weeks after symptom onset, the patient's symptoms were better but not fully resolved.

Although we cannot entirely rule out Epstein-Barr virus as a possible trigger, the length of time between previous infection and onset of neuropsychological symptoms would be unusual. In addition, although it is impossible to exclude contributions of coinfection from other mosquitoborne viruses (e.g., dengue and chikungunya), given the Zika virus positivity on RT-PCR, the patient's condition met criteria for definitive Zika virus infection and the CSF IgM titer was consistent with CNS involvement of Zika virus. The changes on single-photon emission computed tomographs and neuropsychological test scores raise the possibility that Zika virus infection may trigger neuropsychiatric and cognitive symptoms. Although we cannot prove that the patient's symptoms were related to Zika virus, clinicians should be aware of this potential association and the value of closely monitoring patients with Zika virus infection.

K.T., who helped edit the manuscript, is a World Health Organization consultant on neurologic manifestations in the context of the Zika virus outbreak, receives an honorarium as chief consult editor on Zika virus for Medscape consults, and participates in Zika research through the Neuroviruses Emerging in the Americas Study. K.T. receives funding support from the National Institutes of Health, and J.Z. receives funding support from a National Institutes of Health training grant (T32 AI007531).

Dr. Zucker is a postdoctoral fellow in adult and pediatric infectious diseases at Columbia University Medical Center. His research interests include improving prevention and treatment for adolescents and young adults living with, or at risk for, HIV infection, hepatitis C, and sexually transmitted diseases.

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Address for correspondence: Jason Zucker, Columbia University Medical Center, 622 West 168th St, PH4 West–474, New York, NY 10032, USA; email: jz2700@cumc.columbia.edu

Highly Pathogenic Avian Influenza Virus (H5N8) Clade 2.3.4.4 Infection in Migratory Birds, Egypt

Abdullah A. Selim, Ahmed M. Erfan, Naglaa Hagag, Ali Zanaty, Abdel-Hafez Samir, Mohamed Samy, Ahmed Abdelhalim, Abdel-Satar A. Arafa, Mohamed A. Soliman, Momtaz Shaheen, Essam M. Ibraheem, Ibrahim Mahrous, Mohamed K. Hassan, Mahmoud M. Naguib

Author affiliations: National Laboratory for Veterinary Quality Control on Poultry Production, Animal Health Research Institute, Giza, Egypt (A.A. Selim, A.M. Erfan, N. Hagag, A. Zanaty, A.-H. Samir, M. Samy, A. Abdelhalim, A.-S.A. Arafa, M.A. Soliman, M. Shaheen, E.M. Ibraheem, M.K. Hassan, M.M. Naguib); General Organization for Veterinary Services, Giza (I. Mahrous); Friedrich-Loeffler-Institut, Federal Research Institute for Animal Health, Insel-Riems, Germany (M.M. Naguib)

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We isolated highly pathogenic avian influenza virus (H5N8) of clade 2.3.4.4 from the common coot (*Fulica atra*) in Egypt, documenting its introduction into Africa through migratory birds. This virus has a close genetic relationship with subtype H5N8 viruses circulating in Europe. Enhanced surveillance to detect newly emerging viruses is warranted.

Avian influenza is a highly contagious disease of poultry that continues to spread across the globe in bird populations. Occasionally, transmission of a highly pathogenic avian influenza virus (HPAIV) from infected poultry to humans results in a severe public health crisis (*1*).

In 2010, strains of HPAIV (H5N8) of clade 2.3.4.4 were first detected among wild birds in Asia and later spread to domestic birds across China, South Korea, and Japan (*2*,*3*). Most recently, a novel reassortant virus of subtype H5N8 clade 2.3.4.4 was reported in Russia and further spread to many countries in Europe, Asia, and the Middle East (*4*,*5*). The spread of HPAIV (H5N8) strains has been linked to the overlapping flyways of migratory wild birds that come from different continents; this mingling of wild birds poses a major concern worldwide (*4*,*6*).

