

particularly when term approaches. The imbalance in T-cell immunity (Th1/Th2) has been proposed to be implicated in the progression of chronic HEV infection in immunocompromised pigs (7). This imbalance may explain the absence of cytolysis during pregnancy and the increased viral load observed despite discontinuation of infliximab. Conversely, after delivery, restoration of cellular immunity is commonly observed (11) and may have contributed to efficient clearance of the virus by hepatic cytolysis along with the reduced immunosuppression resulting from infliximab discontinuation. Despite reintroduction of infliximab when HEV RNA was still detectable, we observed spontaneous resolution of chronic hepatitis E, although immunosuppressive treatment at that time was identical to that previously implicated in the chronicity of infection.

The risk for HEV vertical transmission seems dependent on viral load (12). In a model of HEV infection in pregnant rabbits, Xia et al. reported severe outcomes and a high level of transmission to offspring (13). In the case we report, despite high viral loads in the mother's plasma throughout pregnancy, we found no HEV RNA in the newborn's plasma. Of note, although mothers in the rabbit model were negative for HEV IgG throughout pregnancy, in the case we report, the mother was IgG positive before pregnancy, which may have helped protect the fetus from infection, although this protective role is inconsistent in previous reports of HEV genotype 3 (HEV3) infection of humans (2–4). Furthermore, despite a high sequence similarity to HEV3, rabbit HEV cross-species infections are restricted to nonhuman primates, and pathogenesis may differ from that of HEV3. In conclusion, our results and those reported by Mallet et al. (5) indicate that chronic HEV3 infection in pregnant women might resolve after pregnancy.

About the Author

Dr. Charre is a microbiologist in the virology laboratory of the Institute of Infectious Agents, Hospices Civils de Lyon, Lyon, France. Her research and teaching interests focus on viral hepatitis and human immunodeficiency virus.

References

- Kamar N, Izopet J, Pavio N, Aggarwal R, Labrique A, Wedemeyer H, et al. Hepatitis E virus infection. *Nat Rev Dis Primers*. 2017;3:17086. <http://dx.doi.org/10.1038/nrdp.2017.86>
- Andersson MI, Hughes J, Gordon FH, Ijaz S, Donati M. Of pigs and pregnancy. *Lancet*. 2008;372:1192. [http://dx.doi.org/10.1016/S0140-6736\(08\)61486-5](http://dx.doi.org/10.1016/S0140-6736(08)61486-5)
- Anty R, Ollier L, Péron JM, Nicand E, Cannavo I, Bongain A, et al. First case report of an acute genotype 3 hepatitis E infected pregnant woman living in South-Eastern France. *J Clin Virol*. 2012;54:76–8. <http://dx.doi.org/10.1016/j.jcv.2012.01.016>
- Tabatabai J, Wenzel JJ, Soboletzki M, Flux C, Navid MH, Schnitzler P. First case report of an acute hepatitis E subgenotype 3c infection during pregnancy in Germany. *J Clin Virol*. 2014;61:170–2. <http://dx.doi.org/10.1016/j.jcv.2014.06.008>
- Mallet V, Le Mener S, Roque-Afonso A-M, Tsatsaris V, Mamzer M-F. Chronic hepatitis E infection cured by pregnancy. *J Clin Virol*. 2013;58:745–7. <http://dx.doi.org/10.1016/j.jcv.2013.09.023>
- Grewal P, Kamili S, Motamed D. Chronic hepatitis E in an immunocompetent patient: a case report. *Hepatology*. 2014;59:347–8. <http://dx.doi.org/10.1002/hep.26636>
- Cao D, Cao QM, Subramaniam S, Yugo DM, Heffron CL, Rogers AJ, et al. Pig model mimicking chronic hepatitis E virus infection in immunocompromised patients to assess immune correlates during chronicity. *Proc Natl Acad Sci U S A*. 2017;114:6914–23. <http://dx.doi.org/10.1073/pnas.1705446114>
- Mansuy JM, Gallian P, Dimeglio C, Saune K, Arnaud C, Pelletier B, et al. A nationwide survey of hepatitis E virus infection in French blood donors. *Hepatology*. 2016;63:1145–54.
- Dalton HR, Pas SD, Madden RG, van der Eijk AA. Hepatitis E virus: current concepts and future perspectives. *Curr Infect Dis Rep*. 2014;16:399.
- Sehgal R, Patra S, David P, Vyas A, Khanam A, Hissar S, et al. Impaired monocyte-macrophage functions and defective Toll-like receptor signaling in hepatitis E virus-infected pregnant women with acute liver failure. *Hepatology*. 2015;62:1683–96. <http://dx.doi.org/10.1002/hep.28143>
- Elenkov IJ, Wilder RL, Bakalov VK, Link AA, Dimitrov MA, Fisher S, et al. IL-12, TNF- α , and hormonal changes during late pregnancy and early postpartum: implications for autoimmune disease activity during these times. *J Clin Endocrinol Metab*. 2001;86:4933–8.
- Sharma S, Kumar A, Kar P, Agarwal S, Ramji S, Husain SA, et al. Risk factors for vertical transmission of hepatitis E virus infection. *J Viral Hepat*. 2017;24:1067–75. <http://dx.doi.org/10.1111/jvh.12730>
- Xia J, Liu L, Wang L, Zhang Y, Zeng H, Liu P, et al. Experimental infection of pregnant rabbits with hepatitis E virus demonstrating high mortality and vertical transmission. *J Viral Hepat*. 2015;22:850–7. <http://dx.doi.org/10.1111/jvh.12400>

