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Circulation of Influenza A(H5N8) Virus, Saudi Arabia

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Highly pathogenic avian influenza A(H5N8) viruses have been detected in several continents. However, limited viral sequence data are available from countries in the Middle East. We report full-genome analyses of highly pathogenic H5N8 viruses recently detected in different provinces in Saudi Arabia.

On December 19, 2017, a high number of dead birds from various species was reported in a live bird market in Riyadh, Saudi Arabia, by the Department of Animal Resources Services, Ministry of Environment, Water, and Agriculture. Oropharyngeal and cloacal swab samples were collected from affected birds and investigated for highly pathogenic avian influenza (HPAI) viruses in Riyadh Veterinary Diagnostic Laboratory using reverse transcription PCR (RT-PCR) (*1*). These tests detected HPAI A(H5N8) virus. After this index outbreak, HPAI was reported in adjacent provinces. Surveillance studies were performed in all provinces (≥1 major poultry market and 10 backyard farms per province) to estimate disease prevalence. As of May 2018, a total of 7,273 birds had been investigated; 805 were positive for H5N8, which was detected in 7 provinces (Riyadh, Eastern, Al-Qasim, Makkah, Al-Madinah, Asir, and Jizan). The highest number of positive results was reported in Riyadh (693 samples), in which different commercial poultry farms (22 farms for laying hens, 2 for broiler breeders, and 1 for quail) were affected. A contingency plan, based on a stamping-out policy, was implemented to control the disease. More than 8.8 million birds were depopulated.

[1]These authors contributed equally to this article.

Positive clinical specimens (N = 14) collected from different settings, different provinces, different avian species or a combination were sent to a World Health Organization H5 reference laboratory in Hong Kong for confirmation. All samples tested positive for membrane protein (M) and hemagglutinin (HA) subtype H5 genes by RT-PCR (online Technical Appendix Table 1, https://wwwnc.cdc.gov/EID/article/24/10/18-0846-Techapp.pdf). Samples that had a cycle threshold value <29 in the M gene assay also tested positive for N8 by RT-PCR. Ten of these samples were positive for virus isolation in embryonated chicken eggs and were associated with death of the chicken embryos by day 3 postinoculation.

We amplified viral RNA extracted from the clinical specimens and virus isolates using a multisegment RT-PCR approach for full-genome amplification (*2*). We subjected the RT-PCR products to next-generation sequencing on an Illumina MiSeq (PE300) platform (Illumina, San Diego, CA, USA). We edited the deduced consensus sequences (average sequence coverage >10,000×) using BioEdit (https://www.mbio.ncsu.edu/BioEdit/bioedit.html) and analyzed them phylogenetically using MEGA7 (https://www.megasoftware.net) (GISAID accession nos. for reference sequences, EPI1215422–EPI1215461, EPI1215137–EPI1215184; http://platform.gisaid.org).

The deduced sequences revealed that H5N8 viruses (n = 11) from different sites in Saudi Arabia are almost identical (sequence identity >99.7%), indicating a common origin for this outbreak. Phylogenetic analyses of HA sequences showed that they belong to clade 2.3.4.4 group B (Figure) (*3*). Polymerase acidic protein (PA), HA, nucleoprotein (NP), neuraminidase (NA), M, and nonstructural protein (NS) segments were genetically similar to those derived from recent group B H5N8 viruses (online Technical Appendix Table 2, Figure 1). No genetic markers associated with mammalian host adaptation, α2,6 receptor-binding specificity, or antimicrobial drug resistance were detected (data not shown) (*4*). The gene constellation of PA, HA, NP, NA, M, and NS segments of these H5N8 viruses is similar to those of some H5N8 viruses detected in wild migratory birds from different geographic areas (e.g., A/Anser_cygnoides/Hubei/FW44/2016 and A/green-winged teal/Egypt/877/2016) (*4*,*5*). The polymerase basic protein (PB) 1 and 2 segments of these viruses are similar to those of HPAI H5N5 viruses detected in the Far East (e.g., A/environment/Kamchatka/18/2016) and Europe (e.g., A/swan/Germany-SN/R10645/2016) (online Technical Appendix Figure 1). H5N5 viruses of this lineage were previously proposed to be reassortants of an H5N8 virus (*6*), with the PB1 and PB2 segments derived from an H10 virus (A/duck/Mongolia/245/2015-like virus) and the PA, HA, M, and NS segments derived from a H5N8 virus. Our results agree with previous observations that H5N8 viruses of this lineage continue to evolve and reassort with other influenza virus subtypes in migratory bird populations (*7*,*8*).

The studied samples were collected from multiple avian species in different settings from 3 provinces (online Technical Appendix Table 1). Of 986 samples from poultry holding sites, 182 (18.5%) tested positive for H5N8 virus. The transmission pathway of H5N8 virus in Saudi Arabia is being investigated. Molecular dating analyses suggest that the most recent common ancestor of these H5N8 viruses emerged in this country in September 2017 (online Technical Appendix Figure 2). The potential roles of wild birds, backyard poultry practices, poultry trading, and other human activities in dissemination of these viruses are yet to be determined. However, our results suggest wide circulation of H5N8 viruses caused by a single introduction.

