

Sub-Saharan Africa and Eurasia Ancestry of Reassortant Highly Pathogenic Avian Influenza A(H5N8) Virus, Europe, December 2019

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We report detection of a highly pathogenic avian influenza A(H5N8) clade 2.3.4.4b virus in Europe. This virus was generated by reassortment between H5N8 subtype virus from sub-Saharan Africa and low pathogenicity avian influenza viruses from Eurasia.

Highly pathogenic avian influenza (HPAI) H5 viruses belonging to clade 2.3.4.4 of the Goose/Guangdong/96 (GS/Gd) lineage continue to pose a threat to poultry and wild birds worldwide (1–6). Reassortment events between HPAI H5 and low pathogenicity avian influenza (LPAI) viruses of wild-bird origin have led to generation of novel variants that might be periodically spread by wild birds across continents (6).

After detection of the unofficially defined clade 2.3.4.4b (7) in May 2016 in Lake Uvs-Nur, Russia (8), and Qinghai Lake, China (9), the virus spread to Europe and Africa, causing one of the largest epizootics reported (1). This virus reached several countries in northern, western, eastern, central, and southern areas of Africa (10). Nigeria, Namibia, South Africa (11), and Egypt reported H5N8 cases throughout 2019, suggesting ongoing circulation of the virus in Africa.

No HPAI H5N8 viruses were detected in Europe during June–November 2019 (12). We report detection of a reassortant HPAI A(H5N8) clade 2.3.4.4b virus in Europe during December 2019.

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The Study

In July 2019, in the framework of active surveillance measures implemented in live bird markets in 18 of the 36 states in Nigeria, the National Veterinary Research Institute in Vom, Nigeria, identified a HPAI H5N8 virus in a guinea fowl in the southwestern state of Ogun. Months later, at the end of December 2019, a suspicion of an HPAI virus was raised in a holding of 14-week-old meat turkeys in Poland, located near water bodies (fish ponds and lakes of the Łęczna-Włodawa Lakeland). A sudden increase in deaths was observed, accompanied by neurologic signs such as trembling, inability to walk, paralysis of the wings, and pedaling movements of the legs. A total of 3,000–5,000 birds died during the first 3 days after the onset of clinical signs. Organ samples submitted to the National Reference Laboratory for Avian influenza at the National Veterinary Research Institute, Pulawy, Poland, were positive for avian influenza virus and were characterized as HPAI H5N8.

We conducted antigenic characterization of the virus isolate by using the hemagglutination inhibition (HI) assay, which showed that the H5N8 virus in Poland had higher antigenic reactivity with European Union Reference Laboratory reference HPAI H5N8 A/turkey/Italy/7898/2014 (IT-7898) chicken antiserum (clade 2.3.4.4, GS/Gd lineage) compared with reactivity determined for European Union Reference Laboratory HPAI H5N1 A/chicken/Scotland/1/59 (SCOT-59) and LPAI H5N3 A/teal/England/7394–2805/06 (ENG-7394) antiserum.

A comparison of HI titers obtained with the IT-7898, SCOT-59 and ENG-7394 homologous antigens and those recorded against the strain from Poland showed differences of 2 log₂, 4 log₂, and 5 log₂, respectively. The H5N1 and H5N3 strains belong to the H5 Eurasian

