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Influenza A(H9N2) Virus, Burkina Faso

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We identified influenza A(H9N2) virus G1 lineage in poultry in Burkina Faso. Urgent actions are needed to raise awareness about the risk associated with spread of this zoonotic virus subtype in the area and to construct a strategy for effective prevention and control of influenza caused by this virus.

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Since their detection in China in 1992, influenza A(H9N2) viruses have caused large economic losses to the poultry industry and have occasionally been transmitted to mammalian species, including humans. Three main genetic lineages were described among the Eurasian H9N2 subtype viruses: G1, Y280, and Y439 (Korean) lineage (*1*). In the past decade, the G1 lineage has spread mostly in gallinaceous birds across Asia, the Middle East, and eventually North Africa, where H9N2 outbreaks were reported in Libya (2006 and 2013) (*2*), Tunisia (2010–2012) (*3*), Egypt (2011–present), and Morocco (2016) (*4*).

The Veterinary Services of Ouagadougou, Burkina Faso, submitted 30 tracheal swab specimens and 10 organ samples collected in January 2017 in Burkina Faso to the World Organisation for Animal Health/Food and Agriculture Organization of the United Nations Reference Laboratory for Avian Influenza, Istituto Zooprofilattico Sperimentale delle Venezie (Legnaro, Padova, Italy). All samples were collected from a layer farm that was experiencing decreased egg production and respiratory signs among its flock; the animals were suspected to have infectious bronchitis virus (IBV).

Molecular analyses of the animal samples showed negative results for IBV and indicated that animals from the farm were infected with avian influenza A(H9N2) virus. The 8 gene segments were obtained for 1 representative virus by using a MiSeq Platform (Illumina, San Diego, CA, USA). Sequences were submitted to GenBank under accession numbers MF510849–56.

The maximum-likelihood phylogenetic tree of the hemagglutinin (HA) gene showed that the H9N2 subtype virus from Burkina Faso belonged to the G1 lineage, which has remarkable zoonotic potential. This virus clustered with H9N2 subtype viruses isolated in Morocco in 2016 (99.2% similarity) and with an H9N2 subtype virus identified in the United Arab Emirates in 2015 (A/chicken/Dubai/D2506.A/2015) (98.7% similarity) (online Technical Appendix Figure 1, https://wwwnc.cdc.gov/EID/article/23/12/17-1294-Teachapp1.pdf). Phylogenetic trees obtained for all other gene segments confirmed clustering with viruses from Morocco and the United Arab Emirates, similar to that observed for HA gene phylogeny.

Phylogeographic analysis (online Technical Appendix) identified multiple introductions of influenza A(H9N2) virus into North Africa from the Middle East and Pakistan. The H9N2 subtype virus identified in Burkina Faso seems to have originated from Morocco, although we cannot rule out the possibility that H9N2 subtype viruses were circulating in unsampled locations (online Technical Appendix Figure 2, Video, https://wwwnc.cdc.gov/EID/article/23/12/17-1294-V1.htm).

Analyses of the deep sequencing data showed that ≈50% of the virus population in the tracheal swab specimen had leucine at position 226 (H3 numbering) of the HA receptor binding site (sequence coverage of 14,152 reads in the indicated position), which enables preferential binding to human-like α2–6-linked sialic acid receptors (*5*). Furthermore, a potential additional glycosylation site (NLS), which had not previously been detected in the G1 lineage, was identified at positions 271–273 (H3 numbering). In the acidic polymerase protein, the H9N2 subtype virus from Burkina Faso had the mutation PA-S409N, which is considered a host specificity marker of human influenza virus (*6*). The same mutation was detected in related viruses from Morocco and Dubai.

Identification of H9N2 subtype virus in West Africa, where highly pathogenic H5 strains of the A/goose/Guangdong/1/1996 lineage (Gs/GD) have been widely circulating since the beginning of 2015, is a concern because of animal health implications, negative effects on local economies, and possible emergence of reassortant viruses with unknown biological properties. Reassortment events between H9N2 and highly pathogenic H5N1 subtype viruses were reported in China in 2005 and 2016 (*7,8*) and in Bangladesh in 2012 (*9*). In December 2013, an H5N1 subtype virus that had an H9N2 subtype polymerase basic 2 gene was reported in a patient in Canada who had returned from China (*10*). Moreover, H5N6 subtype reassortant viruses belonging to clade 2.3.4.4, which contain H9N2 subtype–like internal genes, were identified in China in 2015–2016 (*8*).

