forward projections. N Engl J Med. 2014;371:1481–95. Epub 2014 Sep 22. http://dx.doi.org/10.1056/NEJMoa1411100.
9. Centers for Disease Control and Prevention. Current Ebola treatment centers. 12/31/2014 [cited 2014 Jan 5]. http://www.cdc.gov/vhf/ebola/healthcare-us/preparing/current-treatment-centers.html

Address for correspondence: Gabriel Rainisch, Centers for Disease Control and Prevention, 1600 Clifton Rd NE, Mailstop C18, Atlanta, GA 30333, USA; email: Grainisch@cdc.gov

Highly Pathogenic Avian Influenza A(H5N1) Virus in Poultry, Nigeria, 2015

Isabella Monne,[1] Clement Meseko,[1] Tony Joannis, Ismaila Shittu, Mohammed Ahmed, Luca Tassoni, Alice Fusaro, Giovanni Cattoli

Author affiliations: Istituto Zooprofilattico Sperimentale delle Venezie, Padova, Italy (I. Monne, L. Tassoni, A. Fusaro, G. Cattoli); National Veterinary Research Institute, Vom, Nigeria (C. Meseko, T. Joannis, I. Shittu, M. Ahmed)

DOI: http://dx.doi.org/10.3201/eid2107.150421

To the Editor: In Nigeria, from February 2006 through July 2008, outbreaks of highly pathogenic avian influenza (HPAI) subtype H5N1 virus infection in poultry negatively affected animal and public health as well as the agricultural sector and trade. These outbreaks were caused by viruses belonging to genetic clades 2.2 and 2.2.1 (*1*). In January 2015, seven years after disappearance of the virus, clinical signs of HPAI (swollen head and wattles, hemorrhagic shank and feet) and increased mortality rates were observed among backyard poultry in Kano and in a live bird market in Lagos State, Nigeria. The virus was isolated from 2 samples independently collected from the poultry farm (parenchymatous tissues) and the market (tracheal swab), and H5 subtype virus was identified by reverse transcription PCR. The samples were adsorbed onto 2 Flinders Technology Associates cards (GE Healthcare Life Sciences, Little Chalfont, UK), which were sent to the World Organisation for Animal Health/Food and Agriculture Organization of the United Nations Reference Laboratory for Avian Influenza in Italy for subtype confirmation and genetic characterization. Influenza A(H5N1) virus was detected in both samples, and sequencing of the hemagglutinin (HA) gene showed that the viruses possessed the molecular markers for HPAI viruses with a multibasic amino acid cleavage site motif (PQRERRRKR*G).

The complete genome of the virus from backyard poultry was successfully sequenced from the genetic material extracted from the Flinders Technology Associates cards by using an Illumina MiSeq platform (*2*) and was submitted to the Global Initiative on Sharing All Influenza Data database (http://platform.gisaid.org/) under accession nos. EPI556504 and EPI567299–EPI567305. Maximum-likelihood trees were estimated for all 8 gene segments by using the best-fit general time reversible plus invariant sites plus gamma 4 model of nucleotide substitution with PhyML (*3*). The topology of the phylogenetic tree of the HA gene demonstrated that the H5N1 virus from Nigeria (A/chicken/Nigeria/15VIR339-2/2015) falls within genetic clade 2.3.2.1c (Figure, panel A). In particular, the HA gene sequence clustered with H5 viruses collected in China in 2013 and with an H5N1 virus (A/Alberta/01/2014) isolated from a Canada resident who had returned from China (similarity 99.3%–99.5%) (*4*).

