

Supplementary Materials for

Comparative Pathogenicity and Transmissibility of the H7N9 Highly Pathogenic Avian Influenza Virus and the H7N9 Low Pathogenic Avian Influenza Virus in Chickens

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This PDF file includes:

Tables S1 to S11

Table S1. Different amino acids in the HA gene of the chicken LPAIV H7N9 (A46), the human LPAIV H7N9 and chicken Tennessee/H7N9.

Virus	Amino acid sequence															
	7-13	15-18	29	42	53-54	57-58	61-62	64-67	78-79	91	102	111	115	122	130	150-151
A/chicken/Guangdong/A46/2013	VFALIAI	PTNA	S	V	RT	PR	SK	KRTV	IT	S	S	V	A	E	A	SG
A/Anhui/1/2013	VFALIAI	PTNA	S	V	RT	PR	SK	KRTV	IT	S	S	V	A	E	A	SG
A/chicken/Tennessee/17-007431-3/2017	ALIACML	GAKG	A	I	TA	KK	TQ	RKPT	LI	D	T	T	S	G	S	LD

Virus	Amino acid sequence															
	165-166	178	180	182-183	187	191	195	197-198	211	217	223	235	245	247	255	272
A/chicken/Guangdong/A46/2013	TD	K	T	KS	V	I	V	TA	V	N	V	L	M	N	S	K
A/Anhui/1/2013	TD	K	T	KS	V	I	V	TA	V	N	V	L	M	N	S	K
A/chicken/Tennessee/17-007431-3/2017	SN	R	P	SK	I	V	G	AD	I	K	T	Q	L	D	T	E

Virus	Amino acid sequence															
	274	276	279	281-282	284-285	287	291	293	298	300	307-308	310	321	330	335	338
A/chicken/Guangdong/A46/2013	M	I	G	QV	AN	E	Y	S	I	N	DS	S	R	K	I	G
A/Anhui/1/2013	M	I	G	QV	AN	E	Y	S	I	N	DS	A	R	K	I	G
A/chicken/Tennessee/17-007431-3/2017	L	V	D	PL	SS	K	F	N	V	S	NP	T	T	R	N	T

Virus	Amino acid sequence															
	373	394	396	410	412	414	426-427	447	450	454-455	462-463	486	489	499-500	503	505-506
A/chicken/Guangdong/A46/2013	N	V	E	N	V	K	FI	N	G	KD	KR	D	A	SK	E	AM
A/Anhui/1/2013	T	L	E	N	V	K	SI	I	A	MD	KR	D	A	SK	E	AM
A/chicken/Tennessee/17-007431-3/2017	T	L	D	S	I	Q	SM	I	A	MN	RK	Q	E	TQ	A	SL

Virus	Amino acid sequence				
	517	524	536	541	549
A/chicken/Guangdong/A46/2013	I	V	I	V	V
A/Anhui/1/2013	L	V	I	V	V
A/chicken/Tennessee/17-007431-3/2017	L	I	L	A	I

Table S2. Different amino acids in the NA gene of the chicken LPAIV H7N9 (A46), the human LPAIV H7N9 and chicken Tennessee/H7N9.

Virus	Amino acid sequence															
	16	19	40	45	58	51	53	69-73	80-82	84	112	175	183	189-190	207	215
A/chicken/Guangdong/A46/2013	I	A	G	H	P	T	T	XXXXX	RTS	N	S	V	T	KS	V	A
A/Anhui/1/2013	I	A	G	H	P	T	T	XXXXX	RTS	N	S	V	T	KS	V	A
A/chicken/Tennessee/17-007431-3/2017	V	T	N	N	S	S	A	QISNT	KAN	E	N	I	A	RA	I	T

Virus	Amino acid sequence															
	222	238	247	253	256	269	285-287	334-335	343	354	359	361	371	373	375	392
A/chicken/Guangdong/A46/2013	Q	V	P	D	I	S	ERT	NI	S	L	G	N	L	F	F	L
A/Anhui/1/2013	R	V	S	D	I	S	ERT	NI	P	S	A	T	S	S	Y	I
A/chicken/Tennessee/17-007431-3/2017	R	I	S	E	V	P	VQA	AV	P	S	G	T	S	S	Y	T

Virus	Amino acid sequence											
	401	406	409	413	415	418	422	428	446-447	450	459	463
A/chicken/Guangdong/A46/2013	G	F	F	F	G	E	G	V	GL	F	K	W
A/Anhui/1/2013	A	Y	S	Y	A	D	A	L	VS	S	N	G
A/chicken/Tennessee/17-007431-3/2017	T	Y	S	Y	A	E	A	L	VS	S	N	G

Table S3. Different amino acids in the PB1 genes of the chicken LPAIV H7N9 (A46), the human LPAIV H7N9 and chicken Tennessee/H7N9.