H5 strains belonging to clades 2.3.2.1c and 2.3.4.4 are currently circulating in West Africa. This finding, combined with detection of human-like receptor specificity and 2 mutations typical of human influenza viruses in the H9N2 subtype virus from Burkina Faso, might indicate emergence of a strain capable of infecting humans and warrants additional attention to the avian influenza situation in West Africa. Furthermore, identification of H9N2 subtype viruses in Morocco and Burkina Faso in chickens suggests that commercial poultry trade between North and West Africa might have played a key role in spread of the virus.

Involvement of wild birds in long-distance spread of H9N2 subtype G1 virus seems unlikely because this lineage is strongly adapted to poultry. These observations highlight the difficulty in tracing and containing circulating H9N2 subtype G1 virus and underline the need to review current approaches of disease reporting to understand spread and effects of this virus, which are probably underestimated. Thus, it is imperative to provide strategic guidance to countries in West Africa on technical and policy options for cost-effective surveillance and prevention and control of multiple cocirculating influenza virus strains.

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Influenza A(H9N2) Virus, Burkina Faso

Technical Appendix

Materials and Methods

Genome Amplification and Sequencing

We purified influenza virus RNA from clinical samples by using the Nucleospin RNA Kit (Macherey–Nagel, Duren, Germany). We amplified the complete genome of A/chicken/Burkina_Faso/17RS93–19/2017(H9N2) virus by using the SuperScript III One-Step RT-PCR System and Platinum Taq High Fidelity (Invitrogen, Carlsbad, CA, USA) as described (*1*). The sequencing library was prepared by using the Nextera DNA XT Sample preparation kit (Illumina, San Diego, CA, USA) and quantified by using the Qubit dsDNA High Sensitivity Kit (Invitrogen, Carlsbad, CA, USA). The High Sensitivity DNA Analysis Kit (Agilent Technologies, Alpharetta, GA, USA) was used to determine average fragment length. According to the manufacturer's instructions, the library was sequenced by using Illumina MiSeq (2 × 250-bp paired-end).

Illumina Sequencing Data Analysis

FastQC version 0.11.2 (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/) was used to assess read quality. Raw data were filtered by removal of reads with >10% of undetermined bases, reads with >100 bases with a Q score <7, and duplicated paired-end reads. Remaining reads were clipped from Nextera XT adaptors (Illumina) with scythe version 0.991 (https://github.com/vsbuffalo/scythe) and trimmed with sickle version 1.33 (https://github.com/najoshi/sickle). High-quality reads ≥80 bases were aligned against a reference genome by using BWA version 0.7.12 (*2*). Picard-tools version 2.1.0 (http://picard.sourceforge.net) and GATK version 3.5 (*3*–*5*) were used to correct potential errors, realign reads around indels, and recalibrate base quality. LoFreq version 2.1.2 (*6*) was used to call single-nucleotide polymorphisms. Outputs were used to generate consensus sequences.

Phylogenetic Analyses

Consensus sequences of each gene segment of A/chicken/Burkina_Faso/17RS93–19/2017(H9N2) virus were compared with the most related sequences available in GISAID

(https://www.gisaid.org/) and aligned by using MAFFT version 7 (*7*). Maximum-likelihood phylogenetic trees were obtained by using the best-fit general time-reversible model of nucleotide substitution with gamma-distributed rate variation among sites (with 4 rate categories, Γ4) and a heuristic subtree pruning and regrafting branch-swapping search (*8*) implemented in PhyML version 3.1 (http://www.atgc-montpellier.fr/phyml/versions.php). Bootstrap analysis with 100 replicates was performed for each tree to assess support for nodes. Phylogenetic trees were visualized by using FigTree version 1.4.2 (http://tree.bio.ed.ac.uk/software/figtree/).