Recently, outbreaks of H5N8 viruses were reported in the Middle East (Israel, Iran, Iraq, and Kuwait) (*1*). However, with the exception of a few HA sequences (n = 12), no other H5N8 viral sequences from this region are available in major sequence databases, which has hampered the investigation of H5N8 viruses in this region. Multiple introductions of H5N8 viruses with different gene constellations have been reported in Egypt (*9*,*10*), but their genetic relationship to H5N8 viruses detected in other countries in the Middle East is not clear. Further surveillance using full-genome analyses is urgently needed to identify major risk factors for HPAI H5N8 viruses in the Middle East.

Acknowledgments

The role of the Deputy Ministership for Animal Resources at the Ministry of Environment, Water, and Agriculture (MEWA) of Saudi Arabia in quick reporting and confirmation of the HPAI outbreak is highly appreciated. The authors acknowledge all the teams that supported the H5N8 investigation from MEWA. We acknowledge the authors and the originating and submitting laboratories of the sequences from GISAID's EpiFlu Database (http://platform.gisaid.org) with which this analysis was carried out.

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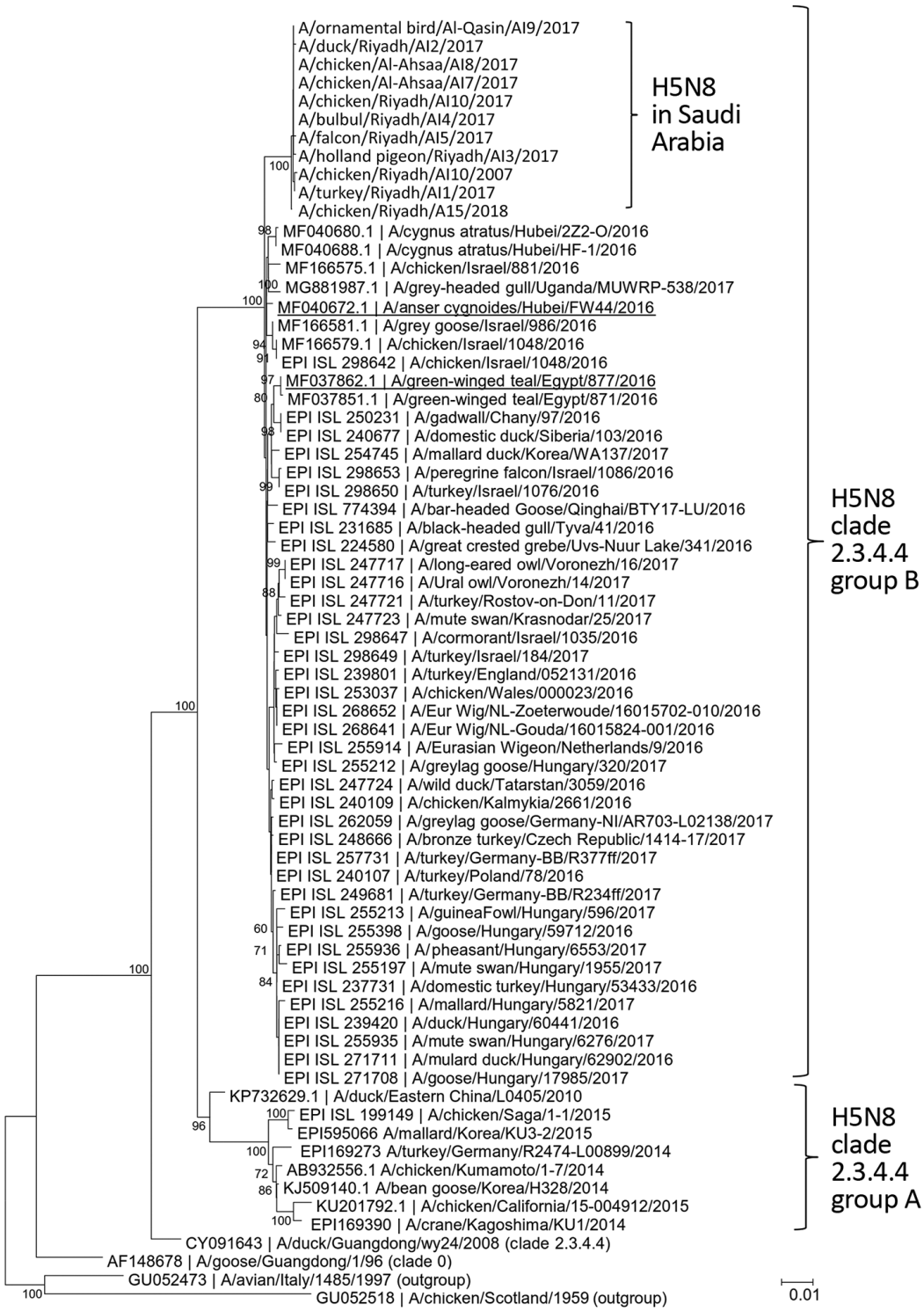