Address for correspondence: Caroline Charre, Laboratoire de Virologie, Hôpital de la Croix-Rousse, Hospices Civils de Lyon, 103 Grande de la Croix Rousse, 69004 Lyon, France; email: caroline.charre@chu-lyon.fr

Multiple Introductions of Influenza A(H5N8) Virus into Poultry, Egypt, 2017

Ahmed H. Salaheldin, Hatem Salah Abd El-Hamid, Ahmed R. Elbestawy, Jutta Veits, Hafez M. Hafez, Thomas C. Mettenleiter, Elsayed M. Abdelwhab

Author affiliations: Alexandria University, Al Buhayrah, Egypt (A.H. Salaheldin); Friedrich-Loeffler-Institut, Insel Riems-Greifswald, Germany (A.H. Salaheldin, J. Veits, T.C. Mettenleiter,

E.M. Abdelwhab); Freie-Universität-Berlin, Berlin, Germany (A.H. Salaheldin, H.M. Hafez); Damanhour University, Damanhour, Egypt (H.S. Abd El-Hamid, A.R. Elbestawy)

DOI: <https://doi.org/10.3201/eid2405.171935>

After high mortality rates among commercial poultry were reported in Egypt in 2017, we genetically characterized 4 distinct influenza A(H5N8) viruses isolated from poultry. Full-genome analysis indicated separate introductions of H5N8 clade 2.3.4.4 reassortants from Europe and Asia into Egypt, which poses a serious threat for poultry and humans.

In Egypt, highly pathogenic avian influenza A(H5N1) clade 2.2.1 virus was introduced to poultry via migratory birds in late 2005 (1) and is now endemic among poultry in Egypt (2). Also in Egypt, the number of H5N1 infections in humans is the highest in the world, and low pathogenicity influenza A(H9N2) virus is widespread among poultry and has infected humans (2). Despite extensive vaccination, H5N1 and H9N2 viruses are co-circulating and frequently reported (2). In 2014, highly pathogenic avian influenza A(H5N8) virus clade 2.3.4.4 was isolated, mostly from wild birds, in several Eurasian countries and was transmitted

to North America. However, in 2016 and 2017, an unprecedented epidemic was reported in Asia, Africa, and Europe (3). In Egypt, during November 30–December 8, 2016, a total of 3 H5N8 viruses were isolated from common coot (*Fulica atra*) (4) and green-winged teal (*Anas carolinensis*) (5). To provide data on the spread of the virus in poultry, we genetically characterized 4 distinct H5N8 viruses isolated from commercial poultry in Egypt in 2017.

During February–May 2017, a high mortality rate was observed for 48 poultry flocks in the Nile Delta, Egypt. Up to 20 tracheal and cloacal swab samples were collected from each flock for initial diagnosis by reverse transcription PCR and virus isolation at the Faculty of Veterinary Medicine, Damanhour University (Damanhour, Egypt). Results were positive for H5N8 virus in samples for 4 flocks not vaccinated for H5 in 3 governorates (Figure). Sudden deaths also occurred in 3 broiler chicken flocks (Ck12, Ck15, Ck21) and 1 duck flock (Dk18); mortality rates were 29%–52% (online Technical Appendix 1 Table 1, <https://wwwnc.cdc.gov/EID/article/24/5/17-1935-Techapp1.pdf>). No epidemiologic links between farms were observed.

Positive samples were spotted onto FTA cards (6) and submitted to Friedrich-Loeffler-Institut (Insel Riems-Greifswald, Germany), where H5N8 virus was confirmed

