

# Characterization of Highly Pathogenic Avian Influenza Virus A(H5N6), Japan, November 2016

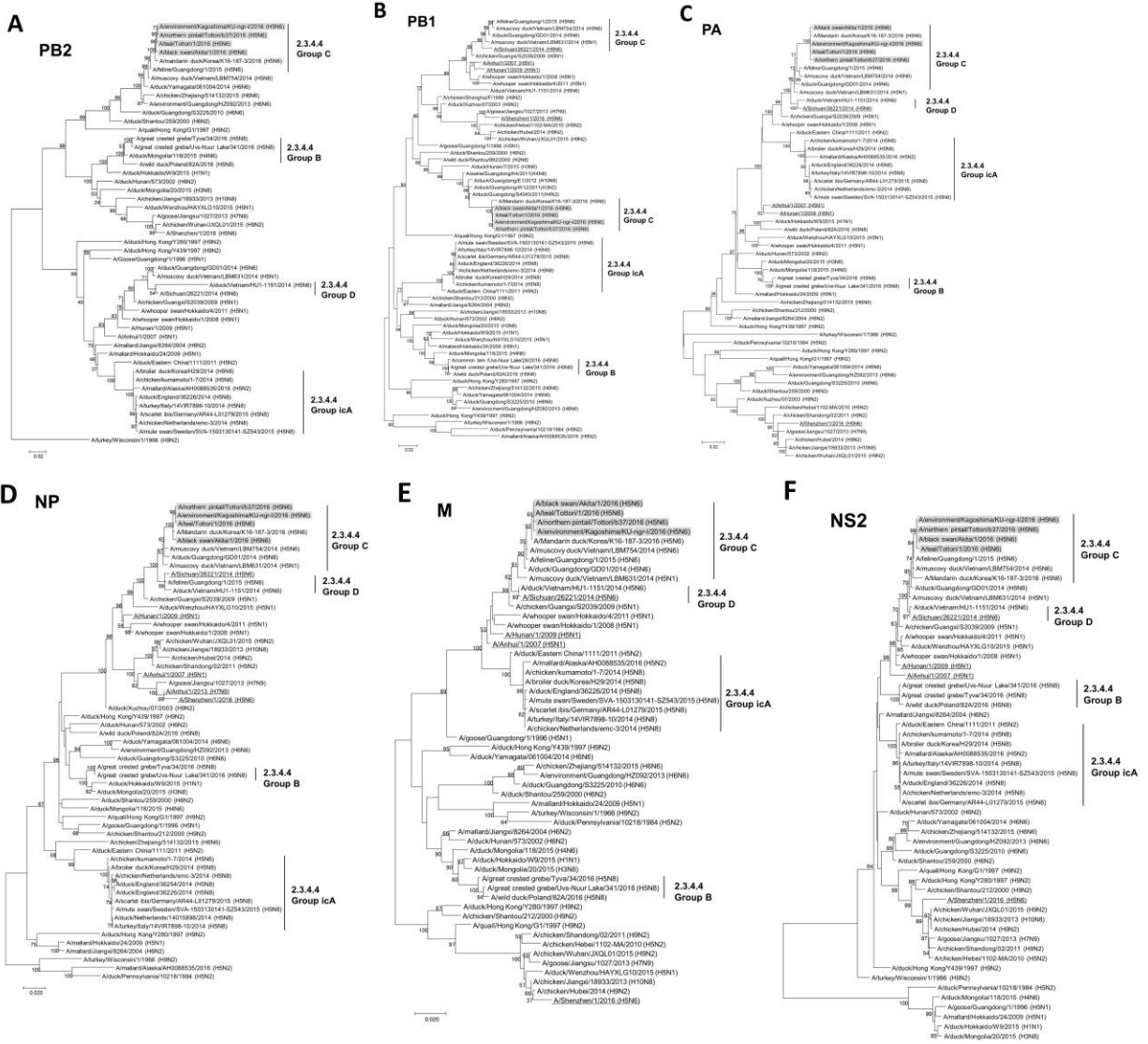
## Technical Appendix

**Technical Appendix Table.** Nucleotide and amino acid mutations in hemagglutinin genes of highly pathogenic avian influenza virus A(H5N6) isolates\*

