

Circulation of Influenza H5N8 Virus, Saudi Arabia

Technical Appendix

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

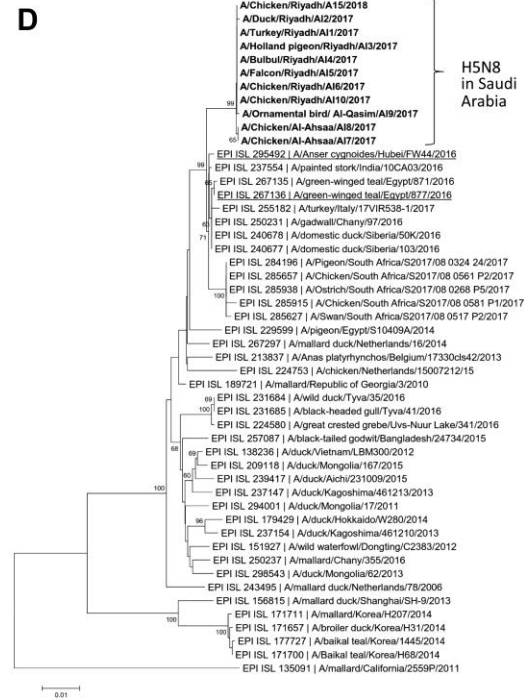
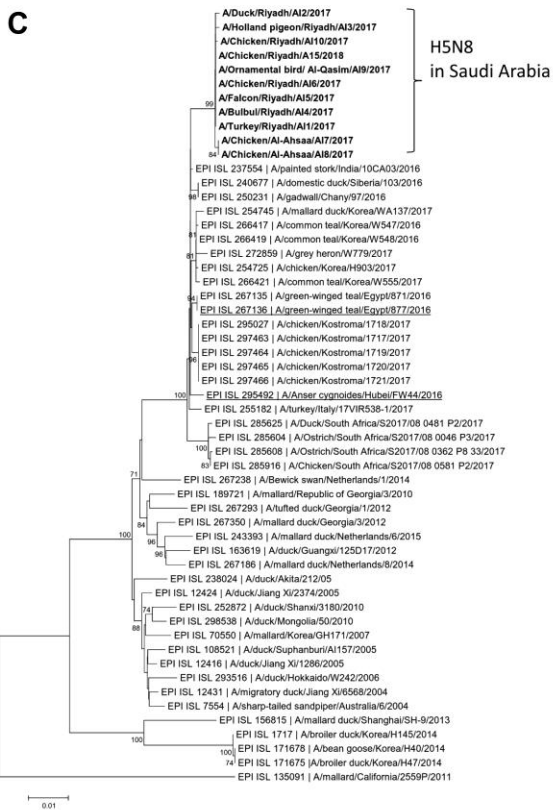
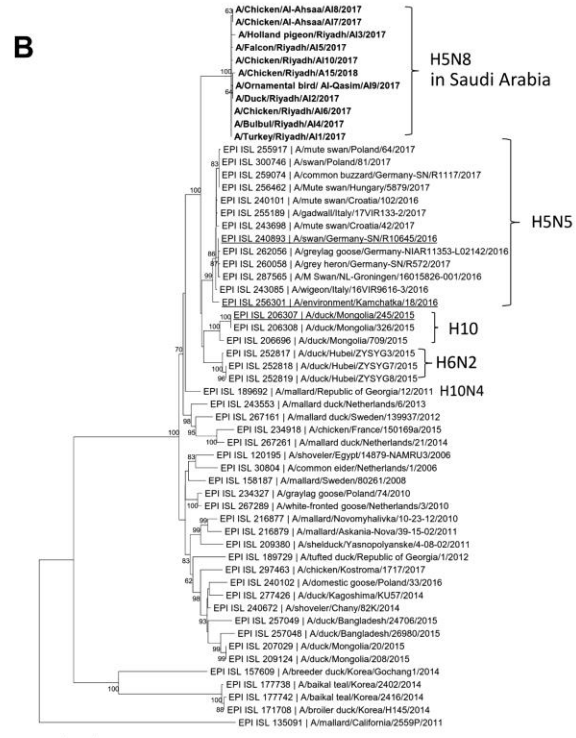
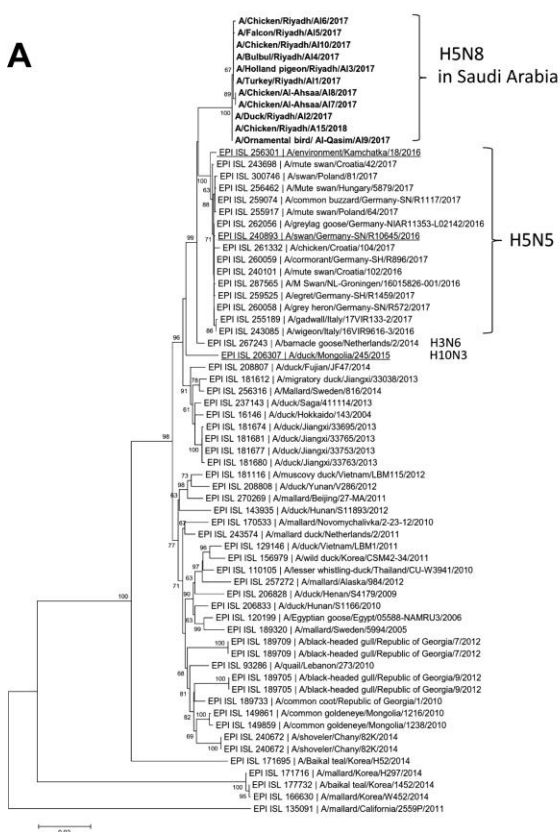
Sample	Sampling province	Sampling site	Sampling date	Type of bird	M†	H5‡	N8§	Virus isolate	NGS
A/Turkey/Riyadh/AI1/2017	Riyadh	Bird market	2017 Dec 21	Poultry	19.6	23.6	18.0	+	+
A/Duck/Riyadh/AI2/2017	Riyadh	Bird market	2017 Dec 21	Poultry	20.7	24.8	19.8	+	+
A/Holland pigeon/Riyadh/AI3/2017	Riyadh	Bird 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/AI15/2018	Riyadh	Bird shop	2018 Jan 3	Poultry	22.8	26.2	21.0	+	+

