Reassortant Clade 2.3.4.4 of Highly Pathogenic Avian Influenza A(H5N6) Virus, Taiwan, 2017

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A highly pathogenic avian influenza A(H5N6) virus of clade 2.3.4.4 was detected in a domestic duck found dead in Taiwan during February 2017. The endemic situation and continued evolution of various reassortant highly pathogenic avian influenza viruses in Taiwan warrant concern about further reassortment and a fifth wave of intercontinental spread.

Since 1996, H5 A/goose/Guangdong/1/1996 (Gs/GD) lineage of highly pathogenic avian influenza viruses (HPAIVs) originating in Asia have caused outbreaks in Asia, Europe, Africa, and North America (1). The H5N1 Gs/GD lineage of HPAIV has evolved into 10 genetically distinct virus clades (0–9) and multiple subclades, including novel H5 clade 2.3.4.4 viruses, which emerged in China (2) and have evolved into 4 distinct genetic groups (2.3.4.4A–D) (3). Four 4 intercontinental waves of Gs/GD lineage HPAIV transmission have occurred: clade 2.2 H5N1 in 2005, clade 2.3.2.1c H5N1 in 2009, clade 2.3.4.4A H5Nx in 2014, and clade 2.3.4.4B H5Nx in 2016 (4). The clade 2.3.4.4A and B H5N8 viruses spread intercontinentally; clade 2.3.4.4A caused outbreaks in Asia, Europe, and North America during 2014–2015, and clade 2.3.4.4B H5N8 caused outbreaks in Asia, Europe, and Africa during 2016–2017 (1,5). In fall 2016, clade 2.3.4.4C H5N6 viruses caused outbreaks in South Korea and Japan (6). Six distinct genotypes of clade 2.3.4.4C H5N6 viruses (designated as C1–C6) were identified in South Korea and Japan during these outbreaks; these genotypes contain different polymerase acidic and nonstructural genes from low pathogenicity influenza viruses from Eurasia (7,8).

We report HPAIV H5N6 detection from a meat-type duck in Taiwan in February 2017. One dead young Pekin-type domestic duck was found on a country road near the Xiuguluan River in Hualien County during wild bird and habitat surveillance for HPAIV by the Wild Bird

Society of Taipei; the carcass was forwarded to the national laboratory of the Animal Health Research Institute (online Technical Appendix 1 Figure 1, https://wwwnc.cdc.gov/EID/article/24/6/17-2071-Techapp1.pdf). We conducted complete genome sequencing and comparative phylogenetic analysis of the detected virus, A/duck/Taiwan/1702004/2017(H5N6) (Dk/Tw/17), to trace the origin and understand its genetic features.

We detected Dk/Tw/17 virus by using reverse transcription PCR and isolated the virus by using egg inoculation as described previously (9). We conducted an intravenous pathogenicity index test according to the World Organisation for Animal Health Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (http://www.oie.int/en/international-standard-setting/terrestrial-manual). We performed full-length genome sequencing by using reverse transcription PCR amplification and Sanger sequencing (9). We estimated maximum-likelihood phylogenies by using RAxML (10) and constructed a median-joining phylogenetic network of the hemagglutinin gene by using NETWORK 5.0 (online Technical Appendix).