The remaining 7 genes were closely related to genes of A/Alberta/01/2014(H5N1), although the 2 viruses differed by 32 aa (online Technical Appendix, http://wwwnc.cdc.gov/EID/article/21/7/15-0421-Techapp1.pdf). Just as for the virus from Canada (*4*), 7 of 8 gene segments of the virus from Nigeria clustered with HPAI A(H5N1) virus circulating in Vietnam and China, while the polymerase basic 2 gene segment (Figure, panel B) resulted from reassortment with viruses circulating in the same Asian countries but belonged to the H9N2 subtype. Differing from the strain from Canada (only 2 aa mutations compared with the 2.3.2.1c candidate vaccine strain; *5*), the strain from Nigeria possesses 6 aa differences: 3 in HA1 and 3 in HA2 (online Technical Appendix). The effect of these mutations on the antigenic relatedness of these strains should be further explored.

Molecular characterization demonstrated that the polymerase basic 2 sequence contains glutamic acid at position 627, establishing the lack of a well-known mammalian adaptation motif (*6*). Mutations associated with increased virulence in mice have been observed in the nonstructural protein 1 (P42S, D87E, L98F, I101M, and the 80–84 deletion) and in the matrix 1 proteins (N30D, T215A). In addition, the substitutions D94N, S133A, S155N (H5 numbering) associated with increased binding to α-2,6 sialic acid have been identified in the HA protein. However, most of these substitutions are present in the H5N1 virus sequences from Asia included in our phylogenetic analyses, suggesting that they may be common among the HPAI H5 virus subtype. Mutations associated with resistance to antiviral drugs have not been detected (*7*).

The results obtained from whole-genome analysis provide evidence that a novel clade of the A(H5N1) virus, specifically clade 2.3.2.1c, has reached Nigeria. Although ascertaining how and exactly when this has happened is difficult, it seems most likely that the virus entered the country in December 2014, as evidenced by unverified

[1]These authors contributed equally to this article.

