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ARTICLE

Pandemic Influenza A/H1N1 Virus Incursion into Africa: Countries, Hosts and Phylogenetic Analysis

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SUMMARY

Swine origin influenza A/H1N1 virus was first detected in Mexico in April 2009. It thereafter spread to over a hundred countries in five continents including Africa and was declared a pandemic by the WHO. The disease was estimated to have caused 18,500 laboratory-confirmed deaths worldwide among millions of infected people within the first year. Though Africa was late in detecting and reporting pandemic H1N1, information on the incursion of the virus into Africa and its molecular features are important for planning control measures between countries and to understand inter-host and genetic relationship of influenza viruses detected in Africa. We studied time and events of the detection of 2009 pandemic Influenza A/HIN1 virus in Africa and selected viral HA and NA gene sequences from the GenBank and GISAID databases. Nucleotide sequence alignment and construction of phylogenetic trees were carried out with MEGA version 5 bioinformatics software and the neighbor-joining ClustalW method with 1000 bootstrap replicates. Earliest human cases of pandemic H1N1 in Africa were detected by June 2009 in Egypt, Morocco, South Africa and later cases in Ghana, Nigeria and Cameroon, with varying phylogenetic clusters. Following cases of Influenza A/H1N1 pandemic in humans, infections in pigs earlier reported in America, Europe and Asia were also observed in Cameroon, Nigeria, Togo and Kenya, apparently transmitted from humans by reverse zoonoses. Adoption of an integrated, cross-sectoral and transboundary approach to zoonotic diseases surveillance and management in the context of One-Health in Africa is advocated. Africa particularly needs to be better prepared to prevent or mitigate outbreaks and spread of emerging diseases.

KEYWORDS: Influenza virus incursion, Pandemic, Africa, Phylogeny, One-Health

INTRODUCTION

The declaration of a pandemic caused by swine origin A/H1N1 influenza virus in 2009 by the World Health Organization (WHO, 2009) re-emphasized the importance of emerging infectious diseases in global public health and economy. Earlier pandemics of influenza occurred in 1918 (H1N1), 1957 (H2N2) and 1968 (H3N2) (Dowdle, 1999; Lina, 2008) but none had the devastating effect of 1918 Spanish flu that was reported to have killed approximately 50 million people (Taubenberger, 2006). Although cases of the 2009 pandemic were mild, the virus spread to 208 countries and territories infecting over 1 million people with 18,500 deaths recorded within the first year (Akhilesh *et al*., 2010; Dawood *et al*., 2012). The rapidity with which infectious diseases are transmitted and spread between countries in the 21st century is unprecedented. This is partly aided by globalization of trade and travels especially those involving international aircraft that move people and commodities, thereby carrying potentially infectious pathogens around the world (Kaferstein *et al*., 1997).

Swine origin influenza virus subtype A/H1N1 was first detected in Mexico and it spread across all continents (Smith *et al*., 2009). It took only few days from the index case in Mexico before the virus was reported in the United States of America (USA), apparently aided by international travels. Mexico and the USA share a very long border with over 600 flights between the two countries daily as well as heavy road traffic (Muula, 2009). In April 2009, the Director General of the World Health Organization raised the alert level of the influenza pandemic to phase five, an indication that the outbreak must be taken seriously because of its capacity to spread rapidly to every country in the world. Expectedly, within one month of circulation it was reported almost everywhere in the world except Africa. Similarly, the WHO influenza A (H1N1 swine flu) update of May 2009 recorded influenza H1N1 virus victims in many countries but none in Africa. However, before the end of 2009, two reports (Archer *et al*., 2009; CDC, 2009) emerged with data on confirmed swine flu cases from South Africa and Kenya indicating that the virus was circulating in Africa but was not reported as readily because of the lack of a rigorous surveillance system (Yazdanbakhsh and Kremsner, 2009). Based on this knowledge and the reports of transmission of pandemic H1N1 virus from humans to pigs in other parts of the world (Howden *et al*., 2009; Hofshagen *et al*., 2009; Pereda *et al*., 2010; Song *et al*., 2010), it is apparent that pandemic H1N1 constitutes a potential public health risk in Africa. This study was therefore aimed at investigating the introduction and spread of pandemic H1N1 virus in humans and animals in Africa by analyzing data on the molecular and phylogenetic features important for planning future interventions and pandemic preparedness strategies. The findings may have economic, public health and political implications for the rest of the world.

