

# Emerging Novel Reassortant Influenza A(H5N6) Viruses in Poultry and Humans, China, 2021

## Appendix

**Appendix Table 1.** Percentage homology of influenza viruses closely related to A/chicken/Chongqing/H1/2021(H5N6).

Segment	Virus	Nucleotide homology (%)
PB2	A/Sichuan/06681/2021(H5N6)	99.4
	A/Environment/Guangxi/28753/2014(H3N2)	97.3
PB1	A/Sichuan/06681/2021(A/H5N6)	98.5
	A/Environment/Guangdong/C18277136/2018(H5N6)	97.8
PA	A/Sichuan/06681/2021(A/H5N6)	99.7
	A/Environment/Guangdong/C17277346/2017(H5N6)	97.6
HA	A/Chongqing/02/2021(H5N6)	99.9
	A/Sichuan/06689/2021(H5N6)	99.8
	A/chicken/Korea/H544/2020(H5N8)	99.6
	A/duck/Korea/H538/2020(H5N8)	99.6
	A/duck/Korea/H499/2020(H5N8)	99.6
NP	A/Sichuan/06681/2021(H5N6)	99.6
	A/Anhui/2021–00011/2020(H5N6)	99.3
	A/Muscovy duck/China/FJFZ21/2020(H5N6)	99.3
NA	A/Chongqing/02/2021(H5N6)	97.5
	A/Sichuan/06689/2021(H5N6)	97.5
	A/Sichuan/06681/2021(H5N6)	97.4
	A/Anhui/2021–00011/2020(H5N6)	97.1
	A/Chongqing/00013/2021(H5N6)	97.0
M	A/Muscovy duck/China/FJFZ21/2020(H5N6)	96.6
	A/Cygnus columbianus/Hubei/49/2020(H5N8)	99.8
	A/wild bird/Korea/H496–3/2020(H5N8)	99.8
	A/chicken/Kostroma/304–10/2020 (H5N8)	99.8
	A/mallard/Kagoshima/KU-d89/2021 (H5N8)	99.8
	A/duck/Northern China/LSP/2020(H5N8)	99.8
	A/turkey/Omsk/0003/2020(H5N8)	99.8

Segment	Virus	Nucleotide homology (%)
NS	A/Sichuan/06681/2021(H5N6)	99.6
	A/Environment/Jiangxi/47054/2016(H4N2)	98.0
	A/Environment/Hunan/34019/2017(H3N2)	97.9

**Appendix Table 2.** The differences in antigenicity sites on HA between A/chicken/Chongqing/H1/2021(H5N6) and Re-11 vaccine strain