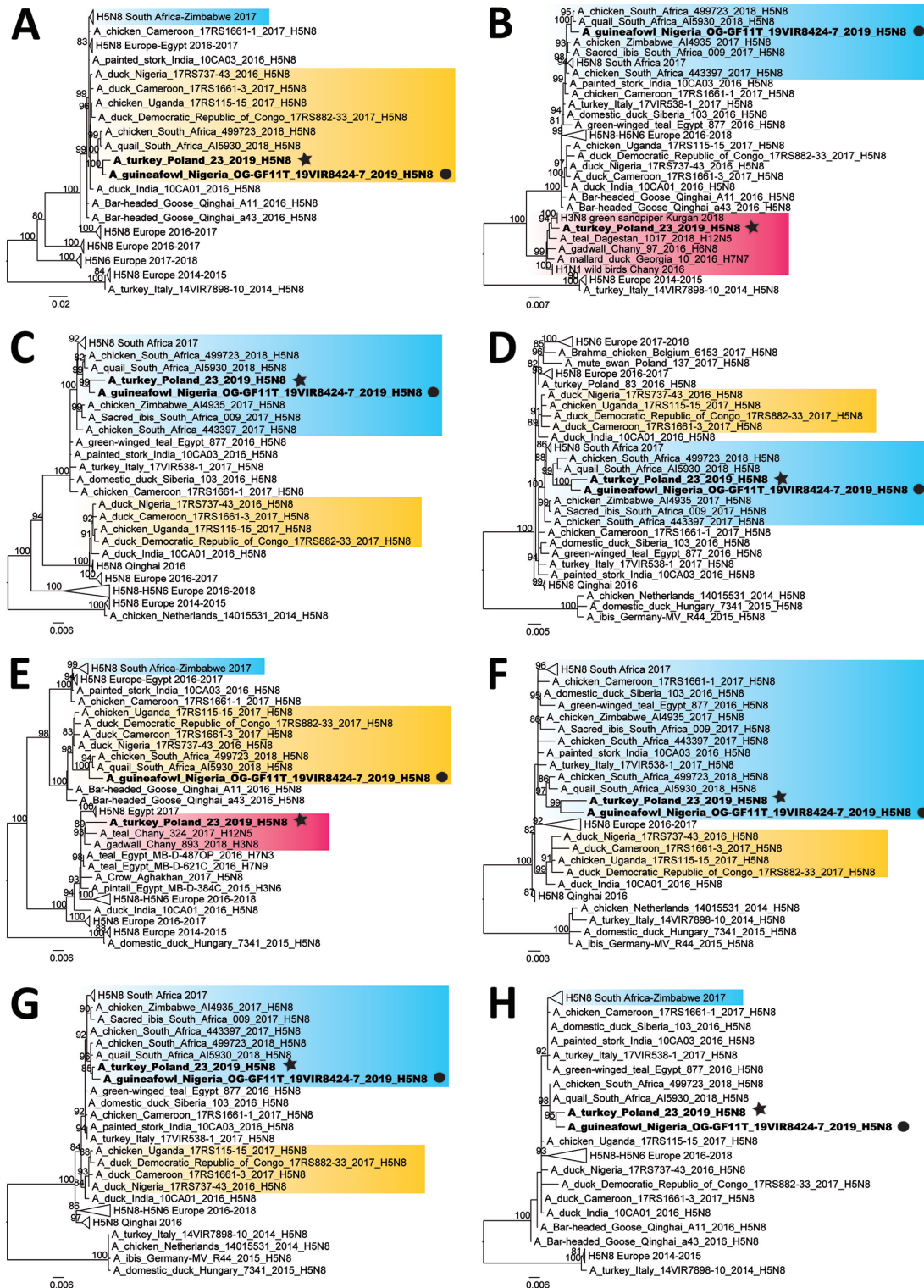


Figure 1. Maximum-likelihood phylogenetic trees of avian influenza A (H5N8) viruses identified in Poland and Nigeria, 2019. A) Polymerase basic protein 2, C) polymerase basic protein 1, C) polymerase acidic protein, D) hemagglutinin, E) nucleoprotein, F) neuraminidase, G) matrix protein, H) nonstructural protein. H5N8 viruses detected in Poland and Nigeria in 2019 are indicated in bold, A/turkey/Poland/23/2019(H5N8) is indicated by a black star, and A/guinea fowl/Nigeria/OG-GF11T_19VIR8424–7/2019(H5N8) is indicated by a black circle. Blue box indicates the South Africa 2017 H5N8 cluster, yellow boxes indicate the West-Central Africa 2016–2017 cluster, and purple box indicates Eurasian LPAI viruses. Numbers next to each node represent ultrafast bootstrap supports (>80). Scale bars indicate nucleotide substitutions per site. LPAI, low pathogenicity avian influenza virus.

lineage and are unrelated to the GS/Gd lineage, which supports the marked difference in reactivity. Pathotyping of the virus from Poland by using the intravenous pathogenicity index recorded a value of 3.0, confirming the highly pathogenic phenotype in chickens.