Figure. Phylogenetic analysis of hemagglutinin sequences of influenza A(H5N8) viruses detected in oropharyngeal and cloacal swab samples from birds in Saudi Arabia. Aligned sequences were analyzed in MEGA7 (http://www.megasoftware.net). We constructed the phylogenetic tree using the neighbor-joining method. Representative viral sequences and viral sequences that are highly similar to those reported in this study were included in the analysis. H5N8 viruses reported in this study are labeled. Bootstrap values ≥60% are shown. Representative viruses sharing a similar gene constellation as the H5N8 viruses found in Saudi Arabia are underlined (see text for details). Virus isolate numbers (EPI ISL) in GISAID (http://platform.gisaid.org) or gene accession numbers in GenBank for corresponding viral sequences are provided. Scale bar indicates estimated genetic distance.

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Severe Respiratory Illness Outbreak Associated with Human Coronavirus NL63 in a Long-Term Care Facility

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We describe an outbreak of severe respiratory illness associated with human coronavirus NL63 in a long-term care facility in Louisiana in November 2017. Six of 20 case-patients were hospitalized with pneumonia, and 3 of 20 died. Clinicians should consider human coronavirus NL63 for patients in similar settings with respiratory disease.

Human coronaviruses (HCoVs) OC43, 229E, NL63, and HKU1 are frequently associated with upper respiratory tract infection but can also cause lower respiratory tract infections (LRTIs), such as pneumonia or bronchitis. Transmission of these viruses primarily occurs through respiratory droplets and indirect contact with secretions from infected persons. Signs and symptoms of illness often include runny nose, headache, cough, sore throat, and fever. LRTI occurs less frequently, but young children, older adults, and persons who are immunosuppressed appear to be at higher risk for these types of infections (*1*–*3*).

A wide range of respiratory viruses are known to circulate in long-term care facilities (LTCFs) and contribute to respiratory illness in the residents who live in them (*4*). Although outbreaks of HCoV-OC43 have been described among elderly populations in long-term care settings (*5*), outbreaks of severe respiratory illness associated with HCoV-NL63 have not, to our knowledge, been documented in LTCF settings.

On November 15, 2017, the Louisiana Department of Health (Baton Rouge, Louisiana, USA) was notified of a possible outbreak of severe respiratory illness by a representative of an LTCF that provides nursing home care and short-term rehabilitation services to 130 residents. At the time of notification, the facility reported 11 residents with chest radiograph–confirmed pneumonia. For this investigation, we defined a case-patient as any LTCF resident with respiratory tract symptoms of new onset in November 2017, and we considered LRTI diagnoses that were based

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Technical Appendix

Technical Appendix Table 1. Influenza A(H5N8) samples reported in this study, Saudi Arabia*