Egypt is one of the most notable migration spots for migratory birds crossing Europe, Asia, and Africa. In early winter each year, thousands of migrating waterfowl use Egypt as a resting stop before they continue their journey southward through the African continent through the East Africa/East Asia and Mediterranean/Black Sea migratory

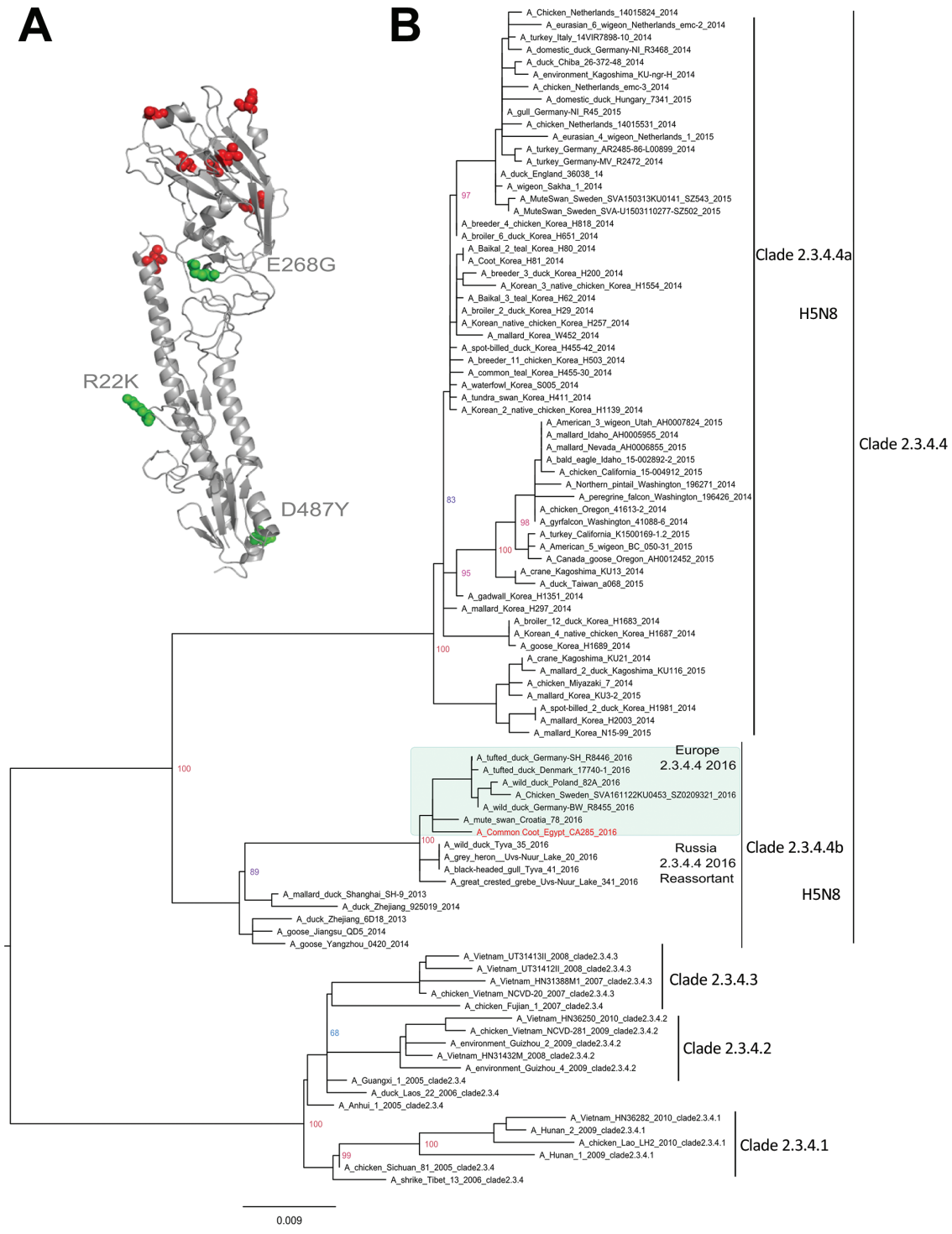

Figure. Structural and phylogenetic modeling of highly pathogenic avian influenza virus (H5N8), EG-CA285, from migratory birds, Egypt, 2016. A) Three-dimensional structural homology model for the hemagglutinin protein of EG-CA285 created by using the ancestral virus of clade 2.3.4.4b (A/duck/Zhejiang/6D18/2013 [H5N8]) as a template. Amino acids distinguishing the EG-CA285 sequence from the modeling template are shown in red; green depicts unique mutations distinguishing this virus from the virus detected in summer 2016 in Russia, A/great crested grebe/Uvs-Nuur-Lake/341/2016. B) Phylogenetic tree of the nucleotide sequences of avian influenza virus hemagglutinin genes. Maximum-likelihood calculations were done with IQ-TREE software (http://iqtree.cibiv.univie.ac.at/) under the best-fit model according to the Akaike criterion (general time reversible plus gamma plus G4 model). Bold indicates strains from Egypt; gray shading indicates strains currently circulating in Europe. Scale bar indicates nucleotide substitutions per site.

flyways. Lake Manzala in northern Egypt is a source of fish and a major refuge for many migratory birds (online Technical Appendix Figure 1, http://wwwnc.cdc.gov/EID/article/23/6/16-2056-Techapp1.pdf1).

