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# Evolution of Avian Influenza Virus (H3) with Spillover into Humans, China

## Appendix

### Method details

#### Environmental sample collection

From January 2009 through June 2022, environmental samples were collected from avian-linked environments (including live poultry markets, poultry farms, backyards, slaughterhouses, and wild bird habitats) monthly across 31 provinces in Chinese mainland according to avian influenza virus (AIV) surveillance guideline of Chinese Center for Disease Control and Prevention (CDC). The environmental samples included poultry feces, sewage, poultry drinking water, surface swabs from poultry cages and chopping boards, etc. Feces or swab samples were maintained in viral transport medium; for liquid samples (sewage, poultry drinking water), 5-ml liquid was collected. The samples were sent to local Chinese National Influenza Surveillance Network laboratories within 48 h and stored at 4°C.

All samples were centrifuged at 3,000 rpm for 10 min, and the supernatant of each sample was harvested for further test. Influenza A viruses were identified by real-time reverse transcription-PCR (real-time RT-PCR). Of these, positive samples were sent to Chinese National Influenza Center (CNIC) for virus isolation.

### **Virus isolation and identification**

Viruses were isolated in 9 to 11-day specific-pathogen-free (SPF) embryonated chicken eggs. After incubation for 48–72 hours at 37°C, the presence of the virus in the allantoic fluids of eggs was identified by a hemagglutination test using 1% turkey red blood cells (TRBC).

### **RNA extraction and genome sequencing**

Virus RNA was extracted from the isolated viruses using the MagMAX CORE Nucleic Acid Purification Kit (Thermo Fisher, Waltham, MA). The extracted RNA was subjected to reverse transcription and amplification using the SuperScript® III One-Step RT-PCR system (Thermo Fisher, Waltham, MA) according to the described method (1). Whole genome sequencing of influenza A virus was implemented on the automatic Applied Biosystems 3730xl DNA Analyzer (Life Technologies, USA) or MiSeq high-throughput sequencing platform (Illumina, Inc., San Diego, CA, USA). The raw data from MiSeq platform were paired reads with length of 150 bp. Low-quality reads were trimmed, and the filtered reads were sampled and de novo-assembled using Velvet (version 1.2.10) (2) and Newbler (version 2.5). Contigs were blasted against a database containing all influenza A virus nucleotide sequences collected from the National Center for Biotechnology Information ([www.ncbi.nlm.nih.gov/genomes/FLU](http://www.ncbi.nlm.nih.gov/genomes/FLU)) and the Global Initiative on Sharing All Influenza Data (GISAID) (<http://www.gisaid.org>). Sequences with the highest similarity were selected as references for mapping of reads using Bowtie 2 (version 2.1.0) (3). The influenza A virus genome sequences were obtained by extracting the consensus sequences from the mapping results, with a coverage depth of at least 30 times at each site on the eight segments.

## **Sequence collection and alignment**

All the sequences of 188 environmental H3 isolates were initially obtained in our surveillance (Appendix Table 1) and 32 of them have been published in our previous study (4). H3, N2, N3, N6, N8 and internal gene sequences of avian-origin viruses were downloaded from the GISAID EpiFlu database as of June 25, 2022. Sequences of two human H3N8 viruses isolated in Henan and Hunan provinces were also included. The resulting sequences of each segment were aligned using MAFFT software v7.222 (5) and manually adjusted to correct frameshift errors in MEGA v7.0 (6). The coding region of each segment was retained and the signal peptide was removed from the HA segment. Sequences with  $\geq 97\%$  of the length of coding region and  $\leq 3$  degenerate bases were included for molecular characterization and phylogenetic analyses. To comprehensively elaborate the evolution of H3 AIVs in China, we extended the selection of Chinese sequences. The sequences collected in China were retained with less than 4 degenerate bases and no less than 90% of the length of coding region of the segment. For the HA gene, sequences with complete HA1 domain were also included.

## **Phylogenetic analyses**

Maximum likelihood (ML) phylogenies of all segments were reconstructed using the FastTree software v2.1.11 (7) under the GTRGAMMA model with 1,000 bootstrap replicates. All H3-HA gene sequences were included in the phylogenetic analyses. The datasets of NA genes and internal genes were reduced using cd-hit to improve the computational efficiency. All H3 virus strains isolated from avian host and environment samples in China (Figure 1; Appendix Figure 15) as well as two human H3N8 virus strains were retained for reconstructing evolutionary relationships. We also retrieved the sequences that shared the highest similarity with Chinese H3 AIVs by BLAST. The resulting trees were classified into divergent lineages or sublineages according to the topology of phylogenetic trees with bootstrap support values  $\geq 70\%$ . Clusters from

Eurasian wild bird gene pool were then manually merged, if necessary, based on the bootstrap support values and reported classification (8). The ggtree package in R(9) and FigTree v1.4.4 (<http://tree.bio.ed.ac.uk/software/figtree/>) were used for visualization and annotation.

### **Genotypic analysis**

There is remarkably frequent occurrence of reassortment in AIV, that formed the wild bird gene pool. Studies on large number of sequences provided little evidence for the elevated fitness of specific gene combinations in AIV isolates from wild birds (10). AIVs maintained in wild birds spill over into novel hosts including domestic gallinaceous poultry, horses, swine, and humans leading to the emergence of lineages/sublineages transmissible in the new host. Hence, we classified NA and internal genes into Eurasian wild bird gene pool, ZJ-5 sublineage (of the wild bird viruses) (8), poultry H5N1/H5N6 sublineage, poultry H9N2 ZJ-HJ/07 sublineage, and/or waterfowl H6 sublineage. For strains with all eight gene segments available, their genotypes were determined by the combination of lineage/sublineage assignments of each of eight segments. Virus genotypes were analyzed for H3 viruses isolated from human, avian and environmental samples in China during 2009-2022. For each NA subtype, genotypes were named G1 to Gn, according to the time of identification.

### **Molecular dating**

To estimate the time to the most recent common ancestor (tMRCA) of human H3N8 viruses, smaller datasets for eight gene segments containing human H3N8 virus strains were selected to run time-measured Bayesian Markov chain Monte Carlo (MCMC) analysis implemented in BEAST v1.10.4 (11). Root-to-tip genetic divergence against sampling dates was analyzed using TempEst v1.5.3 (12) to investigate the temporal signal of our datasets. The best-fitting nucleotide substitution models (GTR+G



for the MP gene, and GTR+G+I for other gene segments) were identified according to the Corrected Akaike Information Criterion in MEGA v7.0 (6). A relaxed clock model with uncorrelated lognormal distribution was used. A Bayesian skyride model with time-aware smoothing was used (13). The default distribution was used for prior CP1 + 2, CP3, and ucl.d.mean. Bayesian MCMC analyses were run for 50 million steps, with sampling every 5,000 steps. Multiple independent MCMC trajectories were computed and combined. The first 10% states were removed. Final effective sample size as assessed in Tracer v1.7.2. were >200 for all parameters. Maximum clade credibility (MCC) trees with median node height were generated using TreeAnnotator v1.10.4. We visualised and annotated the MCC trees using ggtree (9).

### **Evolution details of H3 AIVs**

#### Evolution of China-2 sublineage

After the isolation of H3N2 in Jiangsu in 2004, the China-2 sublineage evolved into 3 subgroups (Figure 2; Appendix Figure 1). The China-2.1 subgroup had a large proportion of viruses isolated from China during 2009-2018. The China-2.2 subgroup mainly comprised environmental H3 viruses (29/31,93.5%) sequenced in this study during 2015-2021. Poultry-origin H3N2 and H3N8 viruses found in Guangxi during 2009-2013 independently formed the China-2.3 subgroup.

#### Evolution of China-3 and China-4 sublineages

A few isolates in this study belonged to the sublineages China-3 (15) and China-4 (7), detected during 2009-2018 (Figure 2; Appendix Figure 1). Most of the viruses in the China-3 sublineage were collected from poultry within the same sampling event and location.

### Evolution of other H3 sublineages

Chinese H3 AIVs were also found scattered in sublineages Asia, Europe-Asia, worldwide-1, and worldwide-2 (Figure 2; Appendix Figure 1). Both poultry and wild bird isolates were found in each sublineage. The worldwide-1 sublineage contained 38 H3 virus strains collected from 1999 through 2022 in China, as well as other H3 viruses covering Asia, Europe, and North America. H3 viruses combined with N1-N8 subtypes were detected, among which H3N8 was predominant. Most of the Chinese H3 strains (31/38) in this sublineage were sampled from poultry or poultry-related environments. The worldwide-2 sublineage contained a smaller cluster of viruses including H3N2, H3N3, H3N5, H3N6, and H3N8 subtypes, spreading across the Eurasian and North American continents. Three H3N2 AIVs in 2011 and 2 H3N3 AIVs in 2000 from China belonged to this sublineage. The viruses in the Europe-Asia sublineage were mainly distributed in Europe and Asia, with a minority (6/230, 2.6%) in Africa and North America. N1, N3, N5, N6, and N8 subtypes were identified. Sixteen H3 strains from poultry and wild birds in China fell into this sublineage, with a transient detection in 4 years. Viruses in the Asia sublineage were mainly sampled from Asian countries except for 7 viruses collected from Russia. H3 viruses with various NA subtypes including N1, N2, N3, N5, N6, N8, and N9 were identified. Fifteen H3 isolates from China belonged to the Asia sublineage.

### **Dynamic reassortment of H3 viruses**

#### Reassortment with N2 genes

The N2 genes of AIVs in the Eurasian lineage could be further classified into 4 major sublineages: Eurasian-1, Eurasian-2, waterfowl H6N2, and poultry H9N2 sublineages (Appendix Figure 2A). The majority of H3N2 viruses in this study were clustered in the Eurasian-2. The N2 genes of this sublineage have circulated in the

poultry and poultry-related environment in China with the earliest isolate detected in 2009. The H3N2 AIVs in China were closely related to the viruses isolated from various species with multiple subtypes, such as H4, H5, H9, etc. Notably, NA genes of 6 H3N2 strains isolated from domestic ducks during 2013-2014 were clustered with chicken H9N2 viruses.

#### Reassortment with N3 genes

The NA genes of the H3N3 AIVs in China were all derived from the Eurasian lineage (Appendix Figure 2B). Notably, one H3N3 strain sequenced in our study, A/Environment/Hunan/13561/2020, showed a close relationship with the NA genes of human-origin H10N3 virus and H10N3 viruses circulating in chicken during 2019-2021. Another H3N3 strain sampled in 2020 was grouped with duck H7N3 viruses circulating in China during 2010-2018.

#### Reassortment with N6 genes

The NA genes of the H3N6 AIVs in China were scattered in the Eurasian lineage (Appendix Figure 2C). H3N6 viruses isolated from poultry and wild bird in Jiangxi, Guangdong, Guangxi, and Hunan provinces during 2014-2015 were clustered with highly pathogenic AIV (HPAIV) H5N6.

#### Reassortment with internal genes

To explore the pattern of reassortment of H3 AIVs in China, we reconstructed the phylogenies for all six internal genes (Appendix Figure 3). The internal genes of all the H3 AIVs sequenced through our surveillance belonged to the Eurasian lineage, except for the M gene of A/Environment/Sichuan/32281/2016(H3N2), as previously described (4). Except for two H3N8 AIVs, almost all the H3 AIVs in China had internal genes derived from the Eurasian wild bird gene pool, ZJ-5 sublineage (of the wild bird viruses), poultry

H5N1/H5N6 sublineage, poultry H9N2 ZJ-HJ/07 sublineage, and/or waterfowl H6 sublineage.

A large proportion of H3 AIVs in China fell into the Eurasian wild bird reservoir (Appendix Figure 3). Many H3 AIVs contained internal genes (especially PA, NP and, M genes) from the ZJ-5 sublineage, of the wild bird viral gene pool, which consisted mainly of viruses isolated from domestic waterfowl in China. Very few H3 AIVs derived their internal genes (PB2 (5), PB1 (1), PA (2) and M (1)) from the waterfowl H6 sublineage. A small proportion of H3 AIVs were grouped into the poultry-established lineages. In the poultry H5N1/H5N6 sublineage, 2-6 H3 AIVs isolated in 2013-2015 were found, and one was isolated in 2004 with NP gene.

#### **Emerging of multiple genotypes**

Seventy-three genotypes (G1-G73) were identified from 212 H3N2 viruses, most of which were transient genotypes (52/73,71.2%) (Appendix Figure 4A). Thirteen genotypes were sporadically detected in 2-3 years while eight genotypes (G6, G23-G25, G39, G54, and G56) were detected in more than 3 years. Obviously, the number of viral genotypes went up and down with the number of viruses detected. The number of genotypes increased gradually from 2009 through 2013, and tend to stabilize during 2014-2019. After 2019, only a few genotypes were detected. Most of the genotypes were found in southern China, particularly in Guangdong and Guangxi provinces which detected twenty-five and twenty-three genotypes, respectively (Appendix Figure 5A). Of the eight common genotypes, G6, G23, and G25 have been circulating for many years and spread to more regions (Appendix Figure 4A and 5A). Notably, the G23, which had been detected as early as 2014, continued circulating and was monitored in 2022. The H3N2 G23 viruses contained HA genes from the China-1 sublineage, NA genes from the Eurasian lineage, M genes from the ZJ-5 sublineage, and other 5 internal genes from the Eurasian wild bird gene pool (Appendix Table 4).

Despite a low reported detection, considerable genomic diversity was identified in H3N3, H3N6, and H3N8 AIVs. Eleven genotypes (G1-G11) from 14 H3N3 genomes, seventeen (G1-G17) from 25 H3N6, and twenty-five (G1-G25) from 33 H3N8 were found (Appendix Figure 4B-D). Most of the strains with identical genotypes were from the same year and location (Appendix Table 4). Only four genotypes, including G7 of H3N3, G2, G4, and G6 of H3N8, have been detected in more than one year (Appendix Figure 4B and 4D) and the former three were found in different provinces (Appendix Figure 5B and 5D).

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**Appendix Table 1.** Detailed information of 188 environmental H3 subtype viruses obtained in our surveillance.

Subtype	Isolate Id	Isolate name*	Province	Collection Date	Sample Type	Sample Site†	Accession numbers‡
H3N2	EPI_ISL_15720733	A/Environment/Guangdong/03/09	Guangdong	2009-04-03	surface swab of cages	D	EPI2210289~EPI2210296
	EPI_ISL_15720738	A/Environment/Guangxi/03045/2012	Guangxi	2011-07-19	sewage	A	EPI2210321~EPI2210328
	EPI_ISL_15720739	A/Environment/Guangxi/03056/2012	Guangxi	2012-06-22	sewage	A	EPI2210329~EPI2210336
	EPI_ISL_15720740	A/Environment/Anhui/03012/2012	Anhui	2012-09-11	sewage	A	EPI2210337~EPI2210344
	EPI_ISL_15720741	A/Environment/Guangxi/03047/2012	Guangxi	2012-09-28	sewage	A	EPI2210345~EPI2210352
	EPI_ISL_15720742	A/Environment/Guangxi/03059/2012	Guangxi	2012-10-11	feces	A	EPI2210353~EPI2210360
	EPI_ISL_15720743	A/Environment/Guangxi/03058/2012	Guangxi	2012-10-11	sewage	A	EPI2210361~EPI2210368
	EPI_ISL_15720744	A/Environment/Guangxi/06232/2013	Guangxi	2013-07-12	others	A	EPI2210369~EPI2210376
	EPI_ISL_15720745	A/Environment/Hunan/26068/2014	Hunan	2014-03-27	feces	A	EPI2210377~EPI2210384
	EPI_ISL_15720746	A/Environment/Hunan/27451/2014	Hunan	2014-04-09	sewage	D	EPI2210385~EPI2210392
	EPI_ISL_15720747	A/Environment/Guangxi/28750/2014	Guangxi	2014-04-14	surface swab of chopping boards	A	EPI2210393~EPI2210400
	EPI_ISL_15720748	A/Environment/Guangdong/77214/2014	Guangdong	2014-08-18	surface swab of cages	A	EPI2210401~EPI2210408
	EPI_ISL_15720749	A/Environment/Hunan/02458/2014	Hunan	2014-12-15	drinking water	A	EPI2210409~EPI2210416
	EPI_ISL_15720750	A/Environment/Chongqing/45115/2015	Chongqing	2015-01-20	surface swab of chopping boards	A	EPI2210417~EPI2210424
	EPI_ISL_15720751	A/Environment/Guangxi/32186/2015	Guangxi	2015-01-21	feces	A	EPI2210425~EPI2210432
	EPI_ISL_15720752	A/Environment/Guangxi/41272/2015	Guangxi	2015-01-28	drinking water	A	EPI2210433~EPI2210440
	EPI_ISL_15720753	A/Environment/Jiangxi/33299/2015	Jiangxi	2015-03-24	surface swab of cages	A	EPI2210441~EPI2210448
	EPI_ISL_15720754	A/Environment/Hunan/37943/2015	Hunan	2015-04-09	drinking water	A	EPI2210449~EPI2210456
EPI_ISL_15720755	A/Environment/Hunan/34583/2015	Hunan	2015-04-10	sewage	A	EPI2210457~EPI2210464	
EPI_ISL_15720756	A/Environment/Hunan/42907/2015	Hunan	2015-08-10	drinking water	C	EPI2210465~EPI2210472	
EPI_ISL_15723330	A/EV/SD/226214/2015	Shandong	2015-10-15	unknown	A	EPI2210473~EPI2210480	
EPI_ISL_15723331	A/EV/SD/226238/2015	Shandong	2015-10-21	unknown	A	EPI2210481~EPI2210488	
EPI_ISL_15723332	A/EV/SD/226236/2015	Shandong	2015-10-21	unknown	A	EPI2210489~EPI2210496	
EPI_ISL_15723333	A/Environment/Guangdong/15077/2015	Guangdong	2015-11-16	feces	A	EPI2210497~EPI2210504	
EPI_ISL_15723334	A/Environment/Yunnan/01456/2015	Yunnan	2015-11-27	feces	D	EPI2210505~EPI2210512	
EPI_ISL_15723335	A/Environment/Guangdong/15131/2015	Guangdong	2015-12-01	feces	A	EPI2210513~EPI2210520	
EPI_ISL_15723337	A/Environment/Hunan/02861/2015	Hunan	2015-12-15	sewage	A	EPI2210529~EPI2210536	
EPI_ISL_15723338	A/Environment/Hunan/10170/2016	Hunan	2016-01-06	drinking water	A	EPI2210537~EPI2210544	
EPI_ISL_15723339	A/Environment/Hunan/11041/2016	Hunan	2016-01-18	drinking water	A	EPI2210545~EPI2210552	
EPI_ISL_15723340	A/Environment/Sichuan/32289/2016	Sichuan	2016-02-04	feces	A	EPI2210553~EPI2210560	
EPI_ISL_15723341	A/Environment/Hunan/31561/2016	Hunan	2016-03-22	feces	A	EPI2210561~EPI2210568	
EPI_ISL_15723342	A/Environment/Sichuan/48404/2016	Sichuan	2016-06-27	feces	A	EPI2210569~EPI2210576	
EPI_ISL_15723343	A/Environment/Jiangsu/06688/2016	Jiangsu	2016-12-21	surface swab of chopping boards	A	EPI2210577~EPI2210583	
EPI_ISL_15723344	A/Environment/Jiangsu/06689/2016	Jiangsu	2016-12-21	sewage	A	EPI2210584~EPI2210591	
EPI_ISL_15723345	A/Environment/Hubei/33702/2017	Hubei	2017-02-09	surface swab of cages	A	EPI2210592~EPI2210599	
EPI_ISL_15723346	A/Environment/Guangxi/23533/2017	Guangxi	2017-03-30	surface swab of cages	A	EPI2210600~EPI2210607	
EPI_ISL_15723347	A/Environment/Guangxi/23558/2017	Guangxi	2017-04-05	drinking water	A	EPI2210608~EPI2210615	
EPI_ISL_15723348	A/Environment/Hunan/34012/2017	Hunan	2017-04-07	sewage	A	EPI2210616~EPI2210623	
EPI_ISL_15723349	A/Environment/Hunan/25648/2017	Hunan	2017-04-11	surface swab of cages	A	EPI2210624~EPI2210631	
EPI_ISL_15723350	A/Environment/Hunan/25643/2017	Hunan	2017-04-11	feces	A	EPI2210632~EPI2210639	