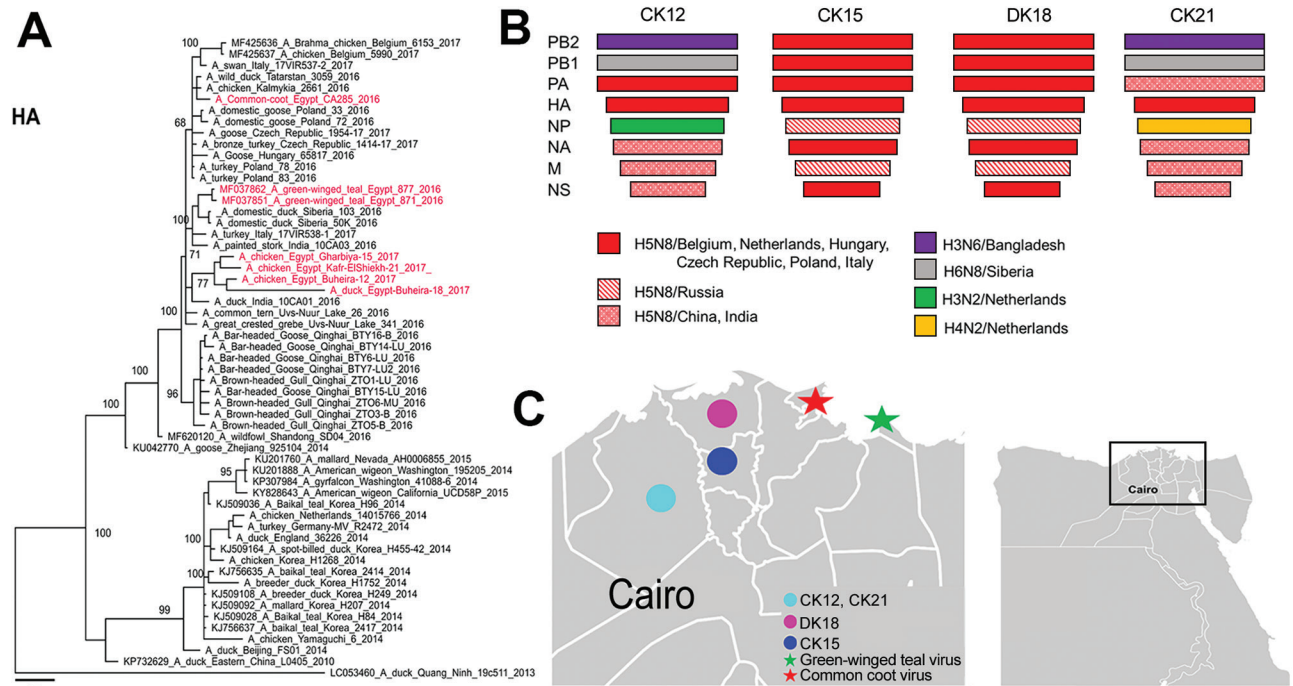


Figure. Characterization of highly pathogenic avian influenza A(H5N8) viruses of clade 2.3.4.4 from Egypt, 2017. A) Phylogenetic relatedness of the HA gene and schematic representation of potential precursors of different H5N8 viruses. The maximum-likelihood midpoint rooted tree was constructed by using MrBayes (<http://mrbayes.sourceforge.net/>). Gray indicates viruses from this study. Scale bar indicates nucleotide substitutions per site. B) Putative ancestors of the different gene segments of H5N8 viruses from Egypt characterized in this study compared with reference viruses. C) Governorates in Egypt where H5N8 viruses had been reported in domestic birds (circles) and where viruses in birds had been previously reported (stars). Inset shows study location in Egypt. Ck, chicken farm; Dk, duck farm; HA, hemagglutinin; M, matrix; NA, neuraminidase; NP, nucleocapsid protein; NS, nonstructural; PA, polymerase acidic; PB, polymerase basic.

by reverse transcription PCR and full-genome sequences (7) from 4 viruses (GISAID [<https://www.gisaid.org/>] accession nos. EPI1104268–EPI1104299) (online Technical Appendix 2, <https://wwwnc.cdc.gov/EID/article/24/5/17-1935-Techapp2.pdf>). We retrieved sequences with high similarity and all H5N8 virus sequences from GISAID and GenBank and aligned them by Multiple Alignment using Fast Fourier Transform (<https://mafft.cbrc.jp/alignment/server/index.html>). The most highly related viruses are summarized in online Technical Appendix 1 Table 2. We calculated sequence identity matrices in Geneious (<https://www.geneious.com/>) (online Technical Appendix 1 Figure 1) and studied phylogenetic relatedness to H5N8 virus isolated in Eurasia and in Egypt by using IQtree (<http://www.iqtree.org/>). Representative viruses were selected for generation of maximum-likelihood midpoint rooted trees by MrBayes (<http://mrbayes.sourceforge.net/>) using a best-fit model (GTR+G) (8) and were further edited by using FigTree (<http://tree.bio.ed.ac.uk/software/figtree/>) and Inkscape (<https://inkscape.org/en/>).

The hemagglutinin (HA) and neuraminidase (NA) genes of the 4 viruses shared 95.8%–99.2% nt and 93.1%–99.4% aa identity and shared 96.5%–99.2% nt and 94.2%–99.7% aa identity with viruses from wild birds in Egypt (4,5). Other segments showed 92.6%–99.6% nt and 96%–99.7% aa identity, where the polymerase acidic (PA) genes and proteins of viruses from Dk18 showed the lowest similarity to those of other viruses (online Technical Appendix 1 Figure 1).

All viruses possess the polybasic HA cleavage site PLREKRRKR/G and contain mammal-adaptation and virulence markers (9) in polymerase basic (PB) 2 (T63I, L89V, G309D, T339K, Q368R, H447Q, R477G), PB1 (A3V, L13P, K328N, S375N, H436Y, M677T), PA (A515T), HA (T156A, A263T; H5 numbering), matrix (M) 1 (N30D, T215A), and nonstructural (NS) 1 (P42S, T127N, V149A) proteins. Therefore, protection of humans and risk assessment of bird-to-human transmission is crucial. The NS1 protein from viruses from Ck15 and Ck18 is 217 aa long because of truncation in the C-terminus, whereas NS1 of the other H5N8 viruses from Egypt are 230 aa long. BLAST (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>) analysis indicated that these 4 viruses differ from viruses isolated from birds in live bird markets in Egypt in 2016 (4,5). Gene segments were closely related to viruses isolated from wild birds, poultry, and zoo birds in Europe (including Belgium, Czech Republic, the Netherlands, Poland, Hungary), Russia, and Asia (including Bangladesh, China, India) (Figure; online Technical Appendix 1 Figures 2, 3).

HA of the 4 H5N8 viruses in this study clustered in 1 distinct branch (Figure), and NA clustered in 2 phylogroups (online Technical Appendix 1 Figure 2). The PB2, nucleoprotein, M, and NS genes of viruses from Ck12 and Ck21

(from chickens in the same governorate, February and May 2017) clustered together, and the same genes from viruses from Dk18 and Ck15 (from ducks and chickens in 2 governorates) clustered in 2 distinct phylogenetic groups. However, viruses from Ck12 and Ck15 have similar but not identical PA gene segments (online Technical Appendix 1 Figure 3).

These data suggest 4 different introductions of H5N8 virus into poultry in Egypt, independent of viruses isolated from captive birds (4,5). Multiple separate introductions of H5N8 virus into Europe also occurred (10). Further studies are needed to identify the source(s) of introduction. The separate introductions of different reassortants of H5N8 clade 2.3.4.4 virus from Europe and Asia into Egypt indicate a serious threat for poultry and human health.