As of January 31, 2020, a total of 20 outbreaks in poultry (commercial and backyard holdings) and 1 case in a wild bird (a dead goshawk found near the index farm) had been detected in different regions from eastern to western Poland. Since the reassortant virus was detected in Poland, the World Organisation for Animal Health has been notified about similar outbreaks in Slovakia, Hungary, Romania, Germany, and the Czech Republic. A greater white-fronted goose (*Anser albifrons*) in Germany, near the border with Poland, was also found to be infected (13).

The genomes of HPAI H5N8 strains from index cases from Nigeria (A/guinea fowl/Nigeria/OG-GF11T_19VIR8424-7/2019) and Poland (A/turkey/Poland/23/2019) have been sequenced (Appendix, <https://wwwnc.cdc.gov/EID/article/26/7/20-0165-App1.pdf>) and submitted to the GISAID EpiFlu database (<https://www.gisaid.org>) under isolate nos. EPI_ISL_405278 and EPI_ISL_402134, respectively. Phylogenetic analysis (Appendix) of the hemagglutinin (HA) gene showed that both viruses belonged to clade 2.3.4.4b (Figure 1).

Topology of the polymerase basic protein 2, polymerase acidic protein, HA, neuraminidase, matrix, and nonstructural protein gene phylogenies showed that the viruses from Poland and Nigeria clustered

together (nucleotide identity 98.9%–99.5%) (Table) and also with two 2018 H5N8 viruses from South Africa (3), which have been demonstrated to be a novel genotype that originated from reassortment events between clade 2.3.4.4b H5N8 viruses from South Africa and West-Central Africa (Figure 1). In contrast, the 2 strains clustered separately for the polymerase basic protein 1 and nucleoprotein gene phylogenies (Figure 1).

For the polymerase basic protein 1 and nucleoprotein genes, the virus from Nigeria grouped with the 2018 viruses from South Africa as for the other gene segments, whereas the virus from Poland clustered with LPAI viruses identified in recent years in the Chany and Kurgan regions of Russia. This finding indicates that the strain from Poland is a reassortant virus derived from LPAI viruses identified in wild birds in those areas of Asia (Figure 2), which represent staging areas for wild birds migrating to Europe. However, where this reassortment event occurred cannot be assessed from the available data. Analysis of molecular markers associated with zoonotic potential demonstrated the absence in the HA and polymerase basic protein 2 genes of major signatures associated with increased replication in humans.

Conclusions

Our data describe a novel HPAI H5N8 genotype of clade 2.3.4.4b in Europe, in which 6 gene segments originated from sub-Saharan Africa HPAI H5N8 clade 2.3.4.4b viruses and 2 gene segments from Eurasia LPAI viruses. It has been shown that Africa might serve as a

Table. Nucleotide identity of A/turkey/Poland/23/2019(H5N8) and A/guinea_fowl/Nigeria/OG-GF11T_19VIR8424-7/2019 influenza virus gene segments with the most similar sequences available in GenBank and EpiFlu*