Subtype	Isolate Id	Isolate name*	Province	Collection Date	Sample Type	Sample Site†	Accession numbers‡
	EPI_ISL_15723351	A/Environment/Guangxi/32152/2017	Guangxi	2017-05-09	sewage	A	EPI2210640~EPI2210647
	EPI_ISL_15723352	A/Environment/Guangxi/32060/2017	Guangxi	2017-05-22	drinking water	A	EPI2210648~EPI2210655
	EPI_ISL_15723353	A/Environment/Guangdong/35458/2017	Guangdong	2017-05-31	drinking water	A	EPI2210656~EPI2210663
	EPI_ISL_15723354	A/Environment/Fujian/36985/2017	Fujian	2017-07-07	sewage	A	EPI2210664~EPI2210671
	EPI_ISL_15723476	A/Environment/Chongqing/36535/2017	Chongqing	2017-08-17	surface swab of cages	A	EPI2210672~EPI2210679
	EPI_ISL_15723477	A/Environment/Guangxi/40947/2017	Guangxi	2017-11-22	feces	A	EPI2210680~EPI2210687
	EPI_ISL_15723478	A/Environment/Guangxi/40934/2017	Guangxi	2017-11-22	feces	A	EPI2210688~EPI2210695
	EPI_ISL_15723479	A/Environment/Guangxi/05048/2017	Guangxi	2017-11-28	others	A	EPI2210696~EPI2210703
	EPI_ISL_15723480	A/Environment/Fujian/04958/2017	Fujian	2017-12-18	feces	A	EPI2210704~EPI2210711
	EPI_ISL_15723481	A/Environment/Guangxi/24894/2018	Guangxi	2018-01-17	surface swab of chopping boards	A	EPI2210712~EPI2210719
	EPI_ISL_15723482	A/Environment/Guangxi/24886/2018	Guangxi	2018-01-17	surface swab of chopping boards	A	EPI2210720~EPI2210727
	EPI_ISL_15723484	A/Environment/Guangxi/24775/2018	Guangxi	2018-02-03	surface swab of cages	A	EPI2210736~EPI2210743
	EPI_ISL_15723485	A/Environment/Guangdong/27940/2018	Guangdong	2018-02-06	feces	A	EPI2210744~EPI2210751
	EPI_ISL_15723486	A/Environment/Guangxi/24790/2018	Guangxi	2018-02-08	feces	A	EPI2210752~EPI2210759
	EPI_ISL_15723487	A/Environment/Guangxi/24805/2018	Guangxi	2018-02-09	drinking water	A	EPI2210760~EPI2210767
	EPI_ISL_15723488	A/Environment/Guangxi/24803/2018	Guangxi	2018-02-09	sewage	A	EPI2210768~EPI2210775
	EPI_ISL_15723489	A/Environment/Guangxi/28076/2018	Guangxi	2018-02-27	feces	A	EPI2210776~EPI2210783
	EPI_ISL_15723490	A/Environment/Guangdong/30984/2018	Guangdong	2018-04-09	feces	A	EPI2210784~EPI2210791
	EPI_ISL_15723491	A/Environment/Hunan/28854/2018	Hunan	2018-04-16	feces	A	EPI2210792~EPI2210799
	EPI_ISL_15723492	A/Environment/Guangdong/30702/2018	Guangdong	2018-05-23	surface swab of cages	A	EPI2210800~EPI2210807
	EPI_ISL_15723493	A/Environment/Zhejiang/31298/2018	Zhejiang	2018-05-23	feces	A	EPI2210808~EPI2210815
	EPI_ISL_15723494	A/Environment/Zhejiang/31299/2018	Zhejiang	2018-05-23	feces	A	EPI2210816~EPI2210823
	EPI_ISL_15723495	A/Environment/Guangxi/32515/2018	Guangxi	2018-08-21	feces	A	EPI2210824~EPI2210831
	EPI_ISL_15723496	A/Environment/Guangxi/32510/2018	Guangxi	2018-08-21	surface swab of cages	A	EPI2210832~EPI2210839
	EPI_ISL_15723497	A/Environment/Guangxi/32507/2018	Guangxi	2018-08-21	feces	A	EPI2210840~EPI2210847
	EPI_ISL_15723498	A/Environment/Guangdong/32693/2018	Guangdong	2018-09-10	others	A	EPI2210848~EPI2210855
	EPI_ISL_15723499	A/Environment/Chongqing/33939/2018	Chongqing	2018-09-13	feces	A	EPI2210856~EPI2210863
	EPI_ISL_15723500	A/Environment/Chongqing/33926/2018	Chongqing	2018-09-17	feces	A	EPI2210864~EPI2210871
	EPI_ISL_15723501	A/Environment/Guangxi/32937/2018	Guangxi	2018-09-25	surface swab of chopping boards	A	EPI2210872~EPI2210879
	EPI_ISL_15723502	A/Environment/Guangxi/32906/2018	Guangxi	2018-09-27	others	A	EPI2210880~EPI2210887
	EPI_ISL_15723503	A/Environment/Guangxi/32562/2018	Guangxi	2018-09-28	feces	A	EPI2210888~EPI2210895
	EPI_ISL_15723504	A/Environment/Guangxi/33582/2018	Guangxi	2018-10-28	surface swab of cages	A	EPI2210896~EPI2210903
	EPI_ISL_15723506	A/Environment/Guangdong/34268/2018	Guangdong	2018-11-06	feces	A	EPI2210912~EPI2210919
	EPI_ISL_15723507	A/Environment/Guangxi/33095/2018	Guangxi	2018-11-07	sewage	A	EPI2210920~EPI2210927
	EPI_ISL_15723508	A/Environment/Guangxi/33056/2018	Guangxi	2018-11-07	sewage	A	EPI2210928~EPI2210935
	EPI_ISL_15723509	A/Environment/Hunan/04235/2018	Hunan	2018-11-12	feces	A	EPI2210936~EPI2210943
	EPI_ISL_15723510	A/Environment/Hunan/05861/2018	Hunan	2018-11-29	feces	A	EPI2210944~EPI2210951
	EPI_ISL_15723511	A/Environment/Guangdong/27250/2018	Guangdong	2018-12-03	sewage	D	EPI2210952~EPI2210959
	EPI_ISL_15723512	A/Environment/Guangdong/27249/2018	Guangdong	2018-12-03	surface swab of chopping boards	D	EPI2210960~EPI2210967
	EPI_ISL_15723522	A/Environment/Guangxi/34661/2018	Guangxi	2018-12-03	drinking water	A	EPI2210968~EPI2210975



Subtype	Isolate Id	Isolate name*	Province	Collection Date	Sample Type	Sample Site†	Accession numbers‡
	EPI_ISL_15723523	A/Environment/Guangxi/35164/2018	Guangxi	2018-12-07	sewage	A	EPI2210976~EPI2210983
	EPI_ISL_15723524	A/Environment/Zhejiang/02021/2018	Zhejiang	2018-12-17	surface swab of chopping boards	A	EPI2210984~EPI2210991
	EPI_ISL_15723525	A/Environment/Guangxi/01878/2018	Guangxi	2018-12-20	others	A	EPI2210992~EPI2210999
	EPI_ISL_15723526	A/Environment/Guangxi/01869/2018	Guangxi	2018-12-20	feces	A	EPI2211000~EPI2211007
	EPI_ISL_15723527	A/Environment/Guangdong/14452/2018	Guangdong	2018-12-24	surface swab of cages	A	EPI2211008~EPI2211015
	EPI_ISL_15723528	A/Environment/Guangdong/14440/2018	Guangdong	2018-12-24	surface swab of cages	A	EPI2211016~EPI2211023
	EPI_ISL_15723529	A/Environment/Guangdong/14438/2018	Guangdong	2018-12-24	feces	A	EPI2211024~EPI2211031
	EPI_ISL_15723530	A/Environment/Guangdong/14430/2018	Guangdong	2018-12-24	sewage	A	EPI2211032~EPI2211039
	EPI_ISL_15723531	A/Environment/Guangdong/14334/2018	Guangdong	2018-12-24	surface swab of cages	A	EPI2211040~EPI2211047
	EPI_ISL_15723532	A/Environment/Guangdong/27641/2018	Guangdong	2018-12-26	others	A	EPI2211048~EPI2211055
	EPI_ISL_15723533	A/Environment/Guangdong/27729/2018	Guangdong	2018-12-26	feces	A	EPI2211056~EPI2211063
	EPI_ISL_15723534	A/Environment/Guangdong/27727/2018	Guangdong	2018-12-26	feces	A	EPI2211064~EPI2211071
	EPI_ISL_15723535	A/Environment/Guangxi/05712/2018	Guangxi	2018-12-26	feces	A	EPI2211072~EPI2211079
	EPI_ISL_15723893	A/Environment/Guangxi/01886/2018	Guangxi	2018-12-26	others	A	EPI2211080~EPI2211087
	EPI_ISL_15723894	A/Environment/Guangxi/01885/2018	Guangxi	2018-12-26	sewage	A	EPI2211088~EPI2211095
	EPI_ISL_15723895	A/Environment/Guangdong/27245/2019	Guangdong	2019-01-03	sewage	A	EPI2211096~EPI2211103
	EPI_ISL_15723896	A/Environment/Guangdong/27246/2019	Guangdong	2019-01-08	surface swab of chopping boards	A	EPI2211104~EPI2211111
	EPI_ISL_15723897	A/Environment/Guangxi/09150/2019	Guangxi	2019-01-17	sewage	A	EPI2211112~EPI2211119
	EPI_ISL_15723898	A/Environment/Hubei/43318/2019	Hubei	2019-01-29	inner wall and outer surface of defeathering machine	C	EPI2211120~EPI2211127
	EPI_ISL_15723899	A/Environment/Guangdong/34622/2019	Guangdong	2019-02-03	drinking water	A	EPI2211128~EPI2211135
	EPI_ISL_15723900	A/Environment/Guangdong/34240/2019	Guangdong	2019-02-13	feces	A	EPI2211136~EPI2211143
	EPI_ISL_15723902	A/Environment/Hunan/40453/2019	Hunan	2019-03-18	sewage	A	EPI2211152~EPI2211159
	EPI_ISL_15723903	A/Environment/Hunan/40442/2019	Hunan	2019-03-18	feces	A	EPI2211160~EPI2211167
	EPI_ISL_15723904	A/Environment/Guangxi/25038/2019	Guangxi	2019-03-25	drinking water	A	EPI2211168~EPI2211175
	EPI_ISL_15723905	A/Environment/Gansu/01469/2019	Gansu	2019-03-27	surface swab of cages	A	EPI2211176~EPI2211183
	EPI_ISL_15723906	A/Environment/Guangxi/32383/2019	Guangxi	2019-04-30	sewage	A	EPI2211184~EPI2211191
	EPI_ISL_15723907	A/Environment/Guangxi/39142/2019	Guangxi	2019-05-31	others	D	EPI2211192~EPI2211199
	EPI_ISL_15723908	A/Environment/Fujian/39244/2019	Fujian	2019-06-05	feces	A	EPI2211200~EPI2211207
	EPI_ISL_15723909	A/Environment/Guangxi/45278/2019	Guangxi	2019-06-12	surface swab of cages	D	EPI2211208~EPI2211215
	EPI_ISL_15723910	A/Environment/Hubei/43399/2019	Hubei	2019-06-20	surface swab of cages	D	EPI2211216~EPI2211223
	EPI_ISL_15723911	A/Environment/Guangxi/39550/2019	Guangxi	2019-07-18	drinking water	D	EPI2211224~EPI2211231
	EPI_ISL_15723912	A/Environment/Guangxi/39500/2019	Guangxi	2019-08-12	feces	D	EPI2211232~EPI2211239
	EPI_ISL_15723913	A/Environment/Guangxi/41094/2019	Guangxi	2019-09-18	sewage	D	EPI2211240~EPI2211247
	EPI_ISL_15723914	A/Environment/Chongqing/41153/2019	Chongqing	2019-10-18	feces	D	EPI2211248~EPI2211255
	EPI_ISL_15723915	A/Environment/Guangxi/45299/2019	Guangxi	2019-10-23	surface swab of cages	D	EPI2211256~EPI2211263
	EPI_ISL_15723916	A/Environment/Guangdong/09210/2019	Guangdong	2019-11-13	cloacal swab	A	EPI2211264~EPI2211271
	EPI_ISL_15723917	A/Environment/Guangdong/08844/2019	Guangdong	2019-11-25	others	D	EPI2211272~EPI2211279
	EPI_ISL_15723918	A/Environment/Fujian/44488/2019	Fujian	2019-11-26	sewage	A	EPI2211280~EPI2211287
	EPI_ISL_15723919	A/Environment/Guangxi/44461/2019	Guangxi	2019-12-09	sewage	D	EPI2211288~EPI2211295
	EPI_ISL_15723920	A/Environment/Guangdong/09270/2019	Guangdong	2019-12-10	feces	A	EPI2211296~EPI2211303
	EPI_ISL_15723921	A/Environment/Guangdong/09263/2019	Guangdong	2019-12-10	cloacal swab	A	EPI2211304~EPI2211311

Subtype	Isolate Id	Isolate name*	Province	Collection Date	Sample Type	Sample Site†	Accession numbers‡
	EPI_ISL_15723922	A/Environment/Guangdong/09230/2019	Guangdong	2019-12-11	oropharyngeal swab	A	EPI2211312~EPI2211319
	EPI_ISL_15723923	A/Environment/Guangxi/03562/2019	Guangxi	2019-12-23	surface swab of cages	D	EPI2211320~EPI2211327
	EPI_ISL_15723925	A/Environment/Guangdong/14024/2020	Guangdong	2020-01-06	others	D	EPI2211336~EPI2211343
	EPI_ISL_15723926	A/Environment/Guangxi/08779/2020	Guangxi	2020-01-06	sewage	D	EPI2211344~EPI2211351
	EPI_ISL_15723929	A/Environment/Hunan/05143/2020	Hunan	2020-12-08	surface swab of cages	D	EPI2211368~EPI2211375
	EPI_ISL_15723930	A/Environment/Jiangxi/13781/2020	Jiangxi	2020-12-08	surface swab of cages	D	EPI2211376~EPI2211383
	EPI_ISL_15723931	A/Environment/Jiangxi/13780/2020	Jiangxi	2020-12-08	feces	D	EPI2211384~EPI2211391
	EPI_ISL_15723932	A/Environment/Chongqing/01452/2021	Chongqing	2021-01-20	surface swab of cages	B	EPI2211392~EPI2211399
	EPI_ISL_15723962	A/Environment/Chongqing/01458/2021	Chongqing	2021-01-20	surface swab of cages	B	EPI2211400~EPI2211407
	EPI_ISL_15723963	A/Environment/Hunan/00548/2021	Hunan	2021-01-21	feces	D	EPI2211408~EPI2211415
	EPI_ISL_15723965	A/Environment/Hunan/05581/2021	Hunan	2021-03-18	feces	D	EPI2211419~EPI2211426
	EPI_ISL_15723966	A/Environment/Guangxi/12090/2021	Guangxi	2021-04-22	others	A	EPI2211427~EPI2211434
	EPI_ISL_15723967	A/Environment/Hunan/05683/2021	Hunan	2021-04-26	feces	D	<i>EPI2211435~EPI2211436</i>
	EPI_ISL_15723968	A/Environment/Sichuan/03175/2021	Sichuan	2021-08-30	feces	C	EPI2211437~EPI2211444
	EPI_ISL_15723969	A/Environment/Sichuan/03131/2021	Sichuan	2021-08-30	feces	C	EPI2211445~EPI2211452
	EPI_ISL_15723970	A/Environment/Guangxi/10369/2021	Guangxi	2021-09-07	sewage	A	EPI2211453~EPI2211460
	EPI_ISL_15723971	A/Environment/Guangxi/04926/2021	Guangxi	2021-10-14	others	A	EPI2211461~EPI2211468
	EPI_ISL_15723973	A/Environment/Guangxi/04909/2022	Guangxi	2022-01-11	sewage	A	EPI2211477~EPI2211484
	EPI_ISL_15723976	A/Environment/Fujian/10650/2022	Fujian	2022-01-25	feces	A	EPI2211501~EPI2211508
	EPI_ISL_15723977	A/Environment/Fujian/10644/2022	Fujian	2022-01-25	feces	A	EPI2211509~EPI2211516
	EPI_ISL_390760	<b>A/Environment/Guangdong/40751/2017</b>	Guangdong	2017-10-16	feces	A	EPI1590009~EPI1590016
	EPI_ISL_390761	<b>A/Environment/Chongqing/36536/2017</b>	Chongqing	2017-08-17	surface swab of cages	A	EPI1590017~EPI1590024
	EPI_ISL_390762	<b>A/Environment/Guangxi/32828/2017</b>	Guangxi	2017-07-04	sewage	A	EPI1590025~EPI1590032
	EPI_ISL_390763	<b>A/Environment/Hunan/34019/2017</b>	Hunan	2017-04-07	sewage	A	EPI1590033~EPI1590040
	EPI_ISL_390764	<b>A/Environment/Hubei/34289/2017</b>	Hubei	2017-01-23	sewage	C	EPI1590041~EPI1590048
	EPI_ISL_390765	<b>A/Environment/Guangxi/14185/2017</b>	Guangxi	2017-01-13	surface swab of chopping boards	A	EPI1590049~EPI1590056
	EPI_ISL_390766	<b>A/Environment/Guangdong/60350/2016</b>	Guangdong	2016-11-23	feces	A	EPI1590057~EPI1590064
	EPI_ISL_390767	<b>A/Environment/Anhui/33167/2016</b>	Anhui	2016-04-30	sewage	A	EPI1590065~EPI1590072
	EPI_ISL_390768	<b>A/Environment/Chongqing/38160/2016</b>	Chongqing	2016-03-18	feces	A	EPI1590073~EPI1590080
	EPI_ISL_390769	<b>A/Environment/Jiangsu/44006/2016</b>	Jiangsu	2016-03-09	feces	A	EPI1590081~EPI1590088
	EPI_ISL_390770	<b>A/Environment/Chongqing/22909/2016</b>	Chongqing	2016-01-19	feces	B	EPI1590089~EPI1590096
	EPI_ISL_390771	<b>A/Environment/Chongqing/22907/2016</b>	Chongqing	2016-01-14	surface swab of cages	A	EPI1590097~EPI1590104
	EPI_ISL_390772	<b>A/Environment/Hunan/11043/2016</b>	Hunan	2016-01-18	sewage	A	EPI1590105~EPI1590112
	EPI_ISL_390773	<b>A/Environment/Sichuan/32281/2016</b>	Sichuan	2016-01-08	sewage	A	EPI1590113~EPI1590120
	EPI_ISL_390774	<b>A/Environment/Inner Mongolia/02114/2015</b>	Inner Mongolia	2015-12-04	others	C	EPI1590121~EPI1590128
	EPI_ISL_390775	<b>A/Environment/Yunnan/01457/2015</b>	Yunnan	2015-11-27	surface swab of cages	D	EPI1590129~EPI1590136
	EPI_ISL_390776	<b>A/Environment/Guangdong/15241/2015</b>	Guangdong	2015-09-15	surface swab of cages	A	EPI1590137~EPI1590144
	EPI_ISL_390777	<b>A/Environment/Hunan/39684/2015</b>	Hunan	2015-04-01	sewage	A	EPI1590145~EPI1590152
	EPI_ISL_390778	<b>A/Environment/Jiangxi/27771/2015</b>	Jiangxi	2015-03-05	feces	A	EPI1590153~EPI1590160
	EPI_ISL_390779	<b>A/Environment/Guangdong/21134/2015</b>	Guangdong	2015-01-20	surface swab of cages	A	EPI1590161~EPI1590168
	EPI_ISL_390780	<b>A/Environment/Hunan/98839/2014</b>	Hunan	2014-10-14	feces	A	EPI1590169~EPI1590176
	EPI_ISL_390781	<b>A/Environment/Guangxi/79509/2014</b>	Guangxi	2014-09-16	sewage	A	EPI1590177~EPI1590184