We classified Dk/Tw/17 as an HPAIV on the basis of the amino acid sequence at the hemagglutinin cleavage site (PLRERRRKR/G) and its high lethality in chickens (intravenous pathogenicity index 3.0). Necropsy and histologic examination revealed virus-specific necrotic and inflammatory lesions in the pancreas, heart, and brain (online Technical Appendix 1 Figure 2). Phylogenetic analyses suggested that the Dk/Tw/17 virus belongs to clade 2.3.4.4C genotype C5 that was found in China, South Korea, and Japan during 2016–2017 (Figure, panel A; online Technical Appendix 1 Figure 1). This virus genotype acquired its polymerase acidic gene of low pathogenicity influenza viruses from Eurasia; its other genes originated in the G1.1.9 and G1.1-like lineages of H5N6 viruses from China (7,8). All 8 gene segments shared high levels of nucleotide identity (99.3%–99.9%) with H5N6 viruses identified from wild birds in Japan and South Korea in November 2016, including A/whooper swan/Korea/Gangjin 49-1/2016 (H5N6), A/spot billed duck/Korea/WB141/2016 (H5N6), and A/teal/Tottori/2/2016 (H5N6) (online Technical Appendix 1 Table). These viruses consistently clustered together with high bootstrap value (>70) in maximum-likelihood phylogenies across all 8 gene segments (online Technical Appendix Figures 3–10).

The genotype C5 comprises 17 H5N6 HPAIVs identified from wild waterfowl in China, Japan, and South Korea during November–December 2016; a virus identified from a chicken farm (A/chicken/Korea/H23/2016 [H5N6]) in South Korea in November 2016; and the Dk/Tw/17 virus. Genotype C5 is phylogenetically distinct from viruses that caused outbreaks in poultry farms in Japan and South

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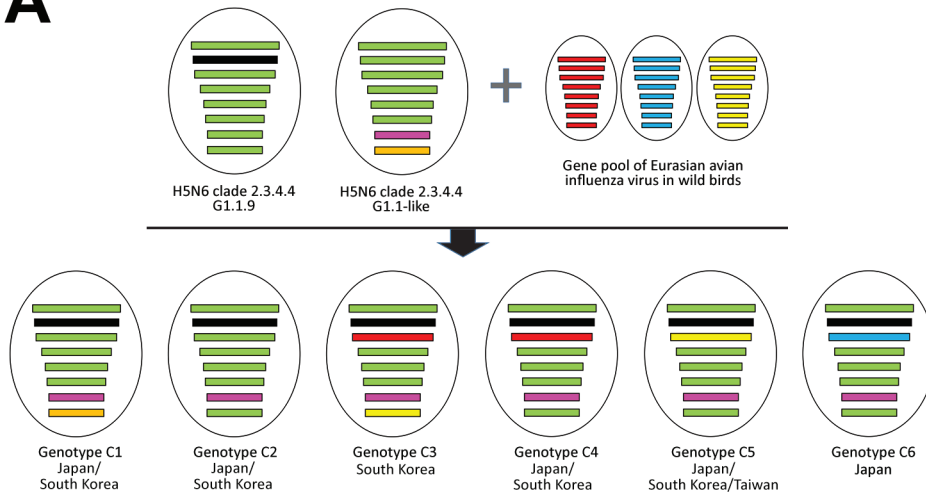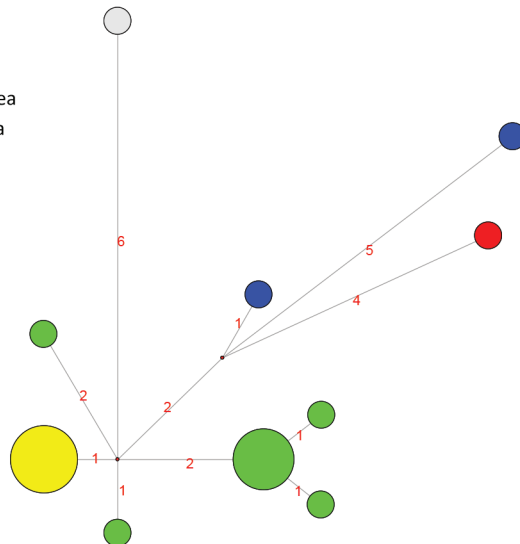
Figure. Genome constellation of influenza A(H5N6) viruses identified in East Asia during 2016–2017 and median-joining phylogenetic network of genotype C5. A) Viruses are represented by ovals containing horizontal bars that represent 8 gene segments (top to bottom: polymerase basic 2, polymerase basic 1, polymerase acidic, hemagglutinin, nucleoprotein, neuraminidase, matrix, and nonstructural). B) Median-joining phylogenetic network of genotype C5 constructed from the hemagglutinin gene and includes all the most parsimonious trees linking the sequences. Each unique sequence is represented by a circle sized relative to its frequency in the dataset. Branch length is proportional to the number of mutations.