During a targeted surveillance for avian influenza viruses (AIVs) conducted in migratory birds by Community Animal Health Outreach (CAHO) program on November 24, 2016, we collected 19 oropharyngeal and cloacal swab samples from diseased (mild depression) and dead migratory birds (common coot, *Fulica atra*; pintail ducks, *Anas acuta*; and Garganey ducks, *A. querquedula*) in a live bird and fish market in the Damietta Governorate in Egypt. (Hunted migratory birds are commonly sold for food in markets in this region.) Two samples from 2 common coots were confirmed positive for AIV and were subtyped as H5N8 by using specific real-time reverse transcription quantitative PCR (RT-qPCR) (online Technical Appendix). On November 30, 2016, the identification of HPAIV (H5N8) from 2 common coots was reported to the World Organisation for Animal Health. Notably, this newly emerged HPAIV (H5N8) was detected in Egypt in the same place, Damietta Governorate, where HPAIV (H5N1) was first identified in 2006 during the global spread of HPAIV (H5N1) viruses of clade 2.2 (*7*). Immediately thereafter, active targeted surveillance for AIV was conducted around Lake Manzala and the surroundings areas for AIV that included wild birds and domestic poultry; however, no more positive cases were detected.

We successfully isolated and characterized 1 HPAIV (H5N8) strain by nucleotide sequencing and phylogenetic analyses on the basis of its hemagglutinin (HA) and neuraminidase (NA) gene segments. The isolate was named A/common coot/Egypt/CA285/2016 (EG-CA285).

The amino acid sequence of the protease cleavage site of EG-CA285 HA protein revealed multiple basic amino acids, PLREKRRKR/GLF, which is characteristic of HPAIV. The receptor-binding pocket of EG-CA285 HA protein showed markers of avian receptor–specific binding: Q222 and G224. We observed 3 amino acid assignment differences in the HA protein, namely, R22K, E268G, and D487Y, which distinguished EG-CA285 from the recent HPAIV (H5N8) clade 2.3.4.4b strain isolated in Russia (A/great-crested-grebe/Uvs-Nuur-Lake/341/2016; GISAID accession no EPI_ISL_224580) (Figure, panel A). In the NA protein, we observed 4 substitution mutations (V8A, V31L, G126E, I407T) that distinguished the EG-CA285 from the subtype found in Russia. Phylogenetic analysis of HA and NA gene sequences revealed that EG-CA285 virus is clustered with clade 2.3.4.4b, along with the recent viruses widely distributed throughout Europe (Figure, panel B; online Technical Appendix Figure 2). Even though the unavailability of a full-length genomic sequence of this virus is a limitation in this study, the genetic and phylogenetic features of the HA and NA gene segments confirm the intercontinental dissemination of HPAIV (H5N8) through wild birds and its introduction into Egypt.

During the evolution of subtype H5Nx viruses of clade 2.3.4.4, frequent reassortment has been noted with other co-circulating HPAIVs and low pathogenicity AIVs in different countries in Europe, North America, and East Asia (*8*). Strains of HPAIV (H5N8) have been involved in multiple independent reassortment events with other AIV subtypes found in wild birds in China, South Korea, the United States, and recently in Russia (*5*,*9*). The probable introduction of HPAIV (H5N8) to poultry populations in Egypt will further complicate disease control and prevention, especially if HPAIV (H5N1) of clade 2.2.1.2 and low pathogenicity AIV (H9N2) strains of G1 lineage are enzootic in poultry (*10*).

In addition, the threat of emergence of a novel reassortants with unpredictable gene constellations of HPAIV (H5N8) strains with enzootic strains of AIV is a public health concern. Therefore, we recommend enhanced surveillance to quickly detect newly emerged viruses. Commercial and backyard poultry owners must follow the recommended biosecurity measures. The detection and immediate reporting of novel HPAIV (H5N8) strains in Egypt will help increase AIV surveillance, detection, and prevention preparedness in other countries of continental Africa.