Subtype	Isolate Id	Isolate name*	Province	Collection Date	Sample Type	Sample Site†	Accession numbers‡
	EPI_ISL_390782	<b><i>A/Environment/Chongqing/79459/2014</i></b>	Chongqing	2014-09-15	surface swab of cages	A	EPI1590185~EPI1590192
	EPI_ISL_390783	<b><i>A/Environment/Guangxi/28753/2014</i></b>	Guangxi	2014-04-14	surface swab of chopping boards	A	EPI1590193~EPI1590200
	EPI_ISL_390784	<b><i>A/Environment/Hunan/26067/2014</i></b>	Hunan	2014-03-27	surface swab of cages	A	EPI1590201~EPI1590208
	EPI_ISL_390785	<b><i>A/Environment/Hunan/18323/2014</i></b>	Hunan	2014-02-27	drinking water	A	EPI1590209~EPI1590216
H3N3	EPI_ISL_15723483	<i>A/Environment/Guangxi/29409/2018</i>	Guangxi	2018-01-23	surface swab of chopping boards	A	EPI2210728~EPI2210735
	EPI_ISL_15723901	<i>A/Environment/Guangdong/34076/2019</i>	Guangdong	2019-03-13	feces	A	EPI2211144~EPI2211151
	EPI_ISL_15723927	<i>A/Environment/Hunan/13561/2020</i>	Hunan	2020-06-30	surface swab of cages	D	EPI2211352~EPI2211359
	EPI_ISL_15723928	<i>A/Environment/Fujian/14131/2020</i>	Fujian	2020-10-28	feces	D	EPI2211360~EPI2211367
	EPI_ISL_390786	<b><i>A/Environment/Fujian/02754/2016</i></b>	Fujian	2016-11-11	drinking water	A	EPI1590217~EPI1590224
	EPI_ISL_390787	<b><i>A/Environment/Hunan/03259/2015</i></b>	Hunan	2015-12-22	feces	A	EPI1590225~EPI1590232
	EPI_ISL_390788	<b><i>A/Environment/Fujian/85141/2014</i></b>	Fujian	2014-10-20	feces	A	EPI1590233~EPI1590240
H3N6	EPI_ISL_15720732	<i>A/Environment/Zhejiang/7/09</i>	Zhejiang	2009-01-01	others	D	EPI2210281~EPI2210288
	EPI_ISL_15723336	<i>A/Environment/Sichuan/03397/2015</i>	Sichuan	2015-12-09	surface swab of chopping boards	A	EPI2210521~EPI2210528
	EPI_ISL_390789	<b><i>A/Environment/Sichuan/03404/2015</i></b>	Sichuan	2015-12-24	feces	B	EPI1590241~EPI1590248
H3N8	EPI_ISL_15720734	<i>A/jilongtumo/Jiangxi/29/09</i>	Jiangxi	2009-10-22	surface swab of cages	A	EPI2210297~EPI2210304
	EPI_ISL_15720735	<i>A/wushui/Chongqing/2/2010</i>	Chongqing	2010-01-27	sewage	A	EPI2210305~EPI2210312
	EPI_ISL_15720736	<i>A/jixue/Zhejiang/04/2010</i>	Zhejiang	2010-04-01	chicken blood	A	EPI2210313~EPI2210320
	EPI_ISL_15723505	<i>A/Environment/Guangxi/32891/2018</i>	Guangxi	2018-10-29	drinking water	A	EPI2210904~EPI2210911
	EPI_ISL_15723924	<i>A/Environment/Guangxi/03561/2019</i>	Guangxi	2019-12-23	sewage	D	EPI2211328~EPI2211335
	EPI_ISL_15723972	<i>A/Environment/Guangxi/04905/2022</i>	Guangxi	2022-01-11	others	A	EPI2211469~EPI2211476
	EPI_ISL_15723974	<i>A/Environment/Fujian/10643/2022</i>	Fujian	2022-01-25	surface swab of cages	A	EPI2211485~EPI2211492
	EPI_ISL_15723975	<i>A/Environment/Fujian/10648/2022</i>	Fujian	2022-01-25	surface swab of cages	A	EPI2211493~EPI2211500
	EPI_ISL_390790	<b><i>A/Environment/Hunan/46780/2015</i></b>	Hunan	2015-11-18	drinking water	A	EPI1590249~EPI1590256
	EPI_ISL_390791	<b><i>A/Environment/Hunan/37939/2015</i></b>	Hunan	2015-04-09	sewage	A	EPI1590257~EPI1590264
H3N0	EPI_ISL_15723964	<i>A/Environment/Guangxi/01993/2021</i>	Guangxi	2021-01-27	sewage	A	<i>EPI2211416~EPI2211418</i>

\*Isolate names of strains published in previous study were in italic bold.

†A, live poultry market; B, poultry farm; C, slaughterhouse; D, unknown, referred to sampling sites without specific information (live poultry market, poultry farm, backyard or slaughterhouse).

‡The accession numbers of strains without complete genomes are in italics.

**Appendix Table 2.** H3 subtype avian influenza viruses sampled from the avian-linked environments

Subtype	Sample site, n (%)				Total
	Live poultry markets	Poultry farms/backyards	Slaughterhouses	Unknown*	
H3N2	133(79.6)	3(1.8)	6(3.6)	25(15.0)	167(88.8)
H3N3	5(71.4)	0	0	2(28.6)	7(3.7)
H3N6	1(33.3)	1(33.3)	0	0	3(1.6)
H3N8	9(0.9)	0	0	1(0.1)	10(5.3)
H3Nx	1(100.0)	0	0	0	1(0.5)
Total	149(79.3)	4(2.1)	6(3.2)	30(16.0)	188(100.0)

\*Unknown referred to sampling sites without specific information (live poultry market, poultry farm, backyard, or slaughterhouse).

**Appendix Table 3.** Host distribution in the sublineages China-1 to China-4

Host	Sublineage, n(%)				
	China-1	China-2	China-3	China-4	Total
Domestic chicken	7(4.2)	4(3.6)	6(5.5)	1(4.3)	18(4.4)
Domestic duck	48(28.9)	63(56.8)	80(72.7)	15(65.2)	206(50.2)
Domestic goose	0	2(1.8)	0	0	2(0.5)
Other poultry	0	1(0.9)	0	0	1(0.2)
Wild bird	1(0.6)	0	4(3.6)	0	5(1.2)
Environment	108(65.1)	41(36.9)	20(18.2)	7(30.4)	176(42.9)
Human	2(1.2)	0	0	0	2(0.5)
Total	166	111	110	23	410(100.0)

**Appendix Table 4.** Genotypes of all H3 subtype AIVs with the whole genome and two human H3N8 viruses sampled in China during 2009-2022\*.

Subtype	Isolate name	Genotype	HA	NA	PB2	PB1	PA	NP	M	NS
H3N2	A/Environment/Guangdong/03/09	G1	China-1	N2-Eurasian lineage	EA	EA	EA	EA	EA	EA(A)
	A/Duck/Guangxi/015D2/2009	G1	China-1	N2-Eurasian lineage	EA	EA	EA	EA	EA	EA(A)
	A/Duck/Guangxi/057D6/2010	G1	China-1	N2-Eurasian lineage	EA	EA	EA	EA	EA	EA(A)
	A/Chicken/Guangxi/073C2/2010	G1	China-1	N2-Eurasian lineage	EA	EA	EA	EA	EA	EA(A)
	A/Duck/Shanghai/27030/2009	G2	Worldwide-1	N2-Eurasian lineage	ZJ-5	EA	ZJ-5	ZJ-5	EA	EA(A)
	A/Duck/Guangdong/W12/2011	G3	China-1	N2-Eurasian lineage	EA	EA	EA	ZJ-5	EA	EA(A)
	A/Environment/Hunan/42907/2015	G3	China-1	N2-Eurasian lineage	EA	EA	EA	ZJ-5	EA	EA(A)
	A/Duck/Hebei/B1645-2/2011	G4	Worldwide-2	N2-Eurasian lineage	EA	EA	EA	EA	EA	EA(A)
	A/Duck/Hebei/B1647-1/2011	G4	Worldwide-2	N2-Eurasian lineage	EA	EA	EA	EA	EA	EA(A)
	A/Duck/Hebei/B1646-2/2011	G4	Worldwide-2	N2-Eurasian lineage	EA	EA	EA	EA	EA	EA(A)
	A/Duck/Guangxi/112D4/2012	G5	China-1	waterfowl H6N2	EA	EA	EA	EA	EA	EA(A)
	A/Pigeon/Guangxi/128P9/2012	G6	China-2	N2-Eurasian lineage	EA	EA	EA	EA	ZJ-5	EA(A)
	A/Goose/Guangxi/139G20/2013	G6	China-2	N2-Eurasian lineage	EA	EA	EA	EA	ZJ-5	EA(A)
	A/Environment/Sichuan/32289/2016	G6	China-2	N2-Eurasian lineage	EA	EA	EA	EA	ZJ-5	EA(A)
	A/Environment/Chongqing/22909/2016	G6	China-2	N2-Eurasian lineage	EA	EA	EA	EA	ZJ-5	EA(A)
	A/Environment/Guangdong/14440/2018	G6	China-2	N2-Eurasian lineage	EA	EA	EA	EA	ZJ-5	EA(A)
	A/Environment/Guangdong/14438/2018	G6	China-2	N2-Eurasian lineage	EA	EA	EA	EA	ZJ-5	EA(A)
	A/Environment/Guangdong/14430/2018	G6	China-2	N2-Eurasian lineage	EA	EA	EA	EA	ZJ-5	EA(A)
	A/Environment/Guangdong/14452/2018	G6	China-2	N2-Eurasian lineage	EA	EA	EA	EA	ZJ-5	EA(A)
	A/Environment/Guangxi/32510/2018	G6	China-2	N2-Eurasian lineage	EA	EA	EA	EA	ZJ-5	EA(A)
	A/Environment/Guangxi/34661/2018	G6	China-2	N2-Eurasian lineage	EA	EA	EA	EA	ZJ-5	EA(A)
	A/Environment/Guangxi/39550/2019	G6	China-2	N2-Eurasian lineage	EA	EA	EA	EA	ZJ-5	EA(A)
	A/Chicken/Guangxi/125C8/2012	G7	China-2	N2-Eurasian lineage	EA	EA	ZJ-5	EA	EA	EA(B)
	A/Environment/Guangxi/03059/2012	G8	China-3	N2-Eurasian lineage	ZJ-5	EA	ZJ-5	EA	EA	EA(A)
	A/Environment/Guangxi/03058/2012	G8	China-3	N2-Eurasian lineage	ZJ-5	EA	ZJ-5	EA	EA	EA(A)
	A/Environment/Guangxi/03047/2012	G8	China-3	N2-Eurasian lineage	ZJ-5	EA	ZJ-5	EA	EA	EA(A)

Subtype	Isolate name	Genotype	HA	NA	PB2	PB1	PA	NP	M	NS
	A/Environment/Anhui/03012/2012	G9	China-3	N2-Eurasian lineage	EA	EA	ZJ-5	ZJ-5	ZJ-5	EA(A)
	A/duck/Guangdong/04.16_SZLGLW009/2015_Mixed	G9	China-3	N2-Eurasian lineage	EA	EA	ZJ-5	ZJ-5	ZJ-5	EA(A)
	A/Environment/Guangxi/41272/2015	G9	China-3	N2-Eurasian lineage	EA	EA	ZJ-5	ZJ-5	ZJ-5	EA(A)
	A/Environment/Guangxi/03056/2012	G10	China-3	N2-Eurasian lineage	EA	EA	ZJ-5	EA	ZJ-5	EA(A)
	A/Duck/Zhejiang/4637/2013	G11	China-1	N2-Eurasian lineage	EA	ZJ-5	EA	EA	EA	EA(A)
	A/Duck/Zhejiang/4613/2013	G11	China-1	N2-Eurasian lineage	EA	ZJ-5	EA	EA	EA	EA(A)
	A/Duck/Zhejiang/4625/2013	G11	China-1	N2-Eurasian lineage	EA	ZJ-5	EA	EA	EA	EA(A)
	A/Duck/Zhejiang/6D7/2013	G12	China-1	N2-Eurasian lineage	EA	EA	ZJ-5	EA	EA	EA(A)
	A/Duck/Zhejiang/D11/2013	G13	Worldwide-1	poultry H9N2	EA	ZJ-5	EA	EA	EA	EA(A)
	A/Duck/Zhejiang/D13/2013	G14	Worldwide-1	poultry H9N2	EA	EA	EA	EA	EA	EA(A)
	A/environment/Shanghai/LPM1/2013	G15	China-2	N2-Eurasian lineage	EA	EA	EA	EA	EA	EA(A)
	A/Environment/Jiangsu/44006/2016	G15	China-2	N2-Eurasian lineage	EA	EA	EA	EA	EA	EA(A)
	A/Environment/Chongqing/36536/2017	G15	China-2	N2-Eurasian lineage	EA	EA	EA	EA	EA	EA(A)
	A/Environment/Chongqing/36535/2017	G15	China-2	N2-Eurasian lineage	EA	EA	EA	EA	EA	EA(A)
	A/Chicken/Guangxi/135C10/2013	G16	China-2	N2-Eurasian lineage	EA	EA	H6	EA	ZJ-5	EA(A)
	A/Duck/Guangxi/135D20/2013	G17	China-2	N2-Eurasian lineage	EA	H6	ZJ-5	EA	ZJ-5	EA(A)
	A/Chicken/Shanghai/LPM2/2013	G18	China-3	N2-Eurasian lineage	EA	EA	EA	EA	ZJ-5	EA(A)
	A/Duck/Shanghai/SH3/2013	G19	China-3	N2-Eurasian lineage	ZJ-5	EA	ZJ-5	ZJ-5	ZJ-5	ZJ-5
	A/Teal/Shanghai/SH-101/2013	G20	China-3	N2-Eurasian lineage	EA	EA	EA	EA	ZJ-5	EA(B)
	A/Teal/Shanghai/SH-104/2013	G20	China-3	N2-Eurasian lineage	EA	EA	EA	EA	ZJ-5	EA(B)
	A/Teal/Shanghai/SH-89/2013	G21	China-3	N2-Eurasian lineage	EA	EA	EA	ZJ-5	EA	EA(A)
	A/Duck/Shanghai/SH1/2013	G22	China-4	N2-Eurasian lineage	ZJ-5	ZJ-5	ZJ-5	ZJ-5	ZJ-5	EA(A)
	A/Duck/Shanghai/SH2/2013	G22	China-4	N2-Eurasian lineage	ZJ-5	ZJ-5	ZJ-5	ZJ-5	ZJ-5	EA(A)
	A/Environment/Guangdong/77214/2014	G22	China-4	N2-Eurasian lineage	ZJ-5	ZJ-5	ZJ-5	ZJ-5	ZJ-5	EA(A)
	A/Duck/Anhui/D293/2014	G22	China-4	N2-Eurasian lineage	ZJ-5	ZJ-5	ZJ-5	ZJ-5	ZJ-5	EA(A)
	A/Environment/Guangxi/28750/2014	G23	China-1	N2-Eurasian lineage	EA	EA	EA	EA	ZJ-5	EA(A)
	A/Environment/Guangxi/28753/2014	G23	China-1	N2-Eurasian lineage	EA	EA	EA	EA	ZJ-5	EA(A)
	A/Chicken/Guangxi/165C7/2014	G23	China-1	N2-Eurasian lineage	EA	EA	EA	EA	ZJ-5	EA(A)
	A/Environment/Guangxi/32186/2015	G23	China-1	N2-Eurasian lineage	EA	EA	EA	EA	ZJ-5	EA(A)
	A/Environment/Guangdong/15131/2015	G23	China-1	N2-Eurasian lineage	EA	EA	EA	EA	ZJ-5	EA(A)
	A/Environment/Guangxi/40947/2017	G23	China-1	N2-Eurasian lineage	EA	EA	EA	EA	ZJ-5	EA(A)
	A/Environment/Guangxi/40934/2017	G23	China-1	N2-Eurasian lineage	EA	EA	EA	EA	ZJ-5	EA(A)
	A/Environment/Guangxi/23533/2017	G23	China-1	N2-Eurasian lineage	EA	EA	EA	EA	ZJ-5	EA(A)
	A/Environment/Fujian/36985/2017	G23	China-1	N2-Eurasian lineage	EA	EA	EA	EA	ZJ-5	EA(A)
	A/Environment/Guangxi/05048/2017	G23	China-1	N2-Eurasian lineage	EA	EA	EA	EA	ZJ-5	EA(A)
	A/Environment/Guangxi/32937/2018	G23	China-1	N2-Eurasian lineage	EA	EA	EA	EA	ZJ-5	EA(A)
	A/duck/China/322D22/2018	G23	China-1	N2-Eurasian lineage	EA	EA	EA	EA	ZJ-5	EA(A)
	A/Environment/Guangdong/34268/2018	G23	China-1	N2-Eurasian lineage	EA	EA	EA	EA	ZJ-5	EA(A)
	A/Environment/Guangxi/35164/2018	G23	China-1	N2-Eurasian lineage	EA	EA	EA	EA	ZJ-5	EA(A)
	A/Environment/Guangdong/27641/2018	G23	China-1	N2-Eurasian lineage	EA	EA	EA	EA	ZJ-5	EA(A)
	A/Environment/Guangxi/01886/2018	G23	China-1	N2-Eurasian lineage	EA	EA	EA	EA	ZJ-5	EA(A)
	A/Environment/Guangxi/24886/2018	G23	China-1	N2-Eurasian lineage	EA	EA	EA	EA	ZJ-5	EA(A)
	A/Environment/Guangxi/24803/2018	G23	China-1	N2-Eurasian lineage	EA	EA	EA	EA	ZJ-5	EA(A)
	A/Environment/Guangxi/24775/2018	G23	China-1	N2-Eurasian lineage	EA	EA	EA	EA	ZJ-5	EA(A)
	A/Environment/Guangxi/05712/2018	G23	China-1	N2-Eurasian lineage	EA	EA	EA	EA	ZJ-5	EA(A)

