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Original Research Article

MOLECULAR ANALYSIS OF INFLUENZA A (H3N2) AND A (H1N1) PDM09 VIRUSES CIRCULATING IN THE DEMOCRATIC REPUBLIC OF CONGO, 2014

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Abstract: Background: Very little is known about influenza viruses circulating in the Democratic Republic of Congo (DRC). We aim to characterize genetically and antigenically Influenza A (H3N2) and A (H1N1) pdm09 viruses circulating in the country. **Methods:** From August to December 2014, specimens were collected from patients with influenza like-illness (ILI) or severe acute respiratory infection (SARI) in various surveillance sites. Specimens were tested using real time reverse transcription polymerase chain reaction (RT-PCR) method for the detection of influenza viruses. Positive influenza samples with a cycle threshold (Ct) <30 were genetically and antigenically characterized. **Results:** 32 samples tested were found positive to influenza A with Ct<30. At CDC Atlanta, 28 out of 32 samples (88%) were tested positive for influenza A virus, including 26 seasonal influenza A viruses subtype H3N2 and 2 pandemic influenza A viruses subtype H1N1pdm 2009. The majority of influenza A (H3N2) viruses were antigenically related to the A/Switzerland/9715293/2013 vaccine virus, while two influenza A (H1N1) pdm09 isolates were antigenically characterized as A/California/07/2009-like. All A (H3N2) and A (H1N1) pdm09 virus isolates characterized were sensitive to oseltamivir and zanamivir.
Conclusion: Two genetically distinct influenza subtypes were co-circulating in the DR Congo. Effective measures against influenza have been suggested.

Keywords: Molecular, Analysis, Influenza viruses, DRCongo

Introduction: Influenza is a contagious respiratory disease caused by the influenza virus, a member of the Orthomyxoviridae family of RNA viruses which have a segmented genome. There are three types of influenza viruses (A, B and C), originally distinguished on the basis of their internal nucleoprotein and matrix proteins. Influenza virus A and B cause annual, recurrent outbreaks in tropical and sub-tropical countries leading to high morbidity and mortality [1], especially in children and the

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elderly. It is estimated that one million deaths each year are due virus infection [2]. According to the World Health Organization (WHO), about20%of all deaths among children aged<5years are attributable to influenza-like illnesses (ILI) [3], and the majority of those deaths occur in Africa and Southeast Asia [3, 4]. Seasonal influenza viruses are a population of virus variants, mainly existing as a result of antigenic drift, the accumulation of point mutations in the hemagglutinin (HA) and neuraminidase (NA) surface glycoprotein due to the lack of virus RNA polymerase complex pro of reading activity [5]. The HA is known to be the main antigenic determinant of the host's neutralizing immune response[6,7,8]and is under selective pressure for antigenic shift ,the process by which new virus variants emerge from genetic reassortment among influenza viruses infecting human and animal populations. Antigenic shift results in virus evasion of the preexisting immunity of the host[9].In addition, genetic mechanisms like nucleic acid substitution, gene insertion and deletion have been reported to contribute with influenza virus variability[9, 10].

Previous studies have shown that influenza is a major contributor of respiratory illness in tropical and subtropical regions worldwide, with more complex patterns of influenza seasonality in Africa and southern Asia [11, 12, and 13]. Tropical countries closer to the equator have year-round influenza activity without a distinct seasonal peak [14, 15, 16, and 17]. However, South Africa, Zambia [18], and Senegal [19] seem to have well-defined patterns of seasonality similar to other countries with temperate climates[17]. Furthermore, Madagascar described a yearly circulation of influenza with two distinct peaks, one that corresponds to the northern hemisphere winter (January–March)and a second that corresponds to the southern hemisphere winter (June–October) [18].Unfortunately, the limited data available from sub-Saharan Africa makes it difficult to comprehensively map and define the seasonality of influenza in the region. The seasonality of influenza coupled with virus genetic and antigenic variation present challenges to the selection of potential virus candidates to be used in the formulation of influenza vaccines. Timely and geographically comprehensive virologic surveillance involving genetic and antigenic analysis of circulating strains is critical to maintaining the effectiveness of the influenza vaccine [20].

Although some countries, such as Madagascar and Senegal, have led national influenza surveillance for many years [21,22], several countries in the African continent began conducting influenza surveillance recently .In DRCongo, the influenza surveillance system based on laboratory analysis was introduced in 2008 and reported that influenza is present year-round associated with some peaks related to the rainy season [23]. Despite the increased interest on improving influenza surveillance in Africa, the available genetic and epidemiological data are still insufficient to understand disease dynamics in the region. Due to the limited knowledge on influenza virus dynamics in Africa and to contribute in the improvement of the DRC surveillance system, we sought to identify and characterize for the first time the influenza viruses circulating in the Democratic Republic of Congo during the period from August to December of 2014.

Materials and Methods

Ethical Considerations: Specimens and data were collected as part of national public health sentinel surveillance systems, which is considered a non research activity. Influenza sentinel surveillance protocols were adapted from World Health Organization (WHO) guidelines with support from the national influenza program at the DRCongo Ministry of health. This protocol was implemented as part of routine public health surveillance by the Ministry of Health and was therefore considered a service and not subject to human subjects review. Before collecting each specimen, physicians explained the purpose of the surveillance system. After that, patients could refuse to participate. Oral consent was documented in the patient's study records. Specimens and data were processed anonymously.

Study area and Sample collection: Nasal, throat and nasopharyngeal swab samples from all enrolled patients presenting with severe acute respiratory infectious (SARI) or influenza-like-illness(ILI)were collected from various surveillance sites selected in DRC and sent to the Influenza National Reference Laboratory at INRB using3-mL cryovials containing Viral Transport Medium (Copan diagnostics-Murrietaca-USA).

Throat and nasopharyngeal swab specimens were collected from the same patient; both swabs were placed in the same cryovial. The specimens were kept refrigerated at 4°C, until they were packaged using a triple packaging system at the sentinel site. The specimens were then sent in refrigerated cool boxes to the INRB within 72 hours after packaging, where they were divided into 3aliquots.One aliquot was used for molecular analysis ;two others were stored at−80°C for further analysis and safeguard.

Laboratory analysis

RNA extraction: Ribonucleic acid (RNA) extractions were performed at INRB, after dividing each sample in three cryovials containing 1 ml aliquots. One aliquot was immediately used for RNA extraction, while the other two aliquots were storedat-80°Cfor future use. Nucleic acid was extracted from 140 µl of aliquot, using the QIAamp®ViralRNA Mini Kit (Qiagen,Germany) according to the manufacturer's instructions. Purified RNA was stored at−80°C in aliquots.

Amplification by Reverse Transcription Polymerase Chain Reaction (RT-PCR)

The RT-PCR technique is used as a rapid and sensitive method for the detection of influenza viruses in clinical and virus isolate samples. All samples were screened for influenza A and B viruses by one step real- time reverse transcription polymerase chain reaction. The influenza A one –step real-time RT-PCR was performed using primers for matrix gene (forward: 5'-GAC CRA TCCTGT CACCTCTGAC -3'; reverse: 5'-AGGGCA TTY TGG ACA AAK CGT CTA -3'; probe: 5'-FAM-TGCAGT CCT CGCTCACTG GGCACG-MGB-3') provided by the CDC in Atlanta, GA and according to the provided CDC protocol. Type A and B influenza matrix genes were amplified.

Reaction mixtures (25µL) were prepared using the Ag- Path-ID^{TM}One-Step RT-PCR Kit (Ambion, USA) and contained final concentrations: 12.5µl of2X RT-PCR Buffer, 0.25µl of 40µM reverse primer, 0.25 µl of40µM forward primers, 0.25 µl of10µM of specific probe, of1µl of25XRT –PCR enzyme (Taq DNA polymerase and reverse transcriptase) and 5 µl of nucleic acid. Amplification was performed on ABI7500 Fast Real-time PCR System (Applied Biosystems, USA) with the following cycling conditions: 40ºC for 10 min, 95ºC for10 min and 95ºC for 15 s followedby55ºC for 60s (45cycles).Each specimen was also tested for the human ribonuclease P gene (constitutive gene) as an internal control. Screening for influenza B was simultaneously done by Flu–B matrix gene specific primers and probe. The positive controls for each virus were made of RNA extracts from non infectious material for use with the CDCRT-PCR method for detection and characterization of human seasonal influenza viruses. Water was used as a negative control.

