .   |
| B/Kenya/100/2011   | .                                  | .  | .  | V   | .   | .   | .   | .   | .   | .   |
| B/Kenya/101/2011   | .                                  | .  | .  | V   | .   | .   | .   | .   | .   | .   |
| B/Kenya/103/2011   | .                                  | .  | I  | V   | .   | .   | .   | .   | .   | .   |
| B/Kenya/104/2011   | .                                  | .  | .  | V   | .   | .   | .   | .   | .   | .   |
| B/Kenya/107/2011   | .                                  | .  | .  | .   | .   | .   | .   | .   | Q   | .   |
| B/Kenya/111/2011   | .                                  | .  | .  | V   | .   | .   | .   | .   | .   | .   |
| B/Kenya/112/2011   | .                                  | T  | .  | .   | I   | .   | E   | .   | .   | .   |
| B/Kenya/113/2011   | .                                  | .  | .  | .   | .   | S   | .   | .   | Q   | .   |
| B/Kenya/115/2011   | .                                  | .  | .  | V   | .   | .   | .   | A   | .   | .   |
| B/Kenya/116/2011   | .                                  | .  | .  | V   | .   | .   | .   | .   | .   | .   |
| B/Kenya/117/2011   | .                                  | .  | .  | V   | .   | .   | .   | .   | .   | .   |

We analyzed 27 influenza A (H1N1) pdm09, 19 A (H3N2) and 16 influenza B viruses isolated from among human influenza viruses which circulated in the country during 2010-2011 season. These isolates are representative of all our sentinel surveillance sites.

### Mutations found in the Kenyan Influenza B viruses

Comparison of the HA1 amino acid sequences of influenza B viruses with the reference vaccine strain B/Brisbane/60/2008 (a B/Victoria/2/87-like virus) revealed substitutions at 10 amino acid positions (**Table 1**). Majority (11/16; 69%) of the Kenyan isolates had a I146V amino acid substitution within the 150-loop antigenic site. The other nine positions that showed polymorphisms were outside of the antigenic sites. These mutations that occurred outside of the antigenic sites were randomly distributed among the isolates. Phylogenetically, majority of Kenyan isolates (69%) clustered together and were characterized by the I146V amino acid change (**Figure 1**). Overall, Kenyan isolates clustered closely with B/Brisbane/60/2008 which was the WHO recommended vaccine strain for 2010-2011 season.



**Figure 1:** Phylogenetic relationship of HA1 hemagglutinin of representative influenza B viruses circulating in Kenya during 2010 to 2011 season in relation to the vaccine strains. The phylogenetic tree was constructed using Mr Bayes 3.1.2. The 2010-2011 northern hemisphere vaccine strain (B/Wisconsin/01/2010) belonging to the Yamagata lineage was used as an outgroup.

### Amino acid substitutions among the Kenyan Influenza A (H1N1) pdm09 viruses

Comparison of HA1 amino acid sequences of influenza A (H1N1) pdm09 Kenyan isolates to A/California/07/2009, the prototype vaccine reference strain identified six parallel amino acid substitutions, common to all the isolates (**Table 2**). They included P83S, D97N, S185T, S203T, I321V and E374K. Amongst these, the P83S change occurred at antigenic site Cb. In contrast, 10 other mutations seemed to occur stochastically in individual isolates. Amongst the non-parallel substitutions, changes including K164F in isolate A/Kenya/146/2011, S190G in isolate A/Kenya/148/2011 and D238E in isolate A/Kenya/138/2011 occurred at antigenic sites Sa, Sb and Ca<sub>1</sub> respectively. Overall, the Kenyan isolates had 17 amino acid substitutions when compared to the vaccine strain. Phylogenetically, all Kenyan isolates were monophyletic and clustered together separately from the vaccine strain on a branch characterized by the six parallel changes described above (**Figure 2**).

