

Because of the social and economic relationships between China and Gabon, the travels of asymptomatic CPE carriers from China to Gabon can be expected to have facilitated the spread of CPE in Gabon. Several multidrug-resistant clones of *K. pneumoniae*, including sequence type 307 (10), have been recognized as having emerging epidemic potential worldwide. The genome analysis of the 3 *bla*<sub>NDM-7</sub>-producing *K. pneumoniae* isolates from Gabon revealed clonal isolates (2 and 5 single-nucleotide polymorphisms between them) of sequence type 307. This result suggests an uncontrolled spread in the hospital intensive care unit.

This description of *bla*<sub>NDM-7</sub> in Africa highlights the international dissemination of carbapenemase determinants and the combination of 2 aggravating factors, resulting in an alarming situation: the identification of *bla*<sub>NDM-7</sub> within a transposon element on a conjugative plasmid with a potentially very high level of transmissibility, and the implication of the presence of *K. pneumoniae*, a pathogen with a high potential to persist and disperse in the hospital environment. Urgent measures are required, including the rational use of antimicrobial drugs, public education on the importance of hygiene, and diligent surveillance to control the spread of these multidrug-resistant organisms in the hospital setting.

Dr. Moussounda is a doctor of pharmacy in the Hôpital d'Instruction des Armées Omar Bongo Ondimba in Libreville, Gabon. He conducted this study in the course of his master's research in tropical infections at the École Doctorale Régionale in Franceville, Gabon.

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## Novel Reassortant Clade 2.3.4.4 Avian Influenza A(H5N8) Virus in Wild Aquatic Birds, Russia, 2016

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The emergence of novel avian influenza viruses in migratory birds is of concern because of the potential for virus dissemination during fall migration. We report the identification of novel highly pathogenic avian influenza viruses of subtype H5N8, clade 2.3.4.4, and their reassortment with other avian influenza viruses in waterfowl and shorebirds of Siberia.

Highly pathogenic avian influenza virus (HPAIV) subtype H5N1 was first isolated from a goose in 1996 in Guangdong China (Gs/GD). This virus evolved into multiple hemagglutinin (HA) genetic clades and underwent reassortment with different neuraminidase and internal genes to generate subtype H5N8 clade 2.3.4.4 Gs/GD HPAIV, which first appeared in an outbreak in poultry in China in 2013 (1), followed closely by outbreaks in South Korea in January 2014 (2). During these outbreaks, 2 distinct groups of H5N8 viruses were identified; group A (Buan-like) and group B (Gochang-like). There have been no further reports of group B virus since its original detection in China and South Korea during 2014 (3,4). In contrast, in early 2014, group A viruses predominated in South Korea (5) and in September of that year were subsequently isolated from a Eurasian wigeon (*Anas penelope*) in Sakha Republic in northeast Siberia (6). On the basis of aquatic bird migration patterns, we hypothesized that HPAIV (H5N8) reached Siberia during the 2014 spring bird migration (7). The virus was probably carried by birds from Siberia to various countries of Asia, Europe, and North America during the fall migration, representing an intercontinental group A (icA) (7). We report detection of novel HPAIV (H5N8) from wild aquatic birds sampled in western Siberia during the summer of 2016.

In June 2016, we collected samples from 13 dead and 30 hunter-harvested wild aquatic birds around Uvs-Nuur Lake (Tyva Republic) at the Russia–Mongolia border. We isolated a total of 11 subtype H5 influenza viruses from birds of various species: the black-headed gull (*Larus ridibundus*), gray heron (*Ardea cinerea*), common tern (*Sterna hirundo*), great crested grebe (*Podiceps cristatus*), and great cormorant (*Phalacrocorax carbo*) (online Technical Appendix Table 1, <http://wwwnc.cdc.gov/EID/article/23/2/16-1252-Techapp1.pdf>). We characterized 3 of the viruses—A/great crested grebe/Uvs-Nuur Lake/341/2016(H5N8), A/common tern/Uvs-Nuur Lake/26/2016(H5N8), and A/gray heron/Uvs-Nuur Lake/20/2016(H5N8)—by sequencing, phylogenetic analysis, and intravenous pathogenicity index (IVPI) testing (online Technical Appendix).

We confirmed that all 3 isolates were HPAIV on the basis of amino acid sequence at the HA proteolytic cleavage site (PLREKRRKR/G) and individual IVPIs of 2.75–2.84 in

chickens (online Technical Appendix Table 1). The 3 isolates shared 99.2%–100% nucleotide identity across all 8 genes: HA, neuraminidase (NA), polymerase basic 2 (PB2), polymerase basic 2 (PB1), polymerase acidic (PA), nucleoprotein (NP), matrix (M), and nonstructural (NS). BLAST (<https://www.ncbi.nlm.nih.gov/blast/>) search results showed that the isolates shared >98% identity with low pathogenicity avian influenza virus (LPAIV) from Mongolia and China over 5 gene segments (PB1, PB2, PA, NP, and M) and >98.5% identity with the 2014 H5N8 clade 2.3.4.4 group B HPAIV for the remaining 3 gene segments (HA, NA, and NS) (Table). Phylogenetic analysis showed that the HA, NA, and NS genes clustered with H5N8 clade 2.3.4.4 group B HPAIV viruses identified in eastern China in 2014 (online Technical Appendix Figure). The PB1, PB2, PA, NP, and M genes clustered with LPAIV identified in Mongolia, China, and Vietnam.