Subsequently ,influenza A positive samples were tested to identify the influenza subtypes A(H3N2) or A (H1N1)pdm09 using a single one step real-time RT-PCR with six pairs of primers and probes for the specific detection of influenza A subtypes. The specific HA genes for each influenza A subtype were amplified: seasonal A(H1N1)and A(H3N2),pandemic A(H1N1)pdm09 and avian A(H5N1) and A (H7N9) according to CDC protocol[24].Influenza isolates with a Cycle threshold (Ct <30) were frozen and shipped in dry ice to the CDC in Atlanta for genetic and antigenic characterization.

Inoculation into Cell Culture

Among all 32 samples sent to the CDC Atlanta, 23 specimens contained sufficient volume to be inoculated into Madin Darby Canine Kidney (MDCK) or MDCK SIAT-1 cell culture.

Hemagglutination and Neuraminidase Inhibition Assays: The antigenic analysis using

the hemagglutination inhibition (HI) assay was performed on all virus isolates having sufficient hemagglutination (HA) titer. Viruses were tested by HI with post-infection ferret antisera to the vaccine strain as well as representative reference viruses from circulating genetic groups. Influenza A (H3N2) viruses were tested with 0.75% guinea pig red blood cells (RBCs) in the presence of 20 µM oseltamivir and A (H1N1) pdm 09viruseswere tested with 0.5%turkeyRBCs.

The neuraminidase inhibition assay was performed to assess the virus susceptibility to the NA inhibitors oseltamivir and zanamivir, as previously described [25].

Sequencing and Genetic Characterization

In collaboration with the INRB, selected influenza A (H3N2) and A (H1N1) pdm09 virus isolates from DRCongo were sent to CDC in Atlanta for sequencing and genetic characterization. The influenza genome was amplified with SuperScript III One- Step RT-PCR with Platinum Taq High Fidelity(Invitrogen) using the Uni/Inf primer set as previously described [26].Indexed paired-end libraries were generated from the amplicons by utilizing Nextera XT Sample Preparation Kit(Illumina, San Diego, CA) according to manufacturer'sprotocol. Libraries were sequenced on the Illumina MiSeqwith a MiSeq v2 300 cycle kit.

Reads were assembled and consensus sequences were generated using Iterative Refinement Meta-Assembler (IRMA) [27].

Phylogenetic analyses were done to genetically characterize influenza virus isolates collected at the DRC and to compare them with the vaccine strains recommended by WHO during the 2014-2016 influenza seasons. First, reference tree data sets for A(H1N1)pdm09andA(H3N2) viruses were created by selecting reference viruses from WHO vaccine selection meeting reports since 2012 and their HA nucleotide sequences were down loaded from the Global Initiative on Sharing Avian Influenza (GISAID) database [28] , as shown in the Supplementary Table 1.In addition, GISAID was searched to download the HA gene sequences of A(H1N1)pdm09 and A(H3N2)virus isolates collected in Africa from August to December, 2014.Virus HA sequences from the DRC and other African countries were aligned against the A(H3N2) or A(H1N1)pdm09 WHO reference data sets using MUSCLE [29]and trimmed to the beginning of the mature HA protein gene sequence using Bio Edit v7.2.5 [30].

The TREESUB phylogenetic program [31] was used to estimate maximum like lihood phylogenetic trees using RAxML and PAML, followed by branch annotation of amino acid substitutions. Briefly, stop codons were trimmed from the aligned sequences in BioEdit and the vaccine virus was placed at the top of the alignment to be used as the tree out group and reference sequence. The general time reversible + Γ(GTR+GAMMA) nucleotide substitution model was selected in RAxMLv.7.3.0f or tree inference [32].Ancestral codon substitutions for each gene were estimated using base ml, as implemented in PAML [33], using the ML trees inferred. Non synonymous substitutions were then transcribed on to the consensus gene phylogenies and visualized in FigTreev1.4.2 [34].).Nucleotide sequences from DR Congo used in this study were entered into the GISAID Epi flu database and accessions are provided in the SupplementaryTable2.

Results

From August to December 2014, 806 samples were collected in DR Congo from which 61were positive for influenza A(Table1,Figure1).During this reporting period, no influenza B specimens were detected(Table1).Only 32 of the 61 influenza A positive samples from different surveillance sites in DRC had a Ct< 30 and these samples were selected and shipped to CDC in Atlanta for antigenic and genetic characterization .Results from this study showed that among the 32 samples tested: 26 were influenza A(H3N2),2 were influenza A(H1N1)pdm09, 2 had insufficient volume for testing and 2 samples were negative for influenza by RT-PCR, as shown in Table 2.

A (H3N2) influenza virus

The majority of A (H3N2) influenza viruses did not have sufficient HA titer to be antigenically characterized by HI. Either full HA sequencing or limited pyrosequencing was done on viruses

with insufficient HA titer for HI testing for genetic characterization only. Limited pyrosequencing was also done on A (H3N2) viruses not recovered in cell culture. Among the four A (H3N2) viruses tested by HI, one was antigenically similar to the A/Texas/50/2012vaccine virus, while three were poorly inhibited by ferret antisera raised against A/Texas/50/2012(Table2). The majority of influenza A (H3N2)viruses tested from the DRC were antigenically related to the A/Switzerland/9715293/2013 vaccine virus .All A(H3N2) viruses tested from DRC were sensitive to oseltamivir and zanamivir.

The H3 phylogenetic tree was divided into7genetic groups based on shared amino acid (AA) changes when compared to the previous vaccine strain A/Perth/16/2009 (data not shown).Genetic analysis shows that genetic group3(V223I) was further divided into subgroups 3A (N144D,N145S,D487N),3B(N145S,A198S,N312S,D487N) and 3C (A198S,N312S).Viruses from group 3C(S45N,T48I) diverged into genetic subgroups 3C.1,3C.2(Q33R,N145S,N278K,D489N) and 3C.3 (Q33R,T128A,R142G,N145S,N278K). Figure2 shows the H3N2 phylogenetic tree since the divergence of groups 3C.2and3C.3 with A/Texas/50/2012vaccine virus as out group. The amino acid differences against A/Texas/50/2012 reference virus are shown in Table3.Group3C.2was divided into two genetic subgroups:3C.2a(L3I,N144S,F159Y,K160T, N225D,Q311H) and3C.2b(with a A changes found in group 3C.2a, except for F159Y). Group3C.3 was split into subgroups 3C.3a (A138S, F159S, N225D, and K326R) and 3C.3b (E62K, K83R, N122D, L157S, R261Q, V347K). Sequencing and phylogenetic analysis of H3N2 isolates from DRC confirmed that these viruses belonged to genetic group 3C. 2a (n=6) and 3C.3 (n=2), as depicted in Table 3 and Figure 2.

A(H1N1)pdm09influenzaviruses

Two influenza A(H1N1)pdm09 isolates from the DR Congo were antigenically characterized as A/California/07/2009-like,the recommended A(H1N1)pdm09 component of the northern and southern hemisphere influenza vaccine formulations since 2009.The A(H1N1) pdm09 phylogenetic tree was divided into 9 major genetic groups(data not shown).The majority of the A(H1N1)pdm09 viruses isolated worldwide in 2014 belonged to genetic group6.Genetic group 6 (D97N,S185T,S203T,E374K,S451N) had diverged into subgroups 6A(H138R,V249L),6B(K163Q,A256T,K283E,E499K)and6C(V2341, K283E,E 499K).The A(H1N1)pdm09 DRC isolates(n=2)were classified as group6B viruses and are represented inTable4.AllA(H1N1)pdm09 viruses tested from DRC were sensitive to oseltamivir and zanamivir.

Discussion:

The main objective of this study was to identify and characterize influenza viruses circulating in the Democratic Republic of Congo in 2014.To the best of our knowledge, this kind of work has never been done in the DRCongo, while other African countries like Madagascar, Morocco and Egypt, have worked on the improvement of their surveillance system capacity to understand the molecular epidemiology and disease burden caused by circulating influenza viruses [35].

