

**Supplementary table 1** Blast analysis of genome sequences of these isolated viruses.

<b>Genes</b>	<b>Virus Strains</b>	<b>Identity (%)</b>
PB2	A/Quail/Guangxi/GX-2/2017 (H5N6)	98.50%
PB1	A/Environment/Guangxi/42586/2015 (H5N6)	98.59%
PA	A/Ostrich/Guangxi/GX-1/2017 (H5N6)	98.84%
HA	A/duck/Guangdong/01.01 SZSGXJK005-G /2016 (H5N6)	98.30%
NP	A/duck/Guangdong/01.01 SZSGXJK005-G /2016 (H5N6)	99.00%
NA	A/duck/Guangdong/01.01 SZSGXJK007-G/2016 (H5N6)	98.41%
M	A/duck/Guangdong/03.27 DGQTSJ133-O/2015 (Mixed)	99.00%
NS	A/duck/Guangdong/01.01 SZSGXJK006-G/2016 (H5N6)	98.40%

**Supplementary table 2** The reference ancestor strains and the estimate divergence time of each gene segment from its tMRCA.

Genes	Reference ancestor strains	Estimated divergence time	95% HPD	
			Upper	Lower
PB2	A/duck/Hunan/04.22 LDDX037-P/2015 (H5N6)	Jun 2014	Feb 2014	Sep 2014
	A/duck/Guangdong/04.23 DGQTXC229-O/2015 (H5N6)			
PB1	A/Environment/Guangxi/44389/2015 (H5N6)	May 2014	Apr 2013	Dec 2014
	A/chicken/Japan/AQ-HE144/2015 (H5N6)			
PA	A/Goose/Qingyuan/16875/2016 (H5N6)	Aug 2015	May 2015	Nov 2015
HA	A/great egret/Hong Kong/00032/2016 (H5N6)	Sep 2015	May 2015	Dec 2015
NP	A/Goose/Qingyuan/16875/2016 (H5N6)	Sep 2015	May 2015	Oct 2015
NA	A/great egret/Hong Kong/00032/2016 (H5N6)	Sep 2015	Jul 2015	Dec 2015
M	A/duck/Guangdong/01.01 SZSGXJK006-G/2016 (H5N6)	Dec 2015	Sep 2015	Feb 2016
NS	A/Muscovy duck/Vietnam/HU7-17/2017 (H5N6)	Jun 2016	Aug 2015	Dec 2016

**Supplementary table 3** Estimated evolutionary rates of each gene segments of H5N6 viruses by Bayesian analysis.

Genes	Substitution rate and 95% HPD ( $10^{-3}$ substitution/site/year)		
	Mean	Lower	Upper
PA	3.55	2.51	4.73
PB1	3.08	2.39	3.68
PB2	2.65	2.05	3.24
HA	4.24	3.14	5.32
NP	2.50	1.81	3.19
NA	4.40	3.47	5.25
M	2.09	1.41	2.71
NS	3.66	2.76	4.52

**Supplementary table 4** Molecular characteristics of isolated virus were analyzed using Influenza Research Database (IRD, <https://www.fludb.org/>)

Viruses <sup>a</sup>	H5 clade	HA <sup>b</sup>			NA	NS1	M2					PB2				PB1			PA	
		Cleavage site	RBS <sup>c</sup>		Stalk deletion	5-aa deletion	Drug-resistance associated aa					Air-borne transmission				aa residues		PB1-F2 truncation	aa residues	
			222	224	58 - 68	80 - 84	26	27	30	31	34	271	591	627	701	99	368		409	672
JS01	2.3.4.4	RERRRKRR	Q	G	Yes	Yes	L	V	A	S	G	T	Q	E	D	H	I	1-39	S	L
JS02	2.3.4.4	RERRRKRR	Q	G	Yes	Yes	L	V	A	S	G	T	Q	E	D	H	I	1-39	S	L
K10	2.3.4.4	RERRRKRR	Q	G	Yes	Yes	L	V	A	S	G	T	Q	E	D	H	I	1-39	S	L
CNIC-21099 <sup>d</sup>	2.3.4.4	REKRRKR	Q	G	Yes	No	L	V	A	S	G	T	Q	E	D	H	I	12-90	S	L
IDCDC-RG43A <sup>d</sup>	2.3.4.4	RERRRKRR	Q	G	-	No	L	V	A	N	G	T	Q	E	D	H	I	1-39	S	F

<sup>a</sup> Abbreviations: CNIC-21099 represents A/Fujian-Sanyuan/21099/2017 (H5N6), IDCDC-RG43A represents A/gyrfalcon/Washington/41088-6/2014 (H5N8).

<sup>b</sup> H5 numbering

<sup>c</sup> RBS: Receptor binding site

<sup>d</sup> Reference viruses were selected based on candidate vaccine viruses recommended by CDC or CCDC

**Supplementary table 5** Dataset size, nucleotide length, best-fit model, and MCMC chain length for each gene segment.

Datasets	Sequence numbers	Nucleotide length (nt)	Substitution models <sup>a</sup>	Relax-clock models <sup>b</sup>	Tree models	MCMC chain length <sup>c</sup>
PB2	180	2280	GTR+G+I	UCED	Bayesian SkyGrid	200
PB1	193	2274	GTR+G+I	UCED	Bayesian SkyGrid	200
PA	192	2151	GTR+G+I	UCED	Bayesian SkyGrid	200
HA	183	1710	GTR+G	UCED	Bayesian SkyGrid	200
NP	164	1496	GTR+G	UCED	Exponential Growth	200
NA	183	1446	GTR+G+I	UCED	Bayesian SkyGrid	200
M	178	981	GTR+G+I	UCED	Bayesian SkyGrid	100
NS	174	837	GTR+G+I	UCED	Bayesian SkyGrid	100

<sup>a</sup> The nucleotide substitution models were determined by jModeltest2.

<sup>b</sup> UCED represents uncorrelated exponential clock model;

<sup>c</sup> The unit was million.