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

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The continuous evolution of avian influenza viruses (AIVs) of subtype H3 in China and the emergence of human infection with AIV subtype H3N8 highlight their threat to public health. Through surveillance in poultry-associated environments during 2009–2022, we isolated and sequenced 188 H3 AIVs across China. Performing large-scale sequence analysis with publicly available data, we identified 4 sublineages of H3 AIVs established in domestic ducks in China via multiple introductions from wild birds from Eurasia. Using full-genome analysis, we identified 126 distinct genotypes, of which the H3N2 G23 genotype predominated recently. H3N8 G25 viruses, which spilled over from birds to humans, might have been generated by reassortment between H3N2 G23, wild bird H3N8, and poultry H9N2 before February 2021. Mammal-adapted and drug-resistance substitutions occasionally occurred in H3 AIVs. Ongoing surveillance for H3 AIVs and risk assessment are imperative for potential pandemic preparedness.

Avian influenza viruses (AIVs) of subtype H3 are highly prevalent among waterfowl globally, causing mild or no apparent signs of illness in birds (*1*–*5*). H3 AIV has shown the potential for cross-species transmission and was the origin of other animal influenza viruses, which caused epidemics in horses, dogs, seals, and pigs (*6*–*9*). In 1968, H3 AIV contributed its hemagglutinin (HA) gene to the human influenza (H3N2) pandemic viruses, and it is still unknown whether an intermediate host was involved (*10*).

In April 2022, the first human infection with AIV (H3N8) was reported; the case was in a 4-year-old boy whose family reared chickens and silky fowls in Henan Province, China (*11*). After infection, the patient exhibited recurrent fever and severe pneumonia. In May 2022, a second case was identified in 5-year-old boy with mild influenza symptoms, who had visited the live poultry market (LPM) in Hunan Province, China (*12*). Those cases raised concern over whether H3N8 AIVs will cause a major public health threat (*13*).

In China, H3 AIVs have been dynamically circulating in poultry and wild birds across multiple regions (*14*). H3 combinations with multiple neuraminidase (NA) subtypes (N1–N8) were reported, among which H3N2 and H3N8 predominated (*14*–*16*). Phylogenetically, those viruses belonged to the Eurasian lineage, which is widespread in wild birds across Eurasia (*3*,*14*,*17*,*18*). Reassortment events often occurred at LPMs (*16*,*19*–*22*). During 2009–2022, we conducted country-level AIV surveillance in poultry-associated environments and performed a large-scale genetic analysis to provide a comprehensive picture of the evolution of H3 AIVs in China.

Methods

During January 2009–June 2022, we collected environmental samples monthly from avian-linked environments across 31 provinces in the China mainland according to AIV surveillance guideline of Chinese Center for Disease Control and Prevention. We isolated and sequenced 188 H3 viruses (32 have been previously published [*15*]). The sequences were deposited in the GISAID EpiFlu database (https://www.gisaid.org; accession nos. EPI2210281–1516) (Appendix Table 1, https://wwwnc.cdc.gov/EID/article/29/6/22-1786-App1.pdf).

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DOI: https://doi.org/10.3201/eid2906.221786

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We performed sequence alignments with available sequences from the GISAID EpiFlu database as of June 25, 2022, by using MAFFT version 7.222 (*23*). We reconstructed maximum-likelihood phylogenies of all segments by using FastTree version 2.1.11 (*24*). The resulting trees were classified into divergent lineages or sublineages. Genotypes were assigned by the combination of lineages for each segment of full-genome viruses.

To estimate the time to the most recent common ancestor (tMRCA) of H3N8 viruses of humans, we used Bayesian Markov chain Monte Carlo analyses for each gene in BEAST version 1.10.4 (*25*). We then generated maximum clade credibility trees (Appendix).