MATERIALS AND METHODS

We studied time and events of the detection of 2009 pandemic Influenza A/HIN1 virus in Africa as reported in published literature and databases. The gene sequences of novel A/H1N1 2009 pandemic viruses were retrieved from the National Center for Biological Information (NCBI) Influenza resources website - http://www.ncbi.nlm.nih.gov/genomes/flu/html (Bao *et al*., 2008) and the GISAID website - http://platform.gisaid.org/epi3/frontend621e1 (Butler, 2006). We searched the PubMed, Google Scholar, Scopus and other databases up till May 2015 using the search terms "influenza A/H1N1", "pandemic", "human", "swine" and countries in Africa to identify articles describing pandemic H1N1 cases. One hundred and fifteen haemagglutinin (HA) and 75 neuraminidase (NA) gene sequences were selected on the basis of earlier deposits in GenBank and GISAID, full or partial gene fragments, country of origin and host species. Multiple sequence deposits and short fragments were excluded. All positions containing gaps

and missing data were eliminated from the dataset (complete deletion option). There were a total of 1693 positions in the final dataset. A consensus for phylogenetic analysis was built by creating an agreement in the data which was achieved by deleting positions 1 – 138 and 1069 – 1752 of the HA gene while positions 1 – 598 and 1318 – 1410 were also deleted in the selected NA gene sequences. Sequences were thereafter aligned using MEGA version 5 bioinformatics software (Tamura *et al*., 2011) while evolutionary history was inferred using the Neighbour-Joining method (Saitou and Nei, 1987).The optimal tree with the sum branch length = 0.05397918 was drawn to scale, with branch lengths being in the same unit as those of the evolutionary distances used to infer the phylogenetic trees.

TABLE I: TIMELINE OF REPORTED CASES OF PANDEMIC INFLUENZA A (H1N1) IN AFRICA

S/N	Country	Date of Reporting	S/N	Country	Date of reporting
1	Egypt	2 June, 2009	24	Madagascar	12 August 2009
2	Morocco	10 June 2009	25	Congo DR	15 August 2009
3	South Africa	14 June 2009	26	Mozambique	17 August 2009
4	Cape Verde	18 June 2009	27	Djibouti	31 August 2009
5	Tunisia	18 June 2009	28	Lesotho	1 September 2009
6	Ethiopia	19 June, 2009	29	Malawi	10 September 2009
7	Algeria	20 June 2009	30	Rwanda	12 October, 2009
8	Cote d'Ivoire	24 June 2009	31	SaoTome/Principe	12 October 2009
9	Kenya (Human)	29 June 2009	32	Nigeria (Human)	29 October 2009
	Kenya (Swine)	5August 2011		Nigeria (Swine)	27 May 2011
10	Mauritius	29 June 2009	33	Sierra Leone	29 October 2009
11	Uganda	30 June 2009	34	Burundi	11 November 2009
12	Angola	July 2009	35	Somalia	13 November 2009
13	Tanzania	July 2009	36	Sudan	December 4, 2009
14	Libya	6 July 2009	37	Mauritania	12 December 2009
15	Seychelles	8 July 2009	38	Guinea	13 December 2009
16	Botswana	10 July 2009	39	Senegal	29 December 2009
17	Zimbabwe	10 July 2009	40	Mali	8 January 2010
18	Zambia	15 July 2009	41	Chad	29 January 2010
19	Namibia	20 July, 2009	42	Niger	2 February 2010
20	Gabon	July 26, 2009	43	Togo (Human)	May, 2010
				Togo (Swine)	01 June 2013
21	Swaziland	29 July 2009	44	Central African Republic	26 July 2010
22	Ghana	2 August 2009	45	Burkina Faso	November 2010
23	Cameroon (Human)	5 August 2009 /20			
	Cameroon (Swine)	January 2010			