Korea during 2016–2017. This genotype has independently evolved and been maintained in wild bird populations in the bird flyway of East Asia, highlighting how wild waterfowl play an important role in the maintenance and dissemination of this HPAIV. In addition, the median-joining phylogenetic network analysis suggests that the A/chicken/Korea/H23/2016 (H5N6) is not the direct ancestor of the Dk/Tw/17 virus, which was likely caused by separate introduction from wild birds (Figure, panel B).

The site where the dead duck was collected is adjacent to a river and located near many ponds used for duck farming. After identification of Dk/Tw/17, intensified active surveillance conducted over 3 months detected additional clade 2.3.4.4C H5N6 HPAIVs from 12 farms in 4 counties (online Technical Appendix 1 Figure 1). Clade 2.3.4.4A H5Nx HPAIVs, mainly H5N2 and H5N8, have caused outbreaks in the poultry industry of Taiwan since January 2015

(9). In 2017, clade 2.3.4.4A H5Nx and 2.3.4.4C H5N6 HPAIVs were detected in domestic poultry. The endemic situation and continued evolution of various reassortant HPAIVs in domestic poultry warrants concern about further reassortment. Enhanced active surveillance in domestic and wild waterfowl is required to monitor the spread and onward reassortment in Taiwan and to inform the design of improved prevention and control strategies.

About the Author

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References

1. Lee DH, Bertran K, Kwon JH, Swayne DE. Evolution, global spread, and pathogenicity of highly pathogenic avian influenza H5Nx clade 2.3.4.4. J Vet Sci. 2017;18(S1):269–80. http://dx.doi.org/10.4142/jvs.2017.18.S1.269
2. Zhao G, Gu X, Lu X, Pan J, Duan Z, Zhao K, et al. Novel reassortant highly pathogenic H5N2 avian influenza viruses in poultry in China. PLoS One. 2012;7:e46183. http://dx.doi.org/10.1371/journal.pone.0046183
3. Lee DH, Bahl J, Torchetti MK, Killian ML, Ip HS, DeLiberto TJ, et al. Highly pathogenic avian influenza viruses and generation of novel reassortants, United States, 2014–2015. Emerg Infect Dis. 2016;22:1283–5. http://dx.doi.org/10.3201/eid2207.160048
4. Sims L, Harder T, Brown I, Gaidet N, Belot G. Dobschuetz Sv, et al. Highly pathogenic H5 avian influenza in 2016 and 2017—observations and future perspectives [cited 2017 Dec 15]. http://agritrop.cirad.fr/585953
5. Global Consortium for H5N8 and Related Influenza Viruses. Role for migratory wild birds in the global spread of avian influenza H5N8. Science. 2016;354:213–7. http://dx.doi.org/10.1126/science.aaf8852
6. Okamatsu M, Ozawa M, Soda K, Takakuwa H, Haga A, Hiono T, et al. Characterization of highly pathogenic avian influenza virus A(H5N6), Japan, November 2016. Emerg Infect Dis. 2017;23:691–5. http://dx.doi.org/10.3201/eid2304.161957
7. Takemae N, Tsunekuni R, Sharshov K, Tanikawa T, Uchida Y, Ito H, et al. Five distinct reassortants of H5N6 highly pathogenic avian influenza A viruses affected Japan during the winter of 2016–2017. Virology. 2017;512:8–20. http://dx.doi.org/10.1016/j.virol.2017.08.035
8. Lee EK, Song BM, Lee YN, Heo GB, Bae YC, Joh SJ, et al. Multiple novel H5N6 highly pathogenic avian influenza viruses, South Korea, 2016. Infect Genet Evol. 2017;51:21–3. http://dx.doi.org/10.1016/j.meegid.2017.03.005
9. Lee MS, Chen LH, Chen YP, Liu YP, Li WC, Lin YL, et al. Highly pathogenic avian influenza viruses H5N2, H5N3, and H5N8 in Taiwan in 2015. Vet Microbiol. 2016;187:50–7. http://dx.doi.org/10.1016/j.vetmic.2016.03.012
10. Stamatakis A. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics. 2014;30:1312–3. http://dx.doi.org/10.1093/bioinformatics/btu033