Bayesian Analysis

A time-scaled Bayesian analysis of the hemagglutinin gene was performed by using the Markov chain Monte Carlo method available in BEAST version 1.8.4 (http://beast.community/2016-06-17_BEAST_v1.8.4_released.html). A Hasegawa-Kishino-Yano 85 + Γ_4 model of nucleotide substitution with 2 data partitions of codon positions (1st and 2nd positions, 3rd position) was used, and base frequencies were unlinked across all codon positions (SRD06 substitution model). We used a relaxed uncorrelated lognormal molecular clock and a Skyride coalescent model in BEAST. Chain lengths were run for 50 million iterations to achieve convergence as assessed by using Tracer version 1.6 (http://beast.bio.ed.ac.uk/Tracer). TreeAnnotator version 1.8.4 (*9*) was used to generate the maximum clade credibility (MCC) phylogenetic tree, and we adopted an appropriate burn-in (10% of trees). The MCC tree was visualized by using FigTree version 1.4.2 (http://tree.bio.ed.ac.uk/software/figtree/). SPREAD version 1.0.6 (https://www.kuleuven.be/aidslab/phylogeography/SPREAD.html) (*10*) was used to visualize the phylogeographic reconstruction resulting from the MCC phylogenetic tree and to identify the well-supported rates, calculating the Bayes factors. An animation of viral spread over time is shown in the video (https://wwwnc.cdc.gov/EID/article/23/12/17-1294-V1.htm).

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Technical Appendix Table 1. Bayes factor test results for significant nonzero rates of influenza A(H9N2) viruses

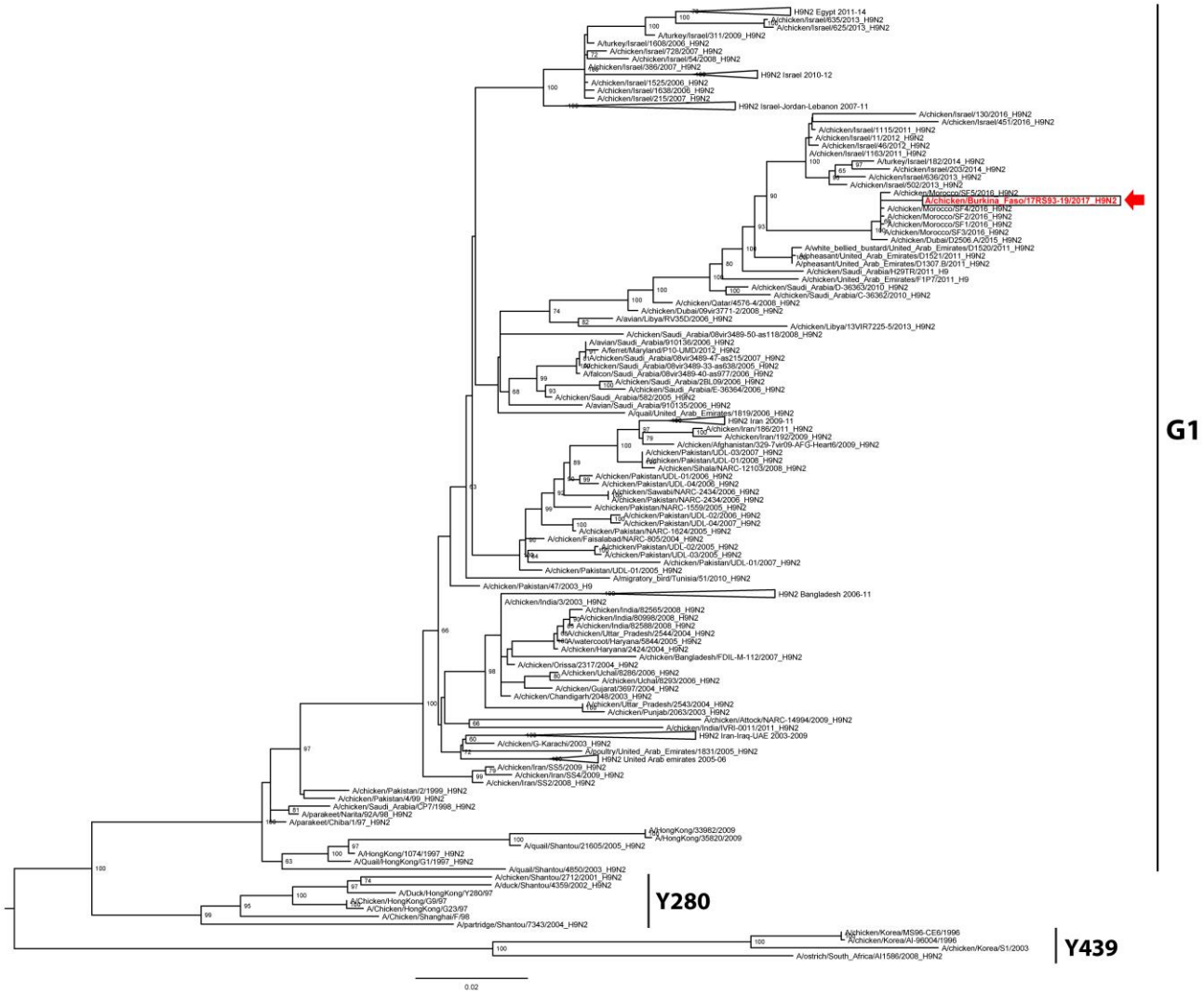
Pairs of locations with Bayes factor >5	Bayes factor
Pakistan-Afghanistan and South Asia	3,795.26
Iran-Iraq and Pakistan-Afghanistan	1,603.01
Saudi Arabia-Qatar and United Arab Emirates	1,436.70
Egypt and Israel-Jordan-Lebanon	658.83
Burkina Faso and Morocco	112.11
Morocco and United Arab Emirates	58.59
Libya and Saudi Arabia-Qatar	22.48
Pakistan-Afghanistan and United Arab Emirates	13.25
Israel-Jordan-Lebanon and United Arab Emirates	13.23
Pakistan-Afghanistan and Tunisia	7.43