Gene segment	Virus 1	Virus 2	Nucleotide identity, %
PB2	H5N8 Poland 2019†	H5N8 Nigeria 2019‡	98.9
	H5N8 Nigeria 2019	H5N8 South Africa 2018§	98.7
PB1	H5N8 Poland 2019	H3N8 Kurgan 2018¶	99.1
	H5N8 Poland 2019	H5N8 Nigeria 2019	94.7
	H5N8 Nigeria 2019	H5N8 South Africa 2018	99.2
PA	H5N8 Poland 2019	H5N8 Nigeria 2019	98.9
	H5N8 Nigeria 2019	H5N8 South Africa 2018	99.2
HA	H5N8 Poland 2019	H5N8 Nigeria 2019	99.1
	H5N8 Nigeria 2019	H5N8 South Africa 2018	98.7
NP	H5N8 Poland 2019	H3N8 Chany 2018#	98.5
	H5N8 Poland 2019	H5N8 Nigeria 2019	92.8
	H5N8 Nigeria 2019	H5N8 South Africa 2018	98.9
NA	H5N8 Poland 2019	H5N8 Nigeria 2019	98.8
	H5N8 Nigeria 2019	H5N8 South Africa 2018	98.9
M	H5N8 Poland 2019	H5N8 Nigeria 2019	99.5
	H5N8 Nigeria 2019	H5N8 South Africa 2018	99.4
NS	H5N8 Poland 2019	H5N8 Nigeria 2019	99.0
	H5N8 Nigeria 2019	H5N8 South Africa 2018	99.2

*HA, hemagglutinin; M, matrix protein; NA, neuraminidase; NP, nucleoprotein; NS, nonstructural protein; PA, polymerase acidic protein; PB1, polymerase basic protein 1; PB2, polymerase basic protein 2.

†A/turkey/Poland/23/2019(H5N8).

‡A/guinea_fowl/Nigeria/OG-GF11T_19VIR8424-7/2019(H5N8).

§A/chicken/South Africa/499723/2018(H5N8).

¶A/green sandpiper/Kurgan/1043/2018(H3N8).

#A/gadwall/Chany/893/2018(H3N8).

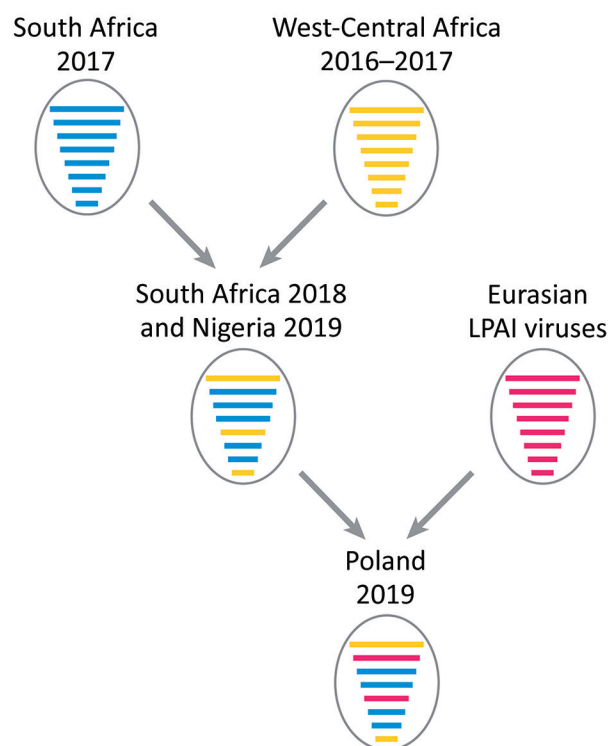


Figure 2. Reassortment events between highly pathogenic avian influenza virus H5N8 viruses from South Africa (2017), HPAI H5N8 viruses from West-Central Africa (2017); and LPAI viruses from Eurasia giving rise to A/guinea_fowl/Nigeria/OG-GF11T_19VIR8424–7/2019(H5N8) (Nigeria 2019) and A/turkey/Poland/23/2019(H5N8) (Poland 2019). Each gene segment is represented by a bar of different length, from top to bottom: polymerase basic protein 2 (PB2), polymerase basic protein 1 (PB1), polymerase acidic protein (PA), hemagglutinin (HA), nucleoprotein (NP), neuraminidase (NA), matrix protein (M), and nonstructural protein (NS). Each color represents a different viral origin: blue, H5N8 South Africa (2017); yellow, H5N8 West-Central Africa (2017); purple, Eurasian LPAI viruses. LPAI, low pathogenicity avian influenza virus.

strong epidemiologic area of circulation for Gs/GD H5 (10). However, despite extensive circulation in poultry in several countries in Africa, virus spread by wild birds from Africa to Europe has not been documented.