Results

Isolation and Sequencing of H3 AIVs

During January 2009–June 2022, we isolated 188 H3 AIVs from the poultry-associated environmental samples: 167 H3N2, 7 H3N3, 3 H3N6, 10 H3N8, and 1 H3 with NA unknown (Appendix Table 1). The H3N2 AIVs were widely distributed across 15 provinces, mainly in southern China (Figure 1, panel A). We isolated H3 AIVs with other NA subtypes (N3, N6, and N8) in 2–8 provinces. More than three quarters of the H3 viruses (149/188, 79.3%) were isolated from the samples collected from LPMs (Appendix Table 2). Before 2014, we isolated and sequenced <6 strains of H3 AIVs per year (Figure 1, panel B). Since 2014, we obtained more isolates, most (48) in 2018. All H3 isolates were sequenced, and we recovered the full genomes from 185 of the isolates (Appendix Table 1).

Evolution of H3 Genes in China

To elucidate the evolution of H3 AIVs in China, we performed a phylogenetic analysis of HA genes of the H3 AIVs sequenced in this study, along with sequences available from the GISAID EpiFlu Database (Figure 2). The HA genes of all viruses in this study were grouped into the Eurasian lineage, sharing a nucleotide homology of 79.2%–100.0%. In brief, the major branch of Eurasian avian H3 lineage containing viruses in recent decades could be further classified into 10 sublineages (named by the geographic distributions: China-1, China-2, China-3, China-4, Asia, Europe-Asia, worldwide-1, worldwide-2, North America-1, and Korea); other minor branches at the bottom of the phylogenetic tree included the North America-2 sublineage and early strains sampled during 1972–1992 (Figure 2; Appendix Figure 1). H3 AIVs collected from wild birds, poultry, or poultry-associated environments in China in recent decades were distributed in 8 sublineages, except sublineages North America-1, North America-2, and Korea, which were only identified in North America and South Korea.

Sublineages China-1, China-2, China-3, and China-4 consisted of AIVs almost all collected from poultry or poultry-associated environments in China in addition to a few viruses from Vietnam (18) and Cambodia (1) (Appendix Figure 1). Domestic ducks acted as the main host for China-1 (48/166), China-2 (63/111), China-3 (80/110), and China-4 (15/23)

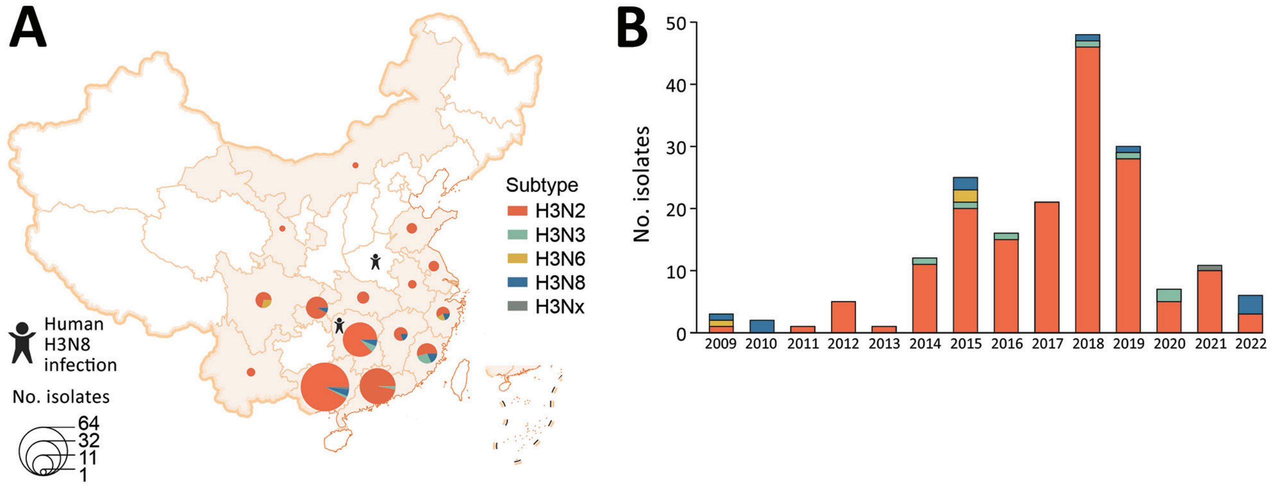

Figure 1. Spatial and temporal distribution of avian influenza virus subtype H3 isolated from poultry-associated environments, China, 2009–2022. A) Spatial distribution of environmental H3 subtype viruses. One H3 isolate without neuraminidase (NA) subtype was designated as H3Nx. Provinces where human infections with H3N8 were reported are noted. B) Number of environmental H3 subtype isolates per year. This figure includes all H3 isolates sequenced by the Chinese National Influenza Center. Additional metadata are available in Appendix Table 1 (https://wwwnc.cdc.gov/EID/article/29/6/22-1786-App1.pdf).