Virus	Amino acid sequence															
	13-14	48	59	113	172	257	368	375	383	387	394	430	525	573	628	638
A/chicken/Guangdong/A46/2013	PV	K	T	I	D	A	V	N	K	E	P	K	V	A	M	D
A/Anhui/1/2013	PV	K	T	I	D	A	V	N	K	E	P	K	V	A	M	D
A/chicken/Tennessee/17-007431-3/2017	KA	Q	S	V	E	T	I	S	E	K	H	R	I	S	L	E

Table S4. Different amino acids in the PB2 genes of the chicken LPAIV H7N9 (A46), the human LPAIV H7N9 and chicken Tennessee/H7N9.

Virus	Amino acid sequence														
	8	191	292	389	392	473	477	508	559	566	598	627	648	676	
A/chicken/Guangdong/A46/2013	R	K	V	K	Q	V	V	R	N	Q	V	E	V	M	
A/Anhui/1/2013	R	K	V	K	Q	M	V	R	N	Q	V	K	V	M	
A/chicken/Tennessee/17-007431-3/2017	K	E	I	R	H	M	I	Q	T	H	T	E	L	T	

Table S5. Different amino acids in the NP genes of the chicken LPAIV H7N9 (A46), the human LPAIV H7N9 and chicken Tennessee/H7N9.

Virus	Amino acid sequence													
	16	34	52	77	105	136	186	352	373	377	406	450	478	482
A/chicken/Guangdong/A46/2013	G	S	N	R	V	L	I	M	A	N	V	S	S	N
A/Anhui/1/2013	G	S	N	R	V	L	I	M	A	N	V	S	S	N
A/chicken/Tennessee/17-007431-3/2017	S	G	Y	K	M	M	V	V	T	S	I	N	F	S

Table S6. Different amino acids in the NS1 genes of the chicken LPAIV H7N9 (A46), the human LPAIV H7N9 and chicken Tennessee/H7N9.

Virus	Amino acid sequence															
	27	47	55	60	63-64	77	80	87	103	106	111-112	124	139	143	172	179-180
A/chicken/Guangdong/A46/2013	M	S	R	E	HM	F	S	p	L	I	IT	V	N	A	K	EI
A/Anhui/1/2013	M	S	R	E	HI	F	S	p	L	I	IT	V	N	A	K	EI
A/chicken/Tennessee/17-007431-3/2017	L	G	E	A	QI	L	T	S	F	M	VA	W	D	T	E	GV

Virus	Amino acid sequence															
	185	193	207	212	215-216	218	221	223	226-228	231	236	241	244	246-249	251	253
A/chicken/Guangdong/A46/2013	F	R	D	S	ST	-	E	E	VKP	R	V	T	I	NYGE	L	A
A/Anhui/1/2013	F	R	D	S	ST	-	E	E	VKP	R	V	T	I	NYGE	L	A
A/chicken/Tennessee/17-007431-3/2017	L	Q	N	P	PP	Q	K	A	IES	-	A	A	T	DHRK	F	T

Virus	Amino acid sequence	
	256	275
A/chicken/Guangdong/A46/2013	F	V
A/Anhui/1/2013	F	V
A/chicken/Tennessee/17-007431-3/2017	I	I

Table S7. Different amino acids in the M genes of the chicken LPAIV H7N9 (A46), the human LPAIV H7N9 and chicken Tennessee/H7N9.

Virus	Amino acid sequence															
	15	37	46	95	101	107	140	142	144	157	166	219	224	227	234	242
<i>A/chicken/Guangdong/A46/2013</i>	I	A	I	K	K	M	A	G	L	A	A	V	N	T	I	N
<i>A/Anhui/1/2013</i>	I	A	I	K	K	M	A	G	L	A	A	V	N	T	I	N
<i>A/chicken/Tennessee/17-007431-3/2017</i>	V	T	L	R	R	T	T	V	F	S	V	T	S	A	L	K

Virus	Amino acid sequence									
	248	254	256-257	259-262	265	284	297	300	306	326
<i>A/chicken/Guangdong/A46/2013</i>	L	A	SR	S - HY	I	F	N	V	S	E
<i>A/Anhui/1/2013</i>	L	A	SR	S - HY	I	F	N	V	S	E
<i>A/chicken/Tennessee/17-007431-3/2017</i>	M	S	RY	RKYH	L	P	S	I	P	G

Table S8. Our study on the pathogenicity and transmissibility of AIVs.