### Amino acid changes found in the Kenyan influenza A (H3N2) viruses

The Kenyan influenza A (H3N2) isolates had amino acid substitutions at fifteen disparate sites when compared to A/Perth/16/2009, the vaccine reference strain (**Table 3**). Out of these fifteen sites, all the Kenyan isolates had three amino acid substitutions (K62E, T212A and S214I) absent in A/Perth/16/2009. Amongst the three substitutions, the K62E substitution is present at antigenic site E. Interestingly; majority of the isolates (89.5%) contained an S45N amino acid

substitution. This residue is located at site C. Furthermore, the substitution at position 45 introduced an N-glycosylation site, which is absent in the reference strain. Two isolates, A/Kenya/008/2010 and A/Kenya/009/2010, had an L157S change. This amino acid residue is located at antigenic site B of the HA1. In this study, all Kenyan viruses isolated in 2010 had a K144N amino acid change. Residue 144 is located at antigenic site A and the substitution from a K to N introduces an N-glycosylation site. Similarly, all A (H3N2) viruses isolated in 2011 had a T128A and K144T amino acid substitutions. All the 2011 isolates had a substitution at position 142 (R142K and R142E in a 50-50 proportion) compared to A/Perth/16/2009. Two of influenza A (H3N2) viruses isolated in 2010 had additional T30A and G78D amino acid substitutions. The G78D amino acid change occurred at site E. Phylogenetic analysis of HA1 genes showed that the Kenyan isolates were distinct from the reference strain A/Perth/16/2009 and clustered on a branch away from this reference strain (**Figure 3**). Within this branch, there were two clusters. The first cluster contained majority of Kenyan isolates (90%), and were characterized by amino acid changes S45N and K144N. This group consisted of two sub-clusters. All viruses isolated in 2011 and characterized by T128A and K144T amino acid changes, clustered into a single group. The other sub-cluster contained viruses isolated in 2010. Viruses isolated in 2011 further formed two sub-groups, each characterized by R142K and R142E amino acid changes. The second cluster was minor (10%) as it comprised only two isolates which had L157S amino acid substitution.

**Table 2:** Comparison of HA1 hemagglutinin of influenza A(H1N1)pdm09 viruses circulating in Kenya during 2010 to 2011 season to the vaccine strains.