Virus	Nucleotide position of hemagglutinin gene														Mutation
	31	33	57	202	208	339	516	520	608	652	658	1125	1527		
A/northern pintail/Tottori/b37/2016 (H5N6)	G	T	T	T	G	C	T	A	C	A	G	G	G	–	
A/environment/Kagoshima/KU-ngr-I/2016 (H5N6)	T	.	.	.	.	.	C	.	.	T	T	A	.	5	
A/black swan/Akita/1/2016 (H5N6)	.	C	C	C	.	.	C	.	T	T	T	.	A	8	
A/teal/Tottori/1/2016 (H5N6)	.	.	.	.	.	T	C	C	.	T	T	.	.	5	
A/crane/Kagoshima/KU-4/2016 (H5N6)	.	.	.	.	A	.	.	.	.	T	T	.	.	3	
Virus	Amino acid position of hemagglutinin (H3 numbering)														Mutation
	(11)†	–	–	63	–	–	163	192	207	209	–	–	–	–	
A/northern pintail/Tottori/b37/2016 (H5N6)	V	I	L	D	D	D	T	T	T	V	G	E	–	–	
A/environment/Kagoshima/KU-ngr-I/2016 (H5N6)	F	.	.	.	.	.	.	.	S	L	.	.	3	–	
A/black swan/Akita/1/2016 (H5N6)	.	.	.	.	.	.	.	I	S	L	.	.	3	–	
A/teal/Tottori/1/2016 (H5N6)	.	.	.	.	.	.	P	.	S	L	.	.	3	–	
A/crane/Kagoshima/KU-4/2016 (H5N6)	.	.	.	N	.	.	.	.	S	L	.	.	3	–	
Character of the location	Signal peptide			Bottom of head			Surface of head		190-helix		Trimeric interface				

\*Dot ( . ) indicates this nucleotide/amino acid is same as that of A/northern pintail/Tottori/b37/2016 (H5N6). Dash indicates no amino acid change in the position.

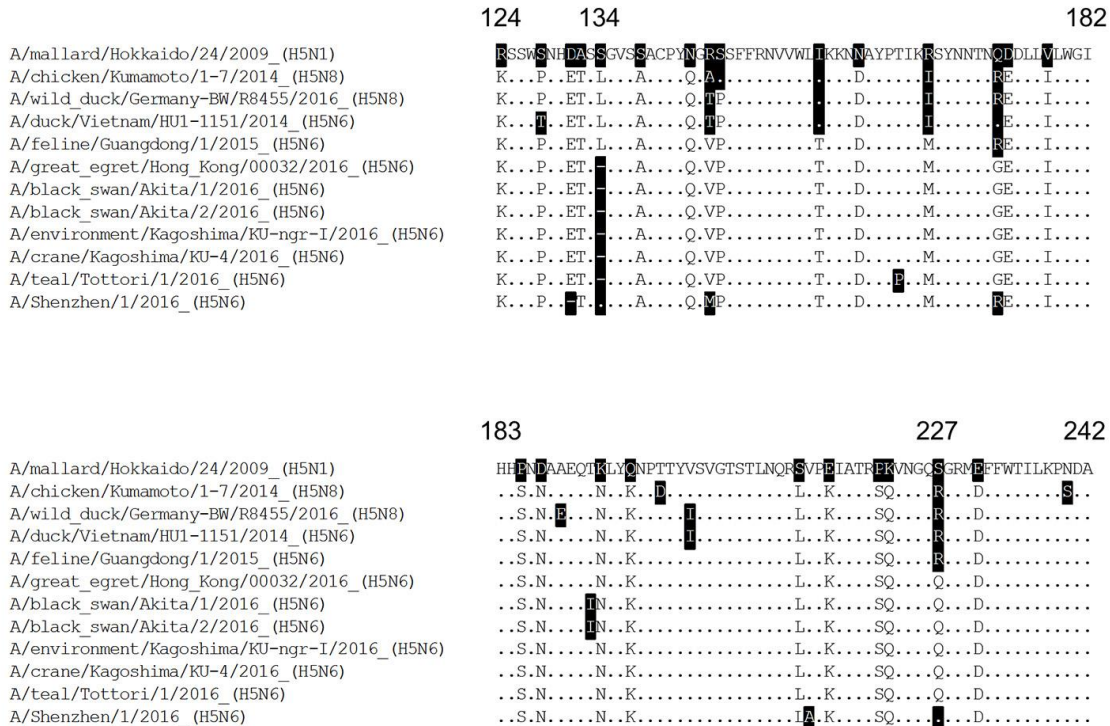
†Numbering from the initiating methionine in H5 hemagglutinin.