Acknowledgments

We acknowledge Günter Strebelow for his assistance with sequencing of viruses in this study, and we thank the colleagues and laboratories who submitted sequence data to GISAID.

A.H.S. is supported by internal funds from Friedrich-Loeffler-Institut, Federal Research Institute for Animal Health.

About the Author

Mr. Salaheldin is a doctoral student at the Institute of Poultry Diseases, Freie-Universität-Berlin. His primary research interests are molecular virology, vaccine development, and epidemiology of avian influenza viruses.

References

1. Saad MD, Ahmed LS, Gamal-Eldein MA, Fouda MK, Khalil F, Yingst SL, et al. Possible avian influenza (H5N1) from migratory bird, Egypt. *Emerg Infect Dis*. 2007;13:1120–1 <http://dx.doi.org/10.3201/eid1307.061222>
2. Abdelwhab EM, Hassan MK, Abdel-Moneim AS, Naguib MM, Mostafa A, Hussein IT, et al. Introduction and enzootic of A/H5N1 in Egypt: virus evolution, pathogenicity and vaccine efficacy ten years on. *Infect Genet Evol*. 2016;40:80–90. <http://dx.doi.org/10.1016/j.meegid.2016.02.023>
3. World Organisation for Animal Health. Update on avian influenza in animals (types H5 and H7): 2017 [cited 2017 Nov 10]. <http://www.oie.int/animal-health-in-the-world/update-on-avian-influenza/>
4. Selim AA, Erfan AM, Hagag N, Zanaty A, Samir AH, Samy M, et al. Highly pathogenic avian influenza virus (H5N8) clade 2.3.4.4 infection in migratory birds, Egypt. *Emerg Infect Dis*. 2017;23:1048–51. <http://dx.doi.org/10.3201/eid2306.162056>
5. Kandeil A, Kayed A, Moatasim Y, Webby RJ, McKenzie PP, Kayali G, et al. Genetic characterization of highly pathogenic avian influenza A H5N8 viruses isolated from wild birds in Egypt. *J Gen Virol*. 2017;98:1573–86. <http://dx.doi.org/10.1099/jgv.0.000847>
6. Abdelwhab EM, Lüscho D, Harder TC, Hafez HM. The use of FTA® filter papers for diagnosis of avian influenza virus. *J Virol Methods*. 2011;174:120–2. <http://dx.doi.org/10.1016/j.jviromet.2011.03.017>
7. Hoffmann E, Stech J, Guan Y, Webster RG, Perez DR. Universal primer set for the full-length amplification of all influenza A

- viruses. Arch Virol. 2001;146:2275–89 <https://doi.org/10.1007/s007050170002>. <http://dx.doi.org/10.1007/s007050170002>
8. Milne I, Lindner D, Bayer M, Husmeier D, McGuire G, Marshall DF, et al. TOPALi v2: a rich graphical interface for evolutionary analyses of multiple alignments on HPC clusters and multi-core desktops. *Bioinformatics*. 2009;25:126–7. <http://dx.doi.org/10.1093/bioinformatics/btn575>
 9. Mertens E, Dugan VG, Stockwell TB, Lindsay LL, Plancarte M, Boyce WM. Evaluation of phenotypic markers in full genome sequences of avian influenza isolates from California. *Comp Immunol Microbiol Infect Dis*. 2013;36:521–36. <http://dx.doi.org/10.1016/j.cimid.2013.06.003>
 10. Fusaro A, Monne I, Mulatti P, Zecchin B, Bonfanti L, Ormelli S, et al. Genetic diversity of highly pathogenic avian influenza A(H5N8/H5N5) viruses in Italy, 2016–17. *Emerg Infect Dis*. 2017;23:1543–7. <http://dx.doi.org/10.3201/eid2309.170539>

Address for correspondence: Elsayed M. Abd El-Whab, Friedrich-Loeffler-Institut, Federal Research Institute for Animal Health, Suedufer 10, 17493 Insel Riems-Greifswald, Germany; email: sayed.abdel-whab@fli.de or sayedabdelwhab@yahoo.com

Fatal Tick-Borne Encephalitis Virus Infections Caused by Siberian and European Subtypes, Finland, 2015

Suvi Kuivanen, Teemu Smura, Kirsi Rantanen, Leena Kämppi, Jonas Kantonen, Mia Kero, Anu Jääskeläinen, Anne J. Jääskeläinen, Jussi Sane, Liisa Myllykangas, Anders Paetau, Olli Vapalahti

Author affiliations: University of Helsinki, Helsinki, Finland (S. Kuivanen, T. Smura, J. Kantonen, M. Kero, A. Jääskeläinen, L. Myllykangas, A. Paetau, O. Vapalahti); Helsinki University Hospital, Helsinki (K. Rantanen, L. Kämppi, A.J. Jääskeläinen, O. Vapalahti); National Institute for Health and Welfare, Helsinki (J. Sane)

DOI: <https://doi.org/10.3201/eid2405.171986>

In most locations except for Russia, tick-borne encephalitis is mainly caused by the European virus subtype. In 2015, fatal infections caused by European and Siberian tick-borne encephalitis virus subtypes in the same *Ixodes ricinus* tick focus in Finland raised concern over further spread of the Siberian subtype among widespread tick species.