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Dr. Selim is the technical manager of the National Laboratory for Quality Control on Poultry Production, Animal Health Research Institute, Egypt. His research interest includes diagnosis and molecular epidemiology of avian influenza viruses.

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Address for correspondence: Mahmoud M. Naguib, Friedrich-Loeffler-Institut Bundesforschungsinstitut fur Tiergesundheit Südufer 10, Greifswald–Insel Riems 17493, Germany; email: Mahmoud.naguib@fli.de

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Highly Pathogenic Avian Influenza Virus (H5N8) Clade 2.3.4.4 Infection in Migratory Birds, Egypt

Technical Appendix

Material and Methods

Samples and Virus Isolation

Nineteen swab samples were collected from the common coot (*Fulica atra*), pintail ducks (*Anas acuta*), and Garganey ducks (*Anas querquedula*) in live bird and fish markets in Damietta Governorate in Egypt. Samples were obtained during a targeted active surveillance of wild birds conducted by Community Animal Health Outreach (CAHO) team. Samples were submitted to the National Laboratory of Veterinary Quality Control on Poultry Production (NLQP) for virus identification and isolation. On November 27, 2016, 2 samples from common coots were confirmed positive for avian influenza virus (AIV) and subtyped as H5N8. On November 30, 2016, the identification of AIV(H5N8) from a common coot was reported to the World Organisation for Animal Health (OIE) as the first case of an outbreak in Egypt in Africa. One virus was successfully isolated through allantoic fluid inoculation of 10-day-old specific-pathogen-free (SPF) embryonated chicken eggs according to the OIE diagnostic manual according to standard protocols (*1*).

RNA Extraction and Molecular Diagnosis

Viral RNA was extracted from the obtained samples by using the QIAamp Viral RNA Mini Kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions. All samples were tested using standard reverse transcription quantitative PCR (RT-qPCR) for the M gene of influenza A viruses (*2*). Positive AIV RNA was subtyped for H5, H7, and H9 subtypes and neuraminase (NA) subtyping by using specific subtyping RT-qPCR (*3*,*4*).

Sequencing and Phylogenetic Analyses

Complete gene segments of the hemagglutin (HA) and NA were amplified by using primers previously described by Hoper et al. (*5*). The gene-specific RT-PCR amplicons were size-separated by agarose gel electrophoresis, excised and purified from gels by using the QIAquick Gel Extraction Kit (QIAGEN). Further, purified PCR products were used directly for cycle sequencing reactions (BigDye Terminator v3.1 Cycle Sequencing Kit; Applied Biosystems, Foster City, CA, USA). Reaction products were purified by using Centrisep spin column (Thermo Fisher, Carlsbad, CA, USA) and sequenced on an ABI PRISM 3100 Genetic Analyzer (Life Technologies, Carlsbad, CA, USA). Thereafter, the obtained sequences of the HA and NA genes were assembled and edited by using the Geneious software, version 9.0.5 (*6*). A BLAST (blast.ncbi.nlm.nih.gov/) search was performed by using GISAID platform, and sequences established in this study have been submitted to the Global Initiative on Sharing All Influenza Data (GISAID) database (accession nos.: EPI868853–4). In addition, genetic sequences of representative subtypes H5N8 and H5Nx were retrieved from the GISAID platform. Alignment and identity matrix analyses were performed by using MAFFT (*7*) and BioEdit (*8*). Phylogenetic analyses were based on maximum likelihood methodology based on Akaike criterion after selection of the best- fit modes (GTR+Γ+G4 and HKY+G4 for the HA and NA, respectively) by using IQ-TREE software version 1.1.3 (*9*). Trees were finally viewed and edited with FigTree v1.4.2 software (http://tree.bio.ed.ac.uk/software/figtree/).

