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Influenza A(H9N2) Virus, Burkina Faso

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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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Dr. Zecchin is a biotechnologist at the Istituto Zooprofilattico Sperimentale delle Venezie, Padova, Italy. Her primary research interests include studying the molecular phylogeny and the evolutionary dynamics of viruses.

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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.
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, $\Gamma_4$) 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 $85 + \Gamma_4$ 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        |
| 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, Salvato A, Valastro V, Schivo A, Amarin NM, Gonzalez C, Ismail MM, Al Blawi MH, Khan OA, Maken Ali AS, Hedayati A, Garcia JG, Ziyai 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, Salvato A, Valastro V, Schivo A, Amarin NM, Gonzalez C, Ismail MM, Al Blawi MH, Khan OA, Maken Ali AS, Hedayati A, Garcia JG, Ziyai 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, Salvato A, Valastro V, Schivo A, Amarin NM, Gonzalez C, Ismail MM, Al Blawi MH, Khan OA, Maken Ali AS, Hedayati A, Garcia JG, Ziyai 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, Salvato A, Valastro V, Schivo A, Amarin NM, Gonzalez C, Ismail MM, Al Blawi MH, Khan OA, Maken Ali AS, Hedayati A, Garcia JG, Ziyai 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, Salvato A, Valastro V, Schivo A, Amarin NM, Gonzalez C, Ismail MM, Al Blawi MH, Khan OA, Maken Ali AS, Hedayati A, Garcia JG, Ziyai 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        | Istituto Zooprofilattico       | 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        | Istituto Zooprofilattico       | 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 |
| EPI355392    | Egypt      | 2011 Jan 1      | A/chicken/Egypt/11vir4453–132/VRLCU/2011 | NA                             | Istituto Zooprofilattico       | NA              |
| EPI355384    | Egypt      | 2011 Mar 5      | A/chicken/Egypt/11vir4453–274/2011 | NA                             | Istituto Zooprofilattico       | NA              |
| EPI223115    | Afghanistan| 2009 Jan 1      | A/chicken/Afghanistan/329–7vir09-AFG-Heart6/2009 | NA                             | Istituto Zooprofilattico       | 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 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).