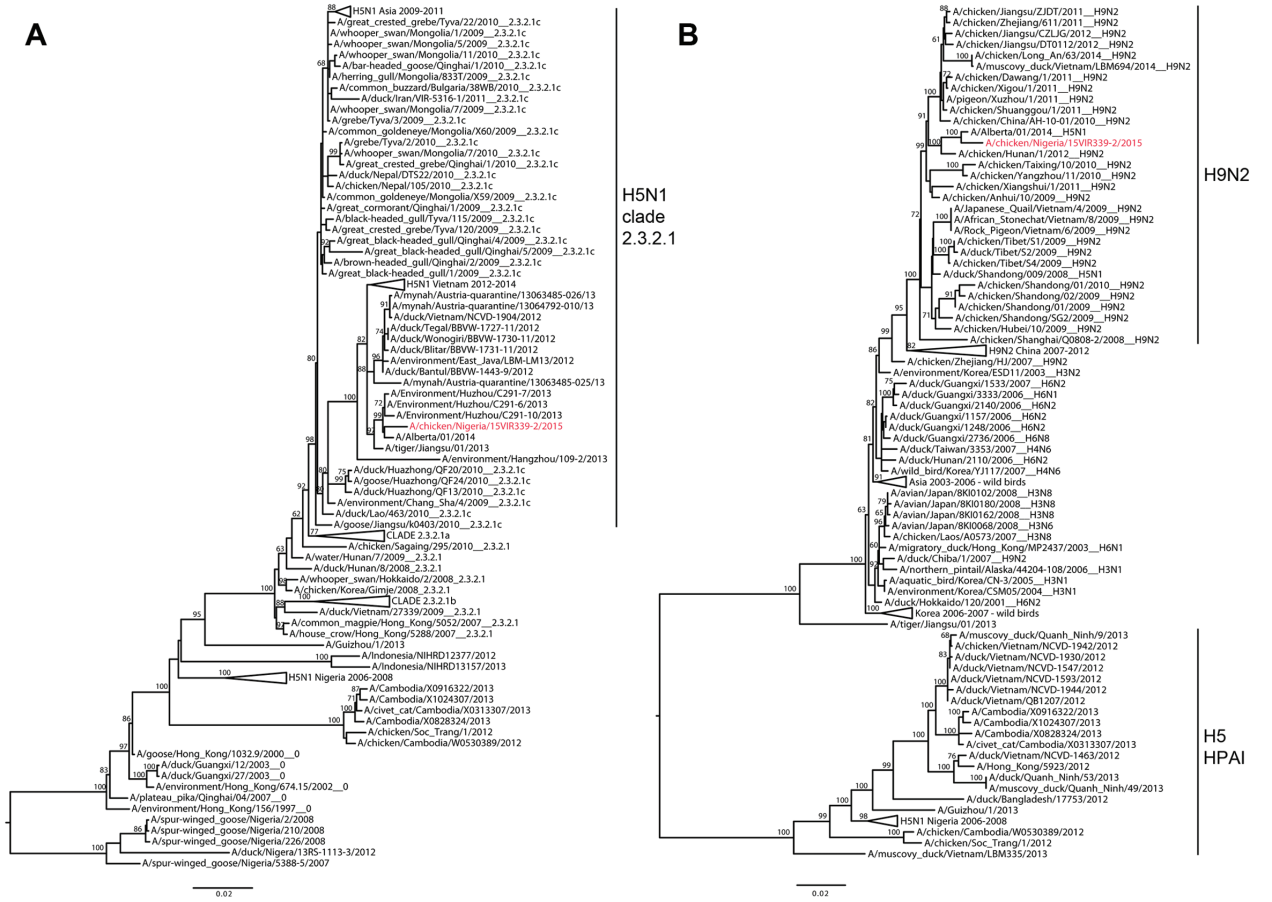


Figure. Maximum likelihood phylogenetic trees of the A) hemagglutinin and B) polymerase basic 2 gene segments of highly pathogenic avian influenza A(H5N1) virus from poultry in Nigeria, 2015 (in red). Bootstrap values (100 replicates) >60 are shown at the nodes. Scale bars indicate nucleotide substitutions per site. HPAI, highly pathogenic avian influenza.

accounts of increased poultry deaths in some live bird markets in Lagos, after the birds had been moved from the north (Kano) to the south during the festive season. The identification of genetic clustering between the strains from Nigeria analyzed here and the HPAI A(H5N1) viruses originally identified in Asia suggests an unknown epidemiologic link between these regions, probably associated with human activities, migratory bird movements, or both.

Considering that this virus is an intersubtype reassortant and has already caused infection in humans, we believe that complete characterization of the strain in terms of virulence and host range is of high priority. Furthermore, because the reemergence of subtype H5N1 virus was followed by epidemiologic amplification (≈265 outbreaks in 18 states as of February 2015; T. Joannis, pers. comm., 2015) for which virus genetic characterization is not yet available, local veterinary and public health services and international organizations should take necessary measures to identify critical control points and stop circulation of this virus.

Acknowledgments

We gratefully acknowledge the contributing authors and the originating and submitting laboratories for the sequences from the Global Initiative on Sharing All Influenza Data EpiFlu database on which this research is based. We also acknowledge Olorunsola Bankole and Idris Ibrahim for sample collection and the Federal Department of Veterinary and Pest Control Services, Federal Ministry of Agriculture, Abuja, Nigeria, for technical support. We thank Silvia Ormelli, Alessia Schivo, and Francesca Ellero for their excellent technical assistance.