TABLE II: AMINO ACID SUBSTITUTIONS IN THE HA GENE OF SELECTED PANDEMIC INFLUENZA A/H1N1 VIRUSES FROM AFRICA

Domain data	202	203	220	222	239	240	251	266	289	300	312	332	338	391
ACP41953.1|A/California/07/2009	S	A	S	R	D	Q	V	V	V	K	I	A	I	E
AFQ37281.1|A/swine/Nigeria/12VIR4047-09/2011	-	T	T	-	-	R	-	-	A	-	V	-	V	K
AEA02269.1|A/swine/Cameroon/11rs149-198/2010	-	-	T	-	-	-	I	-	-	-	-	-	V	-
AIO11674.1|A/swine/Togo/ONA32/2013	T	T	T	-	-	-	I	-	-	-	-	-	V	K
AHY20904.1|A/swine/Kenya/9455/2011	T	-	T	-	-	-	-	-	-	-	-	-	V	K
ADJ41785.1|A/Tunisia/1064/2010	-	-	T	-	**G**	-	-	-	-	-	-	-	-	-
ADM33099.1|A/Lagos/WRAIR1984N/2009	-	-	T	-	-	-	-	M	-	-	-	-	V	-
AEB98608.1|A/South Africa/4901/2009	-	-	T	-	-	-	-	-	-	-	-	-	V	-
AHH25243.1|A/Cameroon/10v-00413/2010	-	-	T	-	E	-	-	-	-	-	-	-	V	-
AFE03092.1|A/Angola/75/2009	-	-	T	-	E	-	-	-	-	-	-	-	-	-
AHY84620.1|A/Uganda/MUWRP-059/2009	-	-	T	-	-	-	-	-	-	-	-	-	V	-
AHH25290.1|A/Ghana/FS-09-1306/2009	-	-	T	-	E	-	-	-	-	-	-	-	V	-
EPI278570|A/Algeria/G350/2009	-	-	T	-	E	-	-	-	-	-	-	-	V	K
	-	-	-	-	-	-	-	-	-	-	-	-	V	-
EPI255219|A/Djibouti/N11092/2009	-	-	T	-	-	-	-	-	-	-	V	-	V	-
EPI235550|A/Egypt/109/2009	-	-	T	-	D	-	-	-	-	-	-	-	-	-
EPI278017|A/Bamako/WR2361N/2009	-	-	T	-	D	-	-	-	-	-	-	-	-	-
EPI516981|A/Togo/LNG/344/2013	T	-	T	-	D	-	I	-	-	-	-	-	-	K

RESULTS

Literature search and information gathered from gene sequences retrieved from public databases revealed that earliest human cases of pandemic H1N1 in Africa were reported by June 2009 in Egypt, Morocco and South Africa with later cases in Ghana, Nigeria, Mali and other West African countries (Table I). As at December 2011, shortly after the peak of the pandemic, 35 countries in Africa officially reported pandemic H1N1 to the World Health Organization. In all, 18,598 laboratory-confirmed human cases of pandemic (H1N1) 2009 including 168 deaths were recorded in Africa (WHO, 2012). Based on curated data from literature, 45 countries in Africa experienced pandemic H1N1 outbreaks. Cases of the pandemic later subsided, as was also the general decline in number of cases reported in other parts of the world. Interestingly, a number of cases were seen in domestic pigs in countries that have had earlier outbreaks of pandemic H1N1 in humans.

A few amino acid substitutions at the HA cleavage site between position 200 to 400 were observed while fewer substitutions were also seen in the antiviral resistance region of the NA gene between amino acid position 200 and 450 compared with the prototype A/California/07/2009 (Table II and III). These substitutions were notably at positions 202, 222, 299 and 240 for the HA gene (Table II) and positions 200, 248, 351 and 369 for the NA gene. Most isolates from individual countries

clustered together on the phylogenetic tree but few countries like Egypt and Ghana had more than one cluster (Fig 1). Isolates from Nigeria, Togo and Kenya where pandemic H1N1 was detected in humans and swine also clustered on the phylogenetic tree.

