Genes	Virus Strains	Identity (%)
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%
Μ	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 1 Blast analysis of genome sequences of these isolated viruses.

Canag	Deference exceptor strains	Estimated	95% HPD			
Genes	Reference ancestor strains	divergence time	Upper	Lower		
PB2 -	A/duck/Hunan/04.22 LDDX037-P/2015 (H5N6)	-1002014	Eab 2014	Sap 2014		
	A/duck/Guangdong/04.23 DGQTXC229-O/2015 (H5N6)	Juli 2014	Fe0 2014	Sep 2014		
PB1 -	A/Environment/Guangxi/44389/2015 (H5N6)	Max 2014	Amm 2012	$D_{22} 2014$		
	A/chicken/Japan/AQ-HE144/2015 (H5N6)	- Way 2014	Apr 2015	Dec 2014		
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		
М	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 2 The reference ancestor strains and the estimate divergence time of each gene segment from its tMRCA.

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						
Μ	2.09	1.41	2.71						
NS	3.66	2.76	4.52						

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

		HA ^b		NA	NS1	M2				PB2						PA				
Viruses ^a	H5 clade	clade Cleavage site		8S°	Stalk deletion	Drug-resistance associated aa				Air-borne transmission				aa PB1-F2 residues truncatio		PB1-F2 truncation	aa 1 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	RERRRKR	Q	G	Yes	Yes	L	V	А	S	G	Т	Q	E	D	Н	Ι	1-39	S	L
JS02	2.3.4.4	RERRRKR	Q	G	Yes	Yes	L	V	А	S	G	Т	Q	Е	D	Н	Ι	1-39	S	L
K10	2.3.4.4	RERRRKR	Q	G	Yes	Yes	L	V	А	S	G	Т	Q	Е	D	Н	Ι	1-39	S	L
CNIC-21099 ^d	2.3.4.4	REKRRKR	Q	G	Yes	No	L	V	А	S	G	Т	Q	Е	D	Н	Ι	12-90	S	L
IDCDC-RG43A ^d	2.3.4.4	RERRRKR	Q	G	-	No	L	V	А	N	G	Т	Q	Е	D	Н	Ι	1-39	S	F

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

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

^b H5 numbering

^c RBS: Receptor binding site

^d Reference viruses were selected based on candidate vaccine viruses recommended by CDC or CCDC

Datasets	Sequence numbers	Nucleotide length (nt)	Substitution models ^a	Relax-clock models ^b	Tree models	MCMC chain length ^c
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
Μ	178	981	GTR+G+I	UCED	Bayesian SkyGrid	100
NS	174	837	GTR+G+I	UCED	Bayesian SkyGrid	100

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

^a The nucleotide substitution models were determined by jModeltest2.

^b UCED represents uncorrelated exponential clock model;

^c The unit was million.