**Technical Appendix Figure 1.** Phylogenetic trees of the PB2 (A), PB1 (B), PA (C), NP (D), M (E), and NS (F) gene segments of highly pathogenic avian influenza virus A(H5N6) isolated in Japan during November 2016 and reference strains. Sequences were analyzed by the maximum-likelihood method along with the corresponding genes of reference strains by using MEGA 7.0 software (<http://www.megasoftware.net/>). Horizontal distances are proportional to the minimum number of nucleotide differences required to join nodes and sequences. Digits at the nodes indicate the probability of confidence levels in a bootstrap analysis with 1,000 replications. The viruses isolated in this study are highlighted in gray. The viruses

isolated in humans are underlined>. Scale bars indicate nucleotide substitutions per site. M, matrix; NP, nucleoprotein; NS, nonstructura; PA, polymerase acidic; PB1, polymerase basic 1; PB2, polymerase basic

2.



**Technical Appendix Figure 2.** Comparison of amino acid position 134 and 227 (H3 numbering) in H5 hemagglutinin (HA). A leucine residue at position 134 was deleted in highly pathogenic avian influenza virus A(H5N6) isolated in Japan during November 2016, compared with the closest relative A/feline/Guangdong/1/2015 (H5N6). The amino acid sequence QQG at positions 226–228, which are located at the receptor-binding site in the HA protein, although the corresponding amino acid sequences of the previous H5 viruses are QSG or QRG.

	51	58	68	100
A/duck/Mongolia/118/2015_ (H4N6)	NETNS	ITTTIINNNTQNNFTNITNIIVTK	EEERTFTNLTKPLCEVNSWHIL	
A/duck/Vietnam/HU1-1151/2014_ (H5N6)	..S	.....HP	.....N..GHL	.....
A/duck/Guangdong/S1419/2011_ (H6N6)	.....P	.....	.....N..N..C	.....
A/black_swan/Akita/1/2016_ (H5N6)	.....P	.....	.....MN.N.....K	.....
A/black_swan/Akita/2/2016_ (H5N6)	.....P	.....	.....MN.N.....K	.....
A/crane/Kagoshima/KU-4/2016_ (H5N6)	.....P	.....	.....MN.N.....K	.....
A/environment/Kagoshima/KU-ngr-I/2016_ (H5N6)	.....P	.....	.....MN.N.....K	.....
A/teal/Tottori/1/2016_ (H5N6)	.....P	.....	.....MN.N.....K	.....

**Technical Appendix Figure 3.** Comparison of amino acid sequence of neuraminidase (NA) stalk. Eleven amino acid deletions (58–68) in the stalk region of the NA protein compared with *A/duck/Vietnam/HU1-1151/2014* (H5N6), a representative virus strain of an N6 NA gene-based group D.