The causative agent of tick-borne encephalitis (TBE), tick-borne encephalitis virus (TBEV), is endemic throughout Europe and Asia; ≈10,000 cases are reported annually (1). TBEV is an enveloped, positive-sense RNA virus in the family *Flaviviridae*, genus *Flavivirus* (2). The westernmost range of the Siberian subtype (TBEV-Sib) extends to Finland and the Baltics, where the European subtype (TBEV-Eur) also circulates. TBEV-Eur is the only subtype found in the rest of Europe (3).

In TBEV-infected patients, neurologic signs appear as the virus passes to the central nervous system; infection is manifested as meningitis, encephalitis, or meningoencephalitis. During 2010–2016, a total of 20 cases of TBE were reported from Kotka archipelago, Finland, a previous TBEV-Sib focus (4). We report 2 fatal TBEV infections acquired 1 month apart in patients on Kuutsalo Island, Kotka archipelago, in 2015.

Patient 1 was a previously healthy 36-year-old woman who had visited Kuutsalo 10 days before fever onset. A week later, she experienced sudden-onset headache, left arm numbness, and impaired vision. Head computed tomography results were unremarkable. Two days later, she experienced disorientation and right hemiparesis and was taken to a tertiary care center. Cerebrospinal fluid (CSF) test results showed pleocytosis. Magnetic resonance images indicated pathologically increased signal in cortical sulcus regions (Figure, panel A). Despite receipt of acyclovir, doxycycline, and ceftriaxone, her condition deteriorated rapidly. Head computed tomography showed cerebellar herniation; the patient had dilated pupils and no pain reaction. CSF and serum were positive for TBEV IgM but negative for TBEV RNA; hemagglutination inhibition results showed a low titer (20) of TBEV-specific antibodies in serum. The patient died 2 weeks after fever onset.

Gross postmortem examination showed widespread and severe signs of viral encephalitis: meningeal and perivascular inflammation, neuronophagy, microglial nodules, endothelial damage, and severe brain edema. The inflammation was evident from the spinal cord to the cerebellum and cortex (Figure, panel B). TBEV (RNA) was detected in brain and spleen (online Technical Appendix Figure 1, panel A, <https://wwwnc.cdc.gov/EID/article/24/5/17-1986-Techapp1.pdf>).

TBEV was isolated from the cerebellum in SK-N-SH neuroblastoma cells, and the whole genome for TBEV-Sib was obtained. A pool of TBEV-Sib-positive *Ixodes ricinus* ticks collected from the neighboring island in 2011 (4) was subjected to viral whole-genome sequencing. This virus and the virus from patient 1 had 3 nt differences resulting in 2 aa mutations, R868K (NS1) and V1452A (NS2B), and clustered together in the Baltic clade of TBEV-Sib (online Technical Appendix Figure 2).

Patient 2 was a 66-year-old man with hypertension, diabetes, and chronic lymphatic leukemia. He had frequently

Multiple Introductions of Influenza A(H5N8) Virus into Poultry, Egypt, 2017

Technical Appendix 1

Technical Appendix 1 Table 1. History of flocks infected with HPAIV H5N8 in 2017 characterized in this study

H5N8 Virus	Birds	Date	Governorate	No. birds	Age	Mortality
A/chicken/Egypt/Buheira-12/2017	Broilers	Mar.	Buheira	2000	29 days	35%
A/chicken/Egypt/Gharbiya/-15/2017	Broilers	Feb.	Gharbiya	1500	32 days	52%
A/duck/Egypt/Kafr Elshiekh-18/2017	Ducks	May	Kafr El-Sheikh	1500	32 days	29%
A/chicken/Egypt/Buheira-21/2017	Broilers	May	Buheira	2000	30 days	32%

Technical Appendix 1 Table 2. Identity (ID) of the Egyptian HPAIV H5N8 isolated in this study and related viruses with highest identity in the GenBank and GISAID databases

Segment	A/chicken/Egypt/Buheira-12/2017	ID%	A/chicken/Egypt/Gharbiya-15/2017	ID%	A/chicken/Egypt/Kafr-Elshiekh-18/2017	ID%	A/duck/Egypt/Buheira-21/2017	ID%
PB2	A/duck/Bangladesh/26920/2015 (H3N6)	99.0	A/eurasian_wigeon/Netherlands/16015653-001/2016_(H5N8)	99.6	A/peacock/Belgium/1017/2017(H5N8)	98.9	A/duck/Bangladesh/26974/2015(H3N6)	98.9
PB1	A/domestic_duck/Siberia/49_feather/2016 (H6N8)	98.4	A/Eur_Wig/NL-Greonterp/16015653-001/2016 (H5N8)	99.6	A/M_Swan/NL-/16014462-019/2016 (H5N8)	99.7	A/domestic_duck/Siberia/49_feather/2016 (H6N8)	98.5
PA	A/Eur_Wig/NL-Greonterp/16015653-001/2016 (H5N8)	98.0	A/Eur_Wig/NL-Greonterp/16015653-001/2016 (H5N8)	99.3	A/Cygnusolor/Belgium/1567/2017(H5N8)	98.6	A/Bar-headedGoose/Qinghai/BTY9-LU/2016 (H5N8)	98.7
HA	A/mute swan/Czech Republic/1296-17_1/2017 (H5N8)	98.9	A/Mallard/Hungary/1574a/2017 (H5N8)	99.1	A/turkey/Poland/83/2016 (H5N8)	98.9	A/mute swan/Czech Republic/1296-17_1/2017 (H5N8)	97.0
NP	A/mallard duck/Netherlands/16/2012(H3N2)	97.6	A/mute swan/Kaliningrad/132/2017 (H5N8)	99.7	A/gadwall/Kurgan/2442/2016 (A/H5N8)	98.3	(A/mallard duck/Netherlands/18/2012 (H4N2))	97.9
NA	A/Brown-headed Gull/Qinghai/ZTO5-K/2016 (H5N8)	99.1	A/Eur_Wig/NL-/16015653-001/2016 (H5N8)	99.4	A/peacock/Belgium/1017/2017(H5N8)	97.3	A/Brown-headed Gull/Qinghai/ZTO6-MU/2016 (H5N8)	98.7
M	A/duck/India/10CA01/2016 (H5N8)	99.7	A/gadwall/Kurgan/2442/2016 (H5N8)	99.2	A/gadwall/Kurgan/2442/2016 (A/H5N8)	99.5	A/duck/India/10CA01/2016 (A/H5N8)	99.6
NS	A/duck/India/10CA01/2016 (A/H5N8)	99.3	A/Mallard/Hungary/1574a/2017 (A/H5N8)	99.9	A/goose/Italy/17VIR6358-3/2017 (A/H5N8)	99.4	A/duck/India/10CA01/2016 (A/H5N8)	99.1