Wild aquatic birds migrate to and congregate in Siberian wetlands for breeding and molting. Major wild aquatic bird migration routes overlap in Siberia, connecting this broad geographic area to the wintering grounds of Eurasia and Africa. This unique ecosystem has been implicated as a pathway for the dissemination of HPAIV during southward autumn migration of waterfowl, as seen in the spread of H5N1 clade 2.2 in 2005–2006 (8) and H5N8 clade 2.3.4.4 in 2014 (6,7). Uvs-Nuur Lake is a key habitat for 46 resident waterfowl species and 215 kinds of birds migrating south from Siberia (9). During widespread dissemination of the HPAIV clade 2.2 in 2006 and clade 2.3.2 in 2009, these viruses were also detected from wild aquatic birds at Uvs-Nuur Lake, suggesting this area is a useful site for surveillance of HPAIV in wild aquatic birds (10). Because numerous species of migratory shorebirds and waterfowl use the summer breeding grounds of Siberia, the identification of HPAIV infection in wild aquatic birds in this area signifies the potential for wide dissemination of these novel reassortant Group B H5N8 viruses during the 2016 fall migration.

Dr. Lee is a postdoctoral researcher at the Southeast Poultry Research Laboratory, USDA Agricultural Research Service, Athens, Georgia, USA. His research interests include molecular epidemiology and host–pathogen interaction of avian influenza viruses.

**Table.** Nucleotide identity of near homologs in GenBank to the influenza A(H5N8) virus from Uvs-Nuur Lake, Russia, as of June 30, 2016\*

Gene	Virus	Classification	% Identity
PB2	A/duck/Mongolia/30/2015(H3N8)	Eurasian LPAI	98.7
PB1	A/chicken/Hunan/S1267/2010(H4N6)	Eurasian LPAI	98.1
PA	A/duck/Mongolia/996/2015(H3N8)	Eurasian LPAI	98.7
HA	A/duck/eastern China/S1109/2014(H5N8)	H5N8 clade 2.3.4.4	99.1
NP	A/duck/Mongolia/129/2015(H3N3)	Eurasian LPAI	98.7
NA	A/duck/eastern China/S1109/2014(H5N8)	H5N8 clade 2.3.4.4	98.9
M	A/duck/Mongolia/179/2015(H3N8)	Eurasian LPAI	98.5
NS	A/duck/eastern China/S1109/2014(H5N8)	H5N8 clade 2.3.4.4	99.3

\*HA, hemagglutinin; LPAI, low pathogenicity avian influenza; MP, matrix; NA, neuraminidase; NP, nucleoprotein; NS, nonstructural; PA, polymerase acidic; PB, polymerase basic.

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## Detection of Vaccinia Virus in Urban Domestic Cats, Brazil

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We investigated possible vaccinia virus (VACV) in urban house cats in Brazil. Serum samples from 6 cats were positive for VACV by PCR, indicating likely VACV circulation among house cats in urban areas of Brazil. This finding highlights the importance of epidemiologic surveillance to avoid outbreaks among urban human populations.

Vaccinia virus (VACV) outbreaks, first reported in Brazil in 1999, affect dairy cattle and humans in rural areas (1). Although studies have shown evidence of VACV circulation among several mammal species (1–3), no consensus exists regarding the role of these animals in the VACV transmission chain or which animal is the natural reservoir. In fact, domestic or wild mammals could be asymptomatic hosts and also contribute to VACV transmission (3).

In contrast to VACV, cowpox virus (CPXV) circulates in urban environments in Europe but also in surrounding wild and rural areas (4). CPXV is transmitted to humans mainly by cats, which play a link between the natural reservoirs and humans in the urban environment (4,5). In cats, the clinical course of CPXV infection varies from no symptoms to widespread skin necrotic lesions and can ultimately lead to death (6). Some studies have shown serologic evidence of orthopoxvirus infection in cats from Europe and have addressed the role of these animals in orthopoxvirus transmission to humans (7,8).

Because VACV and CPXV share some epidemiologic features and cats have a prominent role in the urban CPXV transmission chain, we decided to investigate whether urban domestic cats have evidence of exposure to VACV in Brazil. This study was approved by the Animal Experiments Committee of the Universidade Federal de Minas Gerais (registration protocol 315/2014).

We performed a retrospective study of serum samples from 277 house cats, collected during September 2012–December 2014 in 5 states in Brazil (online Technical Appendix Figure 1, <https://wwwnc.cdc.gov/EID/article/23/2/16-1341-Techapp1.pdf>). The states in this study were those whose veterinary clinics agreed to submit samples. We screened serum samples for neutralizing antibodies by using a  $\geq 70\%$  plaque-reduction neutralization test (9). To detect VACV DNA in serum samples, we performed real-time PCR targeting the C11R and A56R genes (9). We directly sequenced A56R fragments in both orientations and in triplicate by using the Mega-BACE sequencer (GE Healthcare, Buckinghamshire, UK). We used ClustalW (<http://www.genome.jp/tools/clustalw>) and MEGA7 soft-

# Novel Reassortant Clade 2.3.4.4 Avian Influenza A (H5N8) Virus in Wild Aquatic Birds, Russia, 2016

## Technical Appendix

### Materials and Methods

#### Samples

We collected 13 dead and 30 hunter harvested wild birds in the surroundings of Uvs-Nuur Lake (Tyva Republic) located at the Russia-Mongolia border in June 2016. As shown in appendix table 1, a total of 11 H5 viruses were isolated and included viruses obtained from black-headed gull (*Larus ridibundus*), grey heron (*Ardea cinerea*), common tern (*Sterna hirundo*), great crested grebe (*Podiceps cristatus*), and great cormorant (*Phalacrocorax carbo*) by chicken embryo inoculation using 10-day-old chicken embryonating eggs. All viruses caused the death of chicken embryos within 2 days. Isolates were confirmed to be H5 positive by AmpliSens Influenza virus A H5N1-FRT PCR kit (AmpliSens, Russia).