Our findings showed that the influenza activity in DRCongo increased during the month of November of 2014, reaching its peak on December the same year. Of note is that influenza activity was concomitant with the September-April rainy season in DRC, as previously described for the 2009-2011seasons [36].Similar results were found in Senegal (Dakar), Côte d'Ivoire (Abidjan), and Niger (Niamey) probably because of lower temperatures, as described elsewhere for Niger in 2010[36].This same pattern has also been observed in the north eastern part of tropical Brazil (Fortaleza) [37].

A total of 32 virus samples collected at the DRC from August to December of 2014 were shipped to the CDC in Atlanta, from which 20 were genetically characterized by either full genome

sequencing or limited pyrosequencing. Limited pyrosequencing was done in A(H3N2) virus isolates which had insufficient HA titer for HI testing or when viruses were not recovered in cell culture.

Sixteen of the A (H3N2) viruses isolated at the DRC belonged to genetic group3C.2a and two isolates to group3C.3.Four of these isolates were antigenically similar to the A/Switzerland/9715293/2013 virus, the recommended A (H3N2) vaccine component during the 2015-2016 influenza season. In 2013, the majority of A (H3N2) viruses tested was antigenically similar to the A/Texas/50/2012vaccine virus. After the WHO northern hemisphere vaccine selection meeting in February 2014, antigenic drift was detected in Viruses from genetic group 3C, giving rise to subgroups 3C.2a and 3C. 3a. Ferret antisera raised against A/Texas/50/2012vaccine virus showed a reduction in HA titer, with the concomitant worldwide increase of 3C.2a and3C.3a groups from 2014 to date. Despite the genetic diversity, the majority of 3C.2a and 3C.3a viruses were antigenically similar to the new A/Switzerland/9715293/2013vaccine virus.

Other A (H3N2) HA sequences from African countries, including Algiers, Dakar, Madagascar, Ethiopia, Ghana and Tanzania, available from the GISAID Epiflu database, were classified as 3C.3a, and one isolate from Egypt was found to cluster with other 3C.3b viruses (figure2).No DRC A (H3N2) isolates from genetic groups3C.3a and3C.3b were collected in 2014.However, similarly to the DRC, 3C.3viruses were also found in Zambia and Kenya after GISAID Epi flu database searching. Based on the close proximity of East African countries with3C.3a viruses in circulation, we expect the further expansion of the 3C.3a group to the DRC.

Two A (H1N1) pdm09 isolates from the DRC were classified under genetic group6 B and were antigenically indistinguishable to the A/California/07/2009 virus, which remains the WHO A (H1N1) pdm09 influenza vaccine component since 2009.The A (H1N1) pdm09 HA sequences from Ethiopia and Egypt, also available from the GISAID Epiflu database, clustered with other 6B virus sequences as well. Interestingly, Ghana was the only African country found to have viruses from genetic group 6 C in circulation since the summer of 2014 and the reason why further expansion was not observed at the time remains to be elucidated.

Ferret antisera raised against the A/California/07/2009vaccine virus showed reduced HA titers with a small number of isolates during the2014-2015 influenza season. These A (H1N1) pdm09 low-reactors often had amino acid changes in HA positions153-157andmay have acquired these changes through cell culture, which is consistent with results obtained since May2009. African isolates were not found to have amino acid changes in HA positions 153-157, confirming that no antigenic change has taken place, as established by the antigenic analysis data.

Conclusion

Influenza A(H3N2) and A(H1N1)pdm09 viruses have continued to circulate worldwide since their emergence and are expanding at high levels in most countries. Molecular surveillance year-round, especially in sub and tropical areas of Africa, is critical for improving vaccine strategies for the prevention and control of influenza illness. It is also important in contributing to the understanding of genetic variations and in the early detection of antigenic drift.

References:

1. LiCK, Choi BC, Wong TW. Influenza related deaths and hospitalizations in Hong Kong:a sub tropical area. Public Health.2006; 120(6):517-524. doi: 10.1016/j.puhe.2006.03.004
2. Pan American Health Organization. Technical Advisory group(TAG) Meeting on Vaccine Preventable-Diseases, 2004.Finalreport of the XVITAG meeting on vaccine preventable-diseases of the Pan American Health Organization, Mexico City, Mexico, November 3-5, 2004.Available athttp://www.paho.org/English/AD/FCH/IM/TAG16_FinalReport_2004.pdf.
3. World Health Organization. Influenza-associated burden of disease in low and

middle income countries – Examples from Kenya, Bangladesh and China. WHO Peer-reviewed literature. 2012. Available at http://www.who.int/influenza/surveillance_monitoring/updates/2012_05_10_update_GIP_peer_reviewed/en/.
4. Williams BG, Gouws E ,Boschi-Pinto C, Bryce J, Dye C. Estimates of world-wide distribution of child deaths from acute respiratory infections. Lancet Infect Dis 2002;2: 25–32.
5. Webster RG, Laver WG, Air GM, Schild GC. Molecular mechanisms of variation in influenza viruses.Nature1982; 296(5853):115-121.
6. Laver WG, Air GM, Webster RG. Mechanism of antigenic drift in influenza virus .Amino acid sequence changes in an antigenically active region of Hong Kong (H3N2) influenza virus hemagglutinin. JMolBiol1981;145(2):339–361.
7. Sleigh MJ, Both GW, Underwood PA, etal. Antigenic drift in the hemagglutinin of the Hong Kong influenza subtype: correlation of amino acid changes with alterations in viral antigenicity. JVirol1981;37(3):845–853.
8. GW, Sleigh MJ, Cox NJ, Kendal AP. Antigenic drift in influenza virus H3 hemagglutininfrom 1968 to 1980: multiple evolutionary pathways and sequential amino acid changes at key antigenic sites. JVirol1983;48(1):52–60.
9. Lin YP, Gregory V, Bennett M, Hay A. Recent changes among human influenza viruses.VirusRes.2004; 103(1-2):47–52.
10. Buonagurio DA, Nakada S, Parvin JD, Krystal M, Palese P, Fitch WM. Evolution of human influenza A viruses over 50years: rapid, uniform rate of change in NS gene. Science 1986;232(4753):980–982.
11. Shek LP, Lee BW. Epidemiology and seasonality of respiratory tract virus infections in the tropics. Paediatr Respir Rev2003; 4(2):105–111.
12. Viboud C, Alonso WJ, Simonsen L. Influenza in tropical regions. PLoS Med2006; 3(4):e89.
13. Hirve S, Newman LP, Paget J, Azziz-Baumgartner E Fitzner J, Bha N, et al. Influenza Seasonality in the Tropics and Subtropics -When to Vaccinate?PLoSOne.2016;11(4):e0153003. doi: 10.1371/journal.pone.0153003
14. Beckett CG ,Kosasih H, Ma'roef C, Listiyaningsih E, Elyazar IR, Wuryadi S, et al. Influenza surveillance in Indonesia: 1999–2003. Clin Infect Dis. 2004; 39:443–449.
15. Gachara G, Ngeranwa J, Magana JM, Simwa JM, Wango PW, Lifumo SM, et al. Influenza virus strains in Nairobi, Kenya. JClin Virol.2006; 35(1):117–118.
16. Hampson AW. Epidemiological data on influenza in Asian countries. Vaccine. 1999; 17(Suppl.1):S19–S23.
17. Saha S, Chadha M, AlMamun A, Rahman M, Strum-Ramirez K, Chittaganpitch M, et al. Influenza seasonality and vaccination timing in the tropical and subtropical areas of southern and south-eastern Asia.Bull World Health Organ. 2014; 92(5):318-333. doi: 10.2471/BLT.13.124412
18. Gessner BD, Shindo N,Briand S. Seasonal influenza epidemiology in sub-Saharan Africa: a systematic review. Lancet Infect Dis. 2011;11(3):223–235 doi: 10.1016/S1473-
19. Mbayame Ndiaye N, Annick D , Kader Ndiaye, Monique Sagna, Victoria Gregory, Deborah Goudiaby, Alan Hay, and Ousmane M. Diop. Sentinel Surveillance for Influenza in Senegal, 1996–2009. JInfectDis. 2012; 206 (Suppl 1):S129-135.
20. Cox NJ, Bai ZS, Kendal AP. Laboratory-based surveillance of influenza A(H1N1) and A(H3N2) viruses in 1980–81: antigenic and genomic analyses. Bull World Health Organ.1983; 61(1):143–152.
21. Rabarijaona LP, Rakotondrarija NT, RoussetD, Soares JL, Mauclere P. Influenza epidemiologic and virologic surveillance in Antananarivo from 1995 to 2002. Arch Inst Pasteur Madagascar.2003; 69(1-2):20–26.
22. Razanajatovo NH, Richard V,Hoffmann J,et al.Viral etiology of influenza- like illnesses in Antananarivo,Madagascar,July2008 to June 2009.PLoSOne 2011;6(3):e17579.Doi:10.1371/journal.pone.