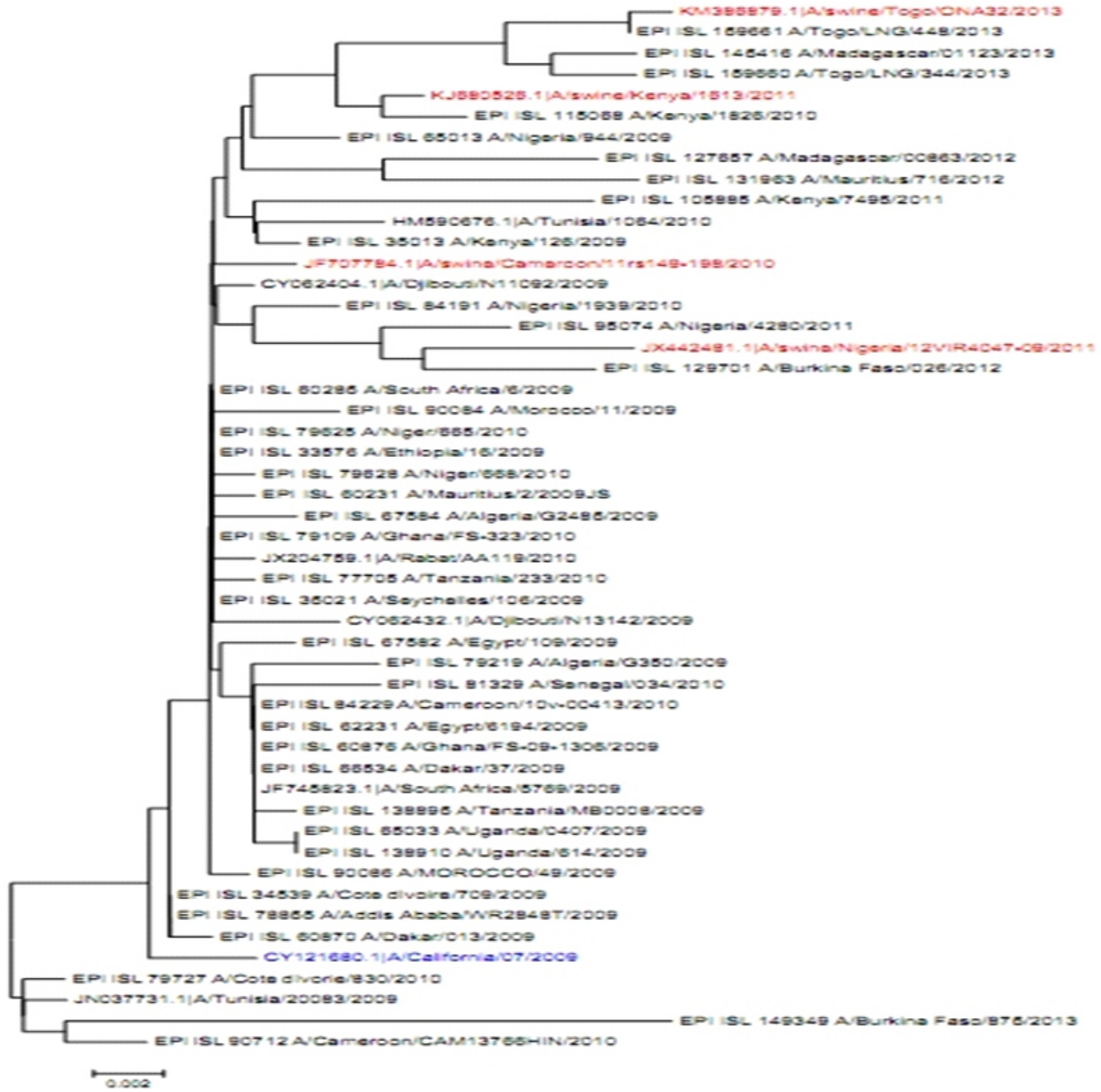
TABLE III: AMINO ACID SUBSTITUTIONS IN THE NA GENE OF SELECTED PANDEMIC INFLUENZA A/H1N1 VIRUSES FROM AFRICA