Possible introduction of an influenza A virus from Africa into Eurasia might be caused by widespread virus circulation in previously unaffected areas, high prevalence of the virus in wild birds in Africa, or alterations of the migratory bird exposure risks after changes in migratory routes caused by unusual weather patterns. Identification of highly related virus strains 1 year apart in 2 countries in Africa >5,000 km apart confirms the high mobility of the HPAI H5N8 virus and suggests a gap in surveillance efforts in these areas. The lack of such surveillance data makes it impossible to determine where this

virus originated and whether it spread from South Africa to Nigeria or to both countries from unsampled locations, and to assess its prevalence in Africa.

Our results suggest that H5N8 virus might have been spread from Africa to Asia or Europe by wild bird migratory movements, likely in 2019, although other routes of virus spread cannot be ruled out. Until its detection in Poland in December 2019, the virus might have circulated in an unknown region of Europe or Asia where it reassorted with local LPAI strains of the Eurasian lineage, although we cannot completely exclude that this reassortment event might have occurred in Africa. Late detection in Europe (end of December 2019), compared with the epidemic wave during 2016–17 (October 2016), might be explained by unusually mild temperatures in molting areas in Russia during November and December 2019 (14,15) and the late westward movement of infected wintering wild birds.

Subclinical infections or insufficient active surveillance efforts in clinically healthy wild population might be the cause of the few detections of the H5N8 virus in wild birds in Eurasia and along the Africa-Eurasia flyways. A better understanding of factors regulating wild bird migrations, as well as increasing wild and domestic bird surveillance in Africa and Eurasia, is needed to improve our ability to early detect and monitor virus spread.

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About the Author

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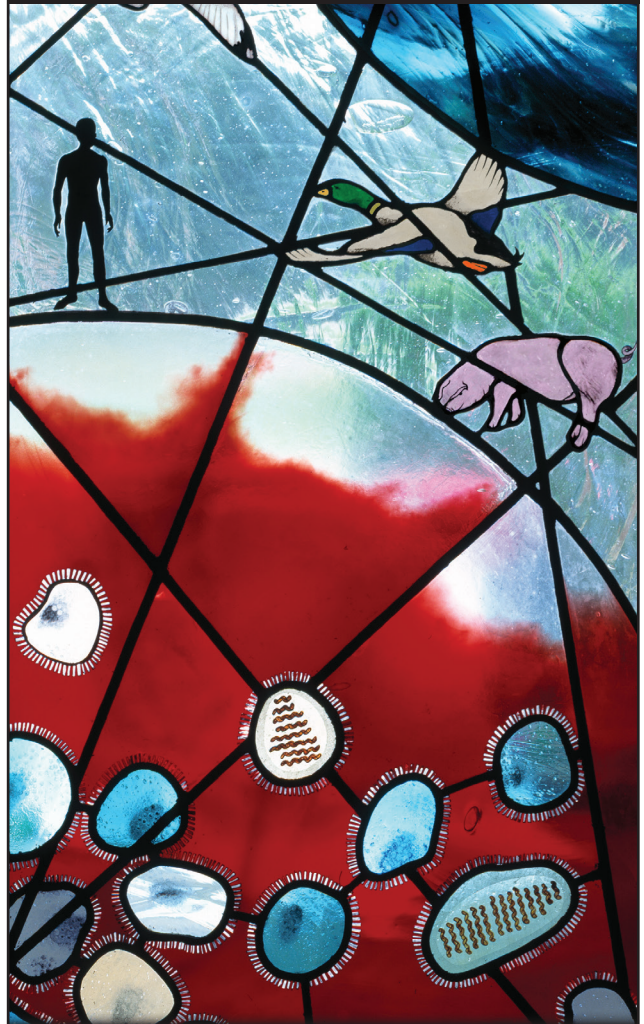
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Sub-Saharan Africa and Eurasia Ancestry of Reassortant Highly Pathogenic Avian Influenza A(H5N8) Virus, Europe, December 2019