0017579
23. Muyembe Tamfum JJ, Nkwembe E, BiShamamba SK ,Bankoshi F,Ilunga BK, Katz KA,et al.Sentinel surveillance for influenza-like illness, severe acute respiratory illness, and laboratory-confirmed influenza in Kinshasa, Democratic Republic of Congo, 2009–2011.JInfectDis. 2012; 206(Suppl 1):S36-40. doi: 10.1093/infdis/jis537
24. Enami K,Qiao Y,Fukuda R, Enami M. An influenza virus Temperature-sensitive mutant defective in the nuclear-cytoplasmic transport of the negative-sense viral RNAs. Virology1993; 194(2):822–827. doi: 10.1006/viro.1993.1324
25. Okomo-Adhiambo M, Mishin VP,SleemanK,Saguar E,GuevaraH, Reisdorf E,et al. Standardizing the influenza neuraminidase inhibition assay among United States public health laboratories conducting virological surveillance. Antiviral Res. 2016;128:28-35.doi: 10.1016/j.antiviral.2016.01.009
26. Zhou B,Wentworth D. Influenza A virus molecular virology techniques. Methods MolBiol.2012;865:175-192. doi: 10.1007/978-1-61779-621-0_11
27. Shepard S. et al. Viral deep sequencing needs an adaptive approach: IRMA, the iterative refinement meta-assembler.BMC Genomics 2016.17:708.doi: 10.1186/s12864-016-3030-6.
28. www.gisaid.org
29. Edgar RC. MUSCLE: multiple sequence alignment with high accuracy and high through put.Nucleic Acid Res. 2004; 32(5):1792–1797. doi: 10.1093/nar/gkh340
30. Hall TA. BioEdit: auser-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT.Nucl Acids Symp Ser.1999; 41:95-98.
31. https://github.com/tamuri/treesub
32. Stamatakis A.RAxML-VI-HPC: Maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models.Bioinformatics.2006; 22(21):2688–2690. doi:10.1093/bioinformatics/btl446
33. YangZ.PAML4: phylogenetic analysis by maximum like lihood .Mol Bio Evol.2007; 24(8):1586-1591.
34. http://tree.bio.ed.ac.uk/software/fig tree
35. World Health Organization. Report ofthe1st Africa Flu Alliance Meeting, Marrakesh, Morocco. June 3-4, 2010. Available at http://www.who.int/influenza/resources/documents/2010_06_3_afa_mtg_marrakesh_mor
36. Jusot JF, Adamou L, Collard JM. Influenza transmission during a one year period (2009–2010) in a Sahelian city: low temperature plays a major role. Influenza Other RespirViruses.2012; 6(2):87-89. doi: 10.1111/j.1750-2659.2011.00286.x
37. Moura FE, Perdigao AC, Siqueira MM. Seasonality of influenza in the tropics: a distinct pattern in northeastern Brazil. Am JTropMedHyg.2009; 81(1):180–183.

Table 1. *DR Congo samples analyzed for influenza by RT-PCR, August to December 2014*

Month	Number of NegativeSamples	Number of Positive Samples	Total	A/H1N1pdm09	A/H3N2	A/unsubtypable
August	118	1	119	1		
September	160	0	160			
October	153	1	154		1	
November	190	6	196	2	4	
December	124	53	177		40	13
	745	**61**	**806**	**3**	**45**	**13**

Table2. *Samples from DRCongo characterized at the CDC Atlanta*

InfluenzaSubtype	lumberofsample	Characterization
A(H1N1)pdm09	2	A/California/07/2009-likeA(H1N1)pdm09
	1	Insufficient volume for testing
A(H3N2)	1	A/Texas/50/2012-likeA(H3N2)GP
	3	A/Texas/50/2012-likeA(H3N2)
	12	Influenza A(H3N2)
	10	Influenza A(H3)
	1	Insufficient volume for testing
Negative	2	Negative by PCR for influenza

Table3. RepresentativeH3aminoacidchangesandgeneticcharacterizationofDRCisolatescomparedto*A/Texas/50/2012*vaccinevirus

HAGroup	StrainName	AminoAcidAnnotationand NumberingafterSignalPeptide[a]																																								
		3	4	38	53	56	62	78	83	94	122	124	128	138	140	142	144	145	157	159	160	168	171	173	186	188	192	198	199	214	219	225	261	262	311	326	347	402	489	510	529	Numberof
3C.1	*A/Texas/50/2012(2013-15Vaccine)*	L	P	N	D	H	E	G	K	Y	N	S	N	A	I	R	N	N	L	F	K	M	N	Q	V	D	I	P	S	I	F	N	R	S	Q	K	V	V	D	G	V	*AADifferences*
3C.2a	**A/Congo/2471/2014Dec**	I	P/T										T				S	S		Y	T		K		G			S			S	D			H				N			14
	A/Congo/2538/2014Dec	I											T				S	S		Y			K		G			S			S	D			H				N			12
	A/Congo/2461/2014Dec	I											T				S	S		Y	T		K		G			S			S	D			H			I	N			14
	A/Congo/2544/2014Dec	I											T				S	S		Y	T		K		G	E		S			S	D			H				N			14
	A/Congo/2458/2014Dec	I											T				S	S		Y	T		K		G			S			S	D			H				N			13
	A/Congo/2495/2014Dec	I											T				S	S		Y	T		K		G			S			S	D			H				N			13
	A/Algiers/249/2014Dec	I											T				S	S		Y	T				G			S			S	D			H				N			12
	A/Mauritius/853/2014Oct	I											T				S	S		Y	T				G			S			S	D			H				N		I	13
	A/Tanzania/594/2014Dec	I											T				S	S		Y	T	V			G			S			S	D			H				N			13
	A/Tanzania/3565/2014Nov	I											T				S	S		Y	T	V			G			S			S	D			H				N			13
	A/Mauritius/912/2014Nov												T				S	S		Y	T				G			S			S	D	Q		H				N			12
3C.3	**A/Congo/2611/2014Dec**			S								N	A			G		S							G			S			S									E		9
	A/Congo/2557/2014Dec	I			N							N	A			G		S							G		V	S			S											10
	A/Zambia/04-303/2014Nov											N	A			G		S						H	G			S			S											8
	A/Zambia/04-288/2014Dec											N	A			G		S						H	G			S			S											8
	A/Zambia/06-024/2014Sep											N	A			G		S							G			S			S											7
	A/Kenya/127/2014Aug							D					A		R	G		S							G			S	I/L		S											9
3C.3a	***A/Switzerland/9715293/2013 (2015Vaccine)***												A	S		G		S		S					G			S			S	D				R						10
	A/Madagascar/3069/2014Oct									H/Y			A	S		G		S		S					G			S			S	D				R						11
	A/Algiers/08/2014Oct												A	S		G		S		S					G						S	D				R						9
	A/Tanzania/1906/2014Dec					H/Y	E/K			H			A	S		G		S		S					G			S			S	D				R						13
	A/Tanzania/3580/2014Nov												A	S		G		S		S					G			S			S	D		N		R						11
	A/Ethiopia/1147/2014Oct									H			A	S		G		S		S					G			S			S	D										10
	A/Ethiopia/1153/2014Nov									H			A	S		G		S		S					G			S			S	D										10
	A/Ghana/DILI-14-1014/2014Nov												A	S		G		S		S					G						S	D							N			9
	A/Ghana/DILI-14-1152/2014Dec												A	S		G		S		S					G						S	D							N			9
	A/Dakar/30/2014Oct												A	S		G		S		S					G			S			S	D										9
3C.3b	*A/Egypt/4870/2014Nov*						K		R		D		A			G		S	S						G			S			S		Q				K					12