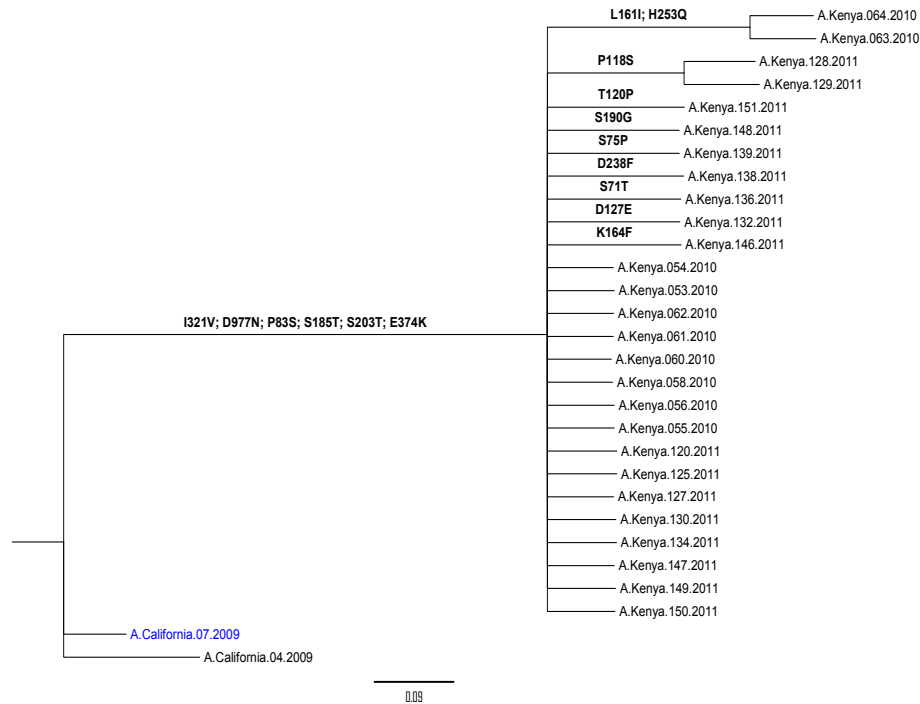
|                      | Amino acid substitution in the HA1 |    |    |    |     |     |     |     |     |     |     |     |     |     |     |     |
|----------------------|------------------------------------|----|----|----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
|                      | 71                                 | 75 | 83 | 97 | 118 | 120 | 127 | 161 | 164 | 185 | 190 | 203 | 238 | 273 | 321 | 374 |
| A/California/07/2009 | S                                  | S  | P  | D  | P   | T   | D   | L   | S   | S   | S   | S   | D   | H   | I   | E   |
| A/Kenya/054/2010     | .                                  | .  | S  | N  | .   | .   | .   | .   | .   | T   | .   | T   | .   | .   | V   | K   |
| A/Kenya/053/2010     | .                                  | .  | S  | N  | .   | .   | .   | .   | .   | T   | .   | T   | .   | .   | V   | K   |
| A/Kenya/064/2010     | .                                  | .  | S  | N  | .   | .   | .   | I   | .   | T   | .   | T   | .   | Q   | V   | K   |
| A/Kenya/063/2010     | .                                  | .  | S  | N  | .   | .   | .   | I   | .   | T   | .   | T   | .   | Q   | V   | K   |
| A/Kenya/062/2010     | .                                  | .  | S  | N  | .   | .   | .   | .   | .   | T   | .   | T   | .   | .   | V   | K   |
| A/Kenya/061/2010     | .                                  | .  | S  | N  | .   | .   | .   | .   | .   | T   | .   | T   | .   | .   | V   | K   |
| A/Kenya/060/2010     | .                                  | .  | S  | N  | .   | .   | .   | .   | .   | T   | .   | T   | .   | .   | V   | K   |
| A/Kenya/058/2010     | .                                  | .  | S  | N  | .   | .   | .   | .   | .   | T   | .   | T   | .   | .   | V   | K   |
| A/Kenya/056/2010     | .                                  | .  | S  | N  | .   | .   | .   | .   | .   | T   | .   | T   | .   | .   | V   | K   |
| A/Kenya/055/2010     | .                                  | .  | S  | N  | .   | .   | .   | .   | .   | T   | .   | T   | .   | .   | V   | K   |
| A/Kenya/120/2011     | .                                  | .  | S  | N  | .   | .   | .   | .   | .   | T   | .   | T   | .   | .   | V   | K   |
| A/Kenya/125/2011     | .                                  | .  | S  | N  | .   | .   | .   | .   | .   | T   | .   | T   | .   | .   | V   | K   |
| A/Kenya/127/2011     | .                                  | .  | S  | N  | .   | .   | .   | .   | .   | T   | .   | T   | .   | .   | V   | K   |
| A/Kenya/128/2011     | .                                  | .  | S  | N  | S   | .   | .   | .   | .   | T   | .   | T   | .   | .   | V   | K   |
| A/Kenya/129/2011     | .                                  | .  | S  | N  | S   | .   | .   | .   | .   | T   | .   | T   | .   | .   | V   | K   |
| A/Kenya/130/2011     | .                                  | .  | S  | N  | .   | .   | .   | .   | .   | T   | .   | T   | .   | .   | V   | K   |
| A/Kenya/132/2011     | .                                  | .  | S  | N  | .   | .   | E   | .   | .   | T   | .   | T   | .   | .   | V   | K   |
| A/Kenya/134/2011     | .                                  | .  | S  | N  | .   | .   | .   | .   | .   | T   | .   | T   | .   | .   | V   | K   |
| A/Kenya/136/2011     | T                                  | .  | S  | N  | .   | .   | .   | .   | .   | T   | .   | T   | .   | .   | V   | K   |
| A/Kenya/138/2011     | .                                  | .  | S  | N  | .   | .   | .   | .   | .   | T   | .   | T   | E   | .   | V   | K   |
| A/Kenya/139/2011     | .                                  | P  | S  | N  | .   | .   | .   | .   | .   | T   | .   | T   | .   | .   | V   | K   |
| A/Kenya/146/2011     | .                                  | .  | S  | N  | .   | .   | .   | .   | F   | T   | .   | T   | .   | .   | V   | K   |
| A/Kenya/147/2011     | .                                  | .  | S  | N  | .   | .   | .   | .   | .   | T   | .   | T   | .   | .   | V   | K   |
| A/Kenya/148/2011     | .                                  | .  | S  | N  | .   | .   | .   | .   | .   | T   | G   | T   | .   | .   | V   | K   |
| A/Kenya/149/2011     | .                                  | .  | S  | N  | .   | .   | .   | .   | .   | T   | .   | T   | .   | .   | V   | K   |
| A/Kenya/150/2011     | .                                  | .  | S  | N  | .   | .   | .   | .   | .   | T   | .   | T   | .   | .   | V   | K   |
| A/Kenya/151/2011     | .                                  | .  | S  | N  | P   | .   | .   | .   | .   | T   | .   | T   | .   | .   | V   | K   |