Technical Appendix Table 2. Hemagglutinin gene segments of 16 influenza virus strains used for analysis of influenza A(H9N2) virus, Burkina Faso*

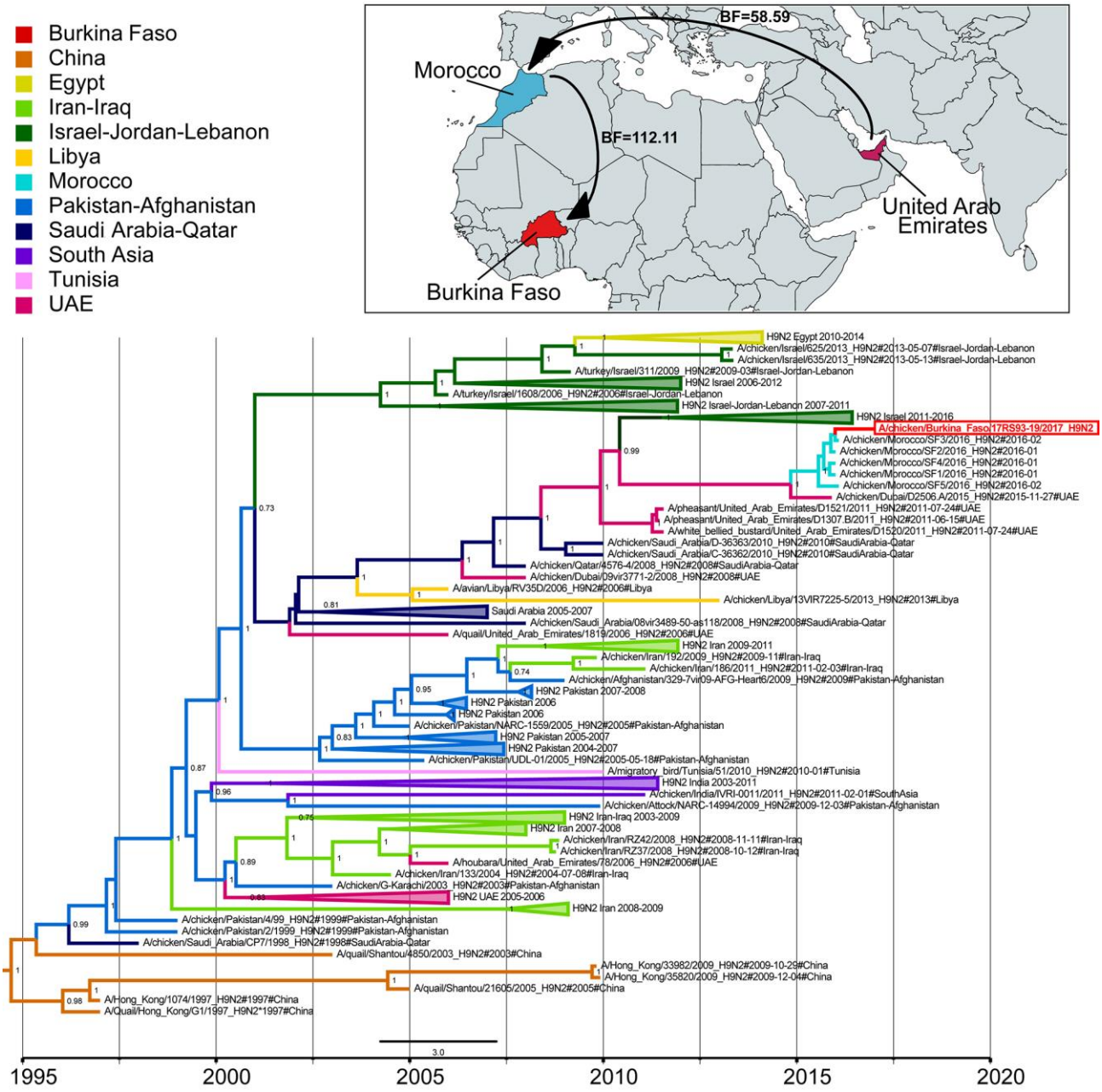
Segment ID	Country	Collection date	Isolate name	Originating laboratory	Submitting laboratory	Authors†
EPI457491	Bangladesh	2009 Mar 5	A/duck/Bangladesh/1009/2009	Institute of Epidemiology Disease Control and Research and Bangladesh National Influenza Centre	Centers for Disease Control and Prevention	NA
EPI557489	Egypt	2013 Feb 14	A/chicken/Egypt/NLQP123VD-AR758/2013		Friedrich-Loeffler-Institut	Naguib MM, Arafa AM, Selim AA, Hassan MK, Beer M, Harder TC
EPI355122	Egypt	2011 Mar 5	A/chicken/Egypt/11vir4453–280/2011	Istituto Zooprofilattico Sperimentale Delle Venezie	Istituto Zooprofilattico Sperimentale Delle Venezie	Monne I, Hussein HA, Fusaro A, Valastro V, Hamoud MM, Rabab A, Noseir S, Capua I, Cattoli G
EPI355114	Egypt	2010 Dec 9	A/chicken/Egypt/11vir4453–276/2010	Istituto Zooprofilattico Sperimentale Delle Venezie	Istituto Zooprofilattico Sperimentale Delle Venezie	Monne I, Hussein HA, Fusaro A, Valastro V, Hamoud MM, Rabab A, Noseir S, Capua I, Cattoli G
EPI355106	Egypt	2011 Mar 5	A/chicken/Egypt/11vir4453–275/2011	Istituto Zooprofilattico Sperimentale Delle Venezie	Istituto Zooprofilattico Sperimentale Delle Venezie	Monne I, Hussein HA, Fusaro A, Valastro V, Hamoud MM, Rabab A, Noseir S, Capua I, Cattoli G
EPI301655	Qatar	2008 Jan 1	A/chicken/Qatar/4576–4/2008	Istituto Zooprofilattico Sperimentale Delle Venezie	Istituto Zooprofilattico Sperimentale Delle Venezie	Fusaro A, Monne I, Salviato A, Valastro V, Schivo A, Amarin NM, Gonzalez C, Ismail MM, Al Blowi MH, Khan OA, Maken Ali AS, Hedayati A, Garcia JG, Ziay GM, Shoushtari A, Al Qahtani KN, Capua I, Holmes EC, Cattoli G