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Reemergence of Human Monkeypox in Nigeria, 2017

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In Nigeria, before 2017 the most recent case of human monkeypox had been reported in 1978. By mid-November 2017, a large outbreak caused by the West African clade resulted in 146 suspected cases and 42 laboratory-confirmed cases from 14 states. Although the source is unknown, multiple sources are suspected.

Human monkeypox is a rare zoonotic infection caused by an orthopoxvirus and characterized by smallpox-like signs and symptoms (1). The disease is endemic to the Democratic Republic of the Congo. Reported outbreaks have occurred mainly in rural rainforest areas of the Congo basin and West Africa, caused by the Central and West African clades of the virus, respectively (1–6). The West African clade is associated with milder disease, fewer deaths, and limited human-to-human transmission. Since 1970, only ≈10 cases in West Africa had been reported; in 2003, a total of 81 cases (41% laboratory confirmed) were reported in the United States (2,7,8). In Nigeria, a case of human monkeypox in a 4-year-old child in the southeastern part

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Technical Appendix

Materials and Methods

The Dk/Tw/17 virus was detected and confirmed by egg inoculation and RT-PCR as described previously (*1*). The intravenous pathogenicity index (IVPI) test was conducted according to the OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (http://www.oie.int/en/international-standard-setting/terrestrial-manual). Full-length genome sequencing was performed by RT-PCR and Sanger sequencing (*1*). Nucleotide sequences have been deposited in GISAID EpiFlu under nos. EPI915867–EPI915874. For phylogenetic analysis, all available sequences of H5N6 HPAIV identified in 2016–2017 were retrieved from the EpiFlu Database on November 29, 2017. A total of 177 nt sequences for each gene were aligned using MAFFT and manual editing of alignments were performed in Geneious 8 software. The ML tree was estimated by RAxML (*2*) using the general time-reversible nucleotide substitution model. Bootstrap support values were generated by using 500 rapid bootstrap replicates. The ML phylogenetic tree was visualized with MEGA 7 software (http://www.megasoftware.net). Bootstrap values >70% are shown at the branch nodes. A median-joining (MJ) phylogenetic network of HA gene was constructed using the NETWORK ver. 5.0 (*3*).

References

1. Lee MS, Chen LH, Chen YP, Liu YP, Li WC, Lin YL, et al. Highly pathogenic avian influenza viruses H5N2, H5N3, and H5N8 in Taiwan in 2015. Vet Microbiol. 2016;187:50–7. PubMed http://dx.doi.org/10.1016/j.vetmic.2016.03.012

2. Stamatakis A. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics. 2014;30:1312–3. PubMed http://dx.doi.org/10.1093/bioinformatics/btu033

3. Bandelt HJ, Forster P, Röhl A. Median-joining networks for inferring intraspecific phylogenies. Mol Biol Evol. 1999;16:37–48. PubMed http://dx.doi.org/10.1093/oxfordjournals.molbev.a026036

Technical Appendix 1 Table. Genetic homologies of the A/duck/Taiwan/1702004/2017(H5N6) isolate to H5N6 HPAIV from Japan and South Korea