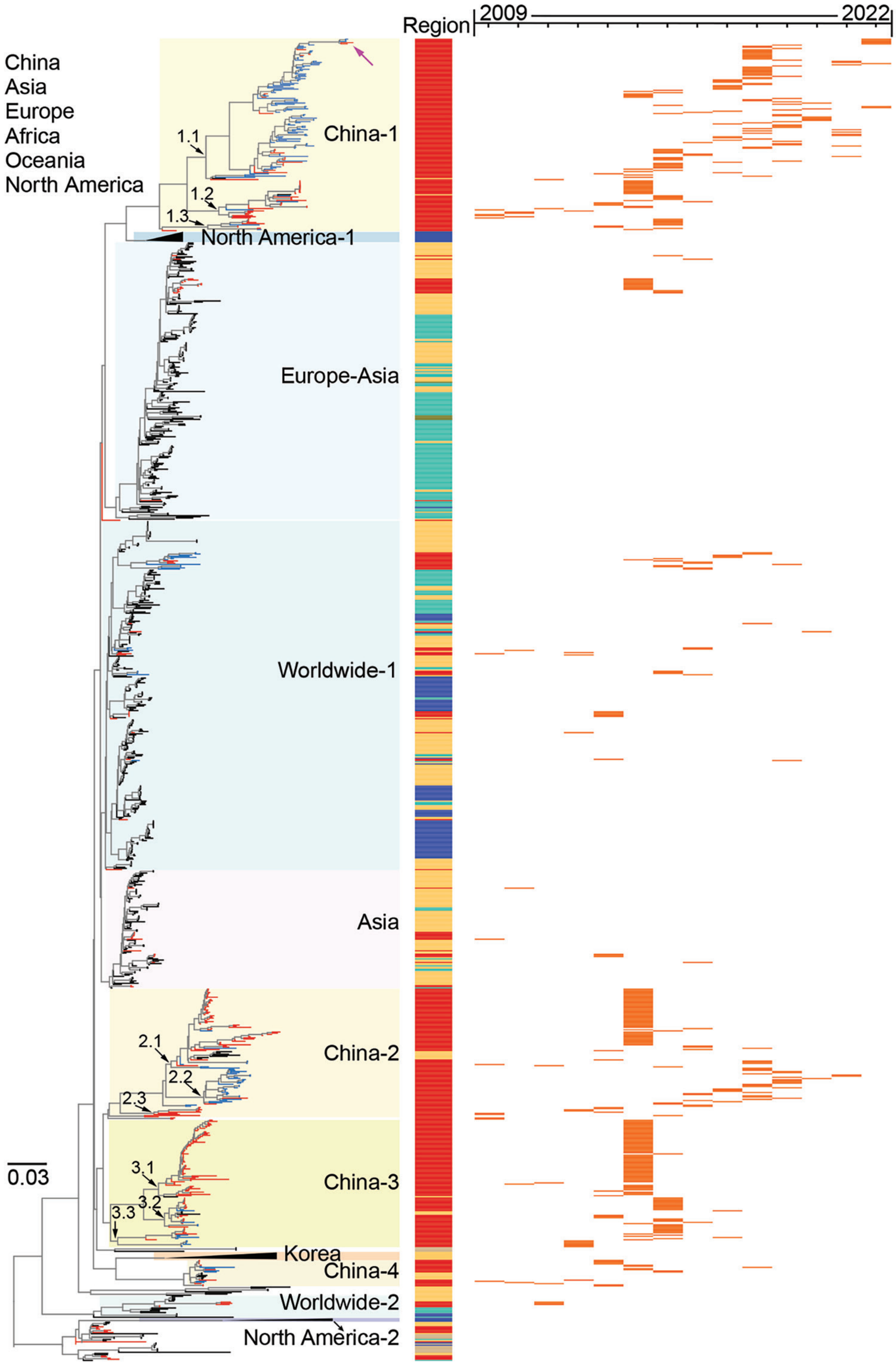

Figure 2. Maximum-likelihood phylogenetic tree of hemagglutinin genes of avian influenza viruses subtype H3 from China (n = 1,291) and reference sequences from GISAID (https://www.gisaid.org). Blue tree sections indicate sequences reported in this study; red tree sections indicate other H3 sequences from China; violet arrow at top of tree indicates human H3N8 virus. For clarity, some clades are collapsed. Sublineages are shown with different background colors on the phylogenetic tree. Subgroups in sublineages China-1, China-2, and China-3 are marked with black arrows at the nodes. The sampling locations are annotated with colored bars adjacent to the tree. For the H3 viruses sampled in China during 2009–2022, the sampling year of each of these viruses is shown on the right panel with orange horizontal bars. The phylogenetic tree of the H3 genes with more detailed information is shown in Appendix Figure 1 (https://wwwnc.cdc.gov/EID/article/29/6/22-1786-App1.pdf). Scale bar indicates nucleotide substitutions per site.

(Appendix Table 3). Each sublineage comprised various NA subtypes (Appendix Figure 1). The most common subtype was H3N2 (270), followed by H3N8 (41), H3N6 (19), H3N3 (12), and H3N9 (1), except for 67 H3 AIVs with NA unknown. A high proportion (397/410, 96.8%) of these viruses have been sampled since 2009, whereas recent isolates were primarily consolidated in the China-1 and China-2 sublineages (Figure 2).

The China-1 sublineage had evolved into 3 distinct subgroups, with prevalence spanning different times. Most of our isolates (101/185, 54.6%) fell into the China-1.1 subgroup, which circulated during