[a]Onlyaminoaciddifferences foundinvirusisolates fromAfricaareshown

Table4. Representative H1pdm09 aminoacid changes and genetic characterization of DRC isolates compared to *A/California/07/2009* vaccine virus

HAGroup	StrainName	AminoAcidAnnotationandNumberingafterSignalPeptide[a]																									TotalNumberof
		30	48	83	84	97	116	163	183	185	186	195	203	222	223	234	256	257	283	321	361	374	379	401	451	496	499	AADifferences
	A/California/07/2009Vaccine	V	A	P	S	D	I	K	S	S	A	A	S	D/G	Q/R	V	A	M	K	I	Y	E	V	E	S	N	E	
6B	**A/Congo/2278/2014Nov**			S	N	N		Q		T		V	T	D	Q		T		E	V		K			N	T	K	16
	A/Congo/2275/2014Nov			F		N		Q		T			T	D	Q		T		E	V		K		D			K	13
	A/Ethiopia/63/2014Nov			S		N		Q		T			T	D	Q		T		E	A/V		K			N		K	14
	A/Ethiopia/66/2014Nov		P	S		N		Q		T			T	D	Q		T		E	V	F/Y	K			N		K	15
	A/Ethiopia/1149/2014Oct		P	S		N		Q		T			T	D	Q		T		E	V		K			N		K	14
	A/Egypt/4372/2014Oct			S		N		Q	P/S	T			T	D	Q		T		E	V		K			N		K	14
	A/Egypt/4758/2014Oct			S		N		Q		T			T		Q		T		E	V		K	A		N		K	13
	A/SouthAfrica/3626/2013Jun			S		N		Q		T			T	D	Q		T		E	V		K			N		K	15
6C	*A/Ghana/FS-14-1022/2014Nov*			S		N	M			T	T		T	D	Q	I	T		E	V		K			N		K	15
	A/Ghana/FS-14-1025/2014Nov	A		S		N	M			T	T		T	D	Q	I			E	V		K			N		K	15
	A/Ghana/FS-14-1069/2014Dec	A		S		N	M			T	T		T	D	Q	I		V	E	V		K			N		K	16
	A/Ghana/Dili-0567/2014Jun	A		S		N	M			T	T		T	D	Q	I		V	E	V		K			N		K	16

[a]Only aminoacid differences found in virus isolates from Africa are shown

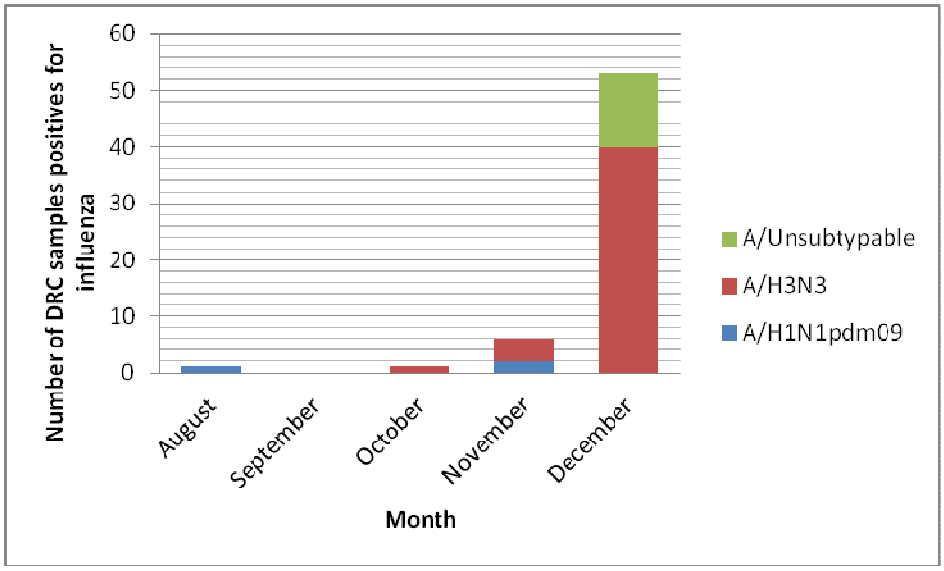

Figure1. Number of DRCongo isolates positive for influenza and characterized at the CDC Atlanta.Thirty-two DRC samples which were positive for influenza A and had Ct <30 were selected for antigenic and genetic characterization.

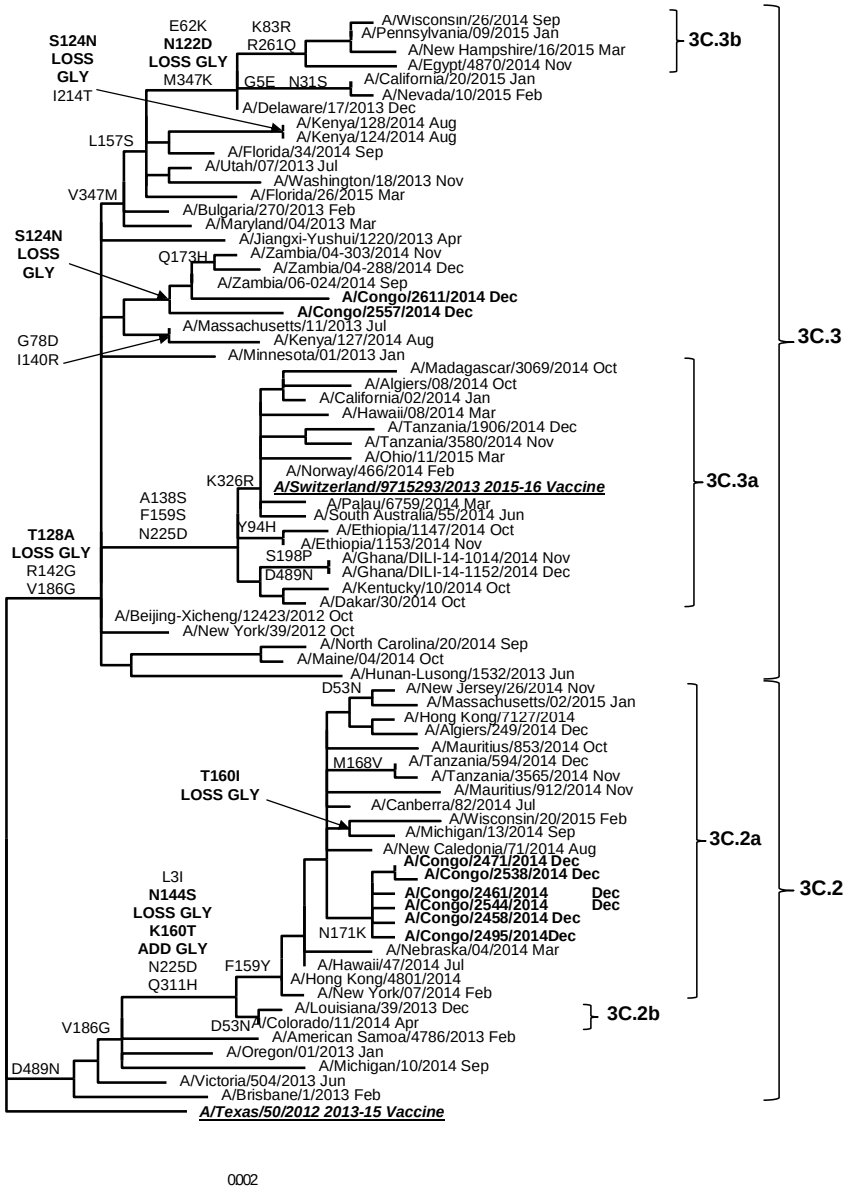

Figure 2. *Representative hemagglutinin ML phylogenetic tree of A(H3N2) viruses collected since 2013. The* A/Texas/50/2012*(*underlined, bold*) was used as reference in TREESUB to estimate MLphylogenetic trees via RAxML and PAML, and for automated annotation of aminoacid substitutions in the nexus format. The consensus HA tree and the transcribed aminoacid substitutions were visualized in Fig Tree. The scalebar represents the average number of nucleotide substitutions per site. Genetic groups 3C.2 and 3C.3, with the irrespective subgroups are represented in the tree. DR Congo isolates(*bold*) grouped with3C.2a and 3C.3 reference viruses. Other African isolates clustered with the* A/Switzerland/9715293/2013*(*underlined, bold*) vaccine virus from genetic group3C.3a.*