#### Genome sequencing and phylogenetic analysis

Complete genome sequencing of A/great crested grebe/Uvs-Nuur Lake/341/2016(H5N8), A/common tern/Uvs-Nuur Lake/26/2016(H5N8), and A/grey heron/Uvs-Nuur Lake/20/2016(H5N8) viruses was performed by next-generation sequencing using the Illumina MiSeq sequencer and Nextera XT DNA Library Preparation kit (Illumina) according to manufacturer`s instructions. The data were analyzed using CLC Genomics Workbench 8.5 (Qiagen, Redwood City, CA). Nucleotide sequences have been deposited in GISAID under no. EPI\_ISL\_224580, EPI\_ISL\_234057, and EPI\_ISL\_234058. We reconstructed the phylogenetic trees using selected representative sequences of Group icA and B and sequences sharing high nucleotide similarity (>98%) available in the GenBank and GISAID. Maximum-likelihood phylogenies for each of the gene segments were generated with RAxML (1) using the general time reversible (GTR) nucleotide substitution model, with among-site rate variation modeled

using a discrete gamma distribution. Bootstrap support values were generated using 1,000 rapid bootstrap replicates.

### Intravenous pathogenicity index (IVPI)

For the intravenous pathogenicity index test of 3 viruses, 0.1 ml of 1:10 dilutions of infectious allantoic fluids were inoculated intravenously into ten 6-week-old specific pathogen free chickens. The IVPI was calculated according to the OIE standard protocol (available at: <http://www.oie.int/international-standard-setting/terrestrial-code/>) and isolates with an IVPI > 1.2 were determined to be HPAI. The challenge study and all experiments with live viruses were conducted in a biosafety level 3 facility.

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**Technical Appendix Table 1.** Summary of influenza test results of 11 wild birds from Uvs-Nurr Lake, Russia, 2016\*

No.	Species	Sample	Status
1	Grey Heron ( <i>Ardea cinerea</i> )	Dead (intestine)	rRT-PCR and isolation positive
2	Grey Heron ( <i>Ardea cinerea</i> )	Dead (intestine)	rRT-PCR and isolation positive; IVPI=2.78; Complete genome sequencing
3	Common Tern ( <i>Sterna hirundo</i> )	Dead (trachea)	rRT-PCR and isolation positive; IVPI=2.75; Complete genome sequencing
4	Common Tern ( <i>Sterna hirundo</i> )	Dead (intestine)	rRT-PCR and isolation positive
5	Great Crested Grebe ( <i>Podiceps cristatus</i> )	Dead (trachea)	rRT-PCR and isolation positive; IVPI=2.84; Complete genome sequencing
6	Black-headed Gull ( <i>Larus ridibundus</i> )	Dead (trachea)	rRT-PCR and isolation positive
7	Black-headed Gull ( <i>Larus ridibundus</i> )	Dead (trachea)	rRT-PCR and isolation positive
8	Great Cormorant ( <i>Phalacrocorax carbo</i> )	Dead (brain)	rRT-PCR and isolation positive
9	Great Cormorant ( <i>Phalacrocorax carbo</i> )	Hunter harvested (cloacal swab)	rRT-PCR and isolation positive
10	Great Cormorant ( <i>Phalacrocorax carbo</i> )	Hunter harvested (cloacal swab)	rRT-PCR and isolation positive
11	Black-headed Gull ( <i>Larus ridibundus</i> )	Hunter harvested (cloacal swab)	rRT-PCR and isolation positive