Appendix

Genome Amplification and Sequencing of H5N8 Virus from Nigeria

Total RNA was purified from allantoic fluid by using the Viral RNA Kit (QIAGEN, <https://www.qiagen.com>). The complete genome of the virus from Nigeria was amplified by using the SuperScript III One-Step RT-PCR System and Platinum Taq High Fidelity (Invitrogen, <https://www.thermofisher.com>) as described (1,2). The sequencing library was prepared by using the Nextera DNA XT Sample Preparation Kit (Illumina, <https://www.illumina.com>) and quantified by using the Qubit dsDNA High Sensitivity Kit (Invitrogen). The average fragment length was assessed by using the High Sensitivity DNA Analysis Kit (Agilent Technologies, <https://www.agilent.com>). The sample was sequenced on an Illumina MiSeq platform, according to the manufacturer's instructions. Read quality was assessed by using FastQC version 0.11.2 (<https://www.bioinformatics.babraham.ac.uk>).

Raw data were filtered by removal of reads with >10% of undetermined bases, reads with >100 bases with a Q score 80 bases were aligned against a reference genome by using BWA version 0.7.12 (3). Potential errors were corrected and base quality was recalibrated by using Picard Tools version 2.1.0 (<http://picard.sourceforge.net>) and GATK version 3.5 (4–6). Single-nucleotide polymorphisms were called by using LoFreq version 2.1.2 (7). Results were used to generate consensus sequences.

Genome Amplification and Sequencing of H5N8 Virus from Poland

RNA was extracted by using RNeasy Mini Kit (QIAGEN) according to the manufacturer's instruction. Complete genome was obtained by using the One Step RT-PCR Kit (QIAGEN) and specific primers as described (8). Nucleotide sequences were determined by using the BigDye Terminator version 3.1 Cycle Sequencing Kit (Applied Biosystems, <https://www.thermofisher.com>) according to the manufacturer's instruction. Capillary electrophoresis was performed by using a 3500

Genetic Analyzer (Applied Biosystems), and sequences obtained were assembled and analyzed by using SeqMan, DNAStar LaserGene version 8.1.3 (<https://www.dnastar.com/software/lasergene>).

Phylogenetic Analyses

Consensus sequences of the 8 gene segments of the viruses from Nigeria and Poland were compared with the most related sequences available in GISAID (<https://www.gisaid.org>) (Appendix Table) and aligned by using MAFFT version 7 (9). Maximum-likelihood phylogenetic trees were generated in IQTREE version 1.6 (<http://www.iqtree.org/release/v1.6.8>) and performing ultrafast bootstrap resampling analysis (1,000 replications) (10,11). Phylogenetic trees were visualized by using FigTree version 1.4.2 (<http://tree.bio.ed.ac.uk>).

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Appendix Table. Information from the GISAID EpiFlu database on hemagglutinin segments used for study of sub-Saharan Africa and Eurasia ancestry of reassortant highly pathogenic avian influenza A(H5N8) virus, Europe, December 2019*