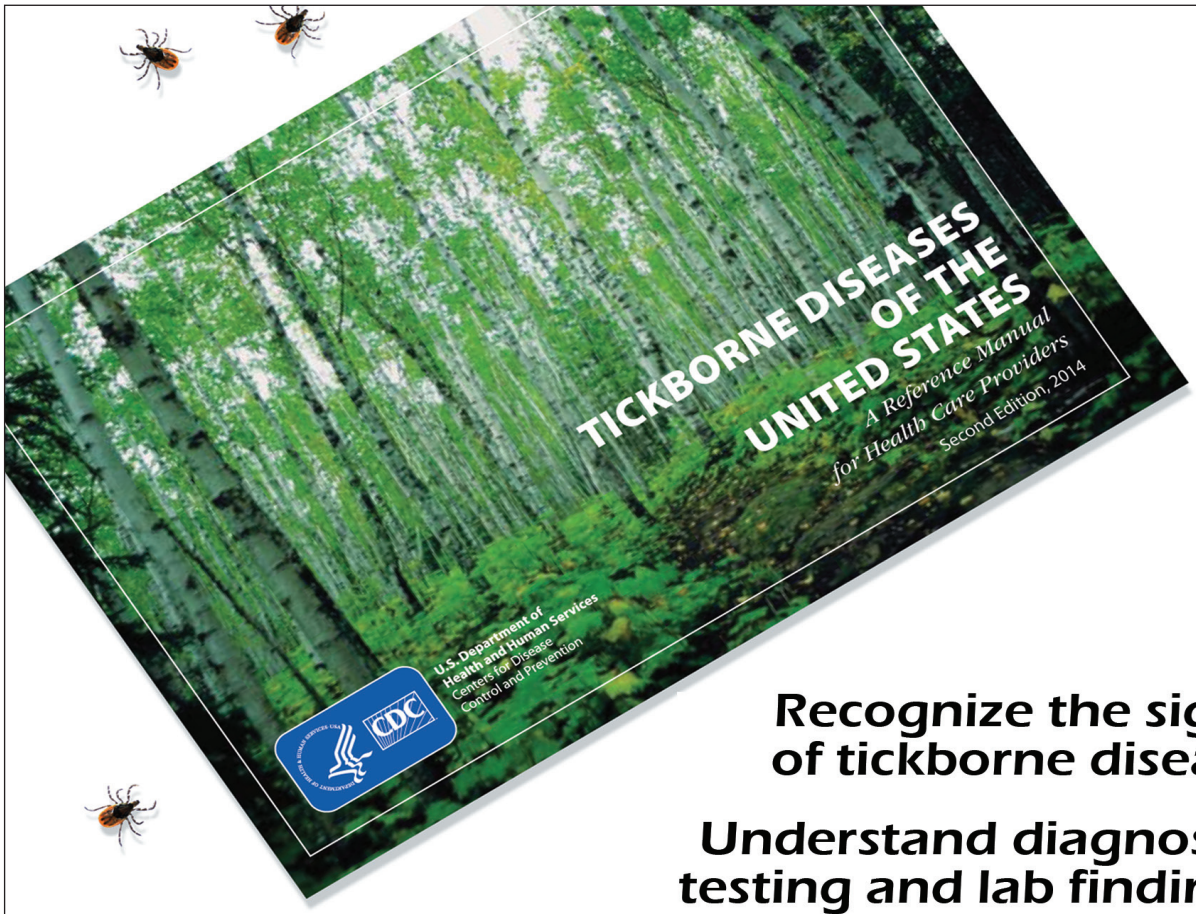
This work was partially supported by the European projects Epi-SEQ (http://www.epi-seq.eu/), FP7 project no. 219235.

References

1. Fusaro A, Nelson MI, Joannis T, Bertolotti L, Monne I, Salviato A, et al. Evolutionary dynamics of multiple sublineages of H5N1 influenza viruses in Nigeria from 2006 to 2008. J Virol. 2010;84:3239–47. http://dx.doi.org/10.1128/JVI.02385-09
2. Monne I, Fusaro A, Nelson MI, Bonfanti L, Mulatti P, Hughes J, et al. Emergence of a highly pathogenic avian influenza virus from a low-pathogenic progenitor. J Virol. 2014;88:4375–88. http://dx.doi.org/10.1128/JVI.03181-13