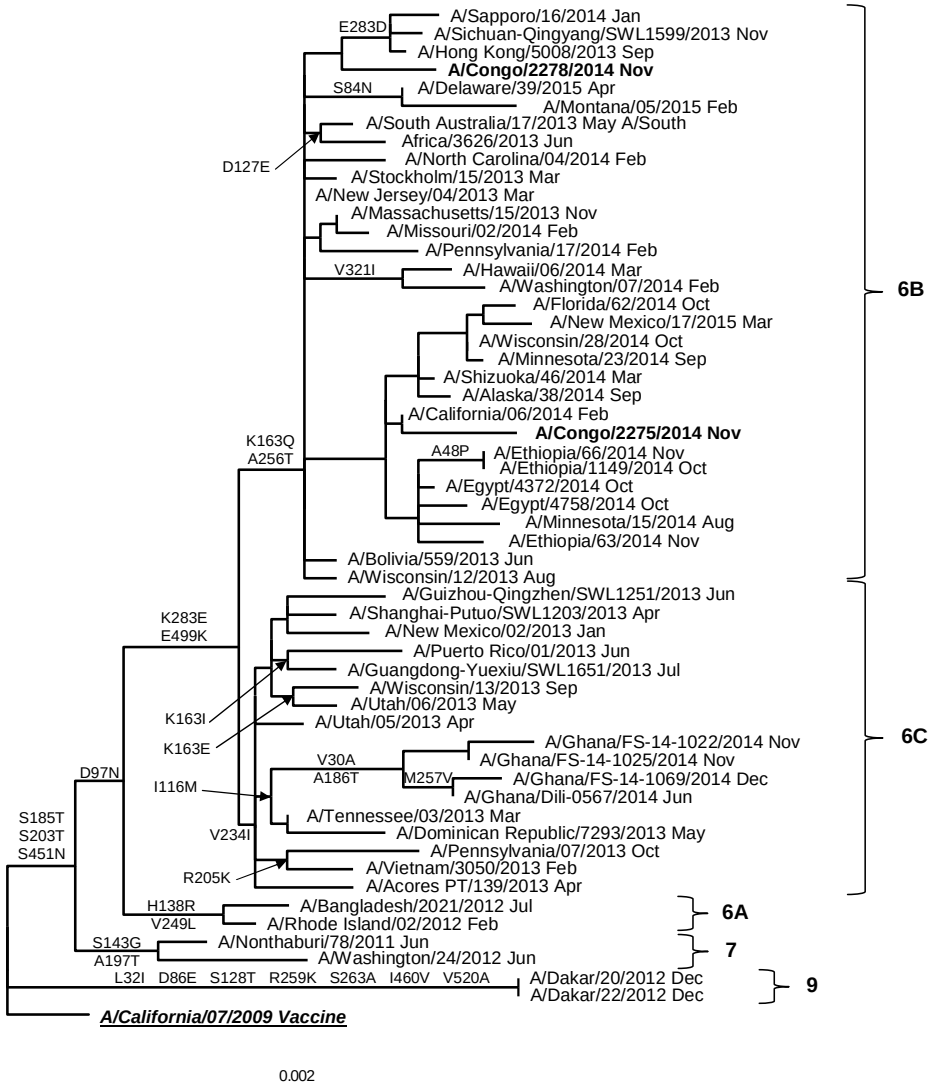

Figure 3. *Representative hemagglutinin MLphylogenetic tree of A(H1N1)pdm09 viruses collected since 2009.The* A/California/07/2009*(*underlined, bold*) was used as reference in TREESUB to estimate MLphylogenetic trees via RAxML and PAML, and for automated annotation of aminoacid substitutions in the nexus format. The consensus HA tree and the transcribed aminoacid substitutions were visualized in Fig Tree. The scalebar represents the average number of nucleotide substitutions per site. Genetic groups 6, 7 and 9 are depicted in the tree. DRCongo isolates (*bold*) clustered with viruses from genetic subgroup 6B.*

Supplementary Tables

Supplementary Table1. Influenza virus sequences downloaded from the GISAID Epiflu database for the phylogenetic analysis

GISAID Epiflu ID	Isolate_Name	Subtype	Collection_Date	Passage	Submitting_Lab
EPI_ISL_173571	A/Alaska/38/2014	H1N1pdm09	2014-09-03	C3	Centers for Disease Control and Prevention
EPI_ISL_131353	A/Bangladesh/2021/2012	H1N1pdm09	2012-07-21	C1/C4	Centers for Disease Control and Prevention
EPI_ISL_145499	A/Bolivia/559/2013	H1N1pdm09	2013-06-08	C2	Centers for Disease Control and Prevention
EPI_ISL_158707	A/California/06/2014	H1N1pdm09	2014-02-03	C1	Centers for Disease Control and Prevention
EPI_ISL_159428	A/California/07/2009	H1N1pdm09	2009-04-09	E3	Centers for Disease Control and Prevention
EPI_ISL_188710	A/Delaware/39/2015	H1N1pdm09	2015-04-13	C1	Centers for Disease Control and Prevention
EPI_ISL_145504	A/Dominican Republic/7293/2013	H1N1pdm09	2013-05-01	C2	Centers for Disease Control and Prevention
EPI_ISL_178484	A/Egypt/4372/2014	H1N1pdm09	2014-10-04	C3/C2	Centers for Disease Control and Prevention
EPI_ISL_178482	A/Egypt/4758/2014	H1N1pdm09	2014-10-20	C3/C2	Centers for Disease Control and Prevention
EPI_ISL_172641	A/Ethiopia/1149/2014	H1N1pdm09	2014-10-30	C1	Centers for Disease Control and Prevention
EPI_ISL_172648	A/Ethiopia/63/2014	H1N1pdm09	2014-11-07	C2	Centers for Disease Control and Prevention
EPI_ISL_172640	A/Ethiopia/66/2014	H1N1pdm09	2014-11-14	C2	Centers for Disease Control and Prevention
EPI_ISL_189819	A/Florida/62/2014	H1N1pdm09	2014-10-28	C3	Centers for Disease Control and Prevention
EPI_ISL_163672	A/Hawaii/06/2014	H1N1pdm09	2014-03-01	C2	Centers for Disease Control and Prevention
EPI_ISL_150295	A/Hong Kong/5008/2013	H1N1pdm09	2013-09-19	CX/C2	Centers for Disease Control and Prevention
EPI_ISL_153089	A/Massachusetts/15/2013	H1N1pdm09	2013-11-13	C3	Centers for Disease Control and Prevention
EPI_ISL_166268	A/Minnesota/15/2014	H1N1pdm09	2014-08-09	C1	Centers for Disease Control and Prevention
EPI_ISL_167881	A/Minnesota/23/2014	H1N1pdm09	2014-09-06	C1	Centers for Disease Control and Prevention
EPI_ISL_159529	A/Missouri/02/2014	H1N1pdm09	2014-02-07	C1	Centers for Disease Control and Prevention
EPI_ISL_176807	A/Montana/05/2015	H1N1pdm09	2015-02-17	C1	Centers for Disease Control and Prevention
EPI_ISL_139900	A/New Jersey/04/2013	H1N1pdm09	2013-03-19	C1	Centers for Disease Control and Prevention
EPI_ISL_138664	A/New Mexico/02/2013	H1N1pdm09	2013-01-06	M1/C2	Centers for Disease Control and Prevention
EPI_ISL_188725	A/New Mexico/17/2015	H1N1pdm09	2015-03-11	M1/C2	Centers for Disease Control and Prevention
EPI_ISL_100059	A/Nonthaburi/78/2011	H1N1pdm09	2011-06-08	C1/C2	Centers for Disease Control and Prevention
EPI_ISL_166344	A/North Carolina/04/2014	H1N1pdm09	2014-02-16	C3	Centers for Disease Control and Prevention
EPI_ISL_156812	A/Pennsylvania/07/2013	H1N1pdm09	2013-10-02	C3	Centers for Disease Control and Prevention
EPI_ISL_159530	A/Pennsylvania/17/2014	H1N1pdm09	2014-02-11	C1	Centers for Disease Control and Prevention
EPI_ISL_145509	A/Puerto Rico/01/2013	H1N1pdm09	2013-06-14	C2	Centers for Disease Control and Prevention