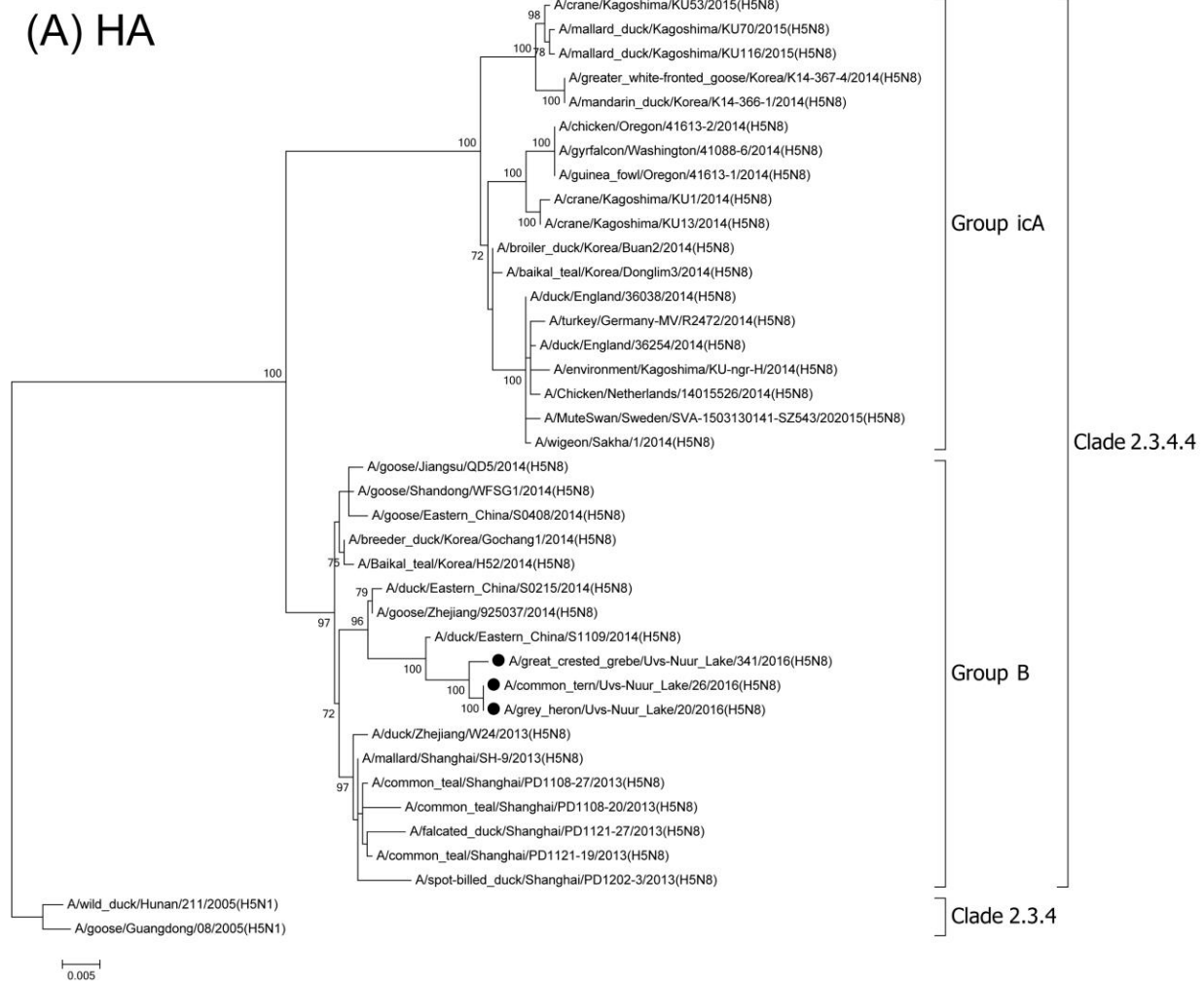
\*rRT-PCR: real-time reverse transcription polymerase chain reaction