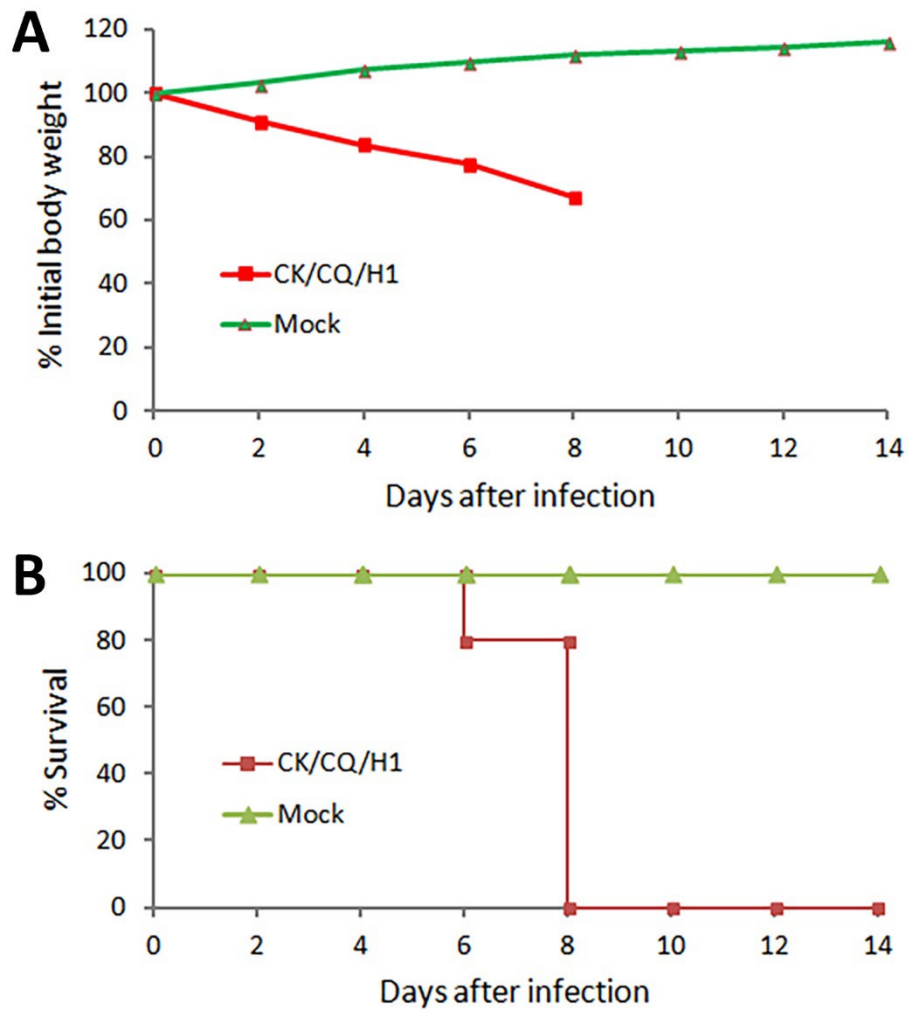
Strains	site A				site B				site C				site D				site E				Other antigenicity associated sites in HA1 (except regions A, B, C, D, E)							
	130	133	144	145	128	155	187	192	276	173	227	238	240	260	262	93	101	119	120	125	166	202	271	272	285			
Re-11	/	S	V	A	S	T	S	T	N	K	H	R	N	I	T	S	N	T	Q	R	M	V	E	I	I			
CK/CQ/H1	E	L	A	P	P	I	N	I	H	R	R	K	D	V	K	A	S	I	L	S	I	I	G	V	V			

**Appendix Table 3.** Protection efficacy of H5 Re-11 vaccine against A/chicken/Chongqing/H1/2021(H5N6) in chickens\*

Group	Vaccine	Challenge test results, by swab type, no. positive birds/no.tested (mean titer±SD)†						No.surviving birds/total no.
		HI titer±SD, log2		3 dpi		5 dpi		
		Re-11	Isolate	Tracheal	Cloacal	Tracheal	Cloacal	
CK/CQ/H1	Re-11	9.3 ± 0.38	4.2 ± 0.32	2/10 (3.67 ± 1.18)	2/10 (3.81 ± 1.32)	2/10 (3.79 ± 1.26)	2/10 (3.92 ± 1.42)	10/10
Mock		ND	ND	4/4 (4.10 ± 1.23)	4/4 (4.63 ± 1.76)	ND	ND	0/10

\*Chickens were immunized with the Re-11 vaccine, and HI antibody titers were determined on day 21 post-vaccination. HI, hemagglutination inhibition assay; dpi, days post-infection; ND, not done.

†Chickens were challenged with 10<sup>6</sup> 50% egg infectious dose (EID<sub>50</sub>) of A/chicken/Chongqing/H1/2021(H5N6) virus; virus titers are expressed as log<sub>10</sub> EID<sub>50</sub>/0.1 ml.



**Appendix Figure.** Virulence of the novel H5N6 virus in Balb/c mice intranasally inoculated with a  $10^6$  EID<sub>50</sub> virus. Bodyweight changes (A) and mortality (B) of BALB/c mice were monitored daily for 14 days.