Domain data	200	241	248	257	313	351	369	385	386	398	416	426
ACT36688.1|A/California/07/2009	N	V	N	R	Q	Y	N	N	N	E	D	L
AFQ37281.1|A/swine/Nigeria/12VIR4047-09/2011	-	-	D	-	-	F	-	-	-	-	-	-
AEA02269.1|A/swine/Cameroon/11rs149-198/2010	-	-	D	-	-	F	-	-	-	-	-	-
AIO11674.1|A/swine/Togo/ONA32/2013	S	I	D	-	H	F	-	-	-	-	-	-
AHY20904.1|A/swine/Kenya/9455/2011	-	I	D	-	-	F	-	-	-	-	-	-
AEH94006.1|A/Tunisia/1064/2010	-	-	D	-	-	F	-	-	-	-	-	-
ADM33101.1|A/Lagos/WRAIR1984N/2009	-	-	D	-	-	F	-	-	-	-	-	-
EPIISL159660_A/Togo/LNG/344/2013	S	I	D	-	-	F	K	-	-	-	-	-
EPIISL84229_A/Cameroon/10v-00413/2010	-	-	D	-	-	F	-	-	-	-	-	-
EPIISL79121_A/Ghana/FS-09-1306/2009	-	-	D	-	-	F	-	-	-	-	-	-
EPIISL79219_A/Algeria/G350/2009	-	-	D	-	-	F	-	-	-	-	-	-
EPIISL34539_A/Cote_dIvoire/709/2009	-	-	D	-	-	F	-	-	-	-	-	-
EPIISL67582_A/Egypt/109/2009	-	-	D	-	-	F	-	-	-	-	-	Q
EPIISL33576_A/Ethiopia/16/2009	-	-	D	-	-	F	-	-	-	-	-	-
EPIISL68458_A/Madagascar/9551/2009	-	-	D	-	-	F	-	-	-	K	-	-
EPIISL77196_A/Zambia/CZC1/2009	-	-	D	-	-	F	-	-	-	-	-	-
EPISL78853_A/Bamako/WR2361N/2009	-	-	D	-	-	F	-	-	-	-	N	-
EPIISL60284_A/South_Africa/2/2009	-	-	D	-	-	F	-	-	-	-	-	-

DISCUSSION

The index case of pandemic H1N1 was reported in Mexico in April 2009 (Smith *et al*., 2009). Few months afterward, Egypt became the first country in Africa to report cases of the pandemic (Ahmed *et al*., 2011). This was closely followed by reported cases in Morocco, South Africa, Cape Verde and Ethiopia while the West African countries of Nigeria, Senegal, Mali, Chad and Togo detected cases in their territories much later (Table I).

According to the World Health Organization, the number of deaths reported by its member States during the influenza A (H1N1) pandemic of 2009/2010 was usually based on laboratory confirmation and widely considered a gross underestimation because, among other reasons, deaths may have occurred without being recognized or tested (WHO, 2011a). Disproportionate number of cases and deaths resulting from infectious diseases often occur in under-developed countries with limited or no laboratory capacity for diagnosis. In Africa, poor surveillance and documentation with ineffective detection in some countries may have caused under-reporting of the 2009/2010 pandemic and resulted in a casualty figure of less than 200 compared to the global fatality of over 18,500 deaths (WHO, 2011a; Grace *et al* 2012; Dawood *et al*., 2012; Meseko *et al*., 2013). However, the wide media attention attracted by the pandemic influenza virus outbreak improved general awareness and encouraged more countries to carry out surveillance for cases particularly at international borders and in hospitals/clinics that served as sentinel sites (Dalhatu *et al*., 2012).


KM386879.1|A/swine/Togo/CNA32/2013
EPI ISL 169661 A/Togo/LNG/448/2013
EPI ISL 145416 A/Madagascar/01123/2013
EPI ISL 169660 A/Togo/LNG/344/2013
KJ590525.1|A/swine/Kenya/1513/2011
EPI ISL 115089 A/Kenya/1826/2010
EPI ISL 65013 A/Nigeria/944/2009
EPI ISL 127657 A/Madagascar/00663/2012
EPI ISL 131963 A/Mauritius/716/2012
EPI ISL 105995 A/Kenya/7495/2011
HM590676.1|A/Tunisia/1064/2010
EPI ISL 35013 A/Kenya/126/2009
JF707794.1|A/swine/Cameroon/11rs149-199/2010
CY062404.1|A/Djibouti/N11092/2009
EPI ISL 84191 A/Nigeria/1939/2010
EPI ISL 95074 A/Nigeria/4280/2011
JX442491.1|A/swine/Nigeria/12VIR4047-09/2011
EPI ISL 129701 A/Burkina Faso/026/2012
EPI ISL 60286 A/South Africa/6/2009
EPI ISL 90094 A/Morocco/11/2009
EPI ISL 79625 A/Niger/665/2010
EPI ISL 33576 A/Ethiopia/16/2009
EPI ISL 79628 A/Niger/668/2010
EPI ISL 60231 A/Mauritius/2/2009JS
EPI ISL 67584 A/Algeria/G2486/2009
EPI ISL 79109 A/Ghana/FS-323/2010
JX204759.1|A/Rabat/AA119/2010
EPI ISL 77705 A/Tanzania/233/2010
EPI ISL 35021 A/Seychelles/106/2009
CY062432.1|A/Djibouti/N13142/2009
EPI ISL 67592 A/Egypt/109/2009
EPI ISL 79219 A/Algeria/G350/2009
EPI ISL 81329 A/Senegal/034/2010
EPI ISL 84229 A/Cameroon/10v-00413/2010
EPI ISL 62231 A/Egypt/6194/2009
EPI ISL 60876 A/Ghana/FS-09-1306/2009
EPI ISL 66534 A/Dakar/37/2009
JF745923.1|A/South Africa/5769/2009
EPI ISL 139896 A/Tanzania/MB0008/2009
EPI ISL 65033 A/Uganda/0407/2009
EPI ISL 139910 A/Uganda/614/2009
EPI ISL 90086 A/MOROCCO/49/2009
EPI ISL 34539 A/Cote d'Ivoire/709/2009
EPI ISL 78865 A/Addis Ababa/WR2848T/2009
EPI ISL 60870 A/Dakar/013/2009
CY121680.1|A/California/07/2009
EPI ISL 79727 A/Cote d'Ivoire/830/2010
JN037731.1|A/Tunisia/20083/2009
EPI ISL 149349 A/Burkina Faso/876/2013
EPI ISL 90712 A/Cameroon/CAM13766HIN/2010
0.002