**Technical Appendix Table 2.** GISAID submitters for influenza virus segments used in this study\*

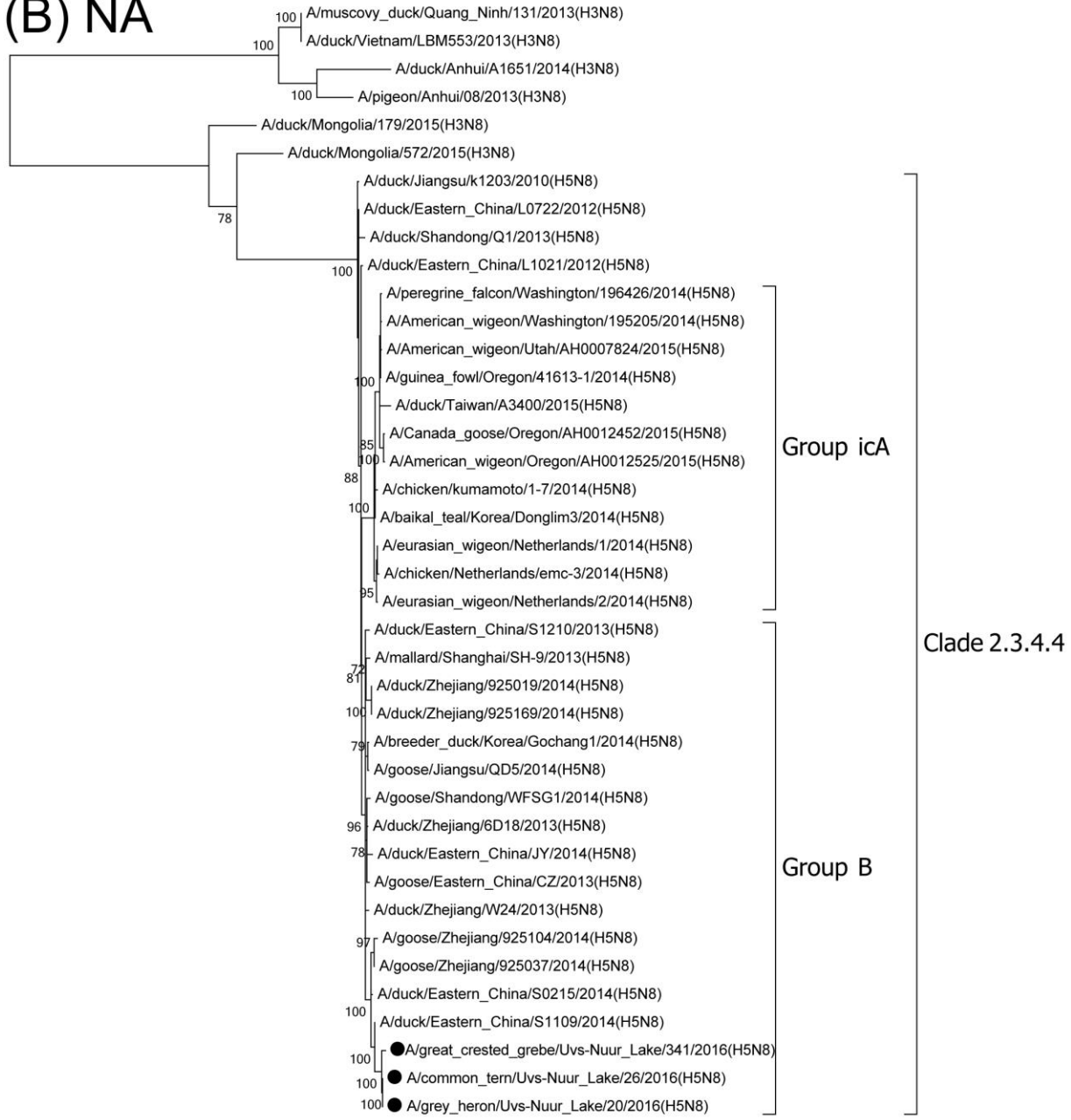
Segment ID	Segment	Country	Collection date	Isolate name	Submitting Lab
EPI595116	HA	Korea, Republic of	2014-Dec-24	A/greater white-fronted goose/Korea/K14-367-4/2014	Konkuk University
EPI576391	HA	Sweden	2015-Mar-05	A/MuteSwan/Sweden/SVA-1503130141-SZ543/2015	National Veterinary Institute
EPI544756	HA	Germany	2014-Nov-04	A/turkey/Germany-MV/R2472/2014	Friedrich-Loeffler-Institut
EPI553208	HA	Japan	2014-Nov-23	A/crane/Kagoshima/KU1/2014	Kagoshima University
EPI573664	HA	Japan	2015-Jan-03	A/crane/Kagoshima/KU53/2015(H5N8)	Kagoshima University
EPI553362	HA	Japan	2014-Dec-01	A/environment/Kagoshima/KU-ngr-H/2014	Kagoshima University
EPI573638	HA	Japan	2014-Dec-07	A/crane/Kagoshima/KU13/2014(H5N8)	Kagoshima University
EPI553349	HA	Russian Federation	2014-Sep-25	A/wigeon/Sakha/1/2014	State Research Center of Virology and Biotechnology Vector
EPI547678	HA	Netherlands	2014-Nov-14	A/Chicken/Netherlands/14015526/2014	Central Veterinary Institute
EPI547673	HA	United Kingdom	2014-Nov-14	A/duck/England/36254/14	Animal and Plant Health Agency (APHA)
EPI550848	HA	United Kingdom	2014-Nov-14	A/duck/England/36038/14	Animal and Plant Health Agency (APHA)
EPI573672	HA	Japan	2015-Jan-14	A/mallard duck/Kagoshima/KU70/2015(H5N8)	Kagoshima University
EPI573680	HA	Japan	2015-Feb-13	A/mallard duck/Kagoshima/KU116/2015(H5N8)	Kagoshima University
EPI595094	HA	Korea, Republic of	2014-Dec-24	A/mandarin duck/Korea/K14-366-1/2014	Konkuk University

\*We acknowledge the authors, originating and submitting laboratories of the sequences from GISAID's EpiFlu™ Database on which this research is based. Contact details of submitters can be found at: <http://platform.gisaid.org/epi3/frontend#39414f>

**Technical Appendix Figure (following pages).** Maximum likelihood phylogenetic trees for the (A) hemagglutinin (HA), (B) neuraminidase (NA), (C) polymerase basic-2 (PB2), (D) polymerase basic-1 (PB1), (E) polymerase acidic (PA), (F) nucleoprotein (NP), (G) matrix (MP), and (H) nonstructural (NS) gene segments for avian influenza virus isolates from Russia and reference isolates. Highly pathogenic and low pathogenic influenza virus sequences from Genbank (<https://www.ncbi.nlm.nih.gov/genbank/>) and the GISAID EpiFlu™ database (<http://platform.gisaid.org/epi3/frontend#39414f>) were used for each phylogenetic comparison. The genetic clusters of highly pathogenic avian influenza viruses are annotated by brackets to the right of the tree. At each branch, the number indicates a bootstrap value (>70%). Black circles indicate the H5N8 viruses sequenced in this study. Scale bar indicates nucleotide substitutions per site.