EPI_ISL_111385	A/Rhode Island/02/2012	H1N1pdm09	2012-02-29	C1	Centers for Disease Control and Prevention
EPI_ISL_175880	A/South Africa/3626/2013	H1N1pdm09	2013-06-06	E1E2/E3	Centers for Disease Control and Prevention
EPI_ISL_142868	A/Tennessee/03/2013	H1N1pdm09	2013-03-26	C1	Centers for Disease Control and Prevention
EPI_ISL_142576	A/Utah/05/2013	H1N1pdm09	2013-04-14	C1	Centers for Disease Control and Prevention
EPI_ISL_143246	A/Utah/06/2013	H1N1pdm09	2013-05-12	C1	Centers for Disease Control and Prevention
EPI_ISL_145097	A/Vietnam/3050/2013	H1N1pdm09	2013-02-28	C3/C1	Centers for Disease Control and Prevention
EPI_ISL_158908	A/Washington/07/2014	H1N1pdm09	2014-02-10	C2	Centers for Disease Control and Prevention
EPI_ISL_128403	A/Washington/24/2012	H1N1pdm09	2012-06-17	C3	Centers for Disease Control and Prevention
EPI_ISL_145252	A/Wisconsin/12/2013	H1N1pdm09	2013-08-04	C1	Centers for Disease Control and Prevention
EPI_ISL_148737	A/Wisconsin/13/2013	H1N1pdm09	2013-09-05	C1	Centers for Disease Control and Prevention
EPI_ISL_167874	A/Wisconsin/28/2014	H1N1pdm09	2014-10-06	C1	Centers for Disease Control and Prevention
EPI_ISL_219303	A/South Australia/17/2013	H1N1pdm09	2013-05-23	E3	WHO Collaborating Centre for Referenceand Research on Influenza
EPI_ISL_148587	A/Guizhou-Qingzhen/SWL1251/2013	H1N1pdm09	2013-06-04	C1/C1/MDCK1	National Institute for Medical Research
EPI_ISL_153261	A/Sichuan-Qingyang/SWL1599/2013	H1N1pdm09	2013-11-03	C2	WHO Chinese National Influenza Center
EPI_ISL_156441	A/Sapporo/16/2014	H1N1pdm09	2014-01-20	MDCK 1	National Institute of Infectious Diseases
EPI_ISL_160497	A/Shizuoka/46/2014	H1N1pdm09	2014-03-04	MDCK 1	National Institute of Infectious Diseases
EPI_ISL_144758	A/Acores_PT/139/2013	H1N1pdm09	2013-04-01	SIAT1/MDCK1	National Institute for Medical Research
EPI_ISL_134399	A/Dakar/20/2012	H1N1pdm09	2012-12-09	SIAT2	National Institute for Medical Research
EPI_ISL_134400	A/Dakar/22/2012	H1N1pdm09	2012-11-03	MDCK/MDCK1	National Institute for Medical Research
EPI_ISL_166054	A/Ghana/DILI-0567/2014	H1N1pdm09	2014-06-16	C1/MDCK1	National Institute for Medical Research
EPI_ISL_174950	A/Ghana/FS-14-1022/2014	H1N1pdm09	2014-11-24	MDCK1	National Institute for Medical Research
EPI_ISL_174951	A/Ghana/FS-14-1025/2014	H1N1pdm09	2014-11-27	MDCK1	National Institute for Medical Research
EPI_ISL_174954	A/Ghana/FS-14-1069/2014	H1N1pdm09	2014-12-19	MDCK1	National Institute for Medical Research
EPI_ISL_144784	A/Stockholm/15/2013	H1N1pdm09	2013-03-26	MDCK0/MDCK1	National Institute for Medical Research
EPI_ISL_145683	A/American Samoa/4786/2013	H3N2	2013-02-22	C3	Centers for Disease Control and Prevention
EPI_ISL_134828	A/Beijing-Xicheng/12423/2012	H3N2	2012-10-04	C3/C4	Centers for Disease Control and Prevention
EPI_ISL_164402	A/California/02/2014	H3N2	2014-01-16	C1S2	Centers for Disease Control and Prevention
EPI_ISL_176747	A/California/20/2015	H3N2	2015-01-20	S1	Centers for Disease Control and Prevention
EPI_ISL_160427	A/Colorado/11/2014	H3N2	2014-04-13	C1	Centers for Disease Control and Prevention
EPI_ISL_153217	A/Delaware/17/2013	H3N2	2013-12-02	C2	Centers for Disease Control and Prevention
EPI_ISL_188884	A/Egypt/4870/2014	H3N2	2014-11-12	C3/S2	Centers for Disease Control and Prevention

GISAID Epiflu ID	Isolate_Name	Subtype	Collection_Date	Passage	Submitting_Lab
EPI_ISL_167413	A/Florida/34/2014	H3N2	2014-09-30	MX	Centers for Disease Control and Prevention
EPI_ISL_172592	A/Ethiopia/1147/2014	H3N2	2014-10-27	S1	Centers for Disease Control and Prevention
EPI_ISL_171380	A/Ethiopia/1153/2014	H3N2	2014-11-11	S1	Centers for Disease Control and Prevention
EPI_ISL_179009	A/Florida/26/2015	H3N2	2015-03-17	MX/S1	Centers for Disease Control and Prevention
EPI_ISL_164418	A/Hawaii/08/2014	H3N2	2014-03-11	S3	Centers for Disease Control and Prevention
EPI_ISL_168126	A/Hawaii/47/2014	H3N2	2014-07-18	S3	Centers for Disease Control and Prevention
EPI_ISL_167396	A/Kentucky/10/2014	H3N2	2014-10-04	MX	Centers for Disease Control and Prevention
EPI_ISL_171375	A/Kenya/124/2014	H3N2	2014-08-27	X1/S2	Centers for Disease Control and Prevention
EPI_ISL_171399	A/Kenya/127/2014	H3N2	2014-08-27	X1/S3	Centers for Disease Control and Prevention
EPI_ISL_171377	A/Kenya/128/2014	H3N2	2014-08-26	X1/S2	Centers for Disease Control and Prevention
EPI_ISL_164406	A/Louisiana/39/2013	H3N2	2013-12-29	C1S2	Centers for Disease Control and Prevention
EPI_ISL_167933	A/Maine/04/2014	H3N2	2014-10-09	S1	Centers for Disease Control and Prevention
EPI_ISL_145503	A/Maryland/04/2013	H3N2	2013-03-06	M1/C2	Centers for Disease Control and Prevention
EPI_ISL_174163	A/Massachusetts/02/2015	H3N2	2015-01-20	S1	Centers for Disease Control and Prevention
EPI_ISL_151025	A/Massachusetts/11/2013	H3N2	2013-07-10	C2	Centers for Disease Control and Prevention
EPI_ISL_167937	A/Michigan/10/2014	H3N2	2014-09-10	M1/S1	Centers for Disease Control and Prevention
EPI_ISL_166983	A/Michigan/13/2014	H3N2	2014-09-18	M1	Centers for Disease Control and Prevention
EPI_ISL_134881	A/Minnesota/01/2013	H3N2	2013-01-08	C1	Centers for Disease Control and Prevention
EPI_ISL_164399	A/Nebraska/04/2014	H3N2	2014-03-11	S4	Centers for Disease Control and Prevention
EPI_ISL_175191	A/Nevada/10/2015	H3N2	2015-02-14	S1	Centers for Disease Control and Prevention
EPI_ISL_178992	A/New Hampshire/16/2015	H3N2	2015-03-03	R1/S1	Centers for Disease Control and Prevention
EPI_ISL_169307	A/New Jersey/26/2014	H3N2	2014-11-17	Original	Centers for Disease Control and Prevention
EPI_ISL_160782	A/New York/07/2014	H3N2	2014-02-10	C2	Centers for Disease Control and Prevention
EPI_ISL_164405	A/New York/39/2012	H3N2	2012-10-20	C2S2	Centers for Disease Control and Prevention
EPI_ISL_167399	A/North Carolina/20/2014	H3N2	2014-09-08	S1	Centers for Disease Control and Prevention
EPI_ISL_179007	A/Ohio/11/2015	H3N2	2015-03-20	S1	Centers for Disease Control and Prevention
EPI_ISL_139166	A/Oregon/01/2013	H3N2	2013-01-11	C1	Centers for Disease Control and Prevention
EPI_ISL_164404	A/Palau/6759/2014	H3N2	2014-03-26	S2	Centers for Disease Control and Prevention
EPI_ISL_182625	A/Pennsylvania/09/2015	H3N2	2015-01-07	S2	Centers for Disease Control and Prevention