Chickens' age	Infected chickens' inoculated doses	Infected chickens' inoculation route	Infection route of uninoculated chicken	H7N9 virus	Pathogenicity					Transmissibility		
					Mortality	Virus replication			Mortality	Virus shedding		
						Specimens	Days	Titer		Routs	Days	Titer
Three to four-week-old	6 log ₁₀ EID ₅₀	Ocular-nasal route	Direct contact	<i>A/chicken/Guangdong/A46/2013</i> (LPAIV)	20%	Kidneys, livers, lungs, trachea & pancreas	1dp	1.58-3.17 log ₁₀ EID ₅₀ /mL	1/5	Oropharynx	3-7 dpi	1.75-2.5 log ₁₀ EID ₅₀ /mL
						Brain, kidneys, livers, lungs, spleen, trachea, ileum, bursa of fabricius & pancreas	3dpi	1.75-4.92 log ₁₀ EID ₅₀ /mL				
						Oropharynx	1-5 dpi	2.06-2.33 log ₁₀ EID ₅₀ /mL				
				<i>A/chicken/Guangdong/Q29/2017</i> (HPAIV)	100%	Cloacae	1-5 dpi	2.13-3.75 log ₁₀ EID ₅₀ /mL	Cloacae	1.75-3.5 log ₁₀ EID ₅₀ /mL		
						Brain, kidneys, livers, lungs, trachea, ileum & bursa of fabricius	1dpi	1.50-6.58 log ₁₀ EID ₅₀ /mL	Oropharynx	3 dpi	2.56 log ₁₀ EID ₅₀ /mL	
						Brain, kidneys, livers, lungs, spleen, trachea, ileum, bursa of fabricius & pancreas	3dpi	3.75-8.25 log ₁₀ EID ₅₀ /mL				
Oropharynx	1-3 dpi	2.38-2.88 log ₁₀ EID ₅₀ /mL	Cloacae	5.38 log ₁₀ EID ₅₀ /mL								
Cloacae	1-3 dpi	2.21-3.13 log ₁₀ EID ₅₀ /mL										

Table S9. Studies on the pathogenicity of H7N9 influenza viruses.

Study	H7N9 virus	Doses	Inoculation route	Chickens' age	Pathogenicity			
					Mortality	Virus replication		
						Specimens	Days	Titer
Ku et al.	A/Anhui/1/2013 (LPAIV)	9.6 log ₁₀ EID ₅₀	Intranasal and intratracheal infection	4-week-old	0	Lungs	3 & 5 dpi	4 - 5 log ₁₀ EID ₅₀ /mL
						Oropharynx	2 days p.i.	2.5 log ₁₀ EID ₅₀ /mL
						Cloacae	1 day p.i.	3.0 log ₁₀ EID ₅₀ /mL
Pantin-Jackwood et al.	A/Anhui/1/2013 (LPAIV)	6.0 log ₁₀ EID ₅₀	Intranasal infection	9-week-old	0	Spleen & intestine	3 dpi	Low
						Kidneys & lungs	3 dpi	0
						Oropharynx	2 & 4 dpi	6.2 log ₁₀ EID ₅₀ /mL
Chowdhury et al.	A/chicken/Tennessee/17-007431-3/2017 (LPAIV)	10 ⁷ pfu/mL	Intranasal infection	4-week-old	0	Cloacae	2 & 4 dpi	3 to 4 units lower than those in oropharynx
						Brain, lungs & spleen	3 days p.i	0
						Oropharynx	3 dpi	Low
Zhang et al.	A/chicken/Shanghai/S1053/2013 (LPAIV)	0.1 mL of a 1:10 dilution	Intravenous infection	6-week-old	0	The IVPI value is 0		
						Tracheal swabs	3 & 5 dpi	1.7-3.0 log ₁₀ EID ₅₀
						Cloacal swabs	5, 7 & 9 dpi	1.7-4.0 log ₁₀ EID ₅₀
Shi et al.	A/chicken/Guangdong/SD008/2017 (HPAIV)	6 log ₁₀ EID ₅₀	Intranasal infection	6-week-old	100%	Tracheal and cloacal swabs, organs, brains, lungs, kidneys, spleens, pancreas, heart, liver, and cecum	3 dpi	About 5.2 to 7.3 log ₁₀ EID ₅₀ /g
						Oropharynx & cloacae	Not stated	About 4.2 to 5.2 log ₁₀ EID ₅₀ /g
Wang et al.	A/chicken/Guangdong/Q1/2016 (HPAIV)	6 log ₁₀ EID ₅₀	Intranasal infection	6-week-old	100%	Brain, kidneys, livers, lungs, spleen & trachea	3 dpi	4.92-7.42 log ₁₀ EID ₅₀ /mL
	A/chicken/Guangdong/Q26/2017 (HPAIV)					Brain, kidneys, livers, lungs, spleen & trachea	3 dpi	6.75-8.42 log ₁₀ EID ₅₀ /mL
	A/chicken/Guangdong/Q39/2017 (HPAIV)					Brain, kidneys, livers, lungs, spleen & trachea	3 dpi	6.33-7.83 log ₁₀ EID ₅₀ /mL