2008–2022. Of note, 3 H3N8 strains sampled in Fujian (2) and Guangxi (1) Provinces in 2022 had a close relationship with 2 human H3N8 strains and together formed a miniature phylogenetic group (Appendix Figure 1). The China-1.2 subgroup was detected during 2009–2016 and the China-1.3 subgroup during 2013–2015 (Figure 2; Appendix Figure 1).

The China-2 and China-3 sublineages have evolved into 3 subgroups, and the China-2.2 subgroup mainly comprised environmental H3 viruses (29/31, 93.5%) sequenced in this study during 2015–2021 (Figure 2; Appendix, Appendix Figure 1). H3 viruses of sublineages Asia, Europe-Asia, worldwide-1, and worldwide-2 were occasionally detected in poultry and wild birds in China, but no stable cluster was established (Appendix, Appendix Figure 1).

Reassortment with NA Genes

We detected multiple NA subtypes in each H3 sublineage. We performed phylogenetic analyses for 4 major NA subtypes: N2, N3, N6, and N8. Almost all NA genes of H3 AIVs in our study were clustered within the Eurasian lineage, and 8 H3N8 AIVs had NA genes derived from the North American lineage (Appendix Figure 2, panels A–D).

The N2 genes of AIVs in the Eurasian lineage could be further classified into sublineages, and most H3N2 viruses in this study were clustered in the Eurasian-2 sublineage (Appendix). We also found H3N3 strains closely related to the human-origin influenza (H10N3) virus and H3N6 closely related to highly pathogenic AIV (HPAIV) subtype H5N6 (Appendix).

Most NA genes of H3N8 viruses (43/59) from China belonged to the North American lineage, closely related to AIVs from different regions (e.g., Russia, Vietnam, South Korea, and North America). Of note, the NA genes of human H3N8 and H10N8 viruses belonged to distinct groups (Figure 3), and 3 environmental strains sequenced in this study were highly homologous to the human H3N8 viruses. Few H3N8 strains from China fell into the Eurasian lineage (Figure 3).