3. Guindon S, Gascuel OA. Simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. Syst Biol. 2003;52:696–704. http://dx.doi.org/10.1080/10635150390235520
4. Pabbaraju K, Tellier R, Wong S, Li Y, Bastien N, Tang JW, et al. Full-genome analysis of avian influenza A(H5N1) virus from a human, North America, 2013. Emerg Infect Dis. 2014;20:887–91. http://dx.doi.org/10.3201/eid2005.140164.
5. World Health Organization. Antigenic and genetic characteristics of zoonotic influenza viruses and development of candidate vaccine viruses for pandemic preparedness [cited 2015 Feb 1]. http://www.who.int/influenza/vaccines/virus/characteristics_virus_vaccines/en/
6. Subbarao EK, London W, Murphy BR. A single amino acid in the PB2 gene of influenza A virus is a determinant of host range. J Virol. 1993;67:1761–4.
7. Centers for Disease Control and Prevention. H5N1 Genetic Changes Inventory: a tool for influenza surveillance and preparedness [cited 2012 Jun 1]. http://www.cdc.gov/flu/avianflu/h5n1-genetic-changes.htm

Address for correspondence: Isabella Monne, Istituto Zooprofilattico Sperimentale delle Venezie, Research & Innovation Department, OIE and National Reference Laboratory for Avian Influenza & Newcastle Disease, FAO Reference Centre for Animal Influenza and Newcastle disease, OIE Collaborating Centre for Diseases at the Human-Animal Interface. Viale dell'Università 10, 35020, Legnaro, Padova, Italy; email: email: imonne@izsvenezie.it



Recognize the signs of tickborne disease

Understand diagnostic testing and lab findings

Quickly find treatment recommendations

Order or download at www.cdc.gov/pubs

Article DOI: http://dx.doi.org/10.3201/eid2107.150421

Highly Pathogenic Avian Influenza A(H5N1) Virus in Poultry, Nigeria, 2015

Technical Appendix

Technical Appendix Table 1. Amino acid comparison among A/chicken/Nigeria/15VIR339-2/2015, A/Alberta/01/2014 and the 2.3.2.1c vaccine candidate A/duck/Vietnam/NCVD-1584/2012*

Protein	Position	H5N1 Nigeria	H5N1 Alberta	2.3.2.1c vaccine candidate
HA1	68	G	D	D
	189	K	K	R
	221	G	R	G
	235	T	P	P
HA2	48	I	V	V
	68	K	R	R
	183	V	I	I
NA	267	E	G	-
	398	M	I	-
PA	319	D	E	-
	369	V	A	-
	474	S	C	-
NS1	59	S	R	-
	72	G	E	-
	124	L	I	-
	134	D	N	-
	166	S	G	-
NS2	36	E	G	-
PB1	179	I	M	-
	384	S	L	-
	744	I	M	-
	758	Q	*	-
PB1-F2	34	S	N	-
	45	T	I	-
	58	W	*	-
PB2	187	K	R	-
	196	C	S	-
	292	V	I	-
	464	M	L	-
	495	A	V	-
	598	T	M	-
	660	R	K	-
	677	G	E	-

*World Health Organization. Antigenic and genetic characteristics of zoonotic influenza viruses and development of candidate vaccine viruses for pandemic preparedness (http://www.who.int/influenza/vaccines/virus/characteristics_virus_vaccines/en/).

Technical Appendix Table 2. Sequences from GISAID's EpiFlu™ Database on which this research is based*