Subtype	Isolate name	Genotype	HA	NA	PB2	PB1	PA	NP	M	NS
	A/Environment/Guangxi/33582/2018	G23	China-1	N2-Eurasian lineage	EA	EA	EA	EA	ZJ-5	EA(A)
	A/Environment/Guangxi/32515/2018	G23	China-1	N2-Eurasian lineage	EA	EA	EA	EA	ZJ-5	EA(A)
	A/Environment/Guangxi/24805/2018	G23	China-1	N2-Eurasian lineage	EA	EA	EA	EA	ZJ-5	EA(A)
	A/Environment/Guangdong/27250/2018	G23	China-1	N2-Eurasian lineage	EA	EA	EA	EA	ZJ-5	EA(A)
	A/Environment/Guangdong/27249/2018	G23	China-1	N2-Eurasian lineage	EA	EA	EA	EA	ZJ-5	EA(A)
	A/Environment/Guangxi/44461/2019	G23	China-1	N2-Eurasian lineage	EA	EA	EA	EA	ZJ-5	EA(A)
	A/Environment/Guangdong/34240/2019	G23	China-1	N2-Eurasian lineage	EA	EA	EA	EA	ZJ-5	EA(A)
	A/Environment/Guangxi/41094/2019	G23	China-1	N2-Eurasian lineage	EA	EA	EA	EA	ZJ-5	EA(A)
	A/Environment/Guangdong/27245/2019	G23	China-1	N2-Eurasian lineage	EA	EA	EA	EA	ZJ-5	EA(A)
	A/Environment/Fujian/44488/2019	G23	China-1	N2-Eurasian lineage	EA	EA	EA	EA	ZJ-5	EA(A)
	A/Environment/Guangxi/12090/2021	G23	China-1	N2-Eurasian lineage	EA	EA	EA	EA	ZJ-5	EA(A)
	A/Environment/Guangxi/04926/2021	G23	China-1	N2-Eurasian lineage	EA	EA	EA	EA	ZJ-5	EA(A)
	A/Environment/Guangxi/04909/2022	G23	China-1	N2-Eurasian lineage	EA	EA	EA	EA	ZJ-5	EA(A)
	A/Environment/Guangxi/79509/2014	G24	China-1	N2-Eurasian lineage	EA	EA	ZJ-5	EA	ZJ-5	EA(A)
	A/Environment/Hunan/27451/2014	G24	China-1	N2-Eurasian lineage	EA	EA	ZJ-5	EA	ZJ-5	EA(A)
	A/Environment/Hunan/11043/2016	G24	China-1	N2-Eurasian lineage	EA	EA	ZJ-5	EA	ZJ-5	EA(A)
	A/Environment/Guangxi/24894/2018	G24	China-1	N2-Eurasian lineage	EA	EA	ZJ-5	EA	ZJ-5	EA(A)
	A/Environment/Guangxi/01869/2018	G24	China-1	N2-Eurasian lineage	EA	EA	ZJ-5	EA	ZJ-5	EA(A)
	A/Environment/Guangdong/08844/2019	G24	China-1	N2-Eurasian lineage	EA	EA	ZJ-5	EA	ZJ-5	EA(A)
	A/Environment/Guangxi/10369/2021	G24	China-1	N2-Eurasian lineage	EA	EA	ZJ-5	EA	ZJ-5	EA(A)
	A/Environment/Hunan/26068/2014	G25	China-1	N2-Eurasian lineage	EA	EA	ZJ-5	ZJ-5	ZJ-5	EA(A)
	A/Environment/Hunan/37943/2015	G25	China-1	N2-Eurasian lineage	EA	EA	ZJ-5	ZJ-5	ZJ-5	EA(A)
	A/duck/Guangdong/04.22_DGCP068-P/2015_Mixed	G25	China-1	N2-Eurasian lineage	EA	EA	ZJ-5	ZJ-5	ZJ-5	EA(A)
	A/duck/Guangdong/04.22_DGCP075-P/2015_Mixed	G25	China-1	N2-Eurasian lineage	EA	EA	ZJ-5	ZJ-5	ZJ-5	EA(A)
	A/Environment/Guangdong/60350/2016	G25	China-1	N2-Eurasian lineage	EA	EA	ZJ-5	ZJ-5	ZJ-5	EA(A)
	A/Environment/Hunan/25648/2017	G25	China-1	N2-Eurasian lineage	EA	EA	ZJ-5	ZJ-5	ZJ-5	EA(A)
	A/Environment/Hunan/05861/2018	G25	China-1	N2-Eurasian lineage	EA	EA	ZJ-5	ZJ-5	ZJ-5	EA(A)
	A/Environment/Chongqing/33939/2018	G25	China-1	N2-Eurasian lineage	EA	EA	ZJ-5	ZJ-5	ZJ-5	EA(A)
	A/Environment/Guangdong/09230/2019	G25	China-1	N2-Eurasian lineage	EA	EA	ZJ-5	ZJ-5	ZJ-5	EA(A)
	A/Environment/Guangdong/09210/2019	G25	China-1	N2-Eurasian lineage	EA	EA	ZJ-5	ZJ-5	ZJ-5	EA(A)
	A/Environment/Hubei/43399/2019	G25	China-1	N2-Eurasian lineage	EA	EA	ZJ-5	ZJ-5	ZJ-5	EA(A)
	A/Environment/Hunan/26067/2014	G26	China-1	N2-Eurasian lineage	EA	EA	EA	ZJ-5	ZJ-5	EA(A)
	A/Environment/Hunan/98839/2014	G26	China-1	N2-Eurasian lineage	EA	EA	EA	ZJ-5	ZJ-5	EA(A)
	A/Environment/Guangxi/32828/2017	G26	China-1	N2-Eurasian lineage	EA	EA	EA	ZJ-5	ZJ-5	EA(A)
	A/Environment/Guangxi/32906/2018	G26	China-1	N2-Eurasian lineage	EA	EA	EA	ZJ-5	ZJ-5	EA(A)
	A/Environment/Guangdong/30702/2018	G26	China-1	N2-Eurasian lineage	EA	EA	EA	ZJ-5	ZJ-5	EA(A)
	A/Environment/Guangxi/32383/2019	G26	China-1	N2-Eurasian lineage	EA	EA	EA	ZJ-5	ZJ-5	EA(A)
	A/Environment/Guangxi/39142/2019	G26	China-1	N2-Eurasian lineage	EA	EA	EA	ZJ-5	ZJ-5	EA(A)
	A/Environment/Guangdong/09270/2019	G26	China-1	N2-Eurasian lineage	EA	EA	EA	ZJ-5	ZJ-5	EA(A)
	A/Environment/Guangdong/09263/2019	G26	China-1	N2-Eurasian lineage	EA	EA	EA	ZJ-5	ZJ-5	EA(A)
	A/Environment/Guangdong/14024/2020	G26	China-1	N2-Eurasian lineage	EA	EA	EA	ZJ-5	ZJ-5	EA(A)
	A/Environment/Hunan/02458/2014	G27	Worldwide-1	N2-Eurasian lineage	EA	EA	ZJ-5	ZJ-5	ZJ-5	EA(A)
	A/duck/Guangxi/04.10_JX050/2015_Mixed	G27	Worldwide-1	N2-Eurasian lineage	EA	EA	ZJ-5	ZJ-5	ZJ-5	EA(A)
	A/Environment/Guangdong/40751/2017	G27	Worldwide-1	N2-Eurasian lineage	EA	EA	ZJ-5	ZJ-5	ZJ-5	EA(A)
	A/chicken/Jiangsu/12.30_WZNHQ013-P/2014_Mixed	G28	China-3	N2-Eurasian lineage	H9N2	ZJ-5	ZJ-5	H9N2	ZJ-5	H9N2

Subtype	Isolate name	Genotype	HA	NA	PB2	PB1	PA	NP	M	NS
	A/duck/Jiangsu/12.18_NJLH1266-P/2014_Mixed	G29	China-3	N2-Eurasian lineage	EA	ZJ-5	ZJ-5	EA	EA	ZJ-5
	A/water/Hunan/140/2014	G30	China-3	N2-Eurasian lineage	ZJ-5	EA	ZJ-5	ZJ-5	ZJ-5	EA(A)
	A/Environment/Hunan/31561/2016	G30	China-3	N2-Eurasian lineage	ZJ-5	EA	ZJ-5	ZJ-5	ZJ-5	EA(A)
	A/Environment/Guangdong/35458/2017	G30	China-3	N2-Eurasian lineage	ZJ-5	EA	ZJ-5	ZJ-5	ZJ-5	EA(A)
	A/Environment/Chongqing/79459/2014	G31	China-3	N2-Eurasian lineage	EA	EA	EA	ZJ-5	ZJ-5	EA(A)
	A/Environment/Yunnan/01456/2015	G31	China-3	N2-Eurasian lineage	EA	EA	EA	ZJ-5	ZJ-5	EA(A)
	A/Environment/Yunnan/01457/2015	G31	China-3	N2-Eurasian lineage	EA	EA	EA	ZJ-5	ZJ-5	EA(A)
	A/Environment/Guangdong/21134/2015	G31	China-3	N2-Eurasian lineage	EA	EA	EA	ZJ-5	ZJ-5	EA(A)
	A/Environment/Hunan/18323/2014	G32	China-4	N2-Eurasian lineage	ZJ-5	ZJ-5	ZJ-5	ZJ-5	ZJ-5	ZJ-5
	A/environment/Jiangxi/05.07_NCJD0096C/2015_Mixed	G33	China-1	N2-Eurasian lineage	EA	ZJ-5	EA	ZJ-5	EA	EA(A)
	A/duck/Guangdong/04.22_DGCP070-O/2015_Mixed	G34	China-1	N2-Eurasian lineage	H6	EA	ZJ-5	ZJ-5	EA	EA(A)
	A/duck/Guangdong/04.22_DGCP064-P/2015_Mixed	G35	China-1	N2-Eurasian lineage	EA	EA	ZJ-5	ZJ-5	EA	EA(A)
	A/duck/Guangdong/04.22_DGCP078-P/2015_Mixed	G35	China-1	N2-Eurasian lineage	EA	EA	ZJ-5	ZJ-5	EA	EA(A)
	A/Duck/Guangdong/04.22_DGCP074-P/2015	G35	China-1	N2-Eurasian lineage	EA	EA	ZJ-5	ZJ-5	EA	EA(A)
	A/Environment/Hunan/10170/2016	G35	China-1	N2-Eurasian lineage	EA	EA	ZJ-5	ZJ-5	EA	EA(A)
	A/Environment/Guangxi/14185/2017	G35	China-1	N2-Eurasian lineage	EA	EA	ZJ-5	ZJ-5	EA	EA(A)
	A/environment/Jiangxi/02.05_YGYXG010/2015	G36	Europe-Asia	N2-Eurasian lineage	EA	EA	ZJ-5	EA	EA	EA(A)
	A/EV/SD/226238/2015	G37	Worldwide-1	N2-Eurasian lineage	EA	EA	EA	EA	EA	EA(A)
	A/EV/SD/226214/2015	G37	Worldwide-1	N2-Eurasian lineage	EA	EA	EA	EA	EA	EA(A)
	A/Environment/Sichuan/48404/2016	G37	Worldwide-1	N2-Eurasian lineage	EA	EA	EA	EA	EA	EA(A)
	A/Environment/Chongqing/45115/2015	G38	China-2	N2-Eurasian lineage	ZJ-5	ZJ-5	ZJ-5	ZJ-5	ZJ-5	ZJ-5
	A/Environment/Zhejiang/31298/2018	G38	China-2	N2-Eurasian lineage	ZJ-5	ZJ-5	ZJ-5	ZJ-5	ZJ-5	ZJ-5
	A/Environment/Hunan/39684/2015	G39	China-2	N2-Eurasian lineage	EA	EA	ZJ-5	ZJ-5	ZJ-5	EA(A)
	A/Environment/Hunan/34019/2017	G39	China-2	N2-Eurasian lineage	EA	EA	ZJ-5	ZJ-5	ZJ-5	EA(A)
	A/Environment/Guangxi/01885/2018	G39	China-2	N2-Eurasian lineage	EA	EA	ZJ-5	ZJ-5	ZJ-5	EA(A)
	A/Environment/Guangxi/01878/2018	G39	China-2	N2-Eurasian lineage	EA	EA	ZJ-5	ZJ-5	ZJ-5	EA(A)
	A/Environment/Guangxi/03562/2019	G39	China-2	N2-Eurasian lineage	EA	EA	ZJ-5	ZJ-5	ZJ-5	EA(A)
	A/Environment/Gansu/01469/2019	G39	China-2	N2-Eurasian lineage	EA	EA	ZJ-5	ZJ-5	ZJ-5	EA(A)
	A/Environment/Guangxi/08779/2020	G39	China-2	N2-Eurasian lineage	EA	EA	ZJ-5	ZJ-5	ZJ-5	EA(A)
	A/Environment/Hunan/34583/2015	G40	China-2	N2-Eurasian lineage	ZJ-5	EA	EA	EA	ZJ-5	EA(A)
	A/duck/Hunan/04.14_YYGK0451-Z-O/2015_Mixed	G41	China-2	N2-Eurasian lineage	H6	EA	ZJ-5	ZJ-5	ZJ-5	EA(A)
	A/environment/Jiangxi/01.08_YGCB009/2015_Mixed	G42	China-3	N2-Eurasian lineage	EA	EA	ZJ-5	EA	EA	EA(A)
	A/Chicken/Jiangxi/01.08_YGCB018-P/2015	G42	China-3	N2-Eurasian lineage	EA	EA	ZJ-5	EA	EA	EA(A)
	A/Chicken/Jiangxi/02.05_YGYXG001-P/2015	G42	China-3	N2-Eurasian lineage	EA	EA	ZJ-5	EA	EA	EA(A)
	A/duck/Jiangxi/01.08_YGCB023-P/2015_Mixed	G42	China-3	N2-Eurasian lineage	EA	EA	ZJ-5	EA	EA	EA(A)
	A/Environment/Jiangxi/27771/2015	G42	China-3	N2-Eurasian lineage	EA	EA	ZJ-5	EA	EA	EA(A)
	A/duck/Jiangxi/01.14_NCJD033-P/2015_Mixed	G43	China-3	N2-Eurasian lineage	EA	ZJ-5	ZJ-5	ZJ-5	ZJ-5	EA(A)
	A/Duck/Jiangxi/01.14_NCJD038-P/2015	G43	China-3	N2-Eurasian lineage	EA	ZJ-5	ZJ-5	ZJ-5	ZJ-5	EA(A)
	A/Duck/Jiangxi/01.14_NCJD035-P/2015	G43	China-3	N2-Eurasian lineage	EA	ZJ-5	ZJ-5	ZJ-5	ZJ-5	EA(A)
	A/duck/Guangdong/04.23_DGQTSJ126-P/2015_Mixed	G44	China-3	N2-Eurasian lineage	ZJ-5	ZJ-5	ZJ-5	ZJ-5	ZJ-5	EA(A)
	A/duck/Guangdong/04.23_DGQTSJ123-P/2015_Mixed	G44	China-3	N2-Eurasian lineage	ZJ-5	ZJ-5	ZJ-5	ZJ-5	ZJ-5	EA(A)
	A/duck/Guangdong/04.23_DGQTSJ122-P/2015_Mixed	G44	China-3	N2-Eurasian lineage	ZJ-5	ZJ-5	ZJ-5	ZJ-5	ZJ-5	EA(A)
	A/environment/Guangdong/04.23_DGQTSJ037/2015_Mixed	G44	China-3	N2-Eurasian lineage	ZJ-5	ZJ-5	ZJ-5	ZJ-5	ZJ-5	EA(A)
	A/duck/Guangdong/04.23_DGQTSJ126-O/2015_Mixed	G45	China-3	N2-Eurasian lineage	H6	ZJ-5	ZJ-5	ZJ-5	ZJ-5	EA(A)

Subtype	Isolate name	Genotype	HA	NA	PB2	PB1	PA	NP	M	NS
	A/Environment/Guangdong/15241/2015	G46	China-4	N2-Eurasian lineage	EA	EA	EA	ZJ-5	ZJ-5	EA(A)
	A/Environment/Guangdong/15077/2015	G47	China-4	N2-Eurasian lineage	ZJ-5	EA	ZJ-5	ZJ-5	ZJ-5	EA(A)
	A/Bean_Goose/Hubei/chenhu_XVI35-1/2016	G48	Europe-Asia	North America-1	EA	EA	EA	EA	EA	EA(A)
	A/Chicken/Ganzhou/GZ157/2016	G49	Worldwide-1	N2-Eurasian lineage	ZJ-5	EA	ZJ-5	ZJ-5	ZJ-5	EA(A)
	A/Chicken/Ganzhou/GZ43/2016	G49	Worldwide-1	N2-Eurasian lineage	ZJ-5	EA	ZJ-5	ZJ-5	ZJ-5	EA(A)
	A/Environment/Anhui/33167/2016	G49	Worldwide-1	N2-Eurasian lineage	ZJ-5	EA	ZJ-5	ZJ-5	ZJ-5	EA(A)
	A/Environment/Sichuan/32281/2016	G50	Worldwide-1	N2-Eurasian lineage	EA	EA	EA	EA	NA	EA(A)
	A/Environment/Chongqing/38160/2016	G51	Worldwide-1	N2-Eurasian lineage	EA	EA	EA	EA	EA	EA(B)
	A/Environment/Chongqing/22907/2016	G51	Worldwide-1	N2-Eurasian lineage	EA	EA	EA	EA	EA	EA(B)
	A/Duck/Jiangsu/YZ916/2016	G52	China-2	N2-Eurasian lineage	ZJ-5	ZJ-5	EA	ZJ-5	ZJ-5	EA(A)
	A/Environment/Jiangsu/06689/2016	G53	China-2	N2-Eurasian lineage	EA	EA	EA	ZJ-5	ZJ-5	EA(A)
	A/Environment/Chongqing/41153/2019	G53	China-2	N2-Eurasian lineage	EA	EA	EA	ZJ-5	ZJ-5	EA(A)
	A/Environment/Guangxi/39500/2019	G53	China-2	N2-Eurasian lineage	EA	EA	EA	ZJ-5	ZJ-5	EA(A)
	A/Environment/Guangxi/32152/2017	G54	China-1	N2-Eurasian lineage	ZJ-5	EA	EA	EA	ZJ-5	EA(A)
	A/Environment/Guangxi/28076/2018	G54	China-1	N2-Eurasian lineage	ZJ-5	EA	EA	EA	ZJ-5	EA(A)
	A/Environment/Fujian/39244/2019	G54	China-1	N2-Eurasian lineage	ZJ-5	EA	EA	EA	ZJ-5	EA(A)
	A/Environment/Guangdong/27246/2019	G54	China-1	N2-Eurasian lineage	ZJ-5	EA	EA	EA	ZJ-5	EA(A)
	A/Environment/Fujian/10644/2022	G54	China-1	N2-Eurasian lineage	ZJ-5	EA	EA	EA	ZJ-5	EA(A)
	A/Environment/Fujian/10650/2022	G54	China-1	N2-Eurasian lineage	ZJ-5	EA	EA	EA	ZJ-5	EA(A)
	A/Environment/Fujian/04958/2017	G55	China-1	N2-Eurasian lineage	EA	ZJ-5	EA	EA	ZJ-5	EA(A)
	A/Environment/Hunan/25643/2017	G56	China-1	N2-Eurasian lineage	ZJ-5	EA	EA	ZJ-5	ZJ-5	EA(A)
	A/Environment/Hunan/34012/2017	G56	China-1	N2-Eurasian lineage	ZJ-5	EA	EA	ZJ-5	ZJ-5	EA(A)
	A/Environment/Hunan/04235/2018	G56	China-1	N2-Eurasian lineage	ZJ-5	EA	EA	ZJ-5	ZJ-5	EA(A)
	A/Environment/Hunan/28854/2018	G56	China-1	N2-Eurasian lineage	ZJ-5	EA	EA	ZJ-5	ZJ-5	EA(A)
	A/Environment/Hunan/40453/2019	G56	China-1	N2-Eurasian lineage	ZJ-5	EA	EA	ZJ-5	ZJ-5	EA(A)
	A/Environment/Hunan/40442/2019	G56	China-1	N2-Eurasian lineage	ZJ-5	EA	EA	ZJ-5	ZJ-5	EA(A)
	A/Environment/Hunan/05143/2020	G56	China-1	N2-Eurasian lineage	ZJ-5	EA	EA	ZJ-5	ZJ-5	EA(A)
	A/Environment/Hunan/00548/2021	G56	China-1	N2-Eurasian lineage	ZJ-5	EA	EA	ZJ-5	ZJ-5	EA(A)
	A/Environment/Hunan/05581/2021	G56	China-1	N2-Eurasian lineage	ZJ-5	EA	EA	ZJ-5	ZJ-5	EA(A)
	A/Environment/Guangxi/23558/2017	G57	Worldwide-1	N2-Eurasian lineage	ZJ-5	EA	EA	ZJ-5	ZJ-5	EA(A)
	A/Environment/Guangxi/32060/2017	G58	Worldwide-1	N2-Eurasian lineage	EA	EA	EA	ZJ-5	EA	EA(B)
	A/Environment/Hubei/33702/2017	G59	China-2	N2-Eurasian lineage	EA	EA	ZJ-5	EA	ZJ-5	EA(A)
	A/Environment/Hubei/34289/2017	G60	China-3	N2-Eurasian lineage	EA	EA	ZJ-5	ZJ-5	EA	EA(A)
	A/Environment/Guangdong/32693/2018	G61	China-1	N2-Eurasian lineage	EA	EA	EA	EA	ZJ-5	EA(B)
	A/Environment/Guangdong/27727/2018	G62	China-1	N2-Eurasian lineage	ZJ-5	ZJ-5	ZJ-5	ZJ-5	ZJ-5	EA(A)
	A/Environment/Guangxi/33095/2018	G62	China-1	N2-Eurasian lineage	ZJ-5	ZJ-5	ZJ-5	ZJ-5	ZJ-5	EA(A)
	A/Environment/Chongqing/01458/2021	G62	China-1	N2-Eurasian lineage	ZJ-5	ZJ-5	ZJ-5	ZJ-5	ZJ-5	EA(A)
	A/Environment/Guangxi/24790/2018	G63	China-1	N2-Eurasian lineage	ZJ-5	EA	ZJ-5	ZJ-5	ZJ-5	EA(A)
	A/Environment/Guangxi/45299/2019	G63	China-1	N2-Eurasian lineage	ZJ-5	EA	ZJ-5	ZJ-5	ZJ-5	EA(A)
	A/Environment/Hubei/43318/2019	G63	China-1	N2-Eurasian lineage	ZJ-5	EA	ZJ-5	ZJ-5	ZJ-5	EA(A)
	A/Environment/Chongqing/01452/2021	G63	China-1	N2-Eurasian lineage	ZJ-5	EA	ZJ-5	ZJ-5	ZJ-5	EA(A)
	A/Environment/Chongqing/33926/2018	G64	Worldwide-1	N2-Eurasian lineage	EA	EA	ZJ-5	EA	ZJ-5	EA(A)
	A/Environment/Guangdong/27940/2018	G65	Worldwide-1	N2-Eurasian lineage	EA	EA	EA	EA	ZJ-5	EA(A)
	A/Environment/Guangdong/30984/2018	G66	China-2	N2-Eurasian lineage	EA	ZJ-5	ZJ-5	EA	ZJ-5	EA(A)
	A/Environment/Guangxi/33056/2018	G67	China-2	N2-Eurasian lineage	ZJ-5	EA	ZJ-5	EA	EA	EA(A)