Gene*	Nucleotide identity, %		
	A/whooper swan/Korea/Gangjin 49 1/2016 collected on 2016 Nov 20	A/spot billed duck/Korea/WB141/2016 collected on 2016 Nov 10	A/teal/Tottori/2/2016(H5N6) collected on 2016 Nov 15
PB2	99.6	99.6	99.3
PB1	99.7	99.7	99.5
PA	99.7	99.7	99.4
HA	99.7	99.5	99.5
NP	99.6	99.7	99.6
NA	99.7	99.6	99.5
MP	99.8	99.8	99.6
NS	99.9	99.8	99.9

*HA, hemagglutinin; MP, matrix; NA, neuraminidase; NP, nucleoprotein; NS, nonstructural; PA, polymerase acidic; PB1 and 2, polymerase basic 1 and 2.

Detection of clade 2.3.4.4c H5N6 HPAIV in Taiwan, 2017

| Date	Type of bird	No of affected farms[A]	Location (County)
2 Feb 2017	Domestic duck	A dead bird on a country road	HUALIEN
9 Feb 2017	Turkey	1	TAINAN
10 Feb 2017	Domestic duck	1	HUALIEN
13 Feb 2017	Native chicken	1	YUNLIN
	Chicken	1	CHIAYI
14 Feb 2017	Chicken	2	CHIAYI
	Chicken	1	CHIAYI
15 Feb 2017	Native chicken	1	YUNLIN
	Goose	1	CHIAYI
18 Feb 2017	Domestic duck	2	HUALIEN
6 Mar 2017	Domestic duck	1	HUALIEN

The map is from Google maps

[A] Confirmed by RT-PCR and Sanger sequencing as previously described (8).