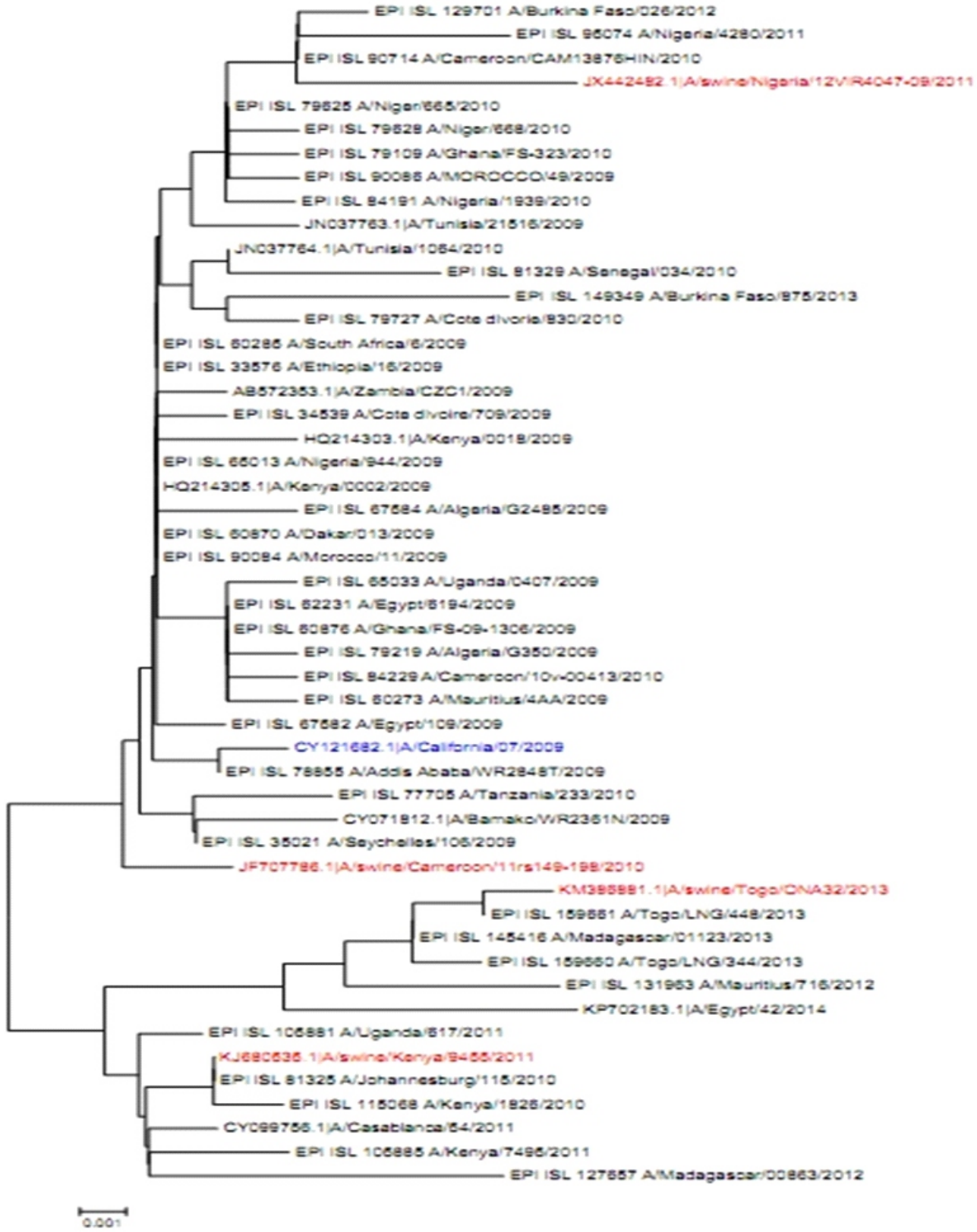


Fig 1: Phylogenetic tree based on amino acid sequences of haemagglutinin (A) and neuraminidase (B) genes of selected pandemic influenza A/H1N1 strains from Africa

Molecular sequences obtained from the NCBI and GISAID databases of pandemic (H1N1) viruses that circulated in Africarevealed certain amino acid substitutions, which were significantly different from the prototype A/California/07/2009. These include mutations at positions 220 and 338 for HA, and positions 248 and 351 for NA, with potential implications for transmission and pathogenicity requiring further investigation. One notable mutation was D222G observed in the HA gene of pandemic (H1N1) 2009 virus isolated from a fatal case in Tunisia. This substitution was observed in a 47 year-old man who died after 3 days without other subjacent pathologies or any known risk factor (Moussi *et al*., 2012). If this mutant virus is transmitted to an animal host, a different pathological and evolutionary scenario may also be presented.

Different clusters of the virus on phylogenetic trees generated using sequences within and between countries indicate a likelihood of dual or multiple sources of infection. Interestingly, the swine isolate from Nigeria clustered together with human isolates from neighbouring Cameroon and Ghana, and another human isolate from Nigeria, suggesting a common origin. This is also similar to observed clustering of human and swine viruses detected in Togo and Kenya (Fig. 1).

Following cases of pandemic influenza A/H1N1 virus in humans, infections were also reported in pigs in America, Asia and Europe (Smith *et al*., 2009). In Africa, Cameroon, Nigeria, Kenya and Togo with earlier cases of H1N1 in humans also reported pandemic H1N1 in free-range and commercial pigs (Bao *et al*., 2008; Njabo *et al*., 2012; Meseko *et al*., 2014; Ducatez *et al*., 2015). According to Peiris *et al.* (2012), cases of pandemic influenza H1N1 in pigs could have originated from humans. This phenomenon of reverse zoonoses is possible in any of the countries and at the human-animal interface where livestock farming is carried out with regular intermingling between people and animals (Howden *et al*., 2009; Nelson & Vincent, 2015).