HA	Ck12	Ck15	Dk18	CK21	Coot	Teal877	Teal871
Ck12	ID	99.1	99	97.4	98.5	98.5	98.4
Ck15	99.4	ID	99.2	97.1	98.7	98.5	98.4
Dk18	98.7	98.9	ID	97	98.8	98.3	98.2
CK21	97.8	97.7	97	ID	96.7	96.5	96.5
Coot	99.2	99.4	99.1	97.5	ID	99.1	99.1
Teal877	99.6	99.4	98.7	97.8	99.6	ID	99.9
Teal871	99.4	99.2	98.5	97.7	99.4	99.8	ID

PA	Ck12	Ck15	Dk18	CK21	Teal877	Teal871
Ck12	ID	98.1	93.6	94.2	94.3	94.3
Ck15	99.1	ID	92.6	93.2	94.3	94.3
Dk18	96.0	96.3	ID	97.7	94.5	94.5
CK21	97.0	97.3	97.2	ID	95.1	95.1
Teal877	98.0	98.3	96.3	97.6	ID	100.0
Teal871	98.0	98.3	96.3	97.6	100.0	ID

NA	Ck12	Ck15	Dk18	CK21	Coot	Teal877	Teal871
Ck12	ID	98	96.3	98.8	98.7	98.7	98.7
Ck15	97.8	ID	96.6	97.7	99.2	98.5	98.5
Dk18	93.8	95.1	ID	95.8	97.1	96.6	96.6
CK21	97.8	97.2	93.1	ID	98.5	98.3	98.4
Coot	98.0	99.7	95.3	97.4	ID	99.2	99.3
Teal877	97.6	98.7	94.2	96.5	98.9	ID	99.9
Teal871	97.8	98.9	94.4	96.8	99.1	99.7	ID

NP	Ck12	Ck15	Dk18	CK21	Teal877	Teal871
Ck12	ID	97.1	96.5	98.0	94.0	93.9
Ck15	96.9	ID	98.5	97.5	95.2	95.1
Dk18	96.5	99.5	ID	97.0	94.8	94.7
CK21	96.7	98.9	98.5	ID	94.6	94.5
Teal877	96.3	99.3	98.9	98.3	ID	99.9
Teal871	96.1	99.1	98.7	98.1	99.7	ID

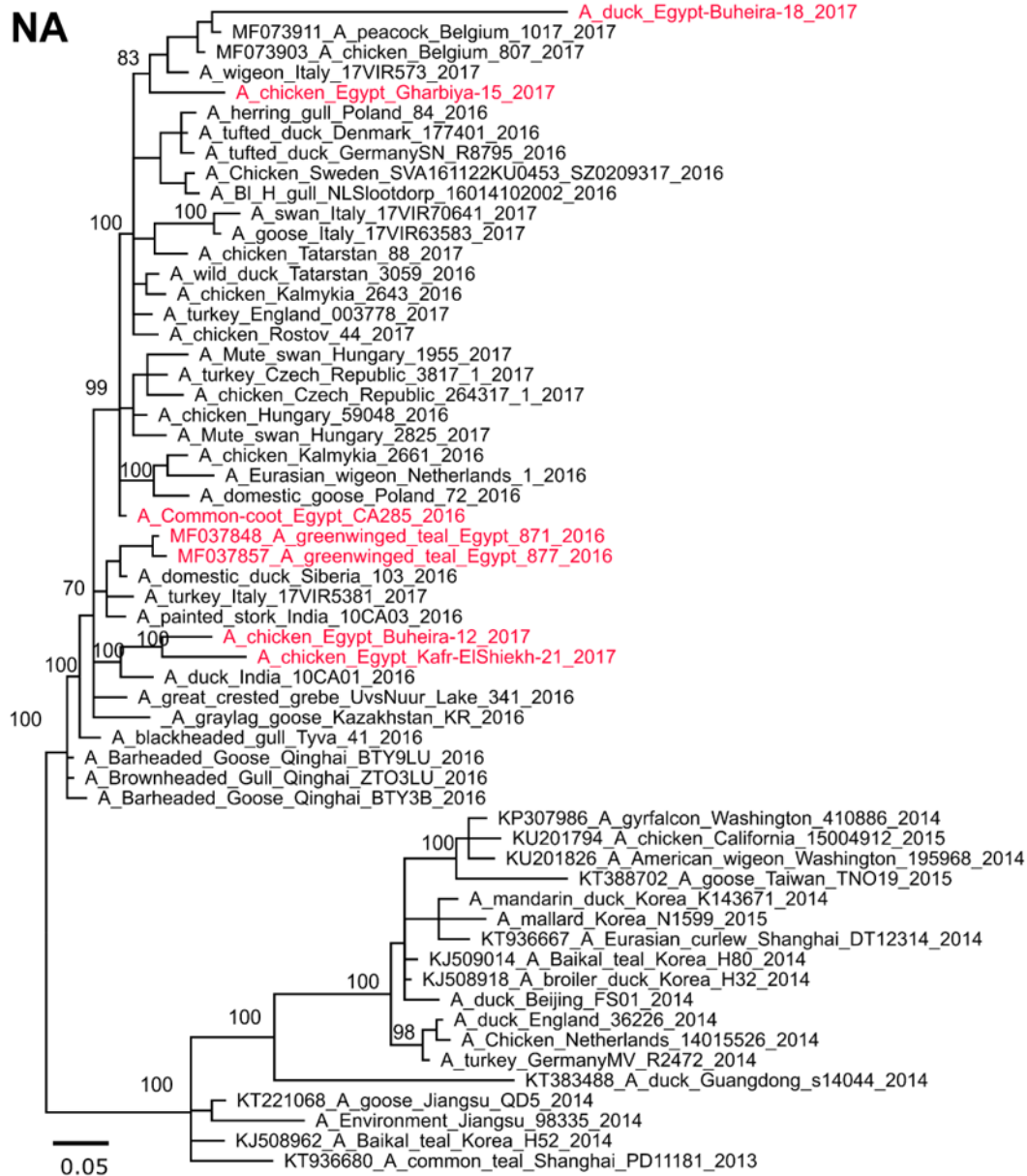
PB2	Ck12	Ck15	Dk18	CK21	Teal877	Teal871
Ck12	ID	98.4	98.0	99.3	97.2	97.1
Ck15	98.6	ID	98.6	98.5	97.0	96.9
Dk18	98.4	98.6	ID	98.2	96.9	96.8
CK21	99.0	98.8	98.5	ID	97.1	97.0
Teal877	98.4	98.4	98.1	98.2	ID	99.9
Teal871	98.2	98.2	98.0	98.1	99.8	ID