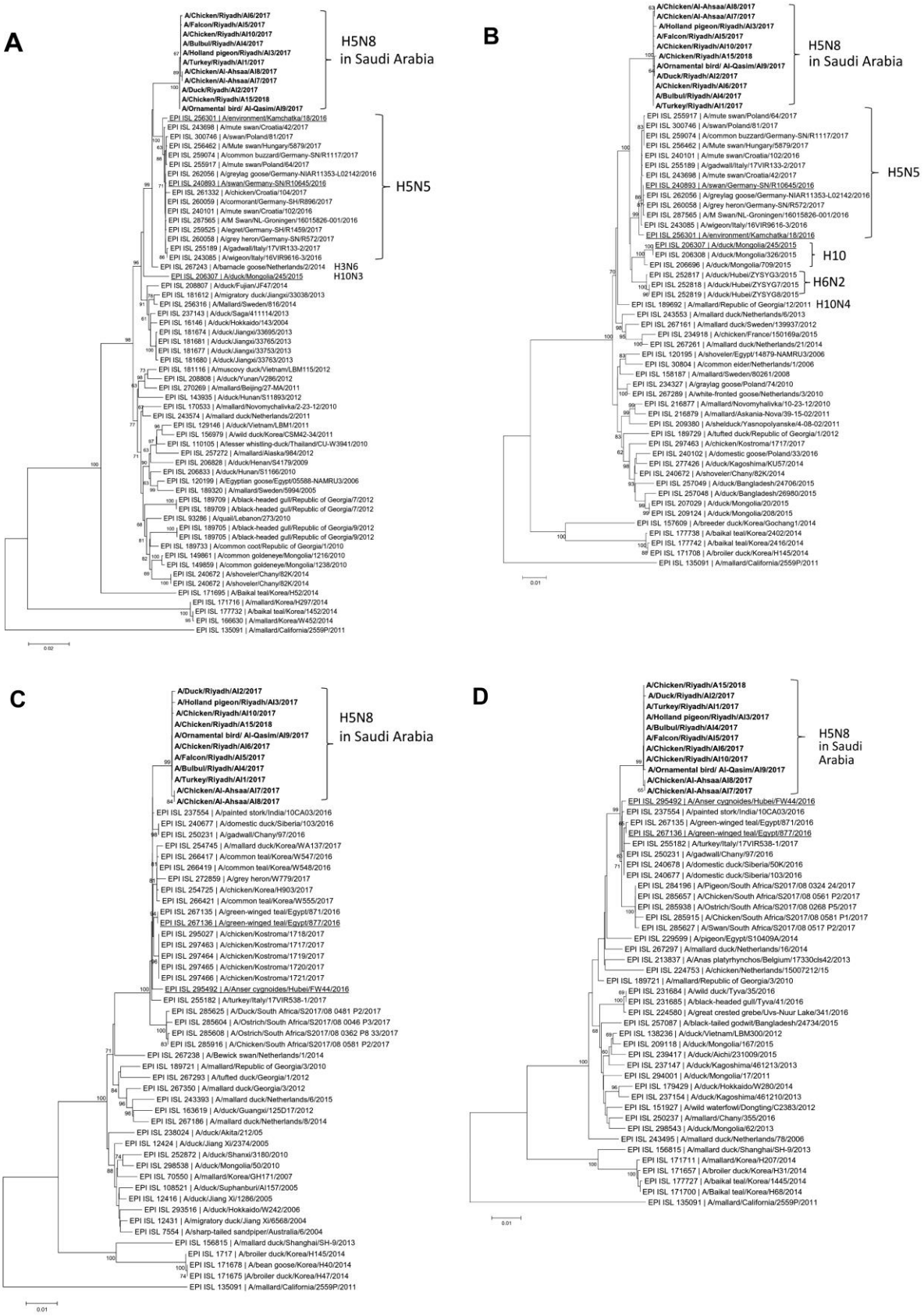
Sample	Sampling province	Sampling site	Sampling date	Type of bird	M†	H5†‡	N8§	Virus isolate	NGS
A/Turkey/Riyadh/AI1/2017	Riyadh	Bird market	2017 Dec 21	Poultry	19.6	23.6	18.0	+	+
A/Duck/Riyadh/AI2/2017	Riyadh	Bird market	2017 Dec 21	Poultry	20.7	24.8	19.8	+	+
A/Holland pigeon/Riyadh/AI3/2017	Riyadh	Bird market	2017 Dec 21	Poultry	26.2	30.9	25.5	+	+¶
A/Bulbul/Riyadh/AI4/2017	Riyadh	Bird shop	2017 Dec 21	Kept bird	30.7	34.0	–#	+	+¶
A/Falcon/Riyadh/AI5/2017	Riyadh	Private owner	2017 Dec 21	Kept bird	24.6	28.0	23.1	+	+¶
A/Chicken/Riyadh/AI6/2017	Riyadh	Poultry farm	2017 Dec 21	Poultry	17.4	20.7	15.7	+	+
A/Chicken/Al-Ahsaa/AI7/2017	Eastern Province	Backyard	2017 Dec 26	Poultry	19.6	23.1	18.7	+	+
A/Chicken/Al-Ahsaa/AI8/2017	Eastern Province	Backyard	2017 Dec 26	Poultry	25.2	28.6	23.8	+	+¶
A/Ornamental bird/Al-Qasim/AI9/2017	Al-Qasim	Backyard	2017 Dec 26	Kept bird	25.0	28.7	24.4	–	+
A/Chicken/Riyadh/AI10/2017	Riyadh	Poultry farm	2017 Dec 28	Poultry	13.9	17.6	12.7	+	+
A/Chicken/Eastern Province/AI11/2017	Eastern Province	Backyard	2017 Dec 28	Poultry	32.5	35.5	–	–	–
A/Goose/Eastern Province/AI12/2017	Eastern Province	Backyard	2017 Dec 28	Poultry	29.5	33.1	–	–	–
A/Duck/Eastern Province/AI13/2017	Eastern Province	Backyard	2017 Dec 28	Poultry	29.4	32.8	–	–	–
A/Chicken/Riyadh/A15/2018	Riyadh	Bird shop	2018 Jan 3	Poultry	22.8	26.2	21.0	+	+