Subtype	Isolate name	Genotype	HA	NA	PB2	PB1	PA	NP	M	NS
	A/Environment/Zhejiang/02021/2018	G68	China-2	N2-Eurasian lineage	ZJ-5	ZJ-5	ZJ-5	ZJ-5	ZJ-5	EA(A)
	A/Environment/Guangdong/27729/2018	G69	China-4	N2-Eurasian lineage	ZJ-5	EA	ZJ-5	EA	ZJ-5	EA(A)
	A/Environment/Guangxi/45278/2019	G70	Worldwide-1	N2-Eurasian lineage	EA	EA	EA	ZJ-5	ZJ-5	EA(A)
	A/Environment/Guangdong/34622/2019	G71	China-2	N2-Eurasian lineage	ZJ-5	EA	ZJ-5	ZJ-5	ZJ-5	EA(A)
	A/duck/China/Influenza_A_virus/2019	G72	China-2	N2-Eurasian lineage	EA	EA	ZJ-5	ZJ-5	EA	EA(A)
	A/Environment/Sichuan/03131/2021	G73	China-2	N2-Eurasian lineage	ZJ-5	EA	EA	ZJ-5	ZJ-5	EA(A)
	A/Environment/Sichuan/03175/2021	G73	China-2	N2-Eurasian lineage	ZJ-5	EA	EA	ZJ-5	ZJ-5	EA(A)
H3N3	A/Duck/Zhejiang/5/2011	G1	China-4	N3-Eurasian lineage	ZJ-5	ZJ-5	ZJ-5	ZJ-5	ZJ-5	ZJ-5
	A/Duck/Zhejiang/D16/2013	G2	Worldwide-1	N3-Eurasian lineage	EA	EA	EA	EA	EA	EA(A)
	A/Duck/Zhejiang/D17/2013	G2	Worldwide-1	N3-Eurasian lineage	EA	EA	EA	EA	EA	EA(A)
	A/Duck/Zhejiang/D18/2013	G2	Worldwide-1	N3-Eurasian lineage	EA	EA	EA	EA	EA	EA(A)
	A/Curlew/Zhuanghe/ZH-47/2013	G3	Asia	N3-Eurasian lineage	EA	EA	EA	EA	EA	EA(A)
	A/environment/Jiangxi/05.07_NCJD0042C/2015_Mixed	G4	China-1	N3-Eurasian lineage	EA	EA	ZJ-5	ZJ-5	ZJ-5	EA(A)
	A/Environment/Hunan/03259/2015	G5	China-1	N3-Eurasian lineage	EA	EA	EA	EA	EA	EA(A)
	A/duck/Jiangxi/01.14_NCJD059-P/2015_Mixed	G6	China-3	N3-Eurasian lineage	ZJ-5	ZJ-5	ZJ-5	EA	ZJ-5	EA(A)
	A/Environment/Fujian/02754/2016	G7	China-1	N3-Eurasian lineage	ZJ-5	ZJ-5	ZJ-5	ZJ-5	ZJ-5	EA(A)
	A/Environment/Hunan/13561/2020	G7	China-1	N3-Eurasian lineage	ZJ-5	ZJ-5	ZJ-5	ZJ-5	ZJ-5	EA(A)
	A/Duck/Fujian/SD063/2017	G8	China-1	N3-Eurasian lineage	ZJ-5	ZJ-5	ZJ-5	EA	ZJ-5	EA(B)
	A/Environment/Guangxi/29409/2018	G9	China-1	N3-Eurasian lineage	EA	EA	EA	EA	ZJ-5	EA(A)
	A/Environment/Guangdong/34076/2019	G10	China-2	N3-Eurasian lineage	EA	EA	EA	ZJ-5	ZJ-5	EA(A)
	A/Environment/Fujian/14131/2020	G11	China-1	N3-Eurasian lineage	EA	ZJ-5	ZJ-5	EA	ZJ-5	EA(B)
H3N6	A/Pigeon/Guangxi/020P/2009	G1	Eurasian lineage	N6-Eurasian lineage	EA	EA	EA	EA	EA	EA(A)
	A/Environment/Zhejiang/7/09	G2	China-4	N6-Eurasian lineage	EA	EA	ZJ-5	ZJ-5	EA	EA(A)
	A/Duck/Jiangsu/4/2010	G3	Asia	N6-Eurasian lineage	EA	EA	EA	EA	H6	EA(A)
	A/Curlew/Zhuanghe/ZH-64/2013	G4	Asia	N6-Eurasian lineage	EA	EA	EA	EA	EA	EA(A)
	A/Curlew/Zhuanghe/ZH-65/2013	G4	Asia	N6-Eurasian lineage	EA	EA	EA	EA	EA	EA(A)
	A/Duck/Zhejiang/D1-1/2013	G5	China-4	N6-Eurasian lineage	ZJ-5	ZJ-5	ZJ-5	ZJ-5	ZJ-5	ZJ-5
	A/Duck/Zhejiang/D1-2/2013	G5	China-4	N6-Eurasian lineage	ZJ-5	ZJ-5	ZJ-5	ZJ-5	ZJ-5	ZJ-5
	A/Duck/Zhejiang/D1-3/2013	G5	China-4	N6-Eurasian lineage	ZJ-5	ZJ-5	ZJ-5	ZJ-5	ZJ-5	ZJ-5
	A/Duck/Guangxi/175D12/2014	G6	China-1	H5N6 sublineage	H5N6	EA	ZJ-5	H5N6	ZJ-5	EA(A)
	A/Duck/Hunan/146/2014	G7	China-2	H5N6 sublineage	H5N6	H5N6	H5N6	H5N6	H5N6	H5N6
	A/duck/Guangdong/04.16_SZLGWL012/2015_Mixed	G8	China-1	H5N6 sublineage	H6	EA	H9N2	H9N2	ZJ-5	H9N2
	A/Duck/Hunan/161/2015	G9	China-1	H5N6 sublineage	ZJ-5	EA	ZJ-5	ZJ-5	ZJ-5	EA(A)
	A/Duck/Hubei/ZYSYF9/2015	G10	China-1	waterfowl H6N6	ZJ-5	ZJ-5	EA	ZJ-5	ZJ-5	EA(A)
	A/Duck/Hubei/ZYSYF2/2015	G10	China-1	waterfowl H6N6	ZJ-5	ZJ-5	EA	ZJ-5	ZJ-5	EA(A)
	A/Duck/Hubei/ZYSYF21/2015	G10	China-1	waterfowl H6N6	ZJ-5	ZJ-5	EA	ZJ-5	ZJ-5	EA(A)
	A/Duck/Hubei/ZYSYF4/2015	G11	China-1	N6-Eurasian lineage	EA	EA	ZJ-5	ZJ-5	ZJ-5	EA(A)
	A/Duck/Hubei/ZYSYF18/2015	G11	China-1	N6-Eurasian lineage	EA	EA	ZJ-5	ZJ-5	ZJ-5	EA(A)
	A/Duck/Hubei/ZYSYF12/2015	G11	China-1	N6-Eurasian lineage	EA	EA	ZJ-5	ZJ-5	ZJ-5	EA(A)
	A/Duck/Hunan/7/2015	G12	China-1	H5N6 sublineage	EA	EA	EA	ZJ-5	ZJ-5	EA(A)
	A/environment/Jiangxi/01.14_NCJD024/2015_Mixed	G13	China-1	waterfowl H6N6	H6	EA	H6	EA	EA	EA(A)
	A/environment/Jiangxi/01.08_YGCB016/2015_Mixed	G14	Europe-Asia	H5N6 sublineage	H6	EA	ZJ-5	EA	EA	H5N6
	A/duck/Jiangxi/01.14_NCJD060-P/2015_Mixed	G15	Europe-Asia	H5N6 sublineage	H5N6	EA	ZJ-5	EA	EA	EA(A)
	A/Environment/Sichuan/03404/2015	G16	Worldwide-1	N6-Eurasian lineage	EA	EA	EA	EA	EA	EA(B)

Subtype	Isolate name	Genotype	HA	NA	PB2	PB1	PA	NP	M	NS
	A/Environment/Sichuan/03397/2015	G16	Worldwide-1	N6-Eurasian lineage	EA	EA	EA	EA	EA	EA(B)
	A/duck/Jiangxi/01.14_NCJD057-P/2015_Mixed	G17	China-3	H5N6 sublineage	ZJ-5	ZJ-5	ZJ-5	ZJ-5	ZJ-5	EA(A)
H3N8	A/Duck/Shanghai/120-1/2009	G1	Asia	N8-Eurasian lineage	EA	EA	EA	EA	ZJ-5	EA(A)
	A/jilongtumo/Jiangxi/29/09	G2	China-2	N8-North American lineage	EA	EA	EA	EA	EA	EA(A)
	A/Duck/Wuxi/7275/2016	G2	China-2	N8-North American lineage	EA	EA	EA	EA	EA	EA(A)
	A/Goose/Guangxi/020G/2009	G3	Eurasian lineage	N8-North American lineage	EA	EA	EA	EA	EA	EA(A)
	A/Duck/Nanjing/A1591-1/2010	G4	China-3	N8-North American lineage	EA	EA	ZJ-5	ZJ-5	ZJ-5	EA(A)
	A/Chicken/Nanjing/B854-2/2011	G4	China-3	N8-North American lineage	EA	EA	ZJ-5	ZJ-5	ZJ-5	EA(A)
	A/Duck/Hunan/199/2014	G4	China-3	N8-North American lineage	EA	EA	ZJ-5	ZJ-5	ZJ-5	EA(A)
	A/jixue/Zhejiang/04/2010	G5	China-4	N8-North American lineage	ZJ-5	EA	ZJ-5	ZJ-5	EA	EA(A)
	A/environment/Hunan/S4350/2011	G6	China-1	N8-North American lineage	EA	EA	EA	EA	EA	EA(A)
	A/Environment/Hunan/37939/2015	G6	China-1	N8-North American lineage	EA	EA	EA	EA	EA	EA(A)
	A/Duck/Hunan/S1256/2012	G7	Worldwide-1	N8-Eurasian lineage	EA	EA	EA	EA	EA	EA(B)
	A/Duck/Hunan/S1824/2012	G8	China-4	N8-North American lineage	EA	EA	EA	EA	EA	EA(A)
	A/Teal/Shanghai/SH-90/2013	G9	Worldwide-1	N8-North American lineage	EA	EA	EA	EA	EA	NA
	A/Duck/Zhejiang/4812/2013	G10	China-2	N8-North American lineage	EA	ZJ-5	ZJ-5	EA	EA	ZJ-5
	A/Duck/Zhejiang/D1-6/2013	G11	China-4	N8-Eurasian lineage	ZJ-5	ZJ-5	ZJ-5	ZJ-5	ZJ-5	ZJ-5
	A/Mallard/Jiangxi/G98/2014	G12	Europe-Asia	N8-Eurasian lineage	EA	H5N6	EA	EA	H5N6	EA(A)
	A/Duck/Guangdong/04.22_DGCP083-P/2015	G13	China-1	N8-North American lineage	EA	EA	EA	ZJ-5	ZJ-5	EA(A)
	A/Duck/Guangdong/03.26_DGCP072-P/2015	G14	China-1	N8-North American lineage	EA	EA	ZJ-5	ZJ-5	EA	EA(A)
	A/duck/Hunan/04.14_YYGK431-P/2015_Mixed	G15	Europe-Asia	N8-North American lineage	EA	EA	EA	EA	EA	EA(A)
	A/Environment/Hunan/46780/2015	G16	Worldwide-1	N8-North American lineage	EA	EA	ZJ-5	ZJ-5	ZJ-5	EA(A)
	A/Chicken/Wuxi/4859/2015	G17	China-2	N8-North American lineage	EA	EA	ZJ-5	H5N1	EA	EA(A)
	A/Mallard/Xuyi/14/2015	G18	China-3	N8-North American lineage	EA	EA	ZJ-5	EA	ZJ-5	EA(A)
	A/duck/Jiangxi/01.14_NCJD031-P/2015_Mixed	G19	China-3	N8-North American lineage	EA	EA	ZJ-5	EA	EA	EA(B)
	A/duck/Jiangxi/01.14_NCJD058-P/2015_Mixed	G20	China-3	N8-North American lineage	ZJ-5	ZJ-5	ZJ-5	ZJ-5	ZJ-5	EA(A)
	A/Duck/Jiangxi/01.14_NCJD028-P/2015	G21	China-3	N8-North American lineage	EA	ZJ-5	ZJ-5	ZJ-5	ZJ-5	EA(A)
	A/duck/Jiangxi/01.14_NCJD027-P/2015_Mixed	G22	China-3	N8-North American lineage	EA	ZJ-5	ZJ-5	ZJ-5	ZJ-5	EA(B)
	A/Environment/Guangxi/32891/2018	G23	China-2	N8-North American lineage	ZJ-5	ZJ-5	ZJ-5	ZJ-5	ZJ-5	EA(A)
	A/Environment/Guangxi/03561/2019	G24	Worldwide-1	N8-Eurasian lineage	EA	EA	EA	EA	EA	EA(A)
	A/Environment/Fujian/10643/2022	G25	China-1	N8-North American lineage	H9N2	H9N2	H9N2	H9N2	H9N2	H9N2
	A/Environment/Fujian/10648/2022	G25	China-1	N8-North American lineage	H9N2	H9N2	H9N2	H9N2	H9N2	H9N2
	A/Environment/Guangxi/04905/2022	G25	China-1	N8-North American lineage	H9N2	H9N2	H9N2	H9N2	H9N2	H9N2
	A/Henan/4-10CNIC/2022	G25	China-1	N8-North American lineage	H9N2	H9N2	H9N2	H9N2	H9N2	H9N2
	A/Changsha/1000/2022	G25	China-1	N8-North American lineage	H9N2	H9N2	H9N2	H9N2	H9N2	H9N2

\*Abbreviation of sublineages in internal genes: ZJ-5, ZJ-5 sublineage (gene pool); H6, waterfowl H6; H9N2, poultry H9N2(ZJ-HJ/07); H5N6, H5N6 sublineage; H5N1, poultry H5N1; EA, Eurasian wild bird gene pool; EA(A), Eurasian wild bird gene pool (allele A); EA(B), Eurasian wild bird gene pool (allele B); NA, North American wild bird gene pool.