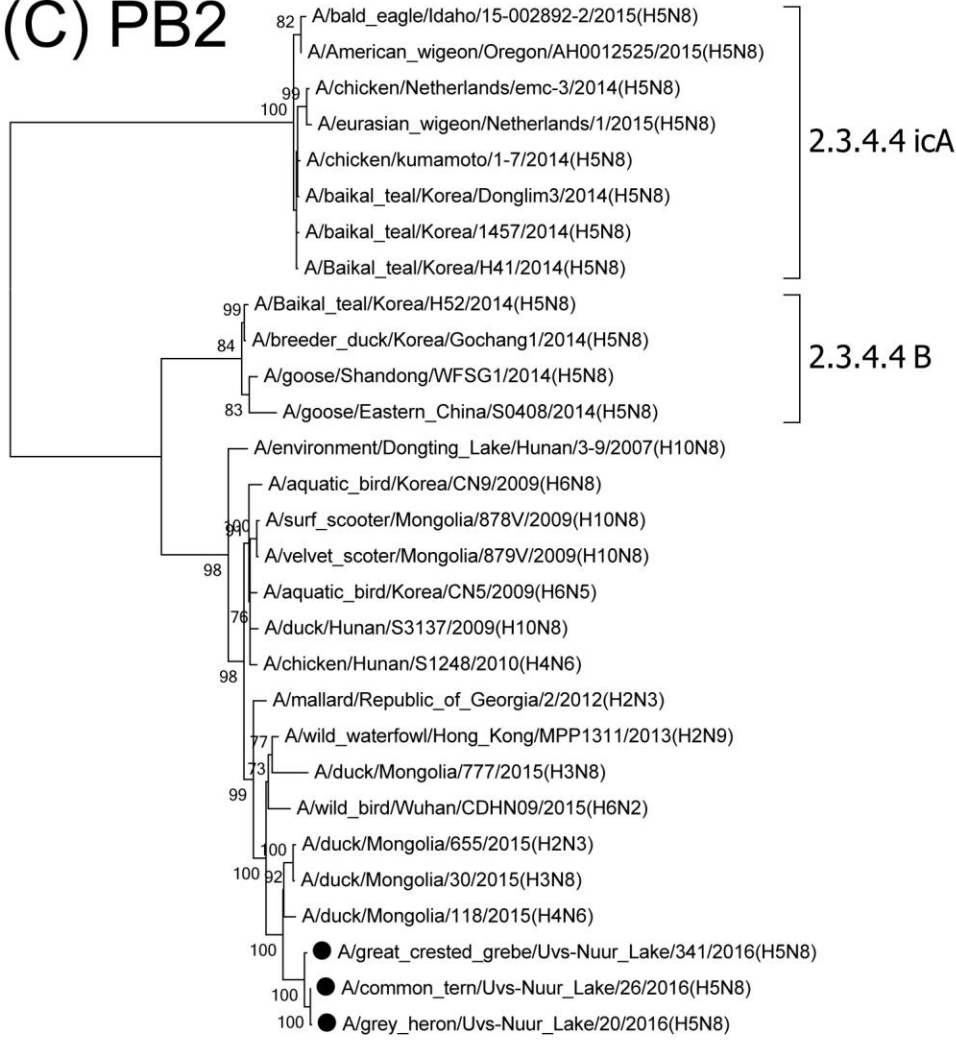
(B) NA



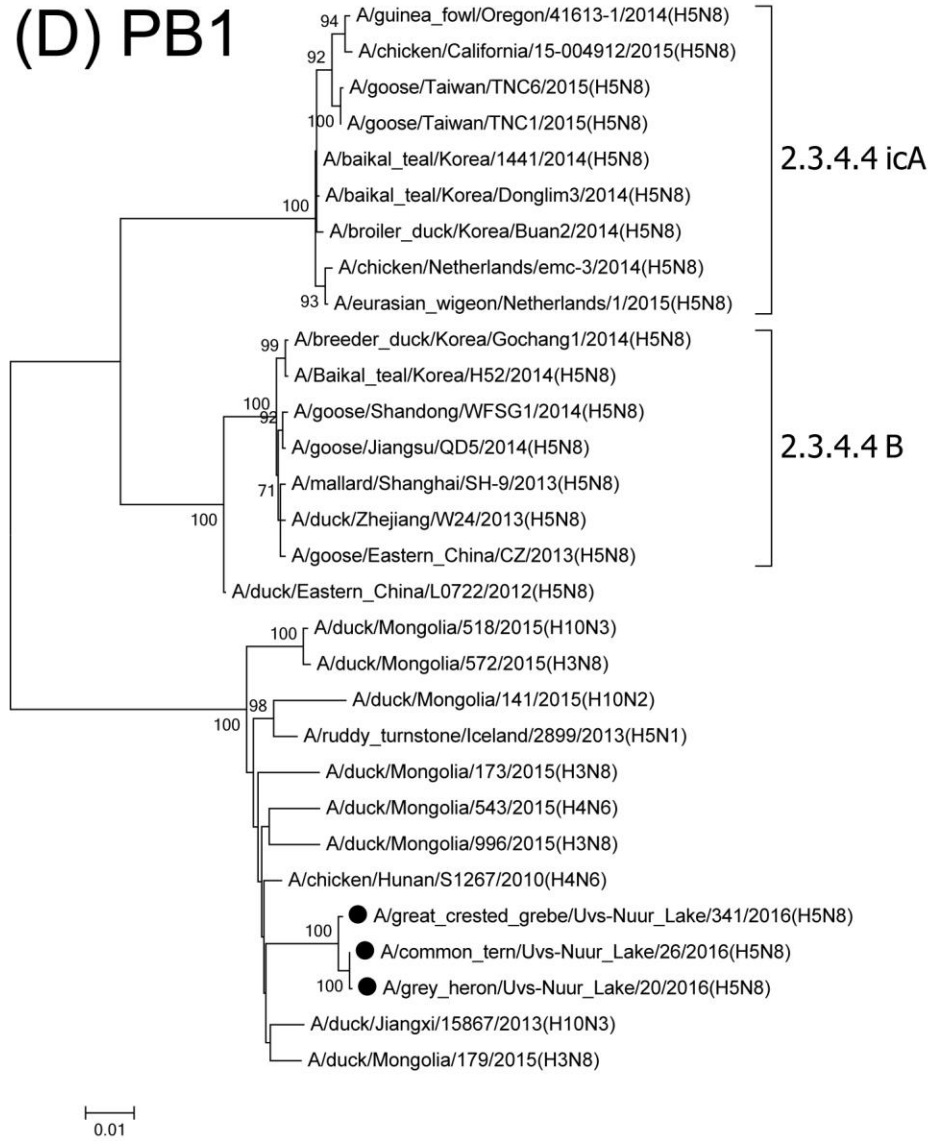