Reassortment with Internal Genes

In the phylogenetic tree of each internal gene, a large proportion of H3 AIVs in China belonged to the Eurasian wild bird reservoir (Appendix Figure 3). Some H3 AIVs had internal genes derived from ZJ-5 sublineage (of the wild bird viruses), poultry H5N1/H5N6 sublineage, poultry H9N2 ZJ-HJ/07 sublineage, or waterfowl H6 sublineage (Appendix). Each internal gene has only 1 or 2 virus sequences that belong to the H9N2 ZJ-HJ/07 sublineage. In 2022, a total of 3 environmental and 2 human H3N8 viruses contained all internal genes belonging to the H9N2 ZJ-HJ/07 sublineage.

Emergence of Multiple Genotypes

Assessment of the diversity of genome constellations indicated that prolific reassortments of the H3 AIVs had occurred in China in past decades. On the basis of the sublineage classification of all 8 gene segments, we identified 126 genotypes among 284 full-genome H3 viruses sampled in China during 2009–2022 (Appendix Figure 4). We found evidence of dynamic emergence for 73 genotypes (G1–G73) from 212 H3N2 genomes, 11 genotypes (G1–G11) from 14 H3N3, 17 (G1–G17) from 25 H3N6, and 25 (G1–G25) from 33 H3N8 (Appendix). H3N2 G23 had been detected in multiple years and provinces during 2014–2022 (Appendix Figure 4, panel A, Figure 5, panel A). H3N8 G25, which had been detected in both environmental and human viruses in 2022, acquired HA genes from the China-1 H3 sublineage, NA genes from the North American N8 lineage, and all 6 internal gene from poultry H9N2 ZJ-HJ/07 sublineage viruses (Appendix Table 4).

Emergence of H3N8 G25 Viruses

We further traced the origin of the H3N8 G25 viruses. When we compared the genetic diversity of G25 genotype viruses, the results showed that these viruses shared a higher similarity in HA (98.4%–99.1%) and NA genes (98.8%–99.3%) and a lower similarity in other internal genes (polymerase basic [PB] 2, 93.9%–100.0%; PB1, 91.6%–99.9%; polymerase acidic [PA], 93.4%–99.6%; nucleocapsid, 94.5%–99.9%; matrix (M), 95.3%–100.0%; and nonstructural, 97.0%–98.7%). This finding indicated that after the emergence of prior H3N8 G25 virus, dynamic reassortment might occur between H3N8 and poultry H9N2 viruses.

To elucidate the timing of H3N8 G25 virus emergence, we performed coalescent analyses and calculated the estimated tMRCA of all 8 segments (Appendix Figures 6–13). The median tMRCA among the HA genes was estimated to be February 2021 (95% highest posterior density [HPD] October 2020–May 2021). The HA genes closely related to those of H3N8 G25 viruses were from H3N2 G23 AIVs isolated from Guangxi and Guangdong Provinces, particularly A/environment/Guangxi/44461/2019 (H3N2), sampled in December 2019 (Figure 4, panels A, C). The median tMRCA among the NA genes of the H3N8 G25 viruses was estimated to be August 2020 (95% HPD November 2019–March 2021). H6N8