EPI301631	Iran	2009 Jan 1	A/chicken/Iran/10VIR854–4/2009	Istituto Zooprofilattico Sperimentale Delle Venezie	Istituto Zooprofilattico Sperimentale Delle Venezie	Fusaro A, Monne I, Salviato A, Valastro V, Schivo A, Amarin NM, Gonzalez C, Ismail MM, Al Blowi MH, Khan OA, Maken Ali AS, Hedayati A, Garcia JG, Ziay GM, Shoushtari A, Al Qahtani KN, Capua I, Holmes EC, Cattoli G
EPI301615	Iran	2009 Jan 1	A/chicken/Iran/10VIR854–3/2009	Istituto Zooprofilattico Sperimentale Delle Venezie	Istituto Zooprofilattico Sperimentale Delle Venezie	Fusaro A, Monne I, Salviato A, Valastro V, Schivo A, Amarin NM, Gonzalez C, Ismail MM, Al Blowi MH, Khan OA, Maken Ali AS, Hedayati A, Garcia JG, Ziay GM, Shoushtari A, Al Qahtani KN, Capua I, Holmes EC, Cattoli G
EPI301607	Iran	2008 Jan 1	A/chicken/Iran/10VIR854–5/2008	Istituto Zooprofilattico Sperimentale Delle Venezie	Istituto Zooprofilattico Sperimentale Delle Venezie	Fusaro A, Monne I, Salviato A, Valastro V, Schivo A, Amarin NM, Gonzalez C, Ismail MM, Al Blowi MH, Khan OA, Maken Ali AS, Hedayati A, Garcia JG, Ziay GM, Shoushtari A, Al Qahtani KN, Capua I, Holmes EC, Cattoli G
EPI301591	United Arab Emirates	2008-Jan-01	A/chicken/Dubai/09vir3771–2/2008	Istituto Zooprofilattico Sperimentale Delle Venezie	Istituto Zooprofilattico Sperimentale Delle Venezie	Fusaro A, Monne I, Salviato A, Valastro V, Schivo A, Amarin NM, Gonzalez C, Ismail MM, Al Blowi MH, Khan OA, Maken Ali AS, Hedayati A, Garcia JG, Ziay GM, Shoushtari A, Al Qahtani KN, Capua I, Holmes EC, Cattoli G