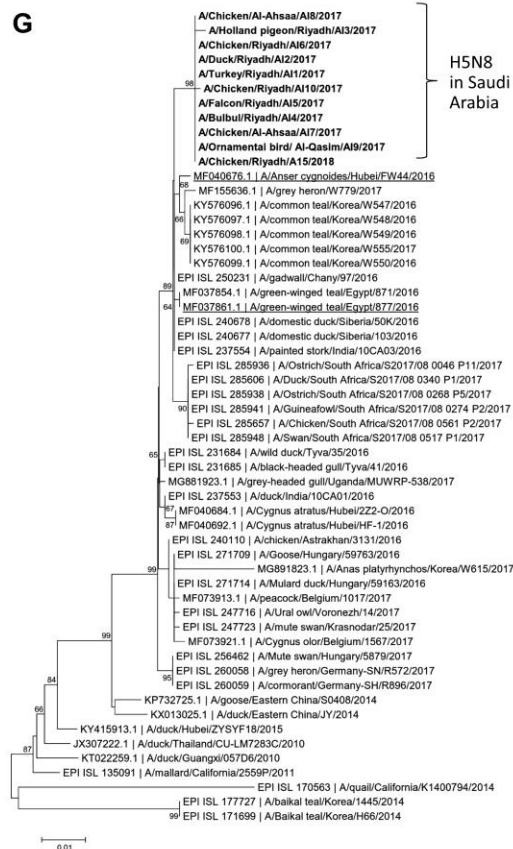
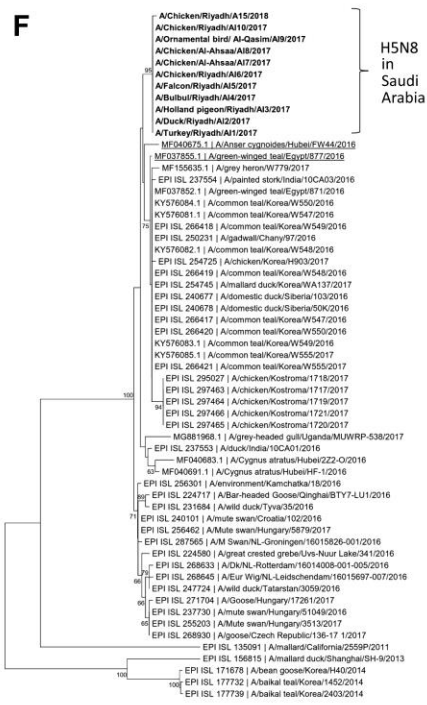
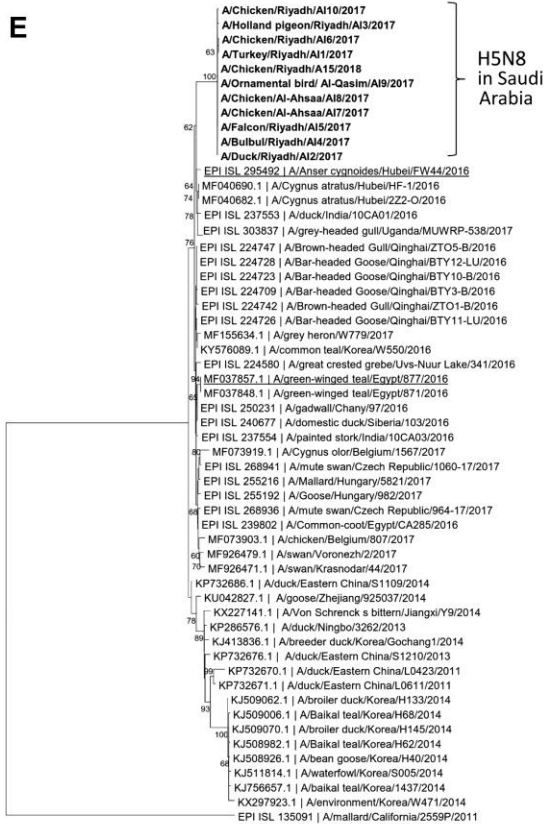
*Numbers denote cycle threshold (CT) values. NGS, next-generation sequencing
†Primer and probe sets were modified from WHO RT-PCR protocols for influenza diagnosis (http://www.who.int/influenza/gisrs_laboratory/molecular_diagnosis/en/). M gene: one-step real-time RT-PCR procedures for the detection of influenza A viruses (protocol 2); H5: one-step real-time RT-PCR procedures for the detection of influenza subtypes H5, H5N9, and H9 (protocol 4).
‡The high Ct values found in this assay were caused by primer mismatches at the reverse primer (5'-AATICCCCTCCAACGCCTCAAAC-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