Segment ID	Segment	Country	Collection date	Isolate name	Originating Lab	Submitting Laboratory	Authors
EPI500778	PB2	Canada	2014-01-03	A/Alberta/01/2014	Provincial Laboratory of Public Health for Southern Alberta	Public Health Agency of Canada (PHAC)	Li, Yan; Bastien, Nathalie; Fonseca, Kevin; Tipples, Graham; Pabbaraju, Kanti; Tellier, Raymond; Wong, Sallene; Tang, Julian W.; Drews, Steven J.
EPI466327	PB2	China	2012-03-24	A/chicken/Jiangsu/CZLJG/2012	Yangzhou University	Chinese Academy of Sciences	Daxin, P
EPI466359	PB2	China	2012-02-12	A/chicken/Jiangsu/DT0112/2012	Yangzhou University	Chinese Academy of Sciences	Daxin, P
EPI466536	PB2	China	2012-09-24	A/chicken/Jiangsu/GY64/2012	Yangzhou University	Chinese Academy of Sciences	Daxin, P
EPI466235	PB2	China	2012-03-24	A/chicken/Jiangsu/WJHDL/2012	Yangzhou University	Chinese Academy of Sciences	Daxin, P
EPI466520	PB2	China	2012-11-09	A/chicken/Jiangsu/YZ4/2012	Yangzhou University	Chinese Academy of Sciences	Daxin, P
EPI466311	PB2	China	2011-05-21	A/chicken/Jiangsu/ZJDT/2011	Yangzhou University	Chinese Academy of Sciences	Daxin, P
EPI425173	PB2	Vietnam	2012-09-01	A/chicken/Vietnam/NCVD-1942/2012	National Centre of Veterinary Diagnostics	Centers for Disease Control and Prevention	
EPI425597	PB2	Vietnam	2012-09-21	A/duck/Vietnam/NCVD-1463/2012	National Centre of Veterinary Diagnostics	Centers for Disease Control and Prevention	
EPI425920	PB2	Vietnam	2012-07-16	A/duck/Vietnam/NCVD-1547/2012	National Centre of Veterinary Diagnostics	Centers for Disease Control and Prevention	
EPI425677	PB2	Vietnam	2012-07-15	A/duck/Vietnam/NCVD-1593/2012	National Centre of Veterinary Diagnostics	Centers for Disease Control and Prevention	
EPI425621	PB2	Vietnam	2012-08-18	A/duck/Vietnam/NCVD-1930/2012	National Centre of Veterinary Diagnostics	Centers for Disease Control and Prevention	
EPI425181	PB2	Vietnam	2012-09-09	A/duck/Vietnam/NCVD-1944/2012	National Centre of Veterinary Diagnostics	Centers for Disease Control and Prevention	
EPI468343	PB2	China	2013-04-12	A/environment/Hangzhou/109-2/2013	Hangzhou Center for Disease Control and Prevention	Hangzhou Center for Disease Control and Prevention	Li, J; Jin, T; Yu, XF; Pu, XY; Pan, JC
EPI423876	PB2	China	2013-02-08	A/Guizhou/1/2013	WHO Chinese National Influenza Center	China National Influenza Centre	
EPI375501	PB2	China	2012-05-28	A/Hong Kong/5923/2012	Public Health Laboratory Services Branch, Centre for Health Protection	Public Health Laboratory Services Branch, Centre for Health Protection	Mak,G.C.; Cheng,P.K.C.; Lo,J.Y.C.
EPI375432	HA	China	2012-05-28	A/Hong Kong/5923/2012	Public Health Laboratory Services Branch,	Public Health Laboratory Services Branch, Centre for	Mak,G.C.; Cheng,P.K.C.; Lo,J.Y.C.