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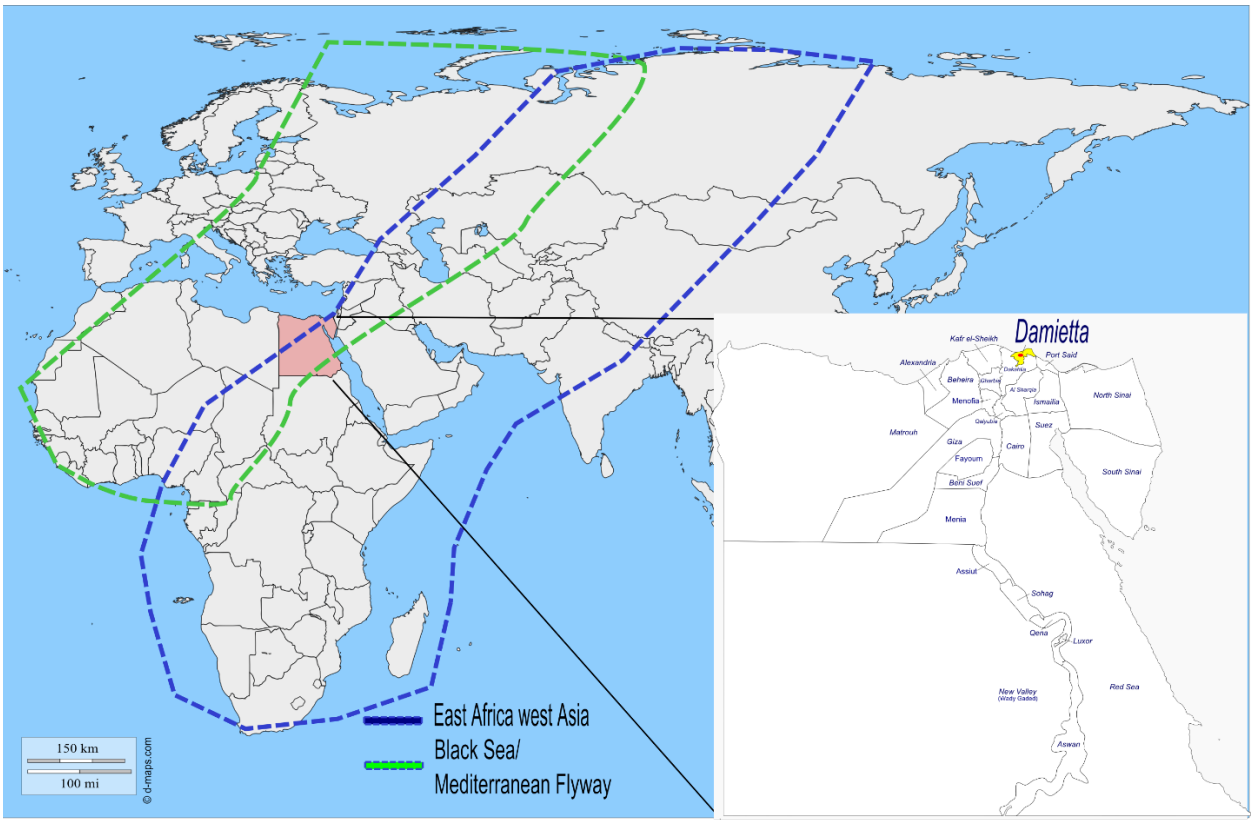
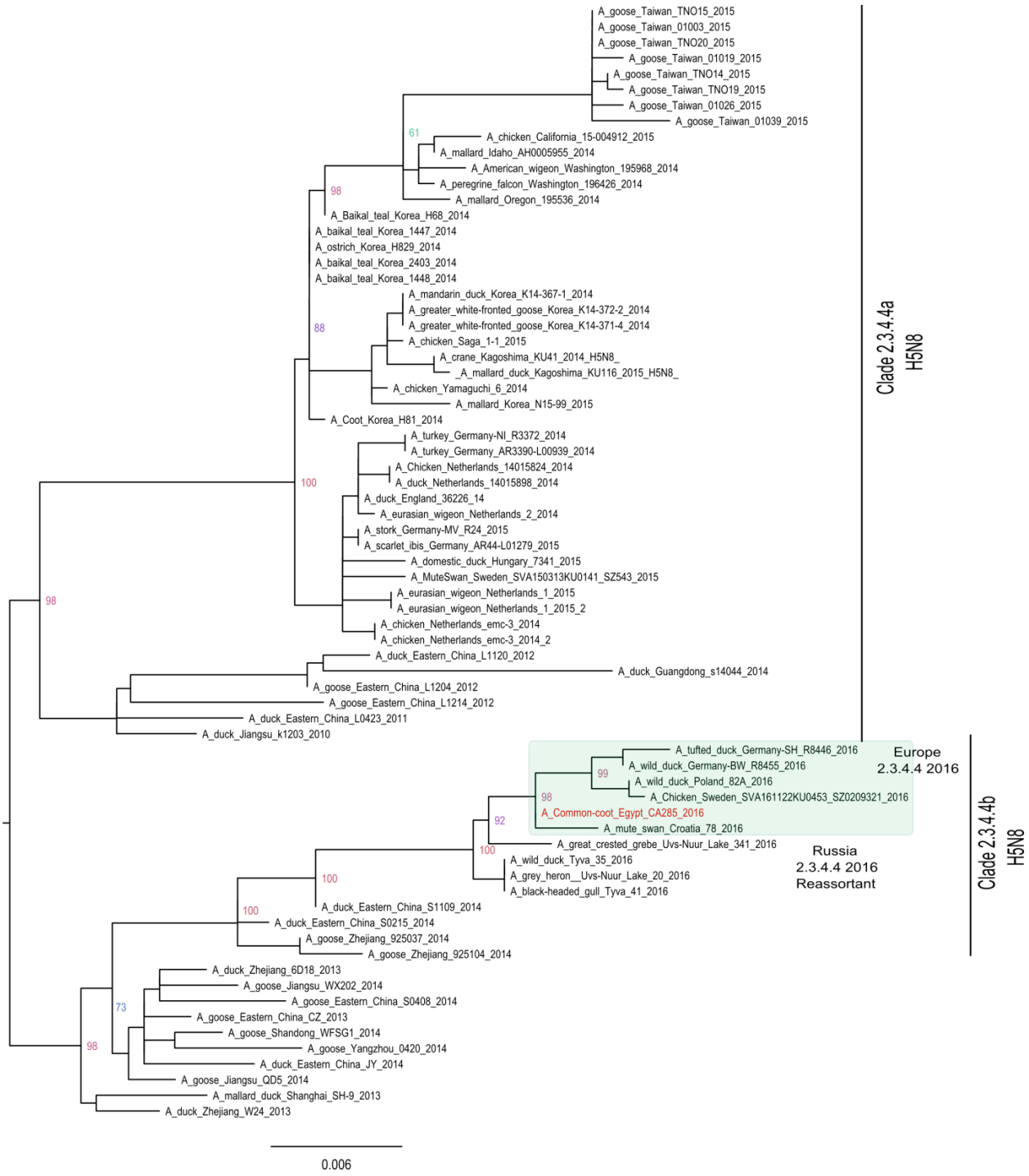
Technical Appendix Table. GISAID submitters of influenza virus segments used in this study