M1	Ck12	Ck15	Dk18	CK21	Teal877	Teal871
Ck12	ID	98.1	98.8	99.6	99.4	99.4
Ck15	98.4	ID	98.5	98.2	98.1	98.1
Dk18	98.4	98.4	ID	98.6	98.5	98.5
CK21	99.6	98.8	98.8	ID	99.3	99.3
Teal877	99.6	98.0	98.0	99.2	ID	100.0
Teal871	99.6	98.0	98.0	99.2	100.0	ID

PB1	Ck12	Ck15	Dk18	CK21	Teal877	Teal871
Ck12	ID	95.0	95.1	99.5	95.1	95.2
Ck15	98.5	ID	99.4	95.1	98.7	99.0
Dk18	98.8	99.7	ID	95.2	98.9	99.2
CK21	99.4	98.8	99.0	ID	95.2	95.3
Teal877	98.0	98.4	98.6	98.2	ID	99.3
Teal871	98.6	99.0	99.3	98.9	98.8	ID

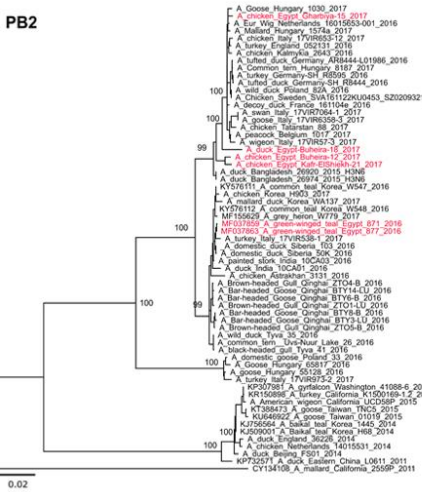
NS1	Ck12	Ck15	Dk18	CK21	Teal877	Teal871
Ck12	ID	98.6	97.7	99.5	99.1	99.1
Ck15	98.6	ID	99.1	98.2	98.6	98.6
Dk18	97.7	99.1	ID	97.3	97.7	97.7
CK21	99.5	98.2	97.3	ID	98.6	98.6
Teal877	99.1	98.6	97.7	98.6	ID	100
Teal871	99.1	98.6	97.7	98.6	100	ID

Technical Appendix 1 Figure 1. Genetic identity of different genes and proteins of HPAIV H5N8 clade 2.3.4.4 isolated from domestic poultry in Egypt in 2017. Given are the nucleotide and amino acid identity matrices ($^{nucleotide}ID_{amino\ acid}$) of H5N8 isolated from poultry in this study compared to the wild bird viruses. ID= identical, Ck12= A/chicken/Egypt/Buheira-12/2017, Ck15= A/chicken/Egypt/Gharbiya-15/2017, Dk18= A/chicken/Egypt/Kafr-Elshiekh-18/2017, Ck21= A/duck/Egypt/Buheira-21/2017, Coot= A/Common coot/Egypt/CA285/2016, Teal 877= A/green winged teal/Egypt/877/2016, Teal 871= A/green winged teal/Egypt/871/2016. Only HA and NA gene sequences are available for the common coot virus. Sequences were aligned and identity matrices were calculated using Geneious version 10.