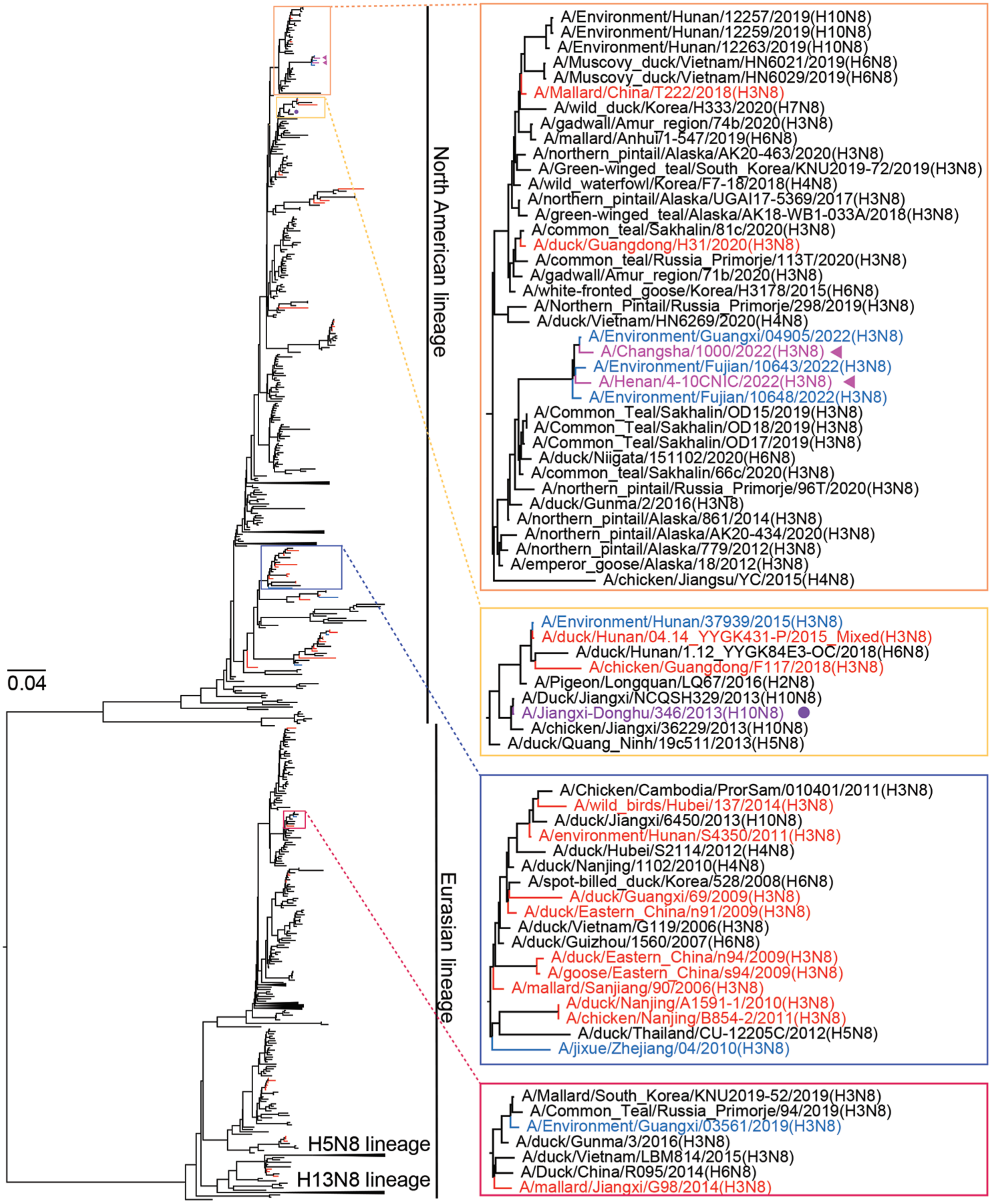

Figure 3. Maximum-likelihood phylogenetic tree of avian influenza virus subtype N8 genes from China (n = 1,106) and reference sequences from GISAID (https://www.gisaid.org). Blue tree sections indicate sequences of H3 subtype viruses reported in this study; red tree sections indicate other H3 subtype viruses from China. For of clarity, some clades are collapsed. Representative clusters are indicated in shaded boxes and magnified on the right. Violet arrows indicate human H3N8 viruses; purple solid circle indicates human H10N8 virus. The phylogenetic tree of N8 genes with more complete information is shown in Appendix Figure 2, panel D (https://wwwnc.cdc.gov/EID/article/29/6/22-1786-App1.pdf). Scale bar indicates nucleotide substitutions per site.