#### 4. Discussion

We found that viruses which circulated in Kenya during the 2010-2011 season were genetic variants of the WHO-recommended vaccine strains within the HA1 gene. Influenza B viruses were the least genetically variable viruses during the season. These viruses had only one major substitution at the antigenic site within the 150-loop. Influenza A (H3N2) viruses displayed the greatest genetic variation compared to the vaccine reference strain. Overall there were seven disparate amino acid substitutions amongst the Kenyan influenza A (H3N2) viruses affecting four of the five antigenic sites within the HA1 gene. Mutations which occur at antibody-receptor binding sites are antigenically

significant because they result in conformational change of the HA molecule, introducing or exposing novel epitopes allowing the virus to evade already existing host immunity (Bulimo et al, 2008).

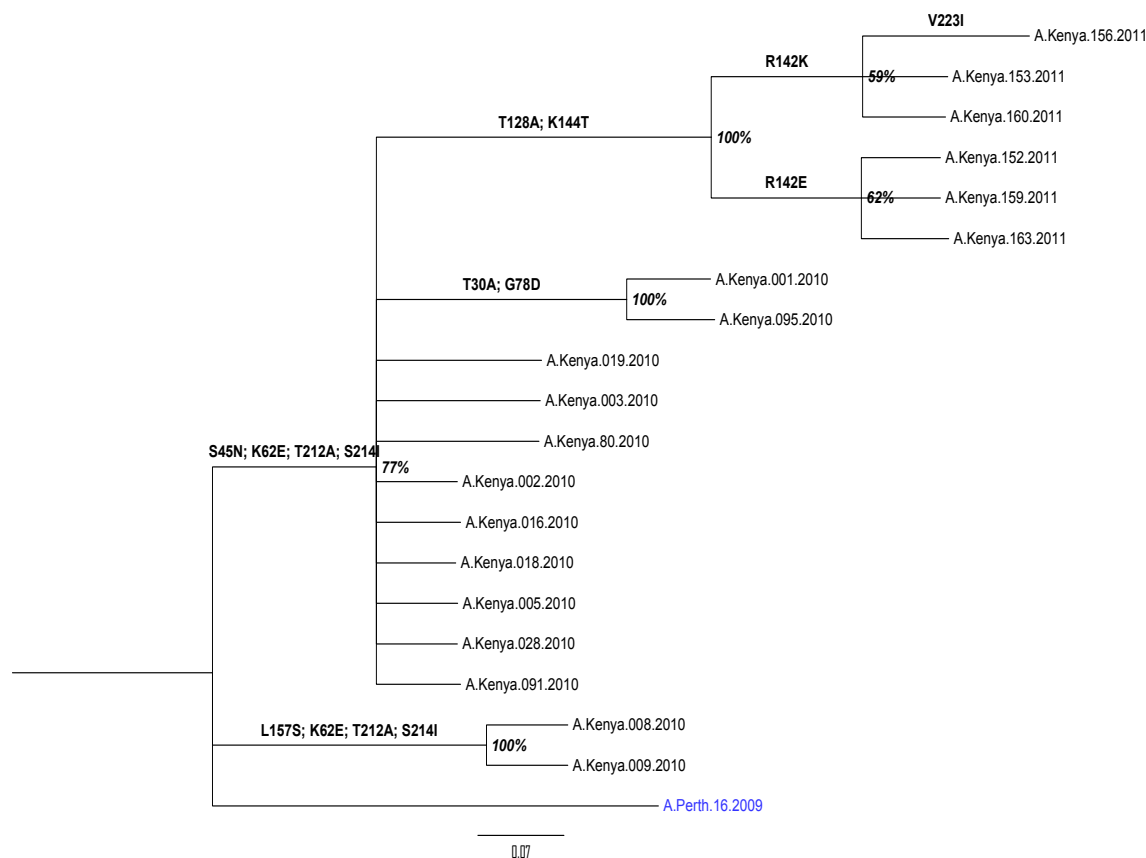
Two new N-glycosylation sites were introduced in HA1 gene of the Kenya A (H3N2) viruses. The first glycosylation was introduced at position 45 within the antigenic site C in majority (~90%) of the 2010-2011 viruses and the second at position 144 in all the 2010 isolates. Introduction of N-glycosylation motifs is also antigenically significant because the glycosyl units introduce new linear and conformational epitopes which interfere with pre-existing neutralizing antibodies to the antigenic sites (Skehel et al, 1984).