**Appendix Table 5.** List of key molecular markers of the avian H3 subtype viruses and human H3N8 viruses in China

Protein	Biological Effect	Mutations	Amino Acids	Avian					Human
				H3N2	H3N3	H3N6	H3N8	H3N0/N1 /N9#	H3N8
HA	Altered receptor specificity	E190D	E	289	19	32	54	80	2
			D	2	0	0	1	0	
		Q226L	Q	291	19	32	54	81	2
		G228S	G	291	19	32	54	81	1
NA	Reduced susceptibility to neuraminidase inhibitors	E119V/A/D*	S/G	0	0	0	0	0	1
			E	309	18	27	49	-	2
		G	1	0	0	0	-	0	
		Q136L*	Q	310	18	27	48	-	2
		L	0	0	0	1	-	0	
		H274Y*	H	309	18	27	49	-	2
PB2	Increase polymerase activity in avian and mammalian cell line	I292V	R	1	0	0	0	-	0
			I	214	14	29	36	2	0
			V	19	2	3	7	0	2
			M	0	0	0	1	0	0
	Efficient replication in mammalian and avian cells, and higher virulence in mice	R389K	T	0	0	0	0	1	0
			R	221	16	32	39	3	0
			K	11	0	0	5	0	2
			A	225	16	25	43	2	0
			T	4	0	5	0	1	0
			V	3	0	2	1	0	2
	Increase the virulence in mammals	V598T/I	T	231	16	32	41	3	0
			V	1	0	0	3	0	2
		E627K	E	232	16	32	44	3	0
			K	0	0	0	0	0	1†
Host signature amino acids (avian to human)	K702R	V	0	0	0	0	0	1‡	
		D	232	16	32	44	3	2	
		K	232	16	32	41	3	1	
		R	0	0	0	3	0	1	
PB1	Increase replication or virulence in mammals	I368V	I	226	10	29	38	2	0
			V	7	6	3	5	0	2
			M	1	0	0	0	0	0
PA	Reduced susceptibility to endonuclease inhibitors	I38M/T/S/L	I	291	17	32	54	81	2
			V	0	2	0	0	0	0
			K	0	15	31	41	2	0
			R	6	0	1	3	0	2
	Host signature amino acids (avian to human)	S409N	S	225	15	28	40	2	0
			N	5	0	4	4	0	2
			D	237	17	33	49	2	2
			N	0	1	0	3	0	2
M1	Altered virulence in mice	N30D	D	237	17	33	49	2	2
			A	237	17	33	49	2	2
	Impacts growth and transmission in the guinea pig model	P41A	A	237	17	33	49	2	2
			A	237	17	33	49	2	2
	Enhanced infectivity	T215A	T	237	17	31	44	2	0
			A	0	0	2	5	0	2
			R	237	16	33	43	2	0
			K	0	1	0	6	0	2
			S	237	16	31	42	2	0
			N	0	1	2	7	0	2
M2	Enhanced infectivity	D21G	K	237	16	33	46	2	0
			N	0	1	0	3	0	2
			D	76	11	27	35	2	0
			G	157	6	6	14	0	2
			V	3	0	0	0	0	0
			D/G	1	0	0	0	0	0
Reduced susceptibility to amantadine and rimantadine	V27A	V	222	17	30	41	2	2	
		I	15	0	2	8	0	0	
		A	0	0	1	0	0	0	
		S	226	16	33	46	2	0	
	S31N/G	S		226	16	33	46	2	0

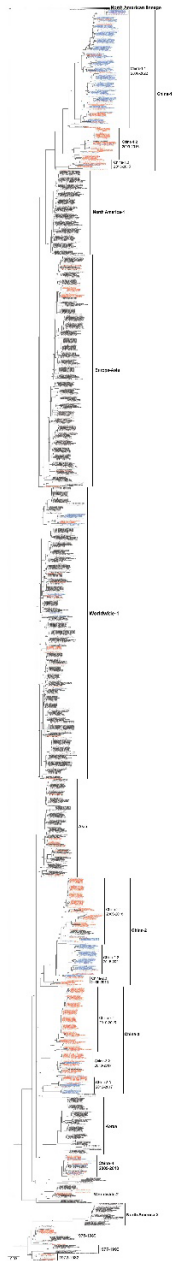
Protein	Biological Effect	Mutations	Amino Acids	Avian				Human	
				H3N2	H3N3	H3N6	H3N8	H3N0/N1 /N9#	H3N8
				N	11	1	0	3	0
NS1	Altered virulence in mice	P42S	S	237	13	31	35	5	2
			A	10	3	2	11	1	0
			L103F	F	234	12	30	32	5
		I106M	Y	10	3	2	11	1	0
			L	3	1	1	3	0	2
			M	246	15	32	43	6	0
		E227R	I	1	1	1	3	0	2
			E	246	15	32	43	6	0
			K	1	1	1	3	0	2
		S228P	S	246	15	32	43	6	0
			P	1	1	1	3	0	2

\*N2 numbering system was used.

#NA genes of H3N1 and H3N9 avian influenza viruses were not included in this analysis. Key molecular markers of each of H3N8 G25 viruses are shown in Appendix Figure 14.

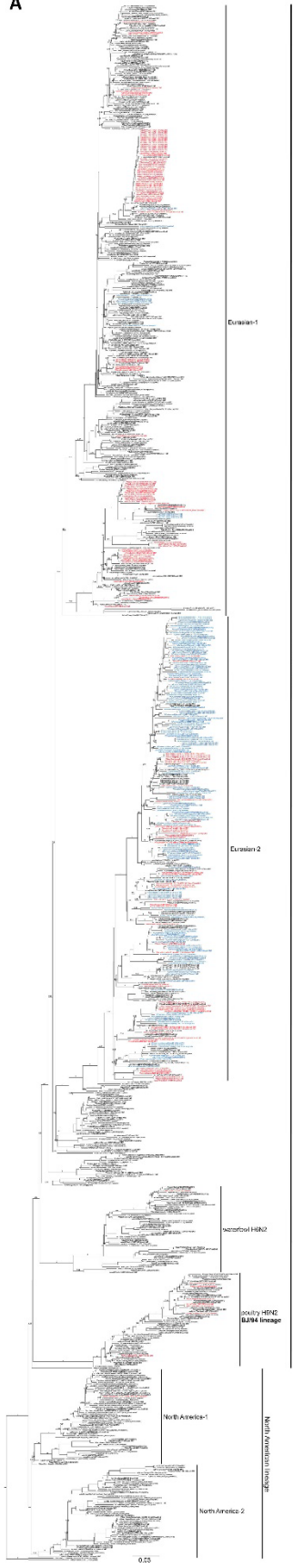
†A/Henan/4-10CNIC/2022(H3N8).

‡A/Changsha/1000/2022(H3N8).



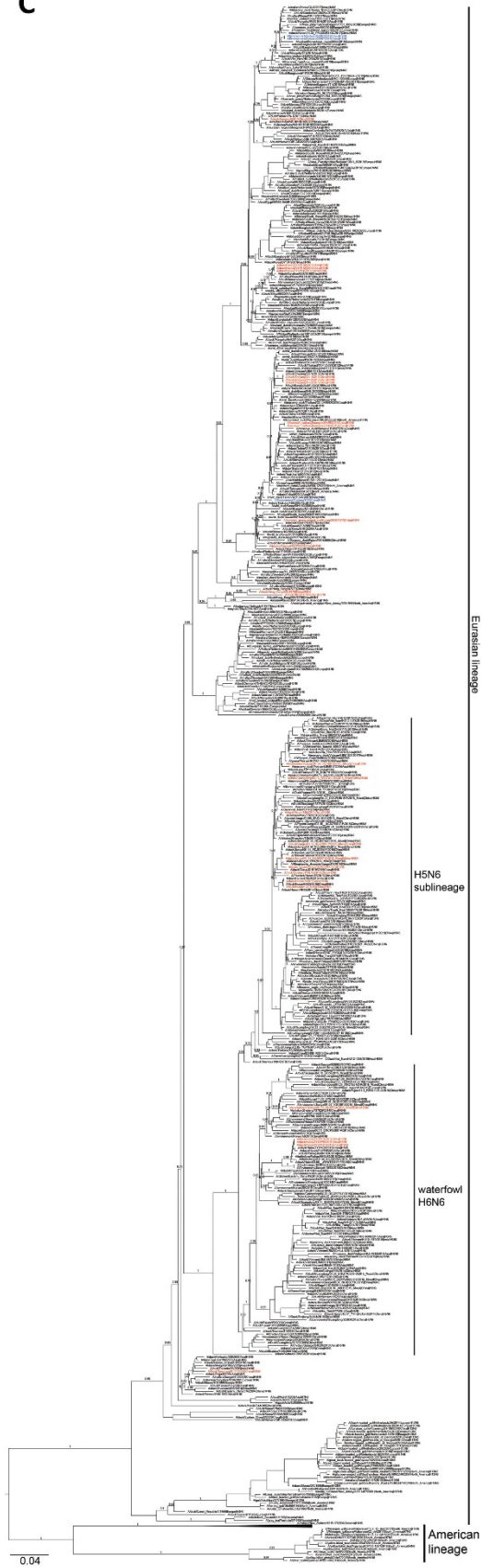
**Appendix Figure 1.** Phylogenetic tree of H3 hemagglutinin (HA) genes. Maximum likelihood tree (n=2871). H3 strains sequenced in this study are in steel blue. Other H3 AIVs in China are in red. Two human viruses are in violet and marked with triangles. A mini group containing two human and three environmental H3N8 viruses is denoted in the dashed box. Branch lengths are scaled according to the numbers of substitutions per site. Branch support values of selected nodes are shown. Sublineages and subgroups are labelled with vertical lines on the right.

A



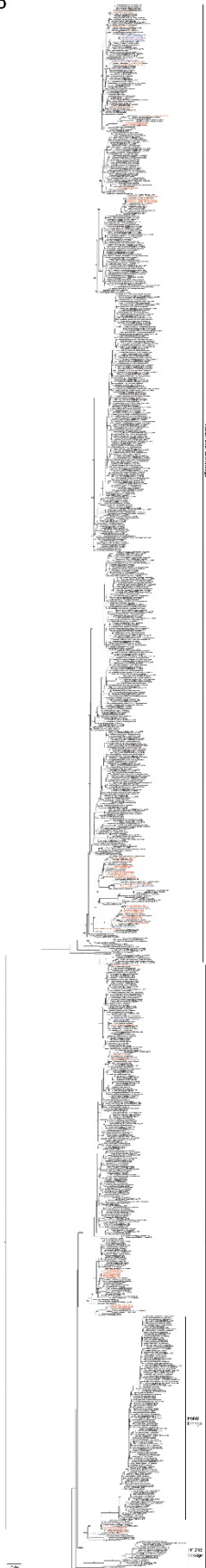


C



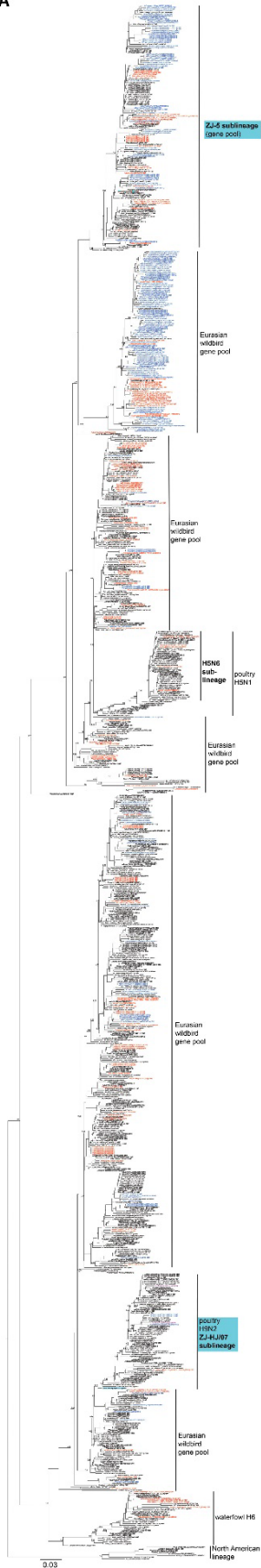


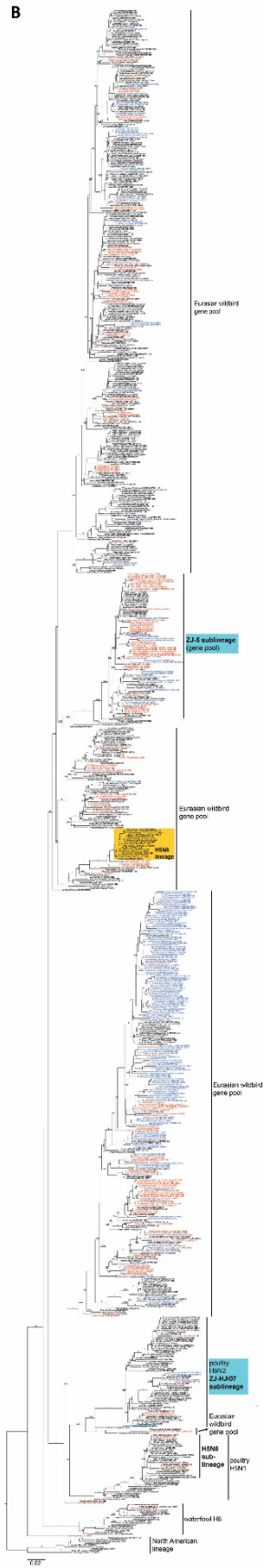
D



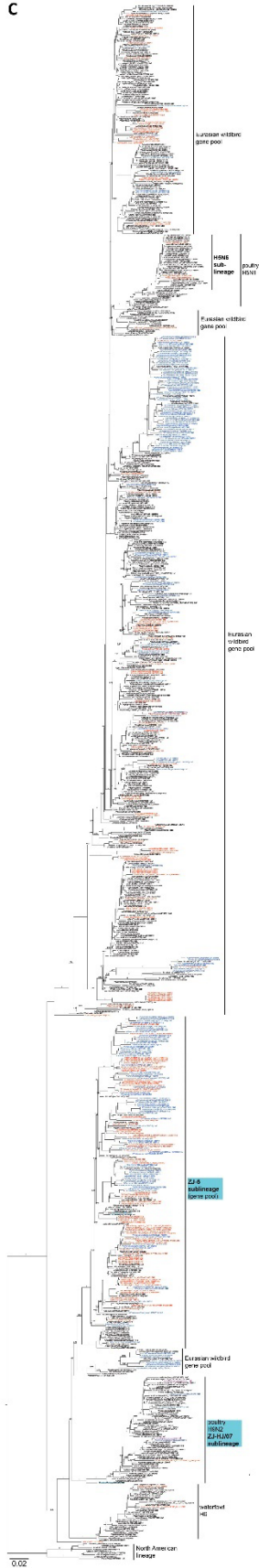
**Appendix Figure 2.** Phylogenetic trees of neuraminidase (NA) genes. Maximum likelihood tree of N2 (n=876, A), N3 (n=253, B), N6 (n=941, C), and N8 (n=1106, D) genes. H3 strains sequenced in this study are in steel blue. Other H3 AIVs in China are in red. For N3 gene (B), human H10N3 virus is indicated by orange and marked with a dot. For N8 gene (D), human H3N8 and H10N8 viruses are indicated by violet (triangles) and purple (dot), respectively. Branch lengths are scaled according to the numbers of substitutions per site. Branch support values of selected nodes are shown. Sublineages and subgroups are labelled with vertical lines on the right.