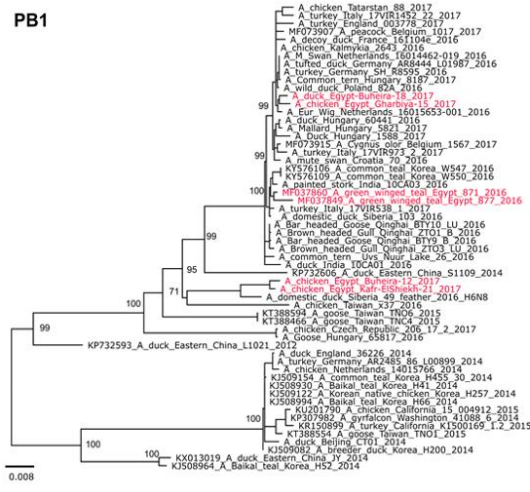


Technical Appendix 1 Figure 2. Phylogenetic relatedness of the NA gene segments of the H5N8 2.3.4.4 viruses from domestic birds in Egypt in 2017. All available sequences of NA of HPAIV H5N8 were retrieved from the GISAID and GenBank databases and aligned using MAFFT. Trees were generated by IQtree and representative viruses were selected for further analysis. Maximum Likelihood mid-point rooted trees were constructed by MrBayes implementing 4 runs, 1000.000 replicates and 10% burn-in using best fit model GTR+G according to BIC value. Egyptian viruses are shown in red.

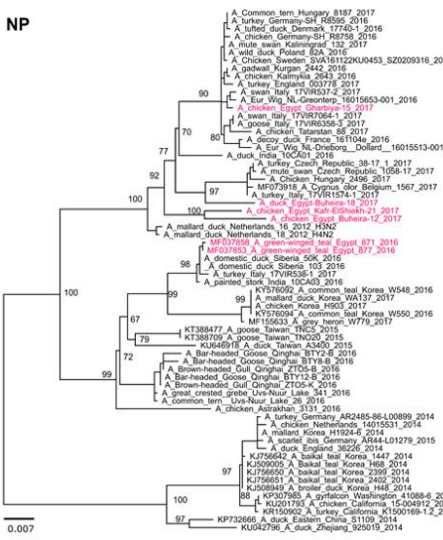
PB2



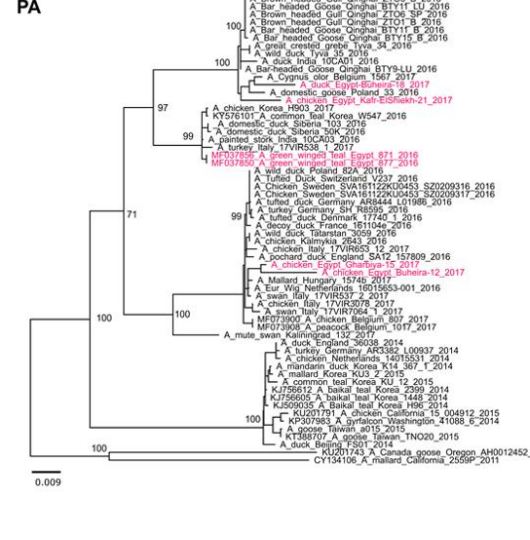
PB1



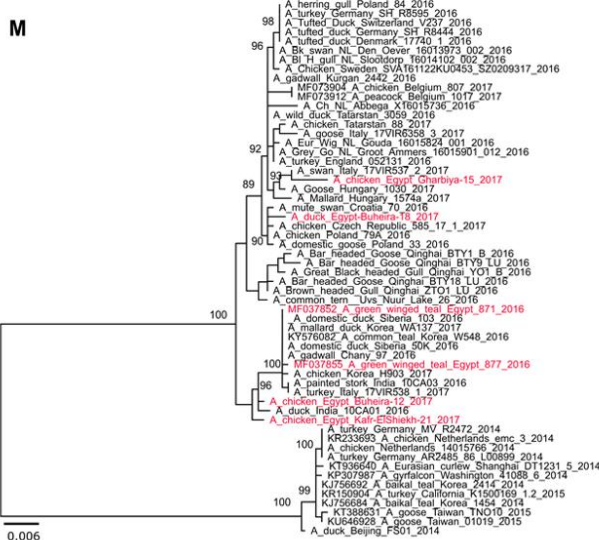
NP



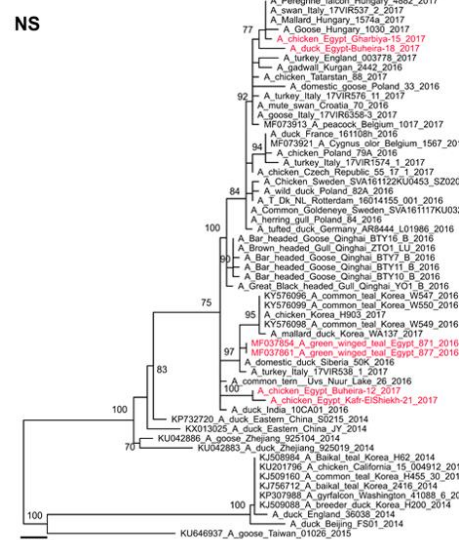
PA



M



NS



Technical Appendix 1 Figure 3. Phylogenetic relatedness of non-HA/NA gene segments of the H5N8 2.3.4.4 viruses from domestic birds in Egypt in 2017. All available sequences of H5N8 viruses were retrieved from the GISAID and GenBank databases. First tree was generated by IQtree and representative viruses were selected for further analysis. Maximum Likelihood mid-point rooted trees were generated by MrBayes using 4 runs each of 1000.000 replicates and 10% burn-in after selection of the best fit model GTR+G according to BIC value as implemented in Topali v2. All trees were edited and prepared for publishing using FigTree and Inkscape.