Technical Appendix 1 Figure 1. Detection of clade 2.3.4.4C H5N6 HPAIV in Taiwan, 2017.
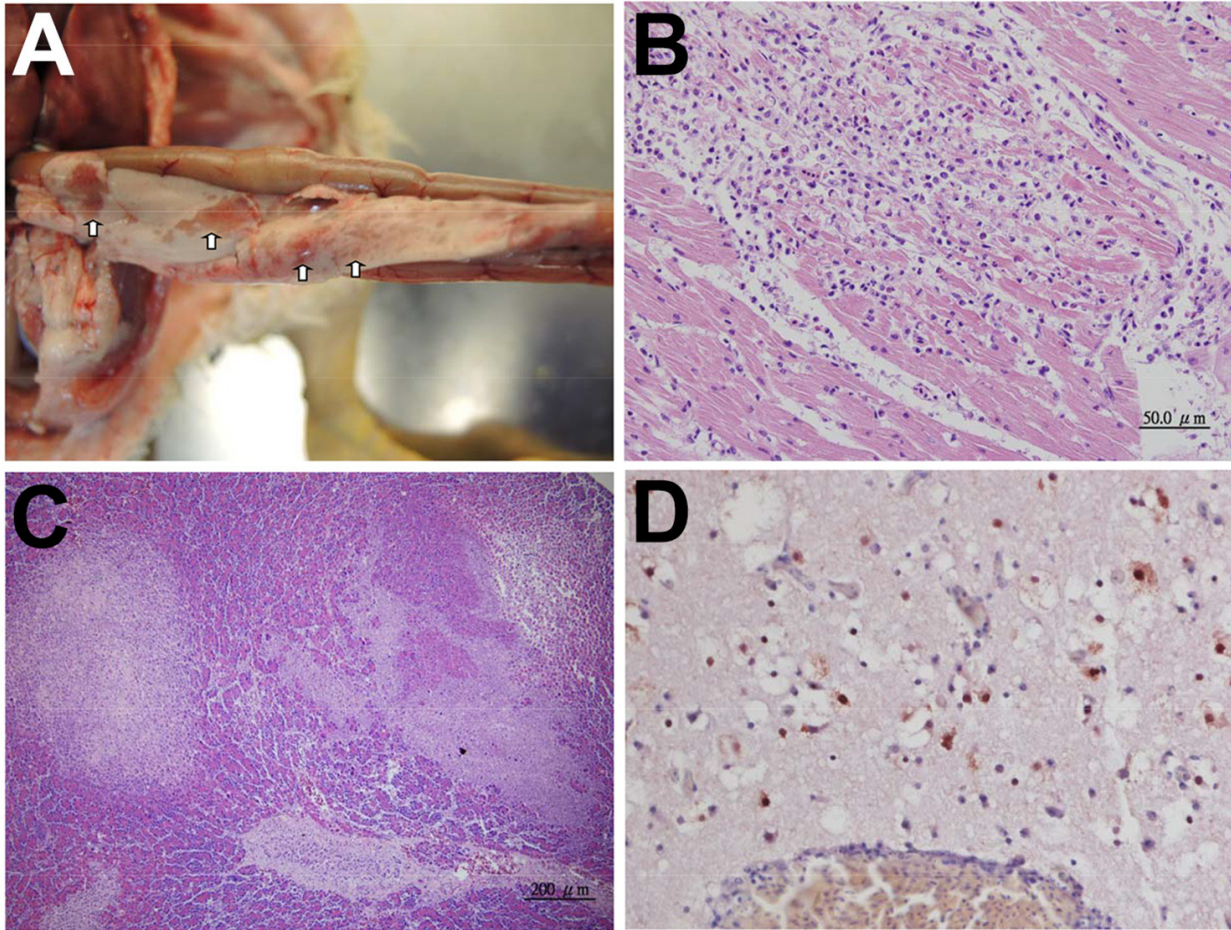
Technical Appendix 1 Figure 2. Necropsy and histopathological findings. A) Multifocal necrosis with hemorrhage in the pancreas (arrow). B) Multifocal coagulative necrosis of the pancreas. C) Heart contains widespread myocyte necrosis with prominent lymphocyte and some heterophil infiltration. D) Mild non-suppurative encephalitis evident as lymphocytic cuffing and endothelial cell hypertrophy and hyperplasia. Viral antigen is localized to neurons.
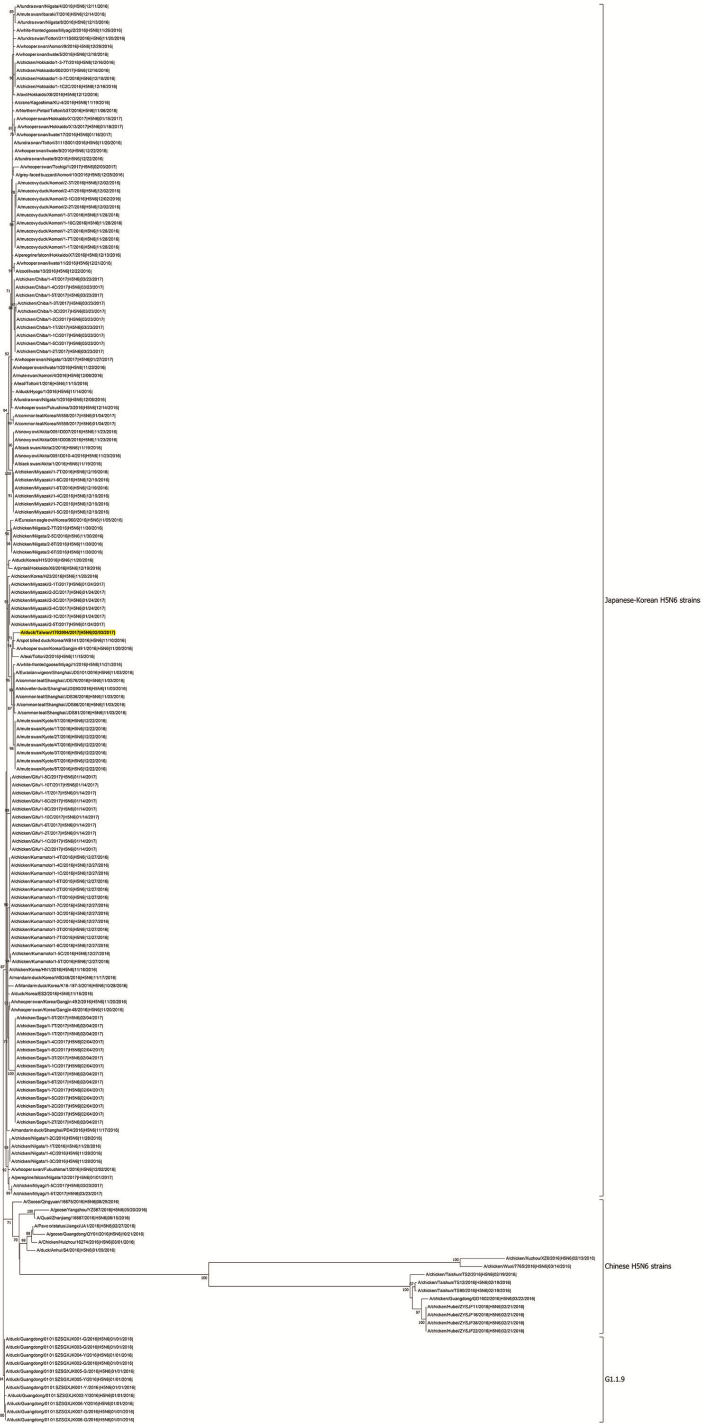
Technical Appendix 1 Figure 3. Maximum-likelihood phylogeny of the PB2 gene of H5N6 viruses identified from East Asia during 2016–2017. The percentages of replicate trees (>70%) in which the associated taxa clustered together in the bootstrap test (500 replicates) are shown next to the branches. The A/duck/Taiwan/1702004/2017(H5N6) virus is highlighted. Scale bar indicates nucleotide substitutions per site.
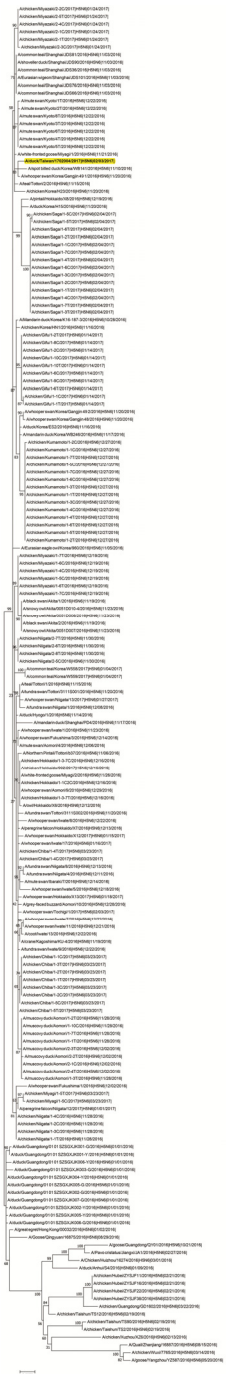
Technical Appendix 1 Figure 4. Maximum-likelihood phylogeny of the PB1 gene of H5N6 viruses identified from East Asia during 2016–2017. The percentages of replicate trees (>70%) in which the associated taxa clustered together in the bootstrap test (500 replicates) are shown next to the branches. The A/duck/Taiwan/1702004/2017(H5N6) virus is highlighted. Scale bar indicates nucleotide substitutions per site.