Segment ID	Country	Collection date	Isolate name	Originating laboratory	Submitting laboratory	Authors†
EPI301498	Jordan	2010 Jan 1	A/chicken/Jordan/436–2/2010	Istituto Zooprofilattico Sperimentale Delle Venezie	Istituto Zooprofilattico Sperimentale Delle Venezie	Fusaro A, Monne I, Salviato A, Valastro V, Schivo A, Amarin NM, Gonzalez C, Ismail MM, Al Blowi MH, Khan OA, Maken Ali AS, Hedayati A, Garcia JG, Ziay GM, Shoushtari A, Al Qahtani KN
EPI301490	Jordan	2010 Jan 1	A/chicken/Jordan/436–1/2010	Istituto Zooprofilattico Sperimentale Delle Venezie	Istituto Zooprofilattico Sperimentale Delle Venezie	Fusaro A, Monne I, Salviato A, Valastro V, Schivo A, Amarin NM, Gonzalez C, Ismail MM, Al Blowi MH, Khan OA, Maken Ali AS, Hedayati A, Garcia JG, Ziay GM, Shoushtari A, Al Qahtani KN, Capua I, Holmes EC, Cattoli G
EPI355392	Egypt	2011 Jan 1	A/chicken/Egypt/11vir4453–132/VRLCU/2011	NA	Istituto Zooprofilattico Sperimentale Delle Venezie	NA
EPI355384	Egypt	2011 Mar 5	A/chicken/Egypt/11vir4453–274/2011	NA	Istituto Zooprofilattico Sperimentale Delle Venezie	NA
EPI223115	Afghanistan	2009 Jan 1	A/chicken/Afghanistan/329–7vir09-AFG-Heart6/2009	NA	Istituto Zooprofilattico Sperimentale Delle Venezie	Valastro V, Salviato A, Fusaro A, Monne I, Habib M, Ziay G, Garcia J, Cattoli G, Capua I