Segment ID	Country	Collection date	Isolate ID	Isolate name	Originating laboratory	Submitting laboratory	Authors
EPI1032508	Hungary	2017 Apr 20	EPI_ISL_271708	A/Goose/Hungary/17985/2017	National Food Chain Safety Office Veterinary Diagnostic Directorate Laboratory for Molecular Biology	Central Agricultural Office Veterinary Diagnostic Directorate	Adam D
EPI1061370	Belgium	2017 Jun 15	EPI_ISL_278200	A/Brahma chicken/Belgium/6153/2017	NA	Public domain	Steensels M, Lambrecht B, Vandenbussche F, Van Borm S.
EPI1144334	United Kingdom	2017 Dec 31	EPI_ISL_292223	A/mute_swan/England/AVP_18_001986/2017	Animal and Plant Health Agency	Animal and Plant Health Agency	Seekings J, Ellis R, Brookes SM, Reid S, Essen S, Lewis N, Brown IH
EPI1144409	China	2016 May 13	EPI_ISL_292235	A/Bar-headed Goose/Qinghai/a43/2016	Wuhan Institute of Virology,	Wuhan Institute of Virology	NA
EPI1145853	China	2016 May 14	EPI_ISL_292496	A/Bar-headed Goose/Qinghai/A11/2016	Wuhan Institute of Virology	Wuhan Institute of Virology	NA
EPI1185001	Poland	2017 Feb 6	EPI_ISL_300703	A/domestic_goose/Poland/124/2017	National Veterinary Research Institut Poland	National Veterinary Research Institut Poland	Swieton E, Smietanka K
EPI1185019	Poland	2017 Mar 8	EPI_ISL_300705	A/turkey/Poland/285/2017	National Veterinary Research Institut Poland	National Veterinary Research Institut Poland	Swieton E, Smietanka K
EPI1185049	Poland	2017 Feb 21	EPI_ISL_300747	A/mute_swan/Poland/137/2017	NA	National Veterinary Research Institut Poland	Swieton E, Smietanka K
EPI1195315	The Netherlands	2018 Feb 26	EPI_ISL_302826	A/Chicken/Netherlands/EMC-14/2018	Erasmus Medical Center	Erasmus Medical Center	Poen MJ, Bestebroer TM, De Meulder D, Vuong O, Scheuer RD; Netherlands Food and Consumer Product Safety Authority, Koopmans MP, Fouchier RA
EPI1208386	The Netherlands	2018 Mar 13	EPI_ISL_305416	A/Domestic_Duck/Netherlands/EMC-2/2018	Erasmus Medical Center	Erasmus Medical Center	Poen MJ, BestebroerTM, De Meulder D, Vuong O, Scheuer RD; Netherlands Food and Consumer Product Safety Authority, Koopmans MP, Fouchier RA
EPI1223834	Democratic Republic of the Congo	2017 May 14	EPI_ISL_308803	A/duck/Democratic Republic of the Congo/17RS882-33/2017	NA	Public domain	Twabela A, Zecchin B, Tshilenge G, Sakoda Y, Kone P, Zamperin G, Drago A, Monne I