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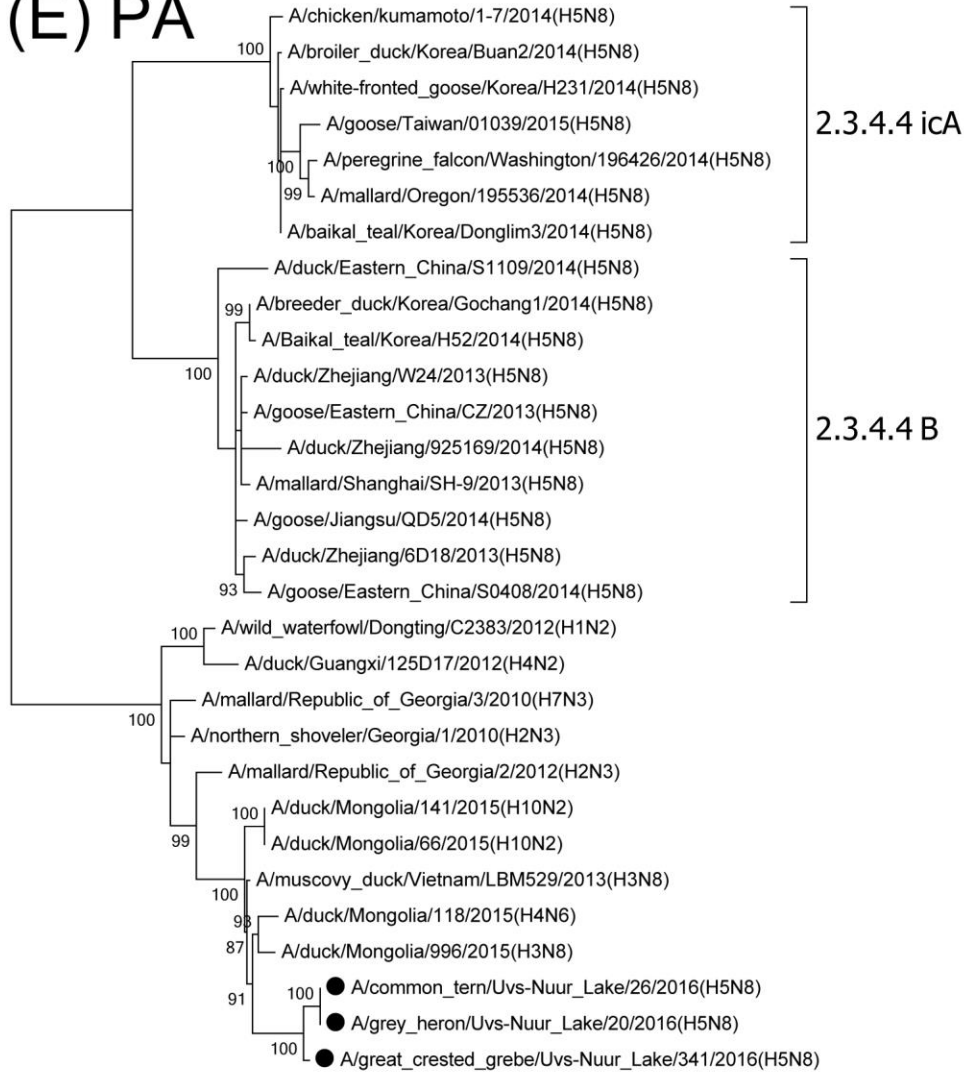
# (C) PB2