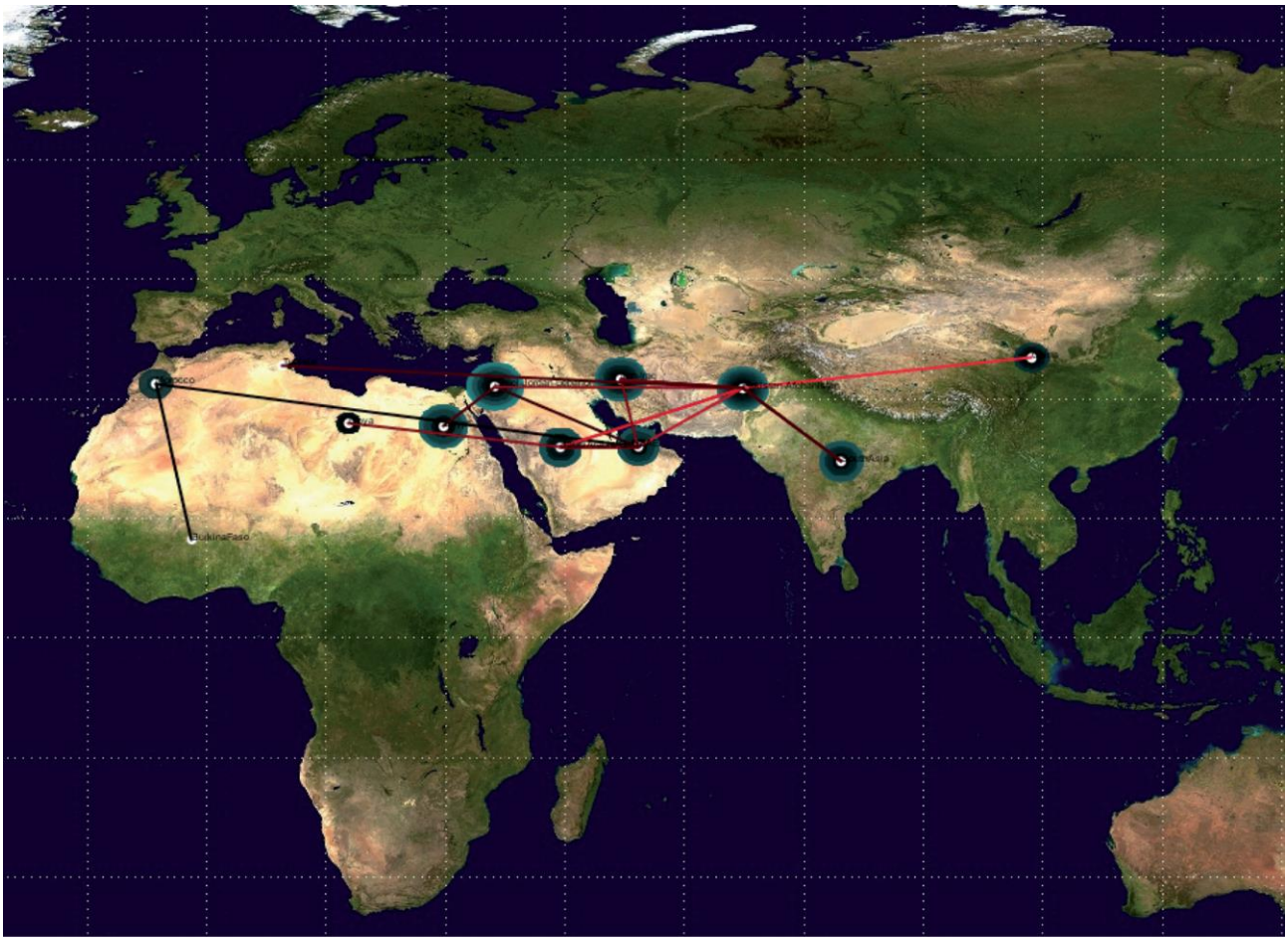
*ID, identification; NA, not available.
†Authors who submitted data may be contacted directly via the GISAID website (https://www.gisaid.org/).



Technical Appendix Figure 1. Maximum-likelihood phylogenetic tree of the hemagglutinin gene of influenza A(H9N2) viruses. Influenza A(H9N2) virus from Burkina Faso is indicated in in red. Bootstrap values >60% are indicated next to nodes. Scale bar indicates nucleotide substitutions per site.



Technical Appendix Figure 2. Maximum clade credibility tree showing evolutionary relationships between A/chicken/Burkina Faso/17RS93–19/2017(H9N2) influenza virus (indicated in red) and influenza A(H9N2) viruses isolated in North Africa, the Middle East, and Asia. Posterior probabilities >70 are provided for each node. Color of each branch indicates location where analyzed viruses were collected. Scale bar indicates nucleotide substitutions per site. Map indicates spread of virus from the United Arab Emirates to Morocco and from Morocco to Burkina Faso. Bayes factors (BF) for significant nonzero rates are indicated next to corresponding arrows. UAE, United Arab Emirates.



Technical Appendix Figure 3. Spread of influenza A(H9N2) virus in Africa and Asia. Phylogeographic reconstruction resulting from the maximum clade credibility phylogenetic tree obtained with SPREAD version 1.0.6 (https://github.com/phylogeography/SPREAD/issues/7).