EPI1223853	Cameroon	2017 Jan 1	EPI_ISL_308806	A/chicken/Cameroon/17RS1661-1/2017	NA	Public domain	Poen MJ, BestebroerTM, De Meulder D, Vuong O, Scheuer RD; Netherlands Food and Consumer Product Safety Authority, Koopmans MP, Fouchier RA
EPI1223854	Cameroon	2017 Jan 1	EPI_ISL_308807	A/duck/Cameroon/17RS1661-3/2017	NA	Public domain	Poen MJ, BestebroerTM, De Meulder D, Vuong O, Scheuer RD; Netherlands Food and Consumer Product Safety Authority, Koopmans MP, Fouchier RA
EPI1279361	Germany	2018 Aug 31	EPI_ISL_322179	A/domestic duck/Germany-MV/AR613-L02727/2018	NA	Friedrich-Loeffler-Institut	NA
EPI1327144	The Netherlands	2018 Aug 24	EPI_ISL_332441	A/Mallard/Netherlands/18012508-017/2018	Wageningen Bioveterinary Research	Wageningen Bioveterinary Research	Beerens N, Heutink R, Harders F, Verschuren-Pritz S, Bossers A, Koch G, Bergervoet S Joannis T, Meseke C, Zamperin G, Milani A, Zecchin B, Fusaro A, Salviato A, Schivo A, Monne I
EPI1398282	Nigeria	2016 Nov 19	EPI_ISL_348279	A/duck/Nigeria/17RS737-43/2016	National Veterinary Research Institute	Istituto Zooprofilattico Sperimentale Delle Venezie	Abolnik C, Rauff DL, Pieterse R, Peyrot B, Choma P, Ebersohn K, Phiri T
EPI1514110	South Africa	2017 Aug 1	EPI_ISL_369339	A/chicken/South Africa/440638A/2017	NA	Public domain	Abolnik C, Rauff DL, Pieterse R, Peyrot B, Choma P, Ebersohn K, Phiri T
EPI1514158	South Africa	2017 Aug 17	EPI_ISL_369345	A/chicken/South Africa/443397/2017	NA	Public domain	Abolnik C, Rauff DL, Pieterse R, Peyrot B, Choma P, Ebersohn K, Phiri T
EPI1514318	South Africa	2017 Sep 29	EPI_ISL_369365	A/chicken/South Africa/451457/2017	NA	Public domain	Abolnik C, Rauff DL, Pieterse R, Peyrot B, Choma P, Ebersohn K, Phiri T
EPI1514326	South Africa	2017 Oct 26	EPI_ISL_369366	A/ostrich/South Africa/002/2017	NA	Public domain	Abolnik C, Rauff DL, Pieterse R, Peyrot B, Choma P, Ebersohn K, Phiri T
EPI1553392	Uganda	2017 Jan 01	EPI_ISL_379227	A/chicken/Uganda/17RS115-15/2017	NA	Public domain	Ndumu D, Zecchin B, Fusaro A, Arinaitwe E, Erechu R, Kidega E, Kayiwa J, Muwanga E, Kirumira M, Kirembe G, Lutwama J, Monne I
EPI1575277	Zimbabwe	2017 May 25	EPI_ISL_387133	A/chicken/Zimbabwe/AI4935/2017	NA	Public domain	Rotherham LS, van Schalkwyk A, Romito M

EPI548623	The Netherlands	2014 Nov 15	EPI_ISL_168075	A/chicken/Netherlands/1401553 1/2014	Wageningen Bioveterinary Research	Wageningen Bioveterinary Research	Heutink R, Harders R, Verschuren-Pritz S, Bossers A, Koch G, Bouwstra R
EPI858836	India	2016 Oct 17	EPI_ISL_237553	A/duck/India/10CA01/2016	National Institute of High Security Animal Diseases	National Institute of High Security Animal Diseases	Nagarajan S, Tripathi S, Kumar M, Murugkar HV, Tosh C, Singh VP
EPI858844	India	2016 Oct 20	EPI_ISL_237554	A/painted stork/India/10CA03/2016	National Institute of High Security Animal Diseases	National Institute of High Security Animal Diseases	Nagarajan S, Tripathi S, Kumar M, Murugkar HV, Tosh C, Singh VP
EPI869929	Poland	2016 Dec 21	EPI_ISL_240106	A/mute swan/Poland/108/2016	NA	National Veterinary Research Institut Poland	Swieton E, Smietanka K
EPI869931	Poland	2016 Dec 19	EPI_ISL_240108	A/turkey/Poland/83/2016	NA	National Veterinary Research Institut Poland	Swieton E, Smietanka K
EPI926613	Russia	2016 Oct 4	EPI_ISL_240677	A/domestic duck/Siberia/103/2016	Research Institute of Experimental and Clinical Medicine	Research Institute of Experimental and Clinical Medicine	Sharshov KA, Kurskaya OG, Alexeev AY, Sobolev IA, Alikina TY, Kabilov MR, Shestopalov AM
EPI954567	Italy	2017 Jan 20	EPI_ISL_255182	A/turkey/Italy/17VIR538-1/2017	Istituto Zooprofilattico Sperimentale Delle Venezie	Istituto Zooprofilattico Sperimentale Delle Venezie	Zecchin B, Fusaro A, Milani A, Schivo A, Salviato A, Zamperin G, Marciano S, Ormelli S, Terregino C, Monne I

*All submitters of data can be contacted directly through the GISAID website (<https://www.gisaid.org>). NA, not available.