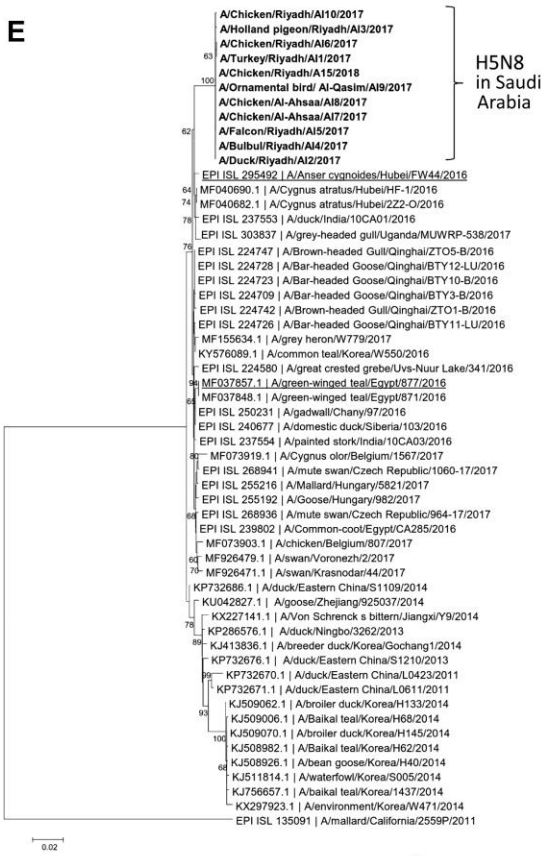
*Numbers denote cycle threshold (CT) values. NGS, next-generation sequencing
†Primer and probe sets were modified from WHO RT-PCR protocols for influenza diagnosis (http://www.who.int/influenza/gisrs_laboratory/molecular_diagnosis/en/). M gene: one-step real-time RT-PCR procedures for the detection of influenza A viruses (protocol 2); H5: one-step real-time RT-PCR procedures for the detection of influenza subtypes H5, H57N9, and H9 (protocol 4).
‡The high Ct values found in this assay were caused by primer mismatches at the reverse primer (5'-AAT<u>T</u>CCCTTCCAAC<u>G</u>GCCTCAAA<u>C</u>-3'; mismatches are underlined).
§The N6 and N8 RT-PCR assays were modified from Hoffman et al. (https://www.nature.com/articles/srep27211).
¶Virus isolates were used as RNA sources for next-generation sequencing.
– indicates that the assay produced a negative result.

Technical Appendix Table 2. Viral sequences with the highest sequence identity to those from A/Turkey/Riyadh/AI1/2017

Segment*	GISAID accession no.	Virus (subtype)	Sequence identity
PB2	EPI1010642	A/barnacle goose/Netherlands/2/2014 (H3N6)	98.3%
PB1	EPI961474	A/environment/Kamchatka/18/2016 (H5N5)	98.5%
PA	EPI858843	A/painted stork/India/10CA03/2016 (H5N8)	99.4%
HA	EPI909452	A/wild duck/Tatarstan/3059/2016 (H5N8)	98.7%
NA	EPI926614	A/domestic duck/Siberia/103/2016 (H5N8)	99.1%
NA	EPI1159827	A/Cygnus atratus/Hubei/HF-1/2016 (H5N8)	98.4%
M	EPI1010490	A/green-winged teal/Egypt/871/2016 (H5N8)	99.5%
NS	EPI926617	A/domestic duck/Siberia/103/2016 (H5N8)	99.5%

*HA, hemagglutinin; M, membrane protein; NA, neuraminidase; NS, nonstructural protein; PA, polymerase acidic protein; PB, polymerase basic protein