**Figure 2:** Phylogenetic relationship of HA1 hemagglutinin of representative influenza A(H1N1)pdm09 viruses circulating in Kenya during 2010 to 2011 season in relation to the vaccine strain. A/California/04/2009 was used as an outgroup. The phylogenetic tree was constructed using MrBayes.

**Table 3:** Comparison of amino acid sequences for the HA1 subunit of influenza A (H3N2) viruses circulating in Kenya during 2010 to 2011 season to the vaccine strain, A/Perth/16/2009.

|                  | Amino acid substitution in the HA1 |    |    |    |     |     |     |     |     |     |     |     |     |     |     |
|------------------|------------------------------------|----|----|----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
|                  | 71                                 | 75 | 83 | 97 | 118 | 120 | 127 | 161 | 164 | 185 | 190 | 203 | 238 | 273 | 321 |
| A/Perth/16/2009  | L                                  | T  | Q  | S  | K   | G   | T   | R   | K   | L   | T   | S   | I   | V   | S   |
| A/Kenya/001/2010 | .                                  | A  | .  | N  | E   | D   | .   | .   | N   | .   | A   | I   | .   | .   | .   |
| A/Kenya/002/2010 | .                                  | .  | .  | N  | E   | .   | .   | .   | N   | .   | A   | I   | .   | .   | .   |
| A/Kenya/016/2010 | .                                  | .  | .  | N  | E   | .   | .   | .   | N   | .   | A   | I   | .   | .   | .   |
| A/Kenya/019/2010 | I                                  | .  | .  | N  | E   | .   | .   | .   | N   | .   | A   | I   | .   | .   | .   |
| A/Kenya/008/2010 | .                                  | .  | .  | .  | E   | .   | .   | .   | N   | S   | A   | I   | .   | .   | .   |
| A/Kenya/018/2010 | .                                  | .  | .  | N  | E   | .   | .   | .   | N   | .   | A   | I   | .   | .   | .   |
| A/Kenya/005/2010 | .                                  | .  | .  | N  | E   | .   | .   | .   | N   | .   | A   | I   | .   | .   | .   |
| A/Kenya/003/2010 | .                                  | .  | K  | N  | E   | .   | .   | .   | N   | .   | A   | I   | .   | .   | .   |
| A/Kenya/009/2010 | .                                  | .  | .  | .  | E   | .   | .   | .   | N   | S   | A   | I   | .   | .   | N   |
| A/Kenya/028/2010 | .                                  | .  | .  | N  | E   | .   | .   | .   | N   | .   | A   | I   | .   | .   | .   |
| A/Kenya/080/2010 | .                                  | .  | .  | N  | E   | .   | .   | .   | N   | .   | A   | I   | T   | .   | .   |
| A/Kenya/091/2010 | .                                  | .  | .  | N  | E   | .   | .   | .   | N   | .   | A   | I   | .   | .   | .   |
| A/Kenya/095/2010 | .                                  | A  | .  | N  | E   | D   | .   | .   | N   | .   | A   | I   | .   | .   | .   |
| A/Kenya/152/2011 | .                                  | .  | .  | N  | E   | .   | A   | E   | T   | .   | A   | I   | .   | .   | .   |
| A/Kenya/153/2011 | .                                  | .  | .  | N  | E   | .   | A   | K   | T   | .   | A   | I   | .   | .   | .   |
| A/Kenya/156/2011 | .                                  | .  | .  | N  | E   | .   | A   | K   | T   | .   | A   | I   | .   | I   | .   |
| A/Kenya/159/2011 | .                                  | .  | .  | N  | E   | .   | A   | E   | T   | .   | A   | I   | .   | .   | .   |
| A/Kenya/160/2011 | .                                  | .  | .  | N  | E   | .   | A   | K   | T   | .   | A   | I   | .   | .   | .   |
| A/Kenya/163/2011 | .                                  | .  | .  | N  | E   | .   | A   | E   | T   | .   | A   | I   | .   | .   | .   |