Technical Appendix 1 Figure 5. Maximum-likelihood phylogeny of the PA gene of H5N6 viruses identified from East Asia during 2016–2017. The percentages of replicate trees (>70%) in which the associated taxa clustered together in the bootstrap test (500 replicates) are shown next to the branches. The A/duck/Taiwan/1702004/2017(H5N6) virus is highlighted. Scale bar indicates nucleotide substitutions per site.
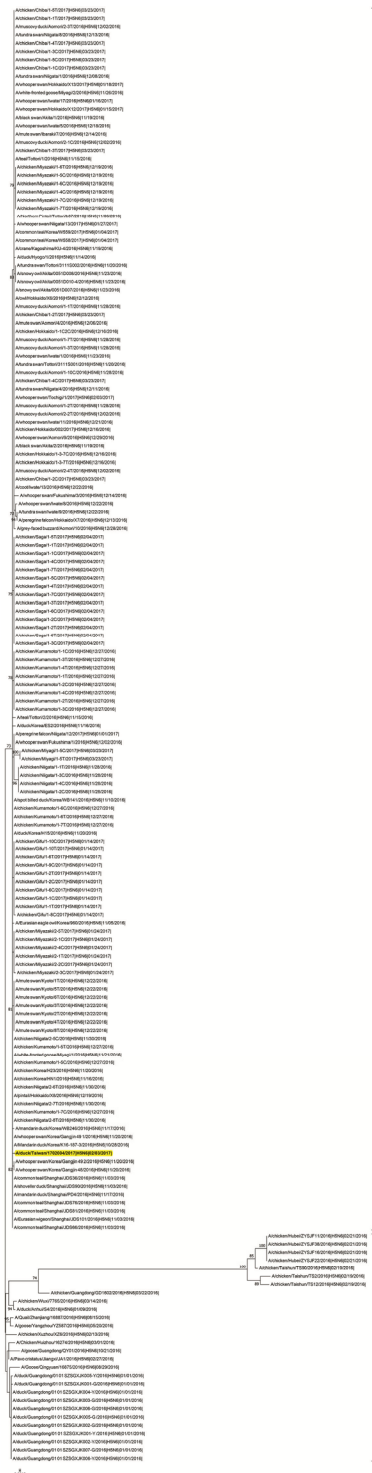
Technical Appendix 1 Figure 6. Maximum-likelihood phylogeny of the HA gene of H5N6 viruses identified from East Asia during 2016–2017. The percentages of replicate trees (>70%) in which the associated taxa clustered together in the bootstrap test (500 replicates) are shown next to the branches. The A/duck/Taiwan/1702004/2017(H5N6) virus is highlighted. Scale bar indicates nucleotide substitutions per site.