A

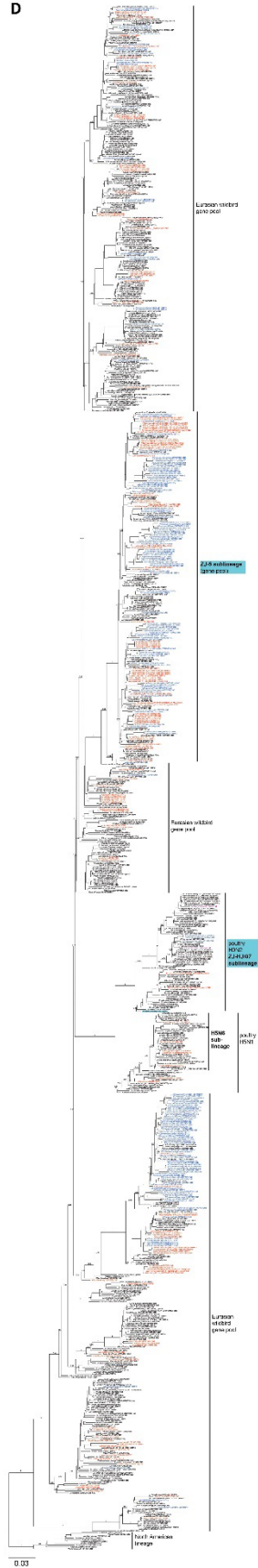


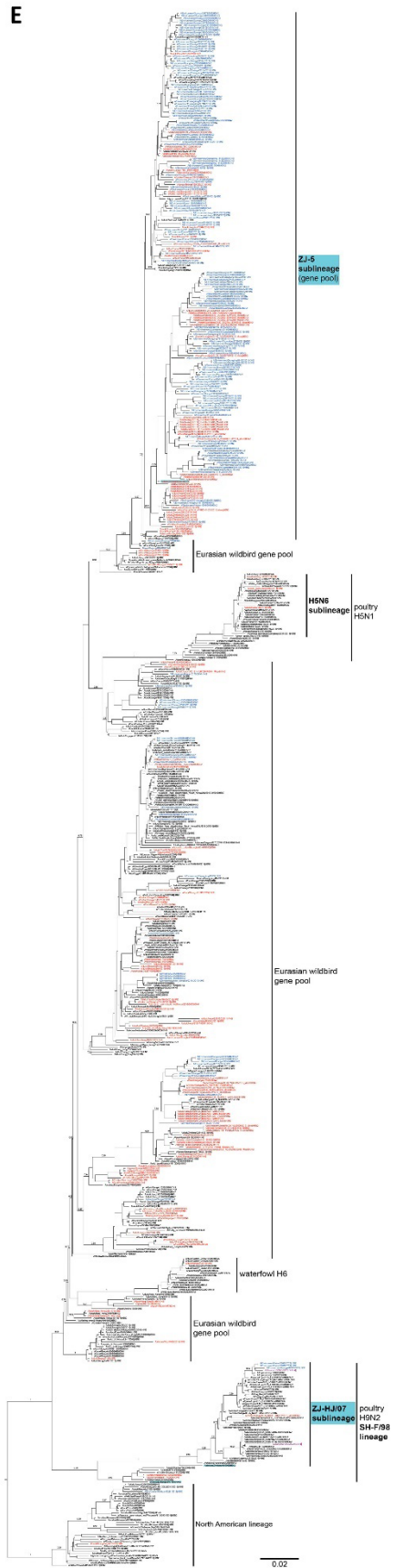


C

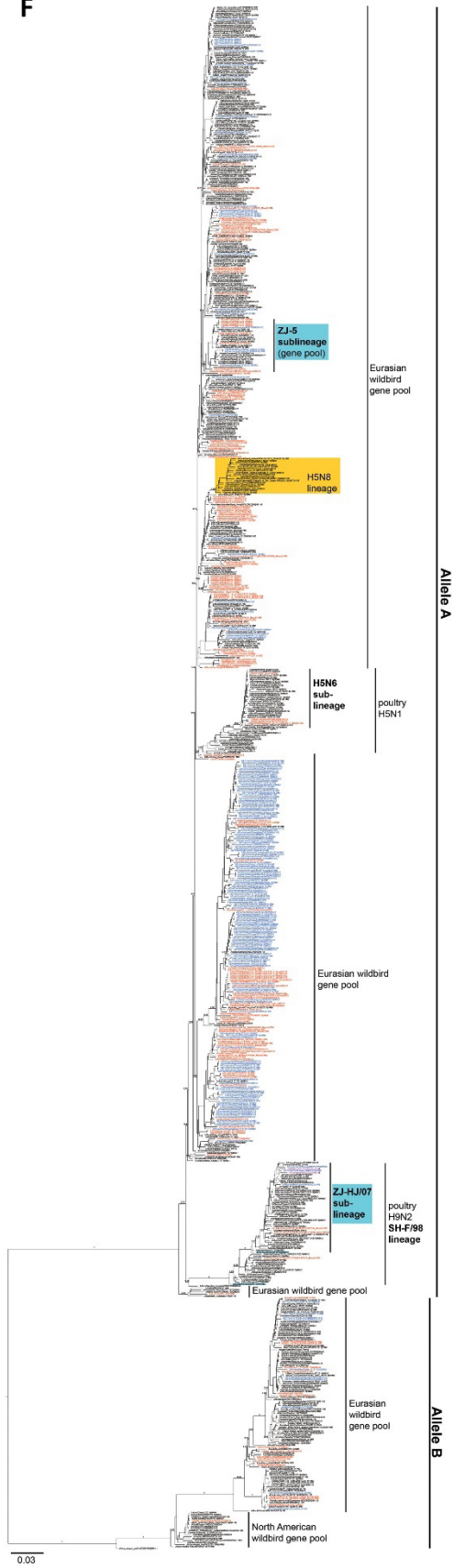


D



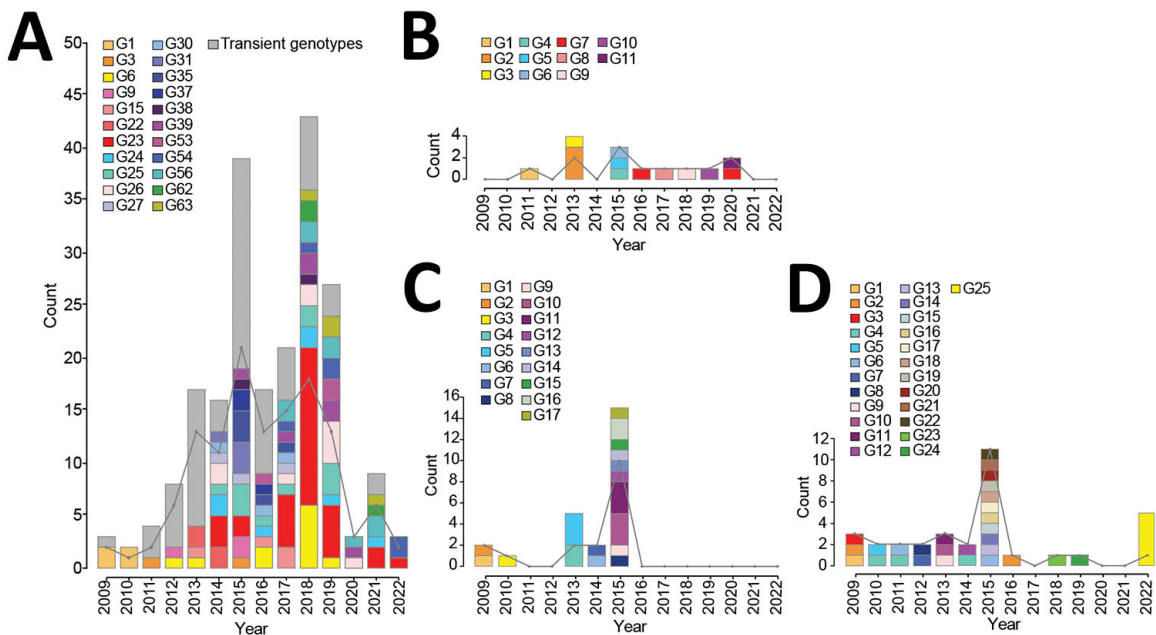


F

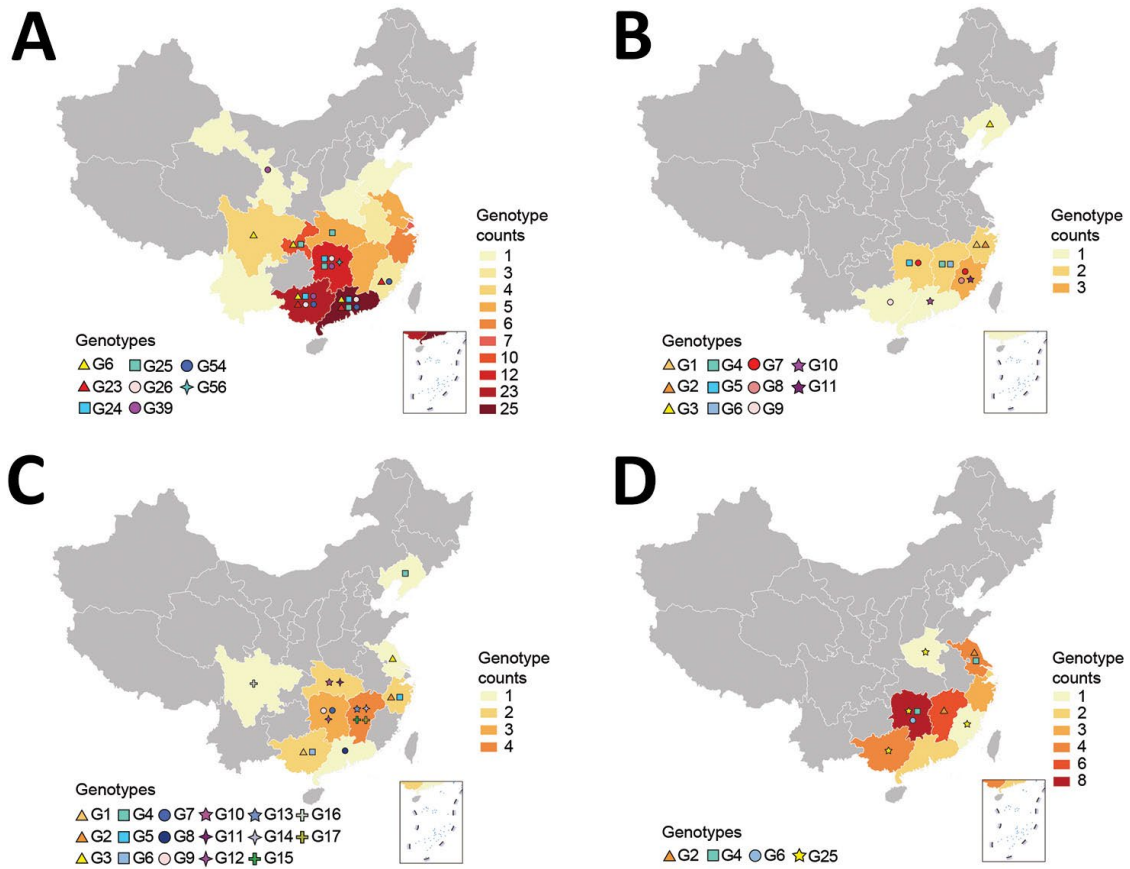




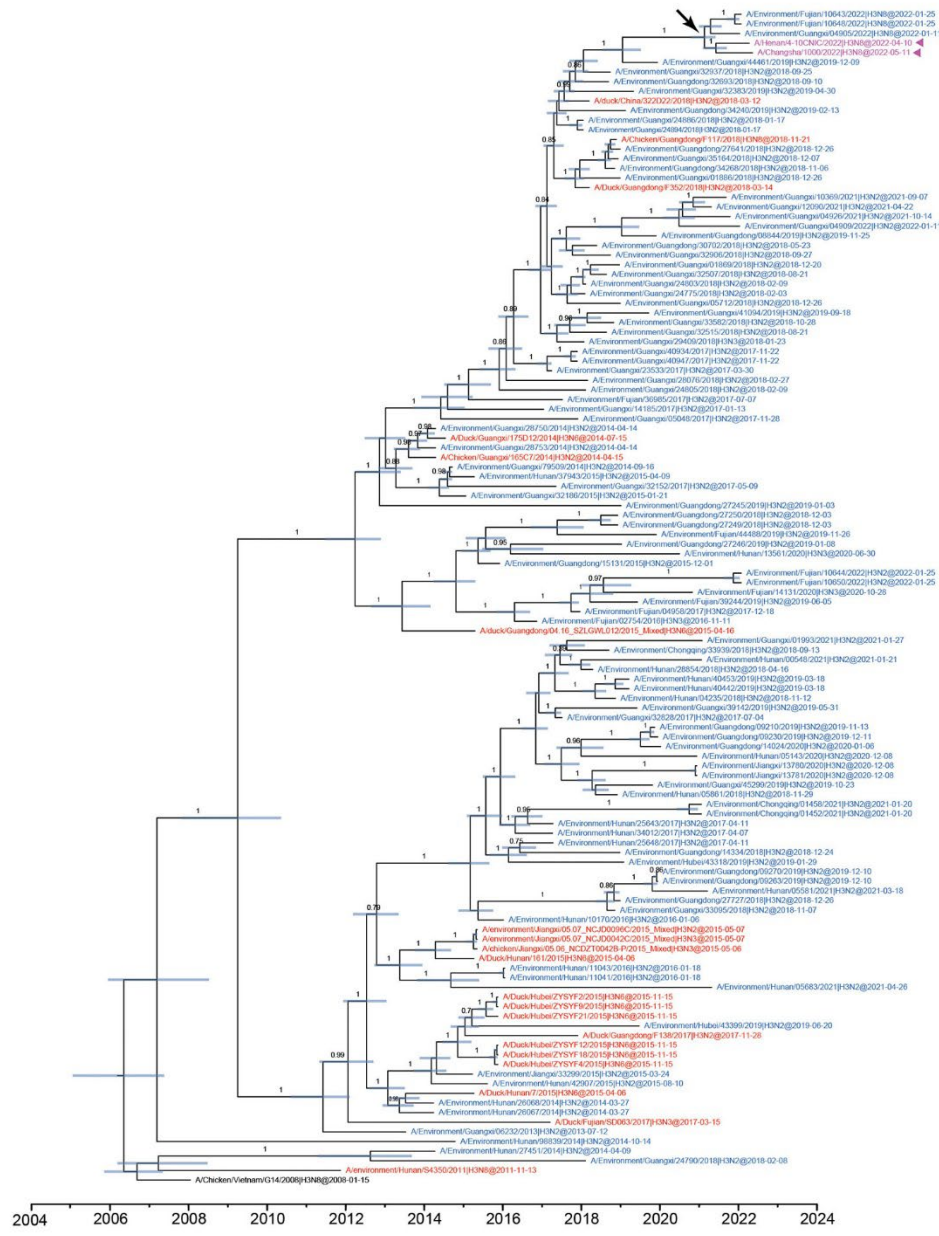
**Appendix Figure 3.** Phylogenetic trees of internal genes. Maximum likelihood trees of PB2 (n=929, A), PB1 (n=841, B), PA (n=841, C), NP (n=856, D), M (n=654, E), and NS (n=776, F) genes. H3 strains sequenced in this study are in steel blue. Other H3 AIVs in China are in red. The human H3N8 viruses are indicated by violet and triangles. The ZJ-5 sublineage and the poultry H9N2 ZJ-HJ/07 sublineage as well as their representative virus strains are highlighted in light-blue blocks. Branch lengths are scaled according to the numbers of substitutions per site. Branch support values of selected nodes are shown. Sublineages and subgroups are labelled with vertical lines on the right.



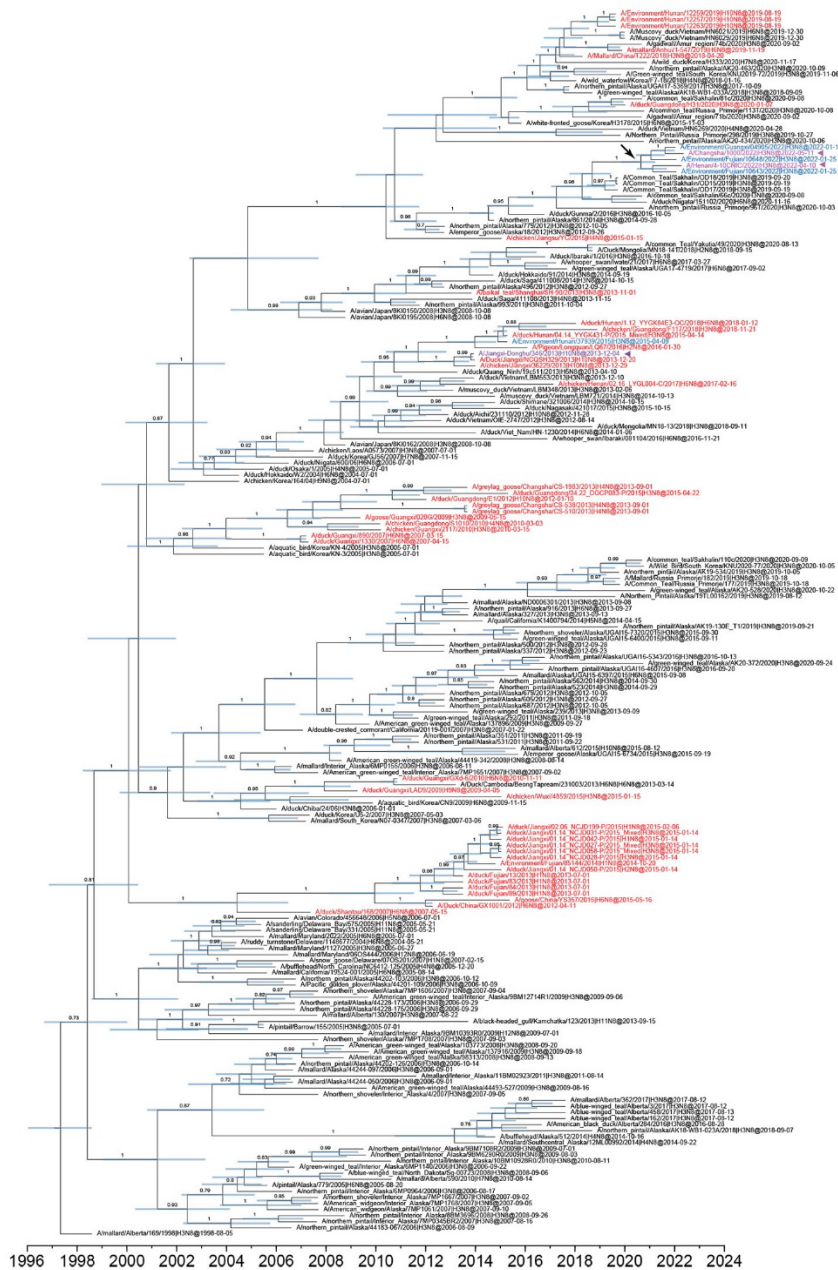
**Appendix Figure 4.** Diversity of genotypes of H3 subtype viruses sampled in China during 2009–2022. (A-D) Temporal distribution of genotypes of H3N2(A), H3N3(B), H3N6 (C), and H3N8 (D) AIVs. The counts of genotypes (line) and isolates of each genotype (column) are shown. For the H3N2 AIVs (A), grey column indicates the sum of isolates of transient genotypes. Detailed information for all genotypes can be found in Appendix Table 4.



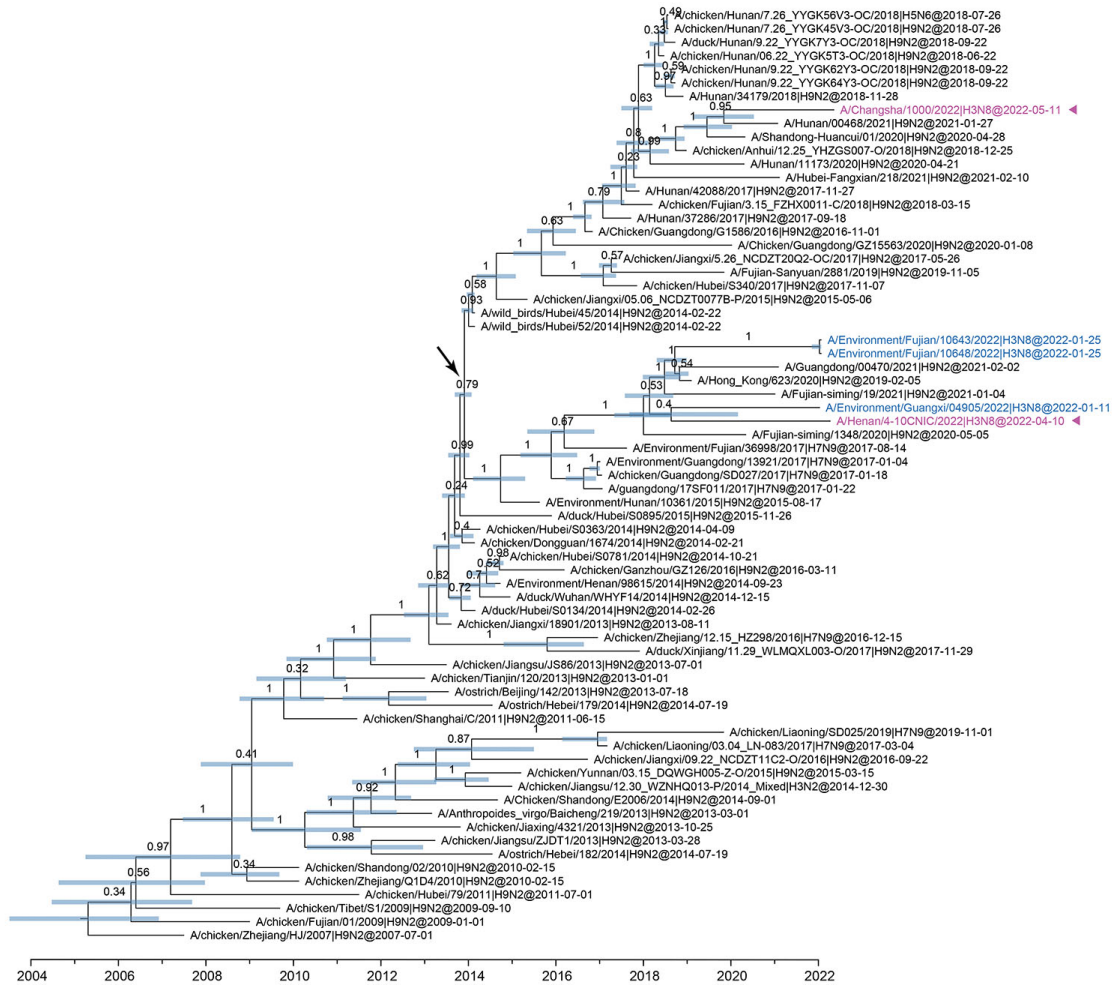
**Appendix Figure 5.** Geographical distribution of genotypes of H3 subtype viruses sampled in China during 2009–2022. (A-D) Geographical distributions of genotypes of H3N2 (A), H3N3 (B), H3N6 (C), and H3N8 (D) AIVs. The color of each province represents the number of genotypes. Provinces without applicable data are in grey. Different genotypes are marked with colored symbols on the map. For H3N2 (A) and H3N8 (D) AIVs, only the major genotypes are shown on the map. Detailed information for all genotypes can be found in Appendix Table 4.



**Appendix Figure 6.** Maximum clade credibility tree of H3 hemagglutinin (HA) sequences of the China-1.1 subgroup viruses (n=122). H3 strains sequenced in this study are in steel blue. Other H3 AIVs in China are in red. Two human H3N8 viruses are indicated by violet and triangles. The timescale bar is provided at the bottom. Clade posterior probabilities and 95% highest posterior density (HPD) of age estimates of selected nodes are shown as numbers and horizontal bars respectively. The most recent common ancestor of H3N8 G25 viruses is marked with arrow.