A
H5N8
in Saudi Arabia
H5N5
H3N6
H10N3
B
H5N8
in Saudi Arabia
H5N5
H10
H6N2
H10N4
C
H5N8
in Saudi Arabia
D
H5N8
in Saudi
Arabia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A/Chicken/Riyadh/AI6/2017
A/Turkey/Riyadh/AI1/2017
A/Chicken/Riyadh/A15/2018
A/Ornamental bird/ Al-Qasim/AI9/2017
A/Chicken/Al-Ahsaa/AI8/2017
A/Chicken/Al-Ahsaa/AI7/2017
A/Falcon/Riyadh/AI5/2017
A/Bulbul/Riyadh/AI4/2017
A/Duck/Riyadh/AI2/2017
H5N8 in Saudi Arabia
EPI ISL 295492 | A/Anser cygnoides/Hubei/FW44/2016
MF040690.1 | A/Cygnus atratus/Hubei/HF-1/2016
MF040682.1 | A/Cygnus atratus/Hubei/2Z2-O/2016
EPI ISL 237553 | A/duck/India/10CA01/2016
EPI ISL 303837 | A/grey-headed gull/Uganda/MUWRP-538/2017
EPI ISL 224747 | A/Brown-headed Gull/Qinghai/ZTO5-B/2016
EPI ISL 224728 | A/Bar-headed Goose/Qinghai/BTY12-LU/2016
EPI ISL 224723 | A/Bar-headed Goose/Qinghai/BTY10-B/2016
EPI ISL 224709 | A/Bar-headed Goose/Qinghai/BTY3-B/2016
EPI ISL 224742 | A/Brown-headed Gull/Qinghai/ZTO1-B/2016
EPI ISL 224726 | A/Bar-headed Goose/Qinghai/BTY11-LU/2016
MF155634.1 | A/grey heron/W779/2017
KY576089.1 | A/common teal/Korea/W550/2016
EPI ISL 224580 | A/great crested grebe/Uvs-Nuur Lake/341/2016
MF037857.1 | A/green-winged teal/Egypt/877/2016
MF037848.1 | A/green-winged teal/Egypt/871/2016
EPI ISL 250231 | A/gadwall/Chany/97/2016
EPI ISL 240677 | A/domestic duck/Siberia/103/2016
EPI ISL 237554 | A/painted stork/India/10CA03/2016
MF073919.1 | A/Cygnus olor/Belgium/1567/2017
EPI ISL 268941 | A/mute swan/Czech Republic/1060-17/2017
EPI ISL 255216 | A/Mallard/Hungary/5821/2017
EPI ISL 255192 | A/Goose/Hungary/982/2017
EPI ISL 268936 | A/mute swan/Czech Republic/964-17/2017
EPI ISL 239802 | A/Common-coot/Egypt/CA285/2016
MF073903.1 | A/chicken/Belgium/807/2017
MF926479.1 | A/swan/Voronezh/2/2017
MF926471.1 | A/swan/Krasnodar/44/2017
KP732686.1 | A/duck/Eastern China/S1109/2014
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KP286576.1 | A/duck/Ningbo/3262/2013
KJ413836.1 | A/breeder duck/Korea/Gochang1/2014
KP732676.1 | A/duck/Eastern China/S1210/2013
KP732670.1 | A/duck/Eastern China/L0423/2011
KP732671.1 | A/duck/Eastern China/L0611/2011
KJ509062.1 | A/broiler duck/Korea/H133/2014
KJ509006.1 | A/Baikal teal/Korea/H68/2014
KJ509070.1 | A/broiler duck/Korea/H145/2014
KJ508982.1 | A/Baikal teal/Korea/H62/2014
KJ508926.1 | A/bean goose/Korea/H40/2014
KJ511814.1 | A/waterfowl/Korea/S005/2014
KJ756657.1 | A/baikal teal/Korea/1437/2014
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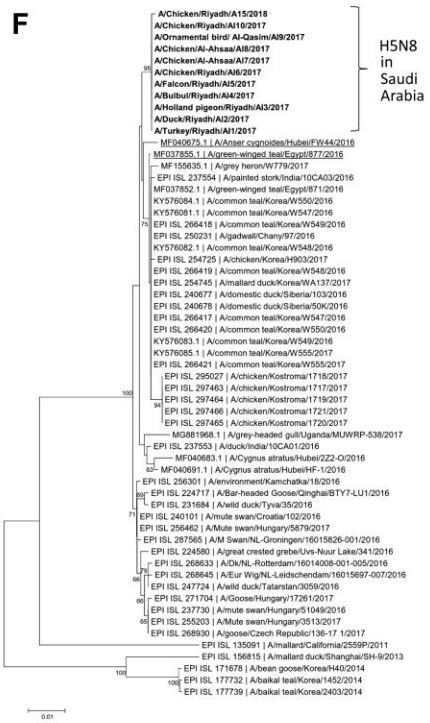
F
A/Chicken/Riyadh/A15/2018
A/Chicken/Riyadh/AI10/2017
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A/Chicken/Al-Ahsaa/AI7/2017
A/Chicken/Riyadh/AI6/2017
A/Falcon/Riyadh/AI5/2017
A/Bulbul/Riyadh/AI4/2017
A/Holland pigeon/Riyadh/AI3/2017
A/Duck/Riyadh/AI2/2017
A/Turkey/Riyadh/AI1/2017
H5N8 in Saudi Arabia
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MF037855.1 | A/green-winged teal/Egypt/877/2016
MF155635.1 | A/grey heron/W779/2017
EPI ISL 237554 | A/painted stork/India/10CA03/2016
MF037852.1 | A/green-winged teal/Egypt/871/2016
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KY576081.1 | A/common teal/Korea/W547/2016
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EPI ISL 254745 | A/mallard duck/Korea/WA137/2017
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EPI ISL 297463 | A/chicken/Kostroma/1717/2017
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MG881968.1 | A/grey-headed gull/Uganda/MUWRP-538/2017