Technical Appendix 1 Figure 7. Maximum-likelihood phylogeny of the NP gene of H5N6 viruses identified from East Asia during 2016–2017. The percentages of replicate trees (>70%) in which the associated taxa clustered together in the bootstrap test (500 replicates) are shown next to the branches. The A/duck/Taiwan/1702004/2017(H5N6) virus is highlighted. Scale bar indicates nucleotide substitutions per site.

Technical Appendix 1 Figure 8. Maximum-likelihood phylogeny of the NA gene of H5N6 viruses identified from East Asia during 2016–2017. The percentages of replicate trees (>70%) in which the associated taxa clustered together in the bootstrap test (500 replicates) are shown next to the branches. The A/duck/Taiwan/1702004/2017(H5N6) virus is highlighted. Scale bar indicates nucleotide substitutions per site.

Technical Appendix 1 Figure 9. Maximum-likelihood phylogeny of the M gene of H5N6 viruses identified from East Asia during 2016–2017. The percentages of replicate trees (>70%) in which the associated taxa clustered together in the bootstrap test (500 replicates) are shown next to the branches. The A/duck/Taiwan/1702004/2017(H5N6) virus is highlighted. Scale bar indicates nucleotide substitutions per site.

Technical Appendix 1 Figure 10. Maximum-likelihood phylogeny of the NS gene of H5N6 viruses identified from East Asia during 2016–2017. The percentages of replicate trees (>70%) in which the associated taxa clustered together in the bootstrap test (500 replicates) are shown next to the branches. The A/duck/Taiwan/1702004/2017(H5N6) virus is highlighted. Scale bar indicates nucleotide substitutions per site.