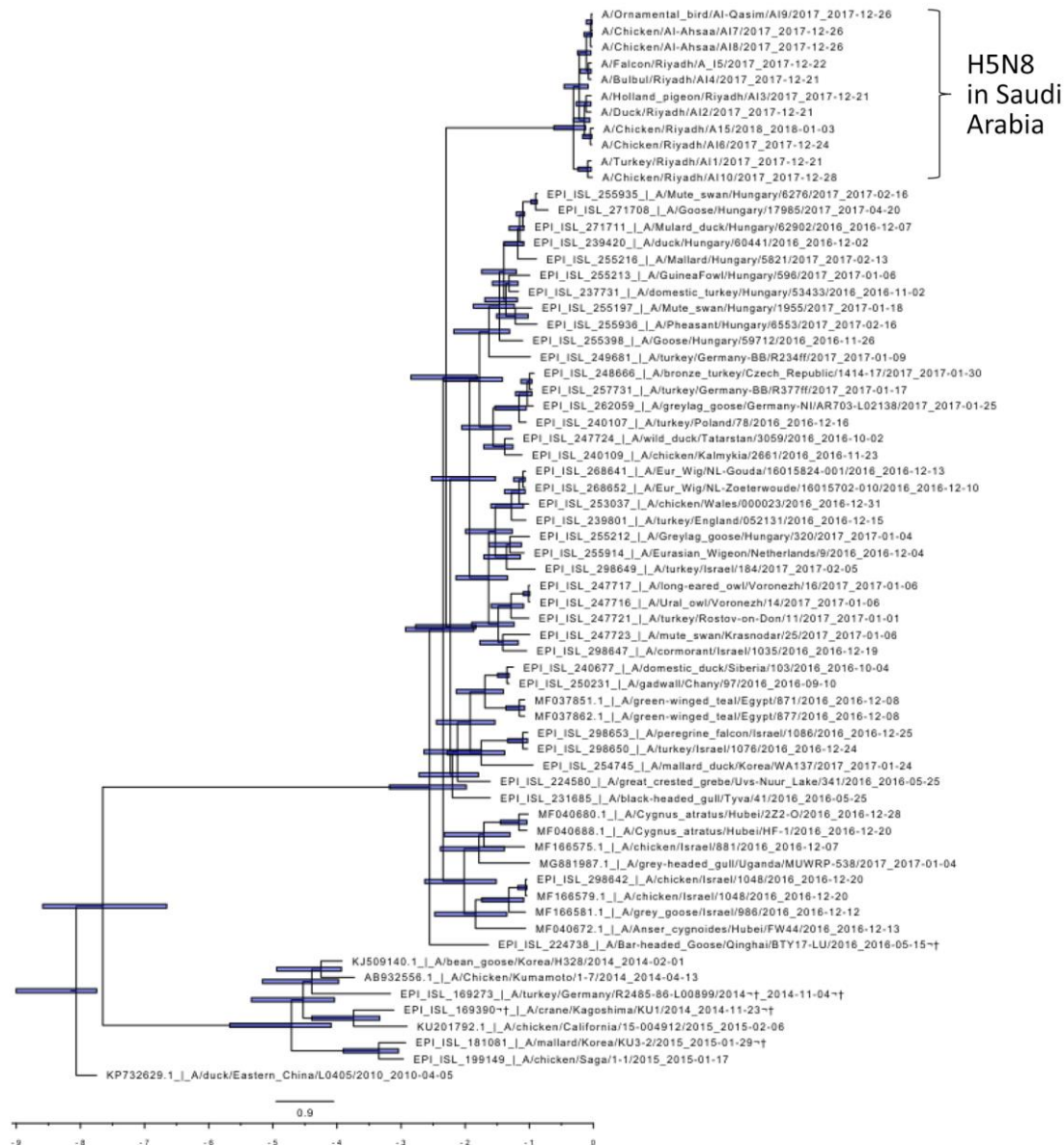
Segment*	GISAIID accession no.	Virus (subtype)	Sequence identity
PB2	EPI1010642	A/barnacle goose/Netherlands/2/2014 (H3N6)	98.3%
PB1	EPI961474	A/environment/Kamchatka/18/2016 (H5N5)	98.5%
PA	EPI858843	A/painted stork/India/10CA03/2016 (H5N8)	99.4%
HA	EPI909452	A/wild duck/Tatarstan/3059/2016 (H5N8)	98.7%
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





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 $\geq 60\%$ 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.