Segment ID	Segment	Country	Collection date	Isolate name	Originating Lab	Submitting Laboratory	Authors
					Centre for Health Protection	Health Protection	
EPI407283	HA	Nepal	2012-10-16	A/chicken/Nepal/PT-16/12	Central Veterinary Laboratory	Animal Health and Veterinary Laboratories Agency (AHVLA)	Puranik, A; Hanna, A; Essen, S; Focosi-Snyman, R; Manvell, R; Bahadur Singh, D; Chapagain, S; Manandhar, S; Bahadur Air, T; Bahadur Kunwar, B; Reid, S
EPI420386	HA	China	2013-02-08	A/Guizhou/1/2013	WHO Chinese National Influenza Center	China National Influenza Centre	
EPI424704	HA	Vietnam	2012-08-20	A/duck/Vietnam/ NCVD-1869/2012	National Centre of Veterinary Diagnostics	Centers for Disease Control and Prevention	
EPI424720	HA	Vietnam	2012-08-23	A/duck/Vietnam/ NCVD-1897/2012	National Centre of Veterinary Diagnostics	Centers for Disease Control and Prevention	
EPI424728	HA	Vietnam	2012-08-27	A/duck/Vietnam/ NCVD-1898/2012	National Centre of Veterinary Diagnostics	Centers for Disease Control and Prevention	
EPI425184	HA	Vietnam	2012-09-09	A/duck/Vietnam/N CVD-1944/2012	National Centre of Veterinary Diagnostics	Centers for Disease Control and Prevention	
EPI425600	HA	Vietnam	2012-09-21	A/duck/Vietnam/ NCVD-1463/2012	National Centre of Veterinary Diagnostics	Centers for Disease Control and Prevention	
EPI442759	HA	Indonesia	2012-06-29	A/Indonesia/ NIHRD12377/2012	National Institute of Health Research and Development	National Institute of Health Research and Development	Pawestri, HA; Ikawati, HD; Setiawaty, V
EPI454493	HA	China	2013-04-12	A/environment/ Hangzhou/109-2/2013	Hangzhou Center for Disease Control and Prevention	Hangzhou Center for Disease Control and Prevention	Li, J; Jin, T; Yu, XF; Pu, XY; Pan, JC
EPI463648	HA	Indonesia	2013-06-18	A/Indonesia/ NIHRD13157/2013	National Institute of Health Research and Development	National Institute of Health Research and Development	HA, Pawestri; AA, Nugraha; V, Setiawaty
EPI475764	HA	Nepal	2013-04-21	A/chicken/Nepal/T-272/13	Central Veterinary Laboratory	Animal Health and Veterinary Laboratories Agency (AHVLA)	Collins, S; Hanna, A; Essen, S; Focosi-Snyman, R; Manvell, R; Jha, VC; Chapagain, S; Koirala, P; Air, TB; Reid, S
EPI448063	HA	Bangladesh	2011-02-15	A/crow/Bangladesh/ 1061/2011	Institute of Epidemiology Disease Control and Research (IEDCR) & Bangladesh National Influenza Centre (NIC)	Centers for Disease Control and Prevention	Gerloff, Nancy; Simpson, Natosha; Poh, Mee;Davis, Todd
EPI353379	HA	Bangladesh	2011-07-17	A/waterfowl/Bangladesh/31935/2011	Institute of Epidemiology Disease Control and Research	Centers for Disease Control and Prevention	Gerloff, Nancy; Simpson, Natosha; Poh, Mee;Davis, Todd

Segment ID	Segment	Country	Collection date	Isolate name	Originating Lab	Submitting Laboratory	Authors
EPI448263	HA	Bangladesh	2012-02-28	A/environment/Bangladesh/1019-G/2012	(IEDCR) & Bangladesh National Influenza Centre (NIC) Institute of Epidemiology Disease Control and Research	Centers for Disease Control and Prevention	Gerloff, Nancy; Simpson, Natosha; Poh, Mee; Davis, Todd
EPI448271	HA	Bangladesh	2012-01-08	A/chicken/Bangladesh/42010/2012	(IEDCR) & Bangladesh National Influenza Centre (NIC) Institute of Epidemiology Disease Control and Research	Centers for Disease Control and Prevention	Gerloff, Nancy; Simpson, Natosha; Poh, Mee; Davis, Todd
EPI448279	HA	Bangladesh	2012-02-20	A/duck/Bangladesh/32077/2012	(IEDCR) & Bangladesh National Influenza Centre (NIC) Institute of Epidemiology Disease Control and Research	Centers for Disease Control and Prevention	Gerloff, Nancy; Simpson, Natosha; Poh, Mee; Davis, Todd
EPI425200	HA	Vietnam	2011-11-22	A/duck/Vietnam/NCVD129-7/2011	(IEDCR) & Bangladesh National Influenza Centre (NIC) National Centre of Veterinary Diagnostics	Centers for Disease Control and Prevention	
EPI425232	HA	Vietnam	2011-12-30	A/duck/Vietnam/NCVD-1160/2011	National Centre of Veterinary Diagnostics	Centers for Disease Control and Prevention	
EPI375432	HA	China	2012-05-28	A/Hong Kong/5923/2012	Public Health Laboratory Services Branch, Centre for Health Protection	Public Health Laboratory Services Branch, Centre for Health Protection	Mak, G.C.; Cheng, P.K.C.; Lo, J.Y.C.
EPI330995	HA	Vietnam	2011-01	A/duck/Vietnam/NCVD-672/2011	National Centre of Veterinary Diagnostics	Centers for Disease Control and Prevention	Davis, Todd; Rivailler, Pierre; Nguyen, Tung
EPI267032	HA	China	2010-06-01	A/Hubei/1/2010		WHO Chinese National Influenza Center	Yu Lan, Wei Wang, Shumei Zou, Zi Li, Leying Wen, Xiaodan Li, Libo Dong, Dexin Li, Yuelong Shu
EPI425312	HA	Vietnam	2012-08-30	A/duck/Vietnam/NCVD-1904/2012	National Centre of Veterinary Diagnostics	Centers for Disease Control and Prevention	
EPI462795	HA	Austria	2013-06-12	A/mynah/Austria-quarantine/13064792-010/13	Institute for Veterinary Disease	Animal Health and Veterinary	Collins, S; Hanna, A; Essen, S; Focosi-Snyman, R; Manvell, R;