EPI ISL 237553 | A/duck/India/10CA01/2016
MF040683.1 | A/Cygnus atratus/Hubei/2Z2-O/2016
MF040691.1 | A/Cygnus atratus/Hubei/HF-1/2016
EPI ISL 256301 | A/environment/Kamchatka/18/2016
EPI ISL 224717 | A/Bar-headed Goose/Qinghai/BTY7-LU1/2016
EPI ISL 231684 | A/wild duck/Tyva/35/2016
EPI ISL 240101 | A/mute swan/Croatia/102/2016
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EPI ISL 268633 | A/Dk/NL-Rotterdam/16014008-001-005/2016
EPI ISL 268645 | A/Eur Wig/NL-Leidschendam/16015697-007/2016
EPI ISL 247724 | A/wild duck/Tatarstan/3059/2016
EPI ISL 271704 | A/Goose/Hungary/17261/2017
EPI ISL 237730 | A/mute swan/Hungary/51049/2016
EPI ISL 255203 | A/Mute swan/Hungary/3513/2017
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EPI ISL 135091 | A/mallard/California/2559P/2011
EPI ISL 156815 | A/mallard duck/Shanghai/SH-9/2013
EPI ISL 171678 | A/bean goose/Korea/H40/2014
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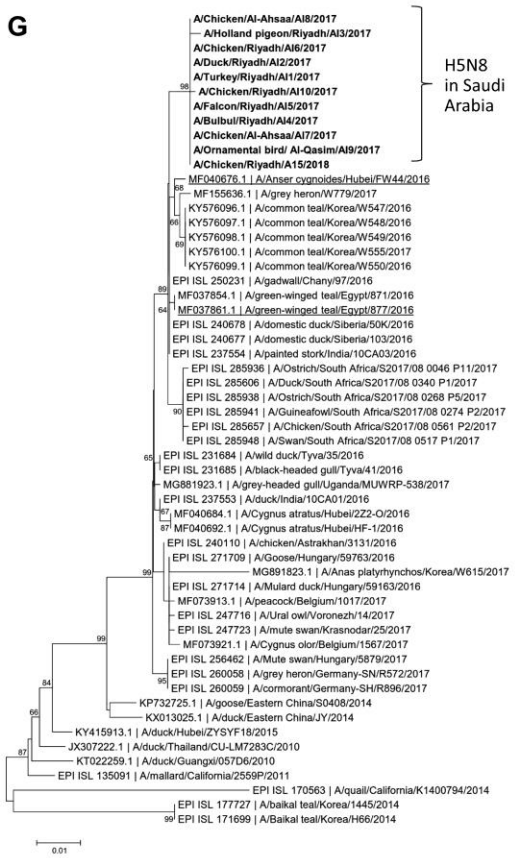
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A/Chicken/Riyadh/AI6/2017
A/Duck/Riyadh/AI2/2017
A/Turkey/Riyadh/AI1/2017
A/Chicken/Riyadh/AI10/2017
A/Falcon/Riyadh/AI5/2017
A/Bulbul/Riyadh/AI4/2017
A/Chicken/Al-Ahsaa/AI7/2017
A/Ornamental bird/ Al-Qasim/AI9/2017
A/Chicken/Riyadh/A15/2018
H5N8 in Saudi Arabia
MF040676.1 | A/Anser cygnoides/Hubei/FW44/2016
MF155636.1 | A/grey heron/W779/2017
KY576096.1 | A/common teal/Korea/W547/2016
KY576097.1 | A/common teal/Korea/W548/2016
KY576098.1 | A/common teal/Korea/W549/2016
KY576100.1 | A/common teal/Korea/W555/2017
KY576099.1 | A/common teal/Korea/W550/2016
EPI ISL 250231 | A/gadwall/Chany/97/2016
MF037854.1 | A/green-winged teal/Egypt/871/2016
MF037861.1 | A/green-winged teal/Egypt/877/2016
EPI ISL 240678 | A/domestic duck/Siberia/50K/2016
EPI ISL 240677 | A/domestic duck/Siberia/103/2016
EPI ISL 237554 | A/painted stork/India/10CA03/2016
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EPI ISL 285606 | A/Duck/South Africa/S2017/08 0340 P1/2017
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EPI ISL 285941 | A/Guineafowl/South Africa/S2017/08 0274 P2/2017
EPI ISL 285657 | A/Chicken/South Africa/S2017/08 0561 P2/2017
EPI ISL 285948 | A/Swan/South Africa/S2017/08 0517 P1/2017
EPI ISL 231684 | A/wild duck/Tyva/35/2016
EPI ISL 231685 | A/black-headed gull/Tyva/41/2016
MG881923.1 | A/grey-headed gull/Uganda/MUWRP-538/2017
EPI ISL 237553 | A/duck/India/10CA01/2016
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MF040692.1 | A/Cygnus atratus/Hubei/HF-1/2016
EPI ISL 240110 | A/chicken/Astrakhan/3131/2016
EPI ISL 271709 | A/Goose/Hungary/59763/2016
MG891823.1 | A/Anas platyrhynchos/Korea/W615/2017
EPI ISL 271714 | A/Mulard duck/Hungary/59163/2016
MF073913.1 | A/peacock/Belgium/1017/2017
EPI ISL 247716 | A/Ural owl/Voronezh/14/2017
EPI ISL 247723 | A/mute swan/Krasnodar/25/2017
MF073921.1 | A/Cygnus olor/Belgium/1567/2017
EPI ISL 256462 | A/Mute swan/Hungary/5879/2017
EPI ISL 260058 | A/grey heron/Germany-SN/R572/2017
EPI ISL 260059 | A/cormorant/Germany-SH/R896/2017
KP732725.1 | A/goose/Eastern China/S0408/2014
KX013025.1 | A/duck/Eastern China/JY/2014
KY415913.1 | A/duck/Hubei/ZYSYF18/2015
JX307222.1 | A/duck/Thailand/CU-LM7283C/2010
KT022259.1 | A/duck/Guangxi/057D6/2010
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EPI ISL 170563 | A/quail/California/K1400794/2014
EPI ISL 177727 | A/baikal teal/Korea/1445/2014
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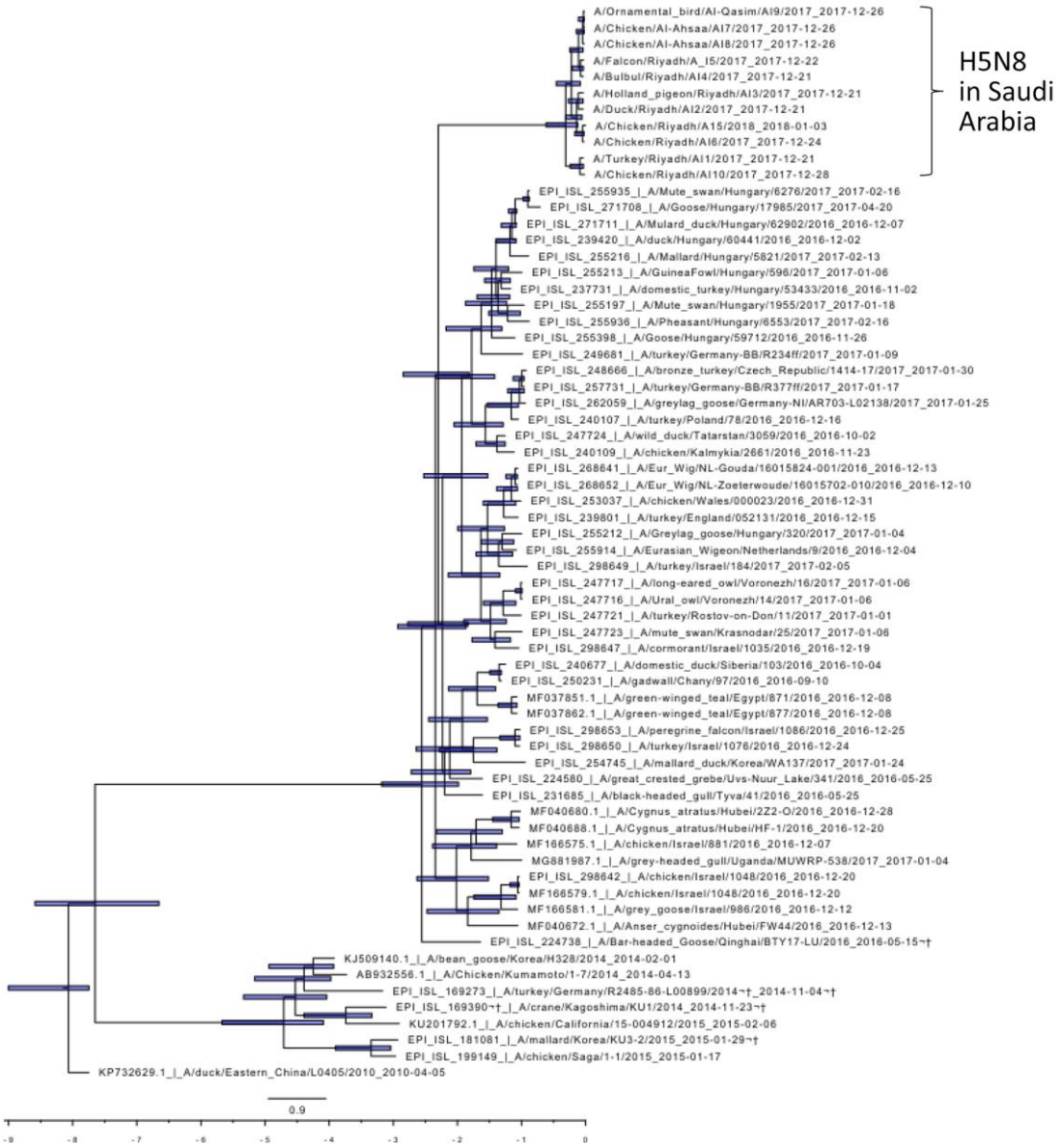
0.01