Segment ID	Segment	Country	Collection date	Isolate name	Submitting laboratory
EPI573173	NA	Netherlands	2014 Nov 20	A/Chicken/Netherlands/14015824/2014	Central Veterinary Institute
EPI573171	HA	Netherlands	2014 Nov 20	A/Chicken/Netherlands/14015824/2014	Central Veterinary Institute
EPI596301	HA	Netherlands	2014 Nov 21	A/chicken/Netherlands/emc-3/2014	Other database Import
EPI596303	NA	Netherlands	2014 Nov 21	A/chicken/Netherlands/emc-3/2014	Other Database Import
EPI573179	HA	Netherlands	2014 Nov 21	A/duck/Netherlands/14015898/2014	Central Veterinary Institute
EPI573181	NA	Netherlands	2014 Nov 21	A/duck/Netherlands/14015898/2014	Central Veterinary Institute
EPI596295	HA	Netherlands	2014 Nov 24	A/eurasian wigeon/Netherlands/2/2014	Other database import
EPI596297	NA	Netherlands	2014 Nov 24	A/eurasian wigeon/Netherlands/2/2014	Other database import
EPI691836	HA	Germany	2014 Dec 1	A/gull Germany-NI/R45/2015	Friedrich-Loeffler-Institut
EPI691837	NA	Germany	2014 Dec 1	A/gull Germany-NI/R45/2015	Friedrich-Loeffler-Institut
EPI687238	NA	Germany	2014 Dec 15	A/turkey/Germany/AR3390-L00939/2014	Friedrich-Loeffler-Institut
EPI687239	HA	Germany	2014 Dec 15	A/turkey/Germany/AR3390-L00939/2014	Friedrich-Loeffler-Institut
EPI687246	NA	Germany	2014 Dec 15	A/turkey/Germany/AR3382-L00937/2014	Friedrich-Loeffler-Institut
EPI687247	HA	Germany	2014 Dec 15	A/turkey/Germany/AR3382-L00937/2014	Friedrich-Loeffler-Institut
EPI584823	HA	Hungary	2015 Feb 23	A/domestic duck/Hungary/7341/2015	Central Agricultural Office Veterinary Diagnostic Directorate
EPI584825	NA	Hungary	2015 Feb 23	A/domestic duck/Hungary/7341/2015	Central Agricultural Office Veterinary Diagnostic Directorate
EPI576393	NA	Sweden	2015 Mar 5	A/MuteSwan/Sweden/SVA150313KU0141/ SZ543/2015	National Veterinary Institute