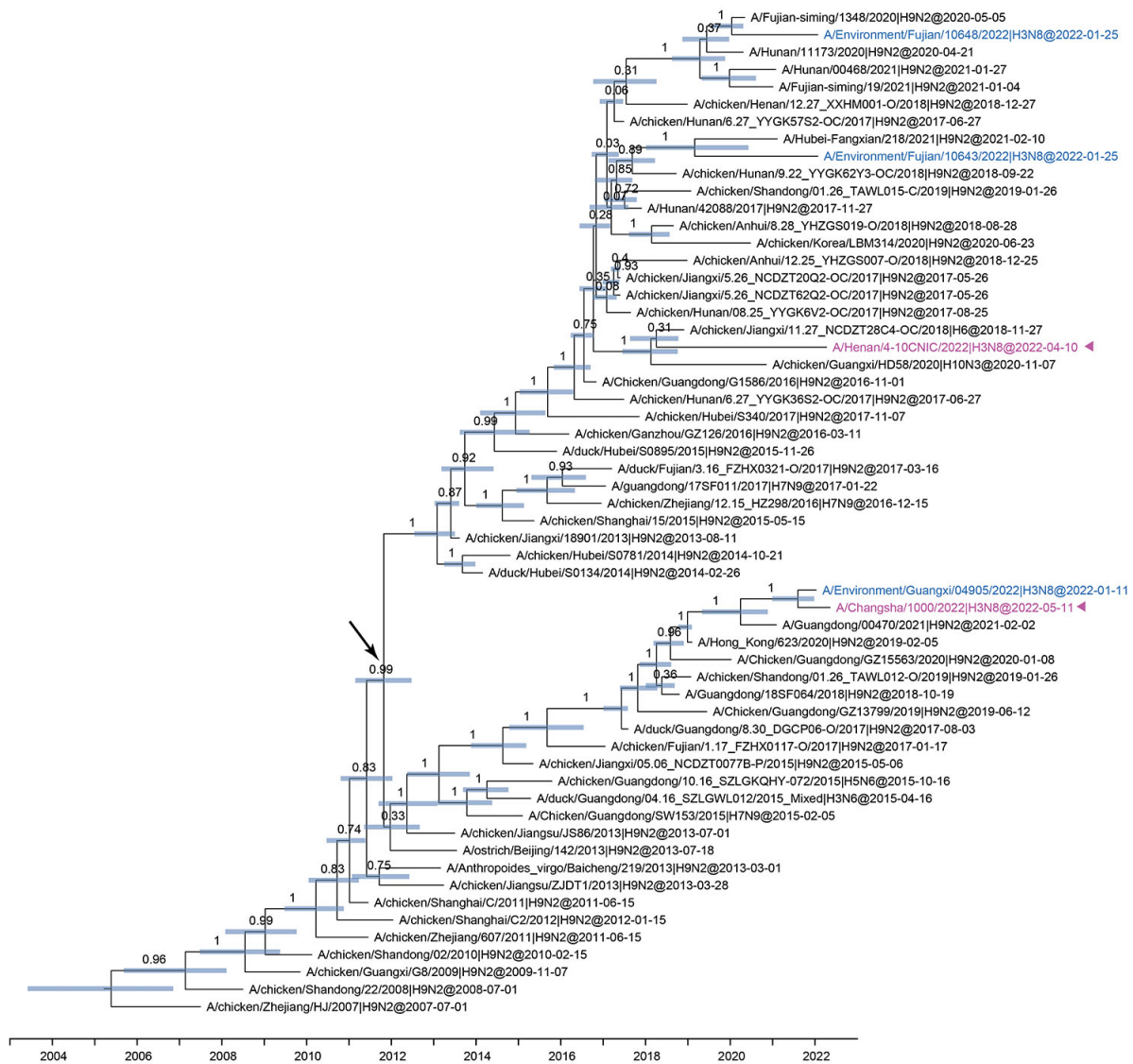
**Appendix Figure 7.** Maximum clade credibility tree of N8 neuraminidase (NA) sequences (n=202). H3 strains sequenced in this study are in steel blue. Other H3 AIVs in China are in red. Human H3N8 and H10N8 viruses are indicated by violet and purple, respectively. The timescale bar is provided at the bottom. Clade posterior probabilities and 95% HPD of age estimates of selected nodes are shown as numbers and horizontal bars respectively. The most recent common ancestor of H3N8 G25 viruses is marked with arrow.



**Appendix Figure 8.** Maximum clade credibility tree of PB2 gene (n=69). H3 strains sequenced in this study are in steel blue. Two human H3N8 viruses are indicated by violet and triangles. The timescale bar is provided at the bottom. Clade posterior probabilities and 95% highest posterior density (HPD) of age estimates of selected nodes are shown as numbers and horizontal bars respectively. The most recent common ancestor of H3N8 G25 viruses is marked with arrow.

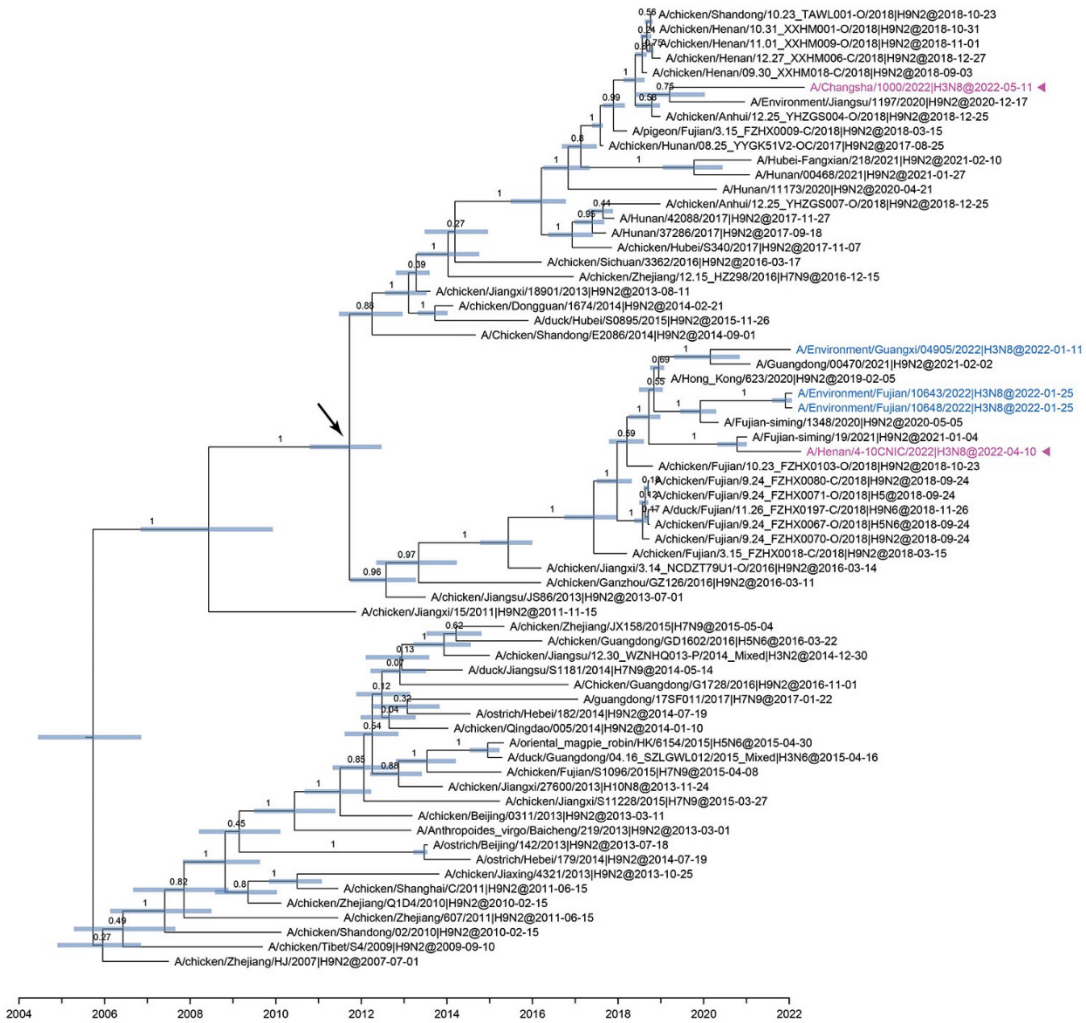






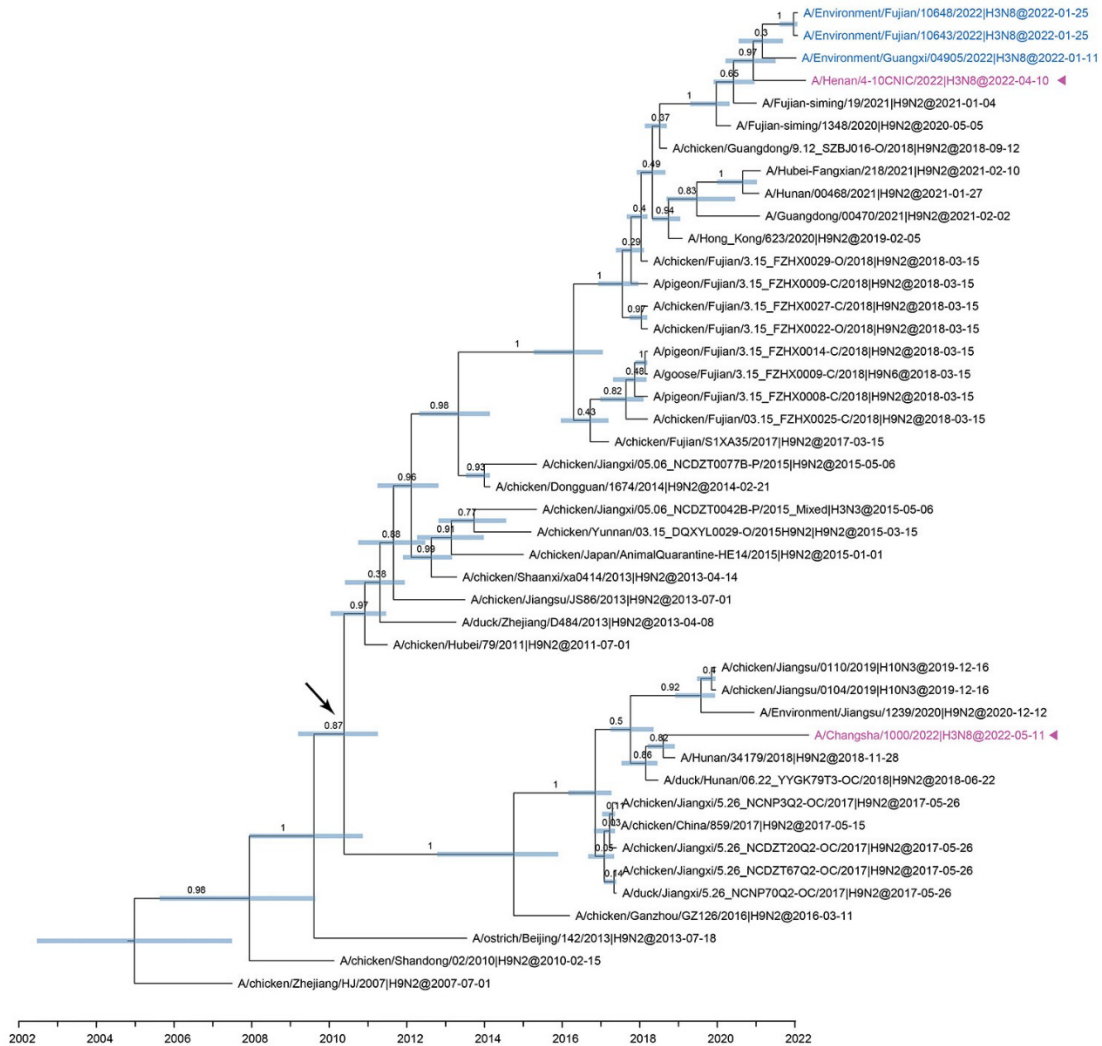
**Appendix Figure 10.** Maximum clade credibility tree of PA gene (n=58). Annotation as in

Appendix Figure 8.

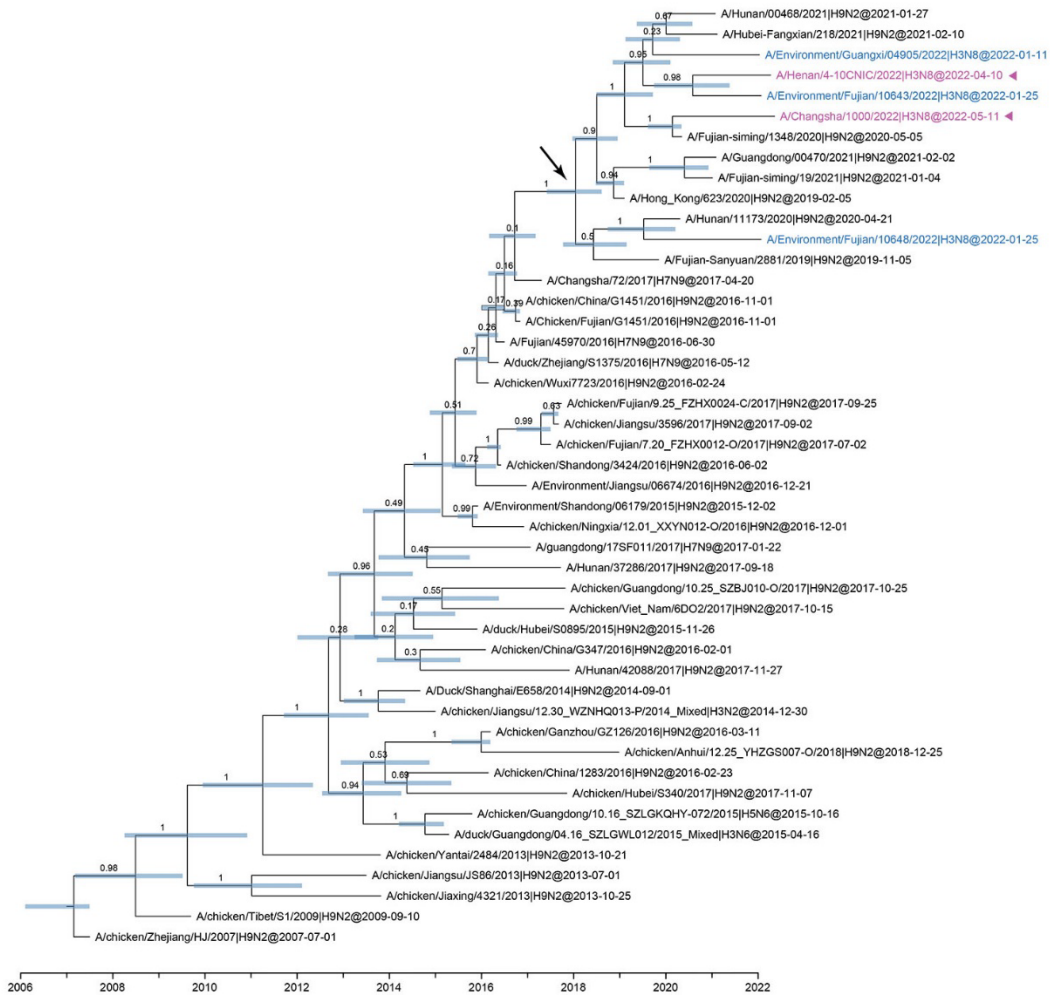


**Appendix Figure 11.** Maximum clade credibility tree of NP gene (n=66). Annotation as in Appendix Figure 8.

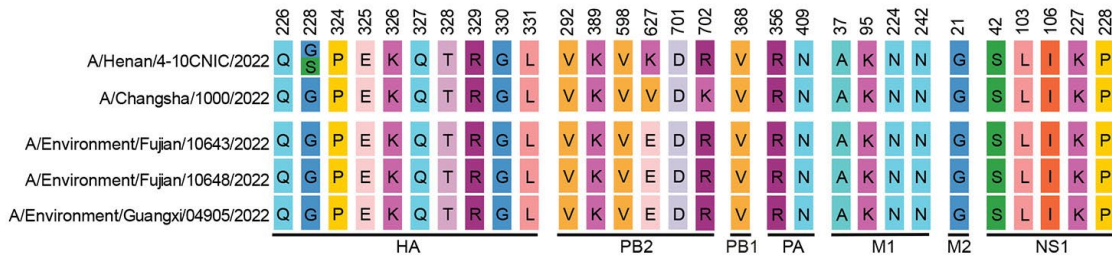




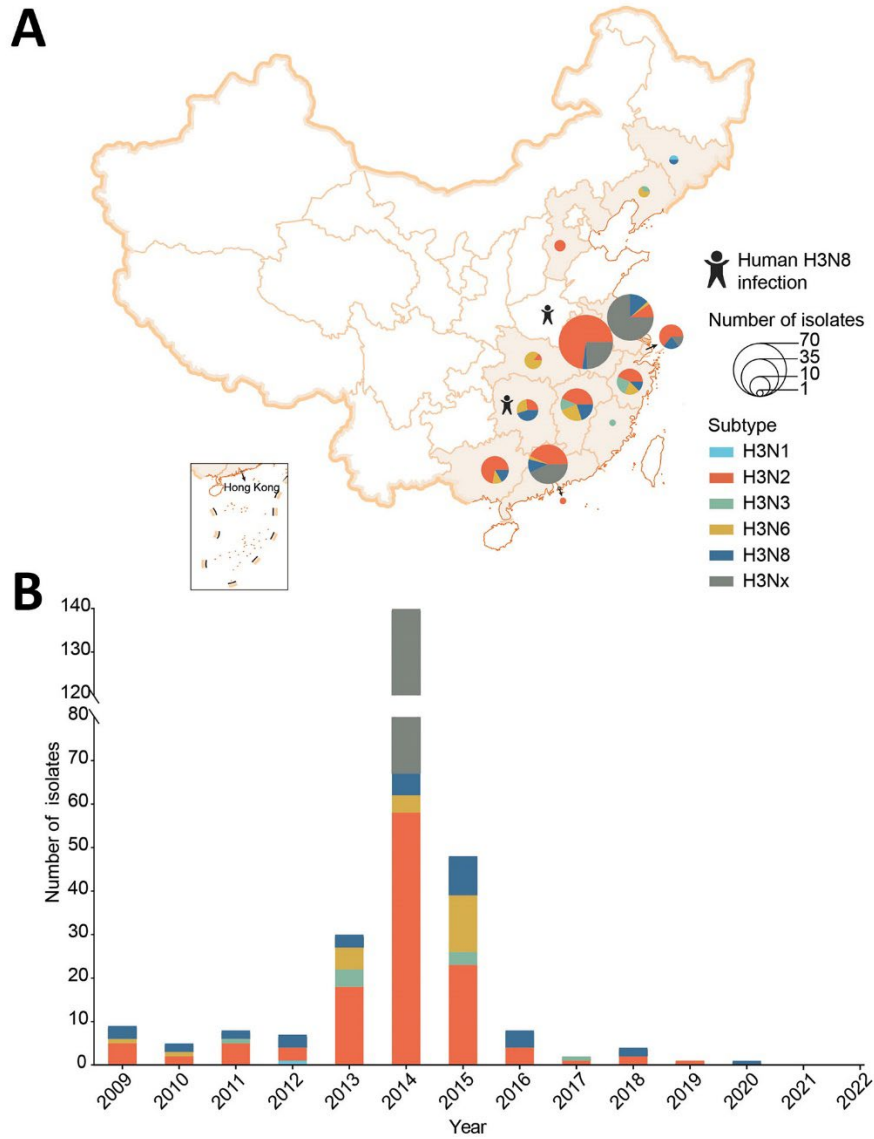
**Appendix Figure 12.** Maximum clade credibility tree of MP gene (n=44). Annotation as in Appendix Figure 8.



**Appendix Figure 13.** Maximum clade credibility tree of NS genes(n=46). Annotation as in Appendix Figure 8.



**Appendix Figure 14.** Key molecular markers of H3N8 G25 viruses. Key molecular markers associated with mammalian adaption are shown.



**Appendix Figure 15.** Spatiotemporal distribution of H3 hemagglutinin (HA) sequences in China during 2009-2022 available from the GISAID EpiFlu Database. (A) Spatial distribution. Annotation as in Figure 1A. (B) Temporal distribution. The colors of each column correspond to those of different NA subtypes in (A).