Technical Appendix Figure 1. Phylogenetic analyses of H5N8 viruses detected in Saudi Arabia: A) polymerase basic protein 2 (PB2); B) PB1; C) polymerase acidic protein (PA); D) nucleoprotein (NP); E) neuraminidase (NA); F) membrane protein (M); G) nonstructural protein (NS). Aligned sequences were analysed by MEGA7 (https://www.megasoftware.net/). Phylogenetic trees were constructed using the neighbor-joining method. Representative viral sequences and viral sequences that are highly similar to those reported in this study were included in these analyses. H5N8 viruses reported in this study are highlighted as shown. Bootstrap values ≥60% are shown. Representative viruses that share a similar gene constellation (PB2 and PB1; PA, HA, NP, NA, M, and NS) of H5N8 viruses found in Saudi Arabia are underlined (see main text for details). GISAID accession numbers for corresponding viral sequences are shown as indicated. Scale bar indicates the estimated genetic distance of these viruses.



Technical Appendix Figure 2. Phylogenetic tree of hemagglutinin (HA) sequences with dating estimated by BEAST (http://beast.community/). Median (in years) and the estimated posterior probabilities of nodes are shown. Node bars indicate 95% highest posterior density regions of node dating. The median date of the most recent common ancestor of H5N8 viruses in Saudi Arabia is estimated to be September 11, 2017 (95% CI May 23–November 20, 2017). GISAID accession numbers of the reference sequences are indicated.