Segment ID	Segment	Country	Collection date	Isolate name	Submitting laboratory
EPI576391	HA	Sweden	2015 Mar 5	A/MuteSwan/Sweden/SVA150313KU0141/ SZ543/2015	National Veterinary Institute
EPI823756	HA	Russian Federation	2016 May 25	A/black-headed gull/Tyva/41/2016	WHO National Influenza Centre Russian Federation
EPI823758	NA	Russian Federation	2016 May 25	A/black-headed gull/Tyva/41/2016	WHO National Influenza Centre Russian Federation
EPI836606	HA	Russian Federation	2016 May 25	A/gray heron /Uvs-Nuur Lake/20/2016	Research Institute of Experimental and Clinical Medicine
EPI836608	NA	Russian Federation	2016 May 25	A/gray heron /Uvs-Nuur Lake/20/2016	Research Institute of Experimental and Clinical Medicine
EPI773759	NA	Russian Federation	2016 May 25	A/great crested grebe/Uvs-Nuur Lake/341/2016	Research Institute of Experimental and Clinical Medicine
EPI773757	HA	Russian Federation	2016 May 25	A/great crested grebe/Uvs-Nuur Lake/341/2016	Research Institute of Experimental and Clinical Medicine
EPI823748	HA	Russian Federation	2016-May-25	A/wild duck/Tyva/35/2016	WHO National Influenza Centre Russian Federation
EPI823750	NA	Russian Federation	2016 May 25	A/wild duck/Tyva/35/2016	WHO National Influenza Centre Russian Federation
EPI859649	NA	Germany	2016 Nov 1	A/wild duck/Germany-BW/R8455/2016	Friedrich-Loeffler-Institut
EPI859650	HA	Germany	2016 Nov 1	A/wild duck/Germany-BW/R8455/2016	Friedrich-Loeffler-Institut
EPI860232	NA	Poland	2016 Nov 2	A/wild duck/Poland/82A/2016	National Veterinary Research Institut Poland, PIWet-PIB
EPI860231	HA	Poland	2016 Nov 2	A/wild duck/Poland/82A/2016	National Veterinary Research Institut Poland, PIWet-PIB
EPI859213	NA	Germany	2016 Nov 7	A/tufted_duck/Germany-SH/R8446/2016	Friedrich-Loeffler-Institut
EPI859212	HA	Germany	2016 Nov 7	A/tufted_duck/Germany-SH/R8446/2016	Friedrich-Loeffler-Institut
EPI860239	HA	Denmark	2016 Nov 8	A/tufted duck/Denmark/17740–1/2016	Technical University of Denmark
EPI861572	HA	Croatia	2016 Nov 12	A/mute swan/Croatia/78/2016	Croatian Veterinary Institute
EPI861573	NA	Croatia	2016 Nov 12	A/mute swan/Croatia/78/2016	Croatian Veterinary Institute
EPI863857	HA	Sweden	2016 Nov 21	A/Chicken/Sweden/SVA161122KU0453/ SZ0209321/2016	National Veterinary Institute
EPI863859	NA	Sweden	2016 Nov 21	A/Chicken/Sweden/SVA161122KU0453/ SZ0209321/2016	National Veterinary Institute



Technical Appendix Figure 1. Map showing various migratory flyways crossing Egypt. The area where highly pathogenic avian influenza virus, subtype H5N8, was detected in Damietta Governorate, Egypt, is indicated in yellow, and the infected live bird market is shown in red.



Technical Appendix Figure 2. Phylogenetic tree of the nucleotide sequences of the neuraminidase gene segments. Maximum likelihood calculations were done with the IQTree software under the best-fit model according to the Akaike criterion (HKY+G4 model). Highly pathogenic avian influenza virus strains (HPAIV), subtype H5N8, in Egypt are shown in red; current circulating HPAIV(H5N8) strains in Europe are highlighted in green.