Table S10. Studies on the pathogenicity of LPAIVs in brain.

Article title	Author	LPAIV	Experimental method	Results	Can it be detected in brain?
Systemic distribution of different low pathogenic avian influenza (LPAI) viruses in chicken	Jacob Post, Eveline D de Geus et al.	H5N2, H7N1, H7N7 & H9N2	1-day-old chickens were inoculated with 0.2 mL (2×10^5 EID ₅₀) of the LPAIVs and their lungs, brain, ileums, blood, livers, kidneys, heart and spleen were collected for RNA extraction to test the systemic distribution.	Viral RNA was found by PCR in lungs, brain, intestine, peripheral blood mononuclear cells, livers, kidneys, heart and spleen from chickens infected with chicken isolated LPAIV H5N2, H7N1, H7N7 or H9N2 at 2 or 4 dpi. But H7N7 virus could not be isolated from brain, which was in agreement with the data from the PCR at 4 dpi.	H5N2, H7N1 & H9N2 can be detected.
H7N9 Avian Influenza Virus Is Efficiently Transmissible and Induces an Antibody Response in Chickens	Peirong Jiao, Yafen Song et al.	H7N9	6-week-old chickens were intranasally inoculated with $8 \log_{10}$ EID ₅₀ of A/chicken/Guangdong/110/2013 (CK110) and A/chicken/Guangdong/134/2013 (CK134) in a 0.2 mL volume, and their brain, spleen, kidneys, lungs, livers, intestine, heart, trachea, and pancreas were collected to detect the virus.	None of the chickens showed any signs of disease, and all survived until the end of the experiment. Viruses can be detected in most organs of the inoculated chickens including brains at 3 and 5 dpi. In the brain, CK110 and CK134 replicated to mean titers of 1.58–2.08 \log_{10} EID ₅₀ /mL at 3 dpi and 1.83–1.92 \log_{10} EID ₅₀ /mL at 5 dpi.	yes

Table S11. Studies on the transmissibility of H7N9 influenza viruses.

Study	H7N9 virus	Infected chickens' inoculated doses	Infected chickens' inoculation route	Infection route of uninoculated chicken	Chickens' age	Transmissibility		
						Mortality	Virus shedding	
							Tested routes	Can it be detected?
Kalthoff et al.	A/Anhui/1/2013 ((LPAIV))	10^6 TCID ₅₀	Oculonasal infection	Direct contact	8-week-old	0	Respiratory route	Yes
B. Vildaña et al.	A/Anhui/1/2013 (LPAIV)	$5 \log_{10}$ EID ₅₀	Intranasal infection	Direct contact	2-week-old	0	Oropharyngeal & cloacal swabs	No
Jiao et al.	A/chicken/Guangdong/110/2013 (LPAIV)	$8 \log_{10}$ EID ₅₀	Intranasal infection	Direct contact	6-week-old	0	Oropharyngeal & cloacal swabs	Yes
	A/chicken/Guangdong/134/2013 (LPAIV)					0	Oropharyngeal & cloacal swabs	Yes
Wang et al.	A/chicken/Guangdong/Q1/2016 (HPAIV)	$6 \log_{10}$ EID ₅₀	Intranasal infection	Direct contact	6-week-old	100%		Yes
	A/chicken/Guangdong/Q26/2017 (HPAIV)					100%	Lungs	Yes
	A/chicken/Guangdong/Q39/2017 (HPAIV)					100%		Yes