EPI_ISL_189817	A/South Australia/55/2014	H3N2	2014-06-29	C1/S3	Centers for Disease Control and Prevention
EPI_ISL_165829	A/Switzerland/9715293/2013	H3N2	2013-12-06	E4/E2	Centers for Disease Control and Prevention
EPI_ISL_172814	A/Tanzania/1906/2014	H3N2	2014-12-09	S1	Centers for Disease Control and Prevention
EPI_ISL_174125	A/Tanzania/3565/2014	H3N2	2014-11-20	S2	Centers for Disease Control and Prevention
EPI_ISL_172737	A/Tanzania/3580/2014	H3N2	2014-11-26	S1	Centers for Disease Control and Prevention
EPI_ISL_172773	A/Tanzania/594/2014	H3N2	2014-12-05	S2	Centers for Disease Control and Prevention
EPI_ISL_127832	A/Texas/50/2012	H3N2	2012-04-15	E4	Centers for Disease Control and Prevention
EPI_ISL_145498	A/Utah/07/2013	H3N2	2013-07-05	C2	Centers for Disease Control and Prevention
EPI_ISL_166309	A/Washington/18/2013	H3N2	2013-11-29	S2	Centers for Disease Control and Prevention
EPI_ISL_176732	A/Wisconsin/20/2015	H3N2	2015-02-24	S1	Centers for Disease Control and Prevention
EPI_ISL_167397	A/Wisconsin/26/2014	H3N2	2014-09-25	S1	Centers for Disease Control and Prevention
EPI_ISL_161267	A/Brisbane/1/2013	H3N2	2013-01-03	MDCK3	WHO Collaborating Centre for Referenceand Research on Influenza
EPI_ISL_168899	A/Canberra/82/2014	H3N2	2014-07-31	MDCK2	WHO Collaborating Centre for Referenceand Research on Influenza
EPI_ISL_168901	A/New Caledonia/71/2014	H3N2	2014-08-13	MDCK1	WHO Collaborating Centre for Reference and Research on Influenza
EPI_ISL_161272	A/Victoria/504/2013	H3N2	2013-06-17	MDCK1	WHO Collaborating Centre for Reference and Research on Influenza
EPI_ISL_149682	A/Hunan-Lusong/1532/2013	H3N2	2013-06-10	C1C1/C2	Centers for Disease Control and Prevention
EPI_ISL_179373	A/Zambia/04-288/2014	H3N2	2014-12-11	MDCK2/SIAT2	Crick Worldwide Influenza Centre
EPI_ISL_172339	A/Algiers/249/2014	H3N2	2014-12-30	SIAT2	National Institute for Medical Research
EPI_ISL_174979	A/Algiers/08/2014	H3N2	2014-10-12	C0/SIAT1	National Institute for Medical Research
EPI_ISL_141668	A/Bulgaria/270/2013	H3N2	2013-02-04	C1/SIAT1	National Institute for Medical Research
EPI_ISL_171746	A/Dakar/30/2014	H3N2	2014-10-07	C2/SIAT1	National Institute for Medical Research
EPI_ISL_175025	A/Ghana/DILI-14-1014/2014	H3N2	2014-11-04	?/SIAT1	National Institute for Medical Research
EPI_ISL_175026	A/Ghana/DILI-14-1152/2014	H3N2	2014-12-10	SIAT1	National Institute for Medical Research
EPI_ISL_165554	A/Hong Kong/4801/2014	H3N2	2014-02-26	SIAT1	National Institute for Medical Research
EPI_ISL_168972	A/Hong Kong/7127/2014	H3N2	2014-07-29	MDCK1/SIAT2	National Institute for Medical Research
EPI_ISL_171756	A/Madagascar/3069/2014	H3N2	2014-10-22	MDCK2/SIAT1	National Institute for Medical Research
EPI_ISL_175044	A/Mauritius/853/2014	H3N2	2014-10-22	MDCK2/SIAT1	National Institute for Medical Research
EPI_ISL_175045	A/Mauritius/912/2014	H3N2	2014-11-10	MDCK2/SIAT1	National Institute for Medical Research
EPI_ISL_162129	A/Norway/466/2014	H3N2	2014-02-03	SIAT2	National Institute for Medical Research
EPI_ISL_171762	A/Zambia/04-303/2014	H3N2	2014-11-14	MDCK2/SIAT1	National Institute for Medical Research
EPI_ISL_175067	A/Zambia/06-024/2014	H3N2	2014-09-15	MDCK2/SIAT2	National Institute for Medical Research

Supplementary Table2.GISAID Epi flu database accession numbers of DRCongo influenza virus isolates genetically characterized at the CDC Atlanta

GISAIDEpifluID	Isolate_Name	Subtype	HAGenetic Group	Collection Date	Passage	CDCID	Characterization
EPI_ISL_175254	A/Congo/2275/2014	H1N1pdm09	6B	2014-11-06	C2	3000095216	A/CALIFORNIA/07/2009-LIKE(H1N1)pdm09
EPI_ISL_175259	A/Congo/2278/2014	H1N1pdm09	6B	2014-11-05	C1	3000095217	A/CALIFORNIA/07/2009-LIKE(H1N1)pdm09
EPI_ISL_176547	A/Congo/2611/2014	H3N2	3C.3	2014-12-29	S2	3000095242	A/TEXAS/50/2012-LIKE(H3N2) A/SWITZERLAND/9715293/2013-LIKE(H3N2)
EPI_ISL_176548	A/Congo/2557/2014	H3N2	3C.3	2014-12-18	S2	3000095233	A/TEXAS/50/2012-LIKE(H3N2)LOW A/SWITZERLAND/9715293/2013-LIKE(H3N2)
EPI_ISL_175217	A/Congo/2461/2014	H3N2	3C.2a	2014-12-08	S1	3000095220	A/TEXAS/50/2012-LIKE(H3N2)LOW A/SWITZERLAND/9715293/2013-LIKE(H3N2)
EPI_ISL_176513	A/Congo/2538/2014	H3N2	3C.2a	2014-12-18	S2	3000095231	A/TEXAS/50/2012-LIKE(H3N2)LOW A/SWITZERLAND/9715293/2013-LIKE(H3N2)
EPI_ISL_175210	A/Congo/2471/2014	H3N2	3C.2a	2014-12-09	S2	3000095222	INSUFFICIENTTITERFORTESTINGINFLUENZAA(H3N2)BYSEQUENCING/PYROSEQUENCING
EPI_ISL_175211	A/Congo/2458/2014	H3N2	3C.2a	2014-12-04	S2	3000095219	INSUFFICIENTTITERFORTESTINGINFLUENZAA(H3N2)BYSEQUENCING/PYROSEQUENCING
EPI_ISL_176515	A/Congo/2544/2014	H3N2	3C.2a	2014-12-17	S3	3000095232	INSUFFICIENTTITERFORTESTINGINFLUENZAA(H3N2)BYSEQUENCING/PYROSEQUENCING
EPI_ISL_189842	A/Congo/2495/2014	H3N2	3C.2a	2014-12-11	S3	3000095227	VIRUSNOTRECOVERED INFLUENZAA(H3N2)BYSEQUENCING/PYROSEQUENCING