# (D) PB1

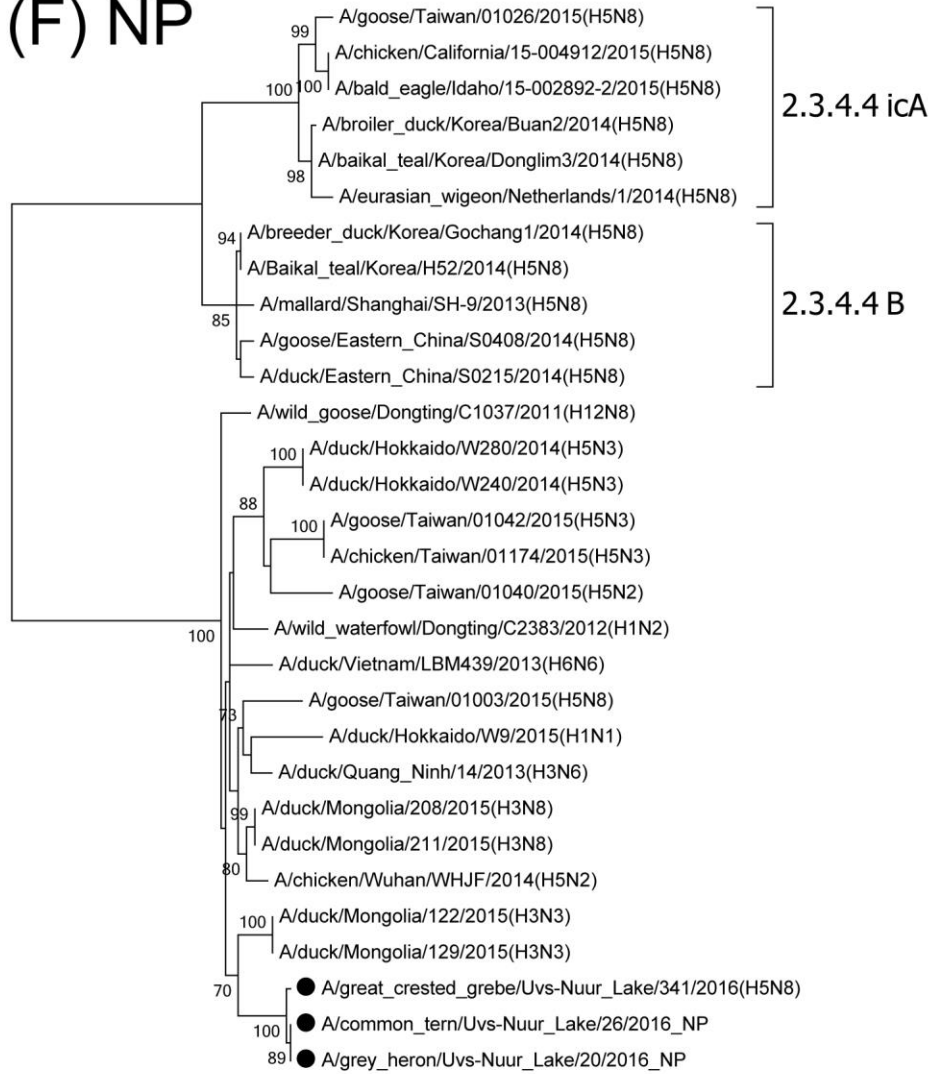


# (E) PA



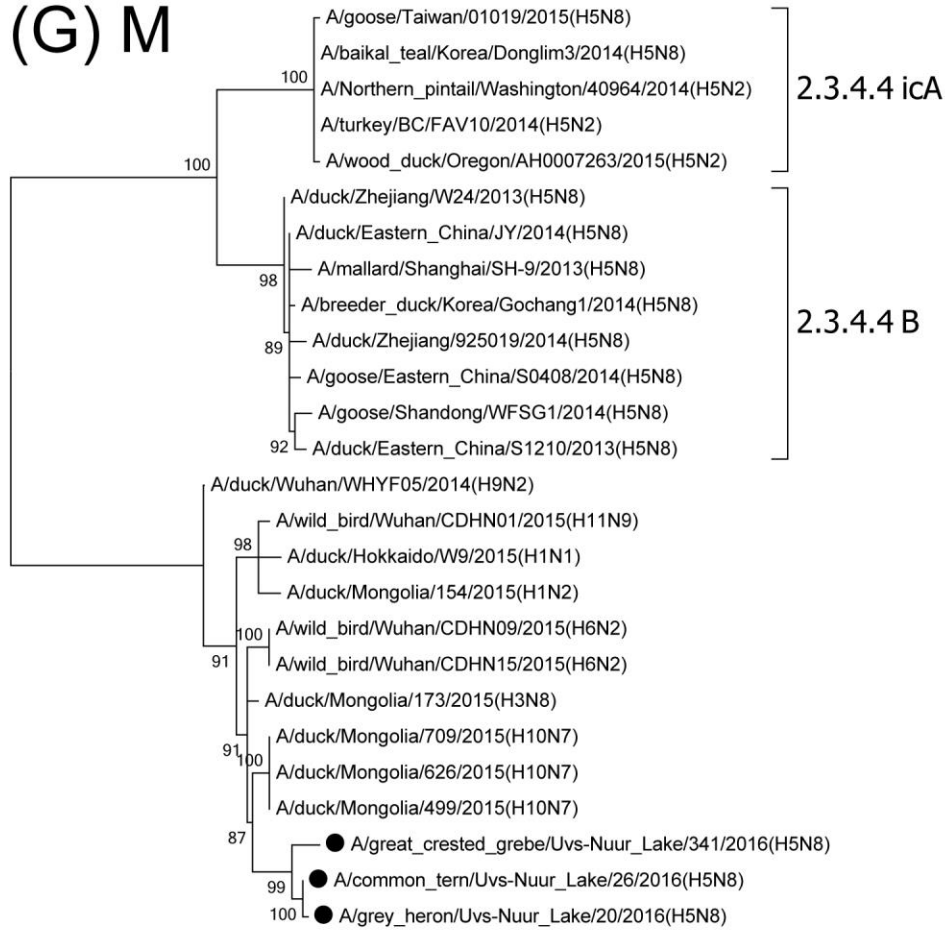
0.005

# (F) NP



0.005

(G) M



0.005

# (H) NS