The index case virus of the influenza (H1N1) 2009 pandemic, described as a lineage of swine influenza virus, has been described as a product of reassortment among avian, swine and human variants of influenza virus (Garten *et al*., 2009). Intermingling of different species of domestic animals with each other and with human handlers in poor biosecurity settings favours such reassortment. Thus, global pandemic preparedness efforts need to take into cognizance this interplay at the human-animal interface.

At the beginning of the outbreak, the virus was initially called swine influenza, which resulted in objectionable dispositions to pigs in some countries. However, FAO/OIE/WHO tripartite committee on pandemic influenza was quick to modify the description of the novel virus from initial swine influenza to the current nomenclature, influenza A (H1N1) pdm09 (WHO, 2011b). This was also to allay initial confusion that the virus was directly transmitted to humans from pigs at the time of the outbreak in April 2009. Nevertheless, authorities in some countries like Egypt already ordered the slaughter of all pigs, a directive that may not be without religious and political influence (ABC News, 2009; Kerr, 2010). The adverse economic consequence of mass culling of livestock in disease control efforts would no doubt jeopardize livelihood if not carefully implemented.

CONCLUSION

The next epidemic or pandemic of influenza may be caused by a reassortant of human seasonal influenza, pandemic or classical swine influenza virus. Poor surveillance, detection and disease reporting that are major problems in Africa may worsen emerging diseases scenario. This may also jeopardize food security and endanger public health,and should therefore be viewed more seriously by relevant authorities in the continent in the context of One-Health.

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