**Figure 3:** Phylogenetic relationship of HA1 hemagglutinin of representative influenza A (H3N2) viruses circulating in Kenya during 2010 to 2011 season in relation to the vaccine strain, A/Perth/16/2009. The phylogenetic tree was constructed using MrBayes methods.

All the Kenyan influenza A (H1N1) pdm09 viruses isolated had six parallel mutations compared to the vaccine strain. Majority of these did not affect an antigenic site hence were antigenically neutral. However, one of these mutations occurred at position 83 and is found at the antigenic site Cb. We variably observed other mutations affecting antigenic sites Ca1 Sa and Sb but these were very few.

Thus, influenza A (H1N1) pdm09 viruses that circulated in Kenya during this period were not significantly divergent antigenically from the vaccine strain and the vaccine component was properly matched. These observations are supported by reports that influenza A(H1N1) pdm09 is antigenically stable (WHO, 2009). Overall, our analyses suggest that due to paucity of mutations affecting known antigenic sites amongst the influenza B and A (H1N1) pdm09 Kenyan viruses, the WHO-recommended vaccine components for these strains were appropriate for Kenya. Our conclusion about the suitability of these vaccine components is supported by the retention of these components by the WHO-vaccine recommendation group in the 2012 southern hemisphere vaccine formulation (WHO, 2012).

In September 2011, the WHO reported that majority of A (H3N2) viruses collected globally from February to August 2011 were antigenically closely related to the vaccine virus A/Perth/16/2009. Phylogenetic analyses of these using the HA genes, grouped the viruses into two phylogenetic clades represented by A/Perth/16/2009 and A/Victoria/208/2009, with the vast majority falling within the A/Victoria/208/2009

clade. However, all Kenyan A (H3N2) viruses we observed belonged to the A/Perth/16/2009 clade. Phylogenetic subgroups have emerged within both clades, two within the A/Perth/16/2009 clade and at least four within the A/Victoria/208/2009 clade. Viruses within all these subgroups remained antigenically similar to A/Perth/16/2009. These observations suggest that the Kenyan viruses were antigenically similar to vaccine strain and that the WHO formulation was appropriate for Kenya. Thus, similar to influenza B and A(H1N1)pdm09 components, our results show that although the influenza A(H3N2) viruses that circulated in Kenya during this period were largely genetically divergent from vaccine component, they remained antigenically similar and were appropriate.

The major limitation of this study is that we did not phenotypically analyze the antigenic properties of the viruses we isolated. However, analyses of HA1 genomic data alone are routinely applied to provide preliminary antigenic understanding of influenza viruses (Ellis et al, 1995; Lindstrom et al, 1996; Soltani et al, 2009).

## 5.0 Conclusion

Our genetic study provides evidence that the WHO vaccine strain recommendations for the southern hemisphere were appropriate for use in Kenya. The study also supports the policy by the Kenya Ministry of Health recommending usage of southern hemisphere WHO influenza vaccine strains formulation in the country. Future work related to this study will involve



antigenic analysis of the Kenyan viruses to determine whether the genetic variations seen among the Kenyan isolates translate to antigenic variants.

### Conflict of Interest declaration

The authors declare no conflict of interest

### Disclaimer

The opinions stated in this paper are those of the authors and do not represent the official position of the U.S. Department of Defense.

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