Segment ID	Segment	Country	Collection date	Isolate name	Originating Lab	Submitting Laboratory	Authors
					Control Moedling, Austrian Agency for Health and Food Safety	Laboratories Agency (AHVLA)	Wodak, E; Revilla-Fernandez, S; Bago, Z; Schmoll, F; Reid, S
EPI462793	HA	Austria	2013-06-07	A/mynah/Austria-quarantine/13063485-026/13	Institute for Veterinary Disease Control Moedling, Austrian Agency for Health and Food Safety	Animal Health and Veterinary Laboratories Agency (AHVLA)	Collins, S; Hanna, A; Essen, S; Focosi-Snyman, R; Manvell, R; Wodak, E; Revilla-Fernandez, S; Bag?, Z; Schmoll, F; Reid, S
EPI462791	HA	Austria	2013-06-07	A/mynah/Austria-quarantine/13063485-025/13	Institute for Veterinary Disease Control Moedling, Austrian Agency for Health and Food Safety	Animal Health and Veterinary Laboratories Agency (AHVLA)	Collins, S; Hanna, A; Essen, S; Focosi-Snyman, R; Manvell, R; Wodak, E; Revilla-Fernandez, S; Bag?, Z; Schmoll, F; Reid, S
EPI464565	HA	China	2013-04-16	A/Environment/Huzhou/C291-7/2013	Huzhou Center for Disease Control and Prevention	Jiangsu University	Han, J. ; Wang, L. ; Liu, J. ; Jin, M. ; Zhang, C. ; Lan, K
EPI464564	HA	China	2013-04-16	A/Environment/Huzhou/C291-6/2013	Huzhou Center for Disease Control and Prevention	Jiangsu University	Han, J. ; Wang, L. ; Liu, J. ; Jin, M. ; Zhang, C. ; Lan, K
EPI464558	HA	China	2013-04-16	A/Environment/Huzhou/C291-10/2013	Huzhou Center for Disease Control and Prevention	Jiangsu University	Han, J. ; Wang, L. ; Liu, J. ; Jin, M. ; Zhang, C. ; Lan, K
EPI500771	HA	Canada	2014-01-03	A/Alberta/01/2014	Provincial Laboratory of Public Health for Southern Alberta	Public Health Agency of Canada (PHAC)	Li, Yan; Bastien, Nathalie; Fonseca, Kevin; Tipples, Graham; Pabbaraju, Kanti; Tellier, Raymond; Wong, Sallene; Tang, Julian W.; Drews, Steven J.

*We gratefully acknowledge the authors, originating and submitting laboratories of sequences from GISAID's EpiFlu Database, on which this research is based All Submitters of data may be contacted directly via the GISAID website www.gisaid.org