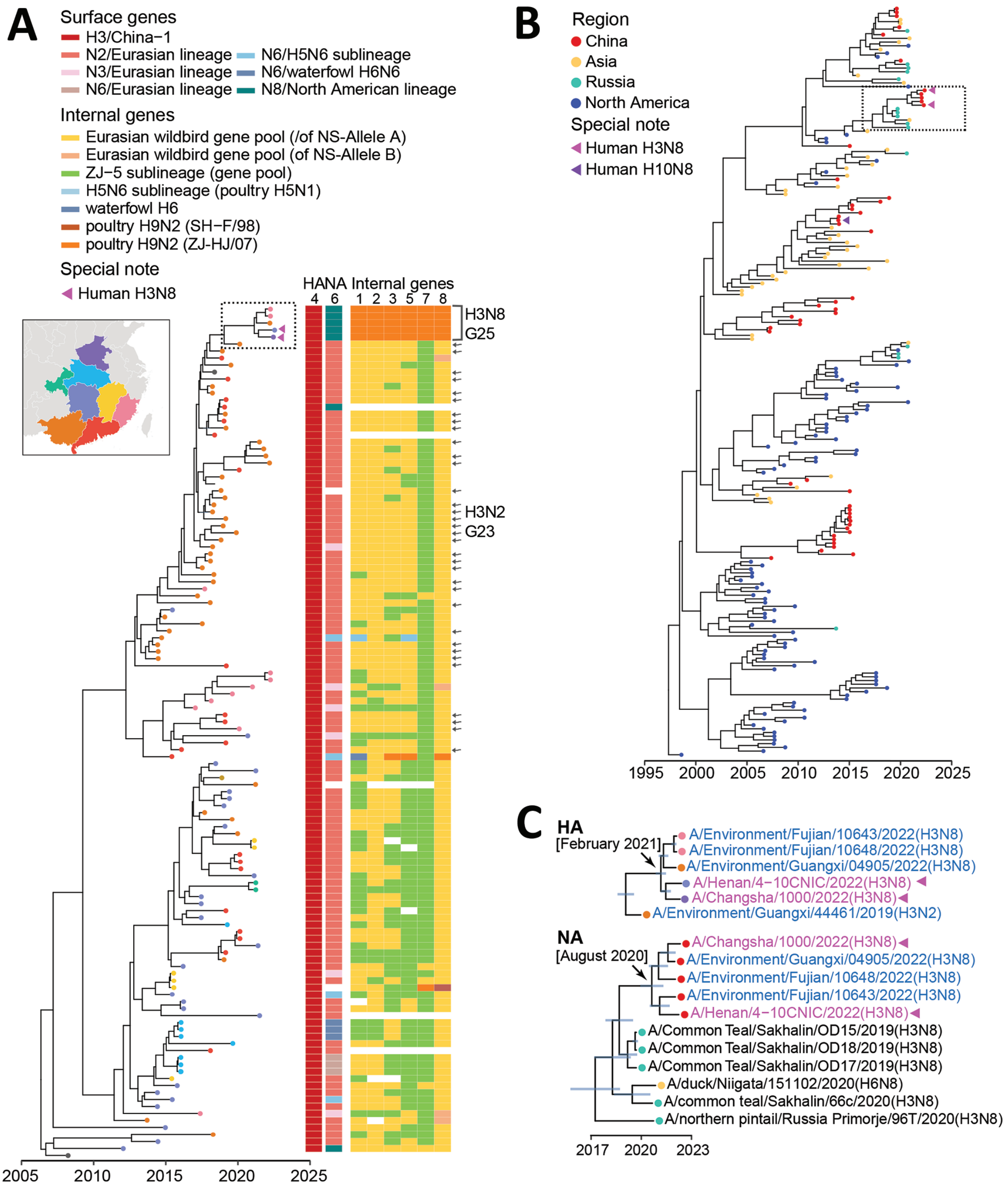

Figure 4. Bayesian time-resolved phylogenetic tree of hemagglutinin (HA) genes from avian influenza subtype H3 viruses and neuraminidase (NA) genes from subtype N8 viruses from China and reference sequences from GISAID (https://www.gisaid.org). A) Maximum clade credibility tree of HA genes of the China-1.1 H3 subgroup (n = 122). Tip points are colored by provinces (corresponding to the fill color in the map). Violet triangles indicate human H3N8 viruses. The lineage origins of each gene segment of H3 AIVs are represented by different colored tiles adjacent to the tree; the tile is blank if the sequence is unavailable. H3N2 G23 viruses are indicated with arrows. H3N8 G25 viruses are indicated within the bracket. The fully resolved tree with detailed information is depicted in Appendix Figure 6 (https://wwwnc.cdc.gov/EID/article/29/6/22-1786-App1.pdf). B) Maximum clade credibility tree of N8 genes (n = 202). Tip points are colored by region. Violet triangles indicate human H3N8 viruses; purple triangle indicates human H10N8 virus. Virus names of the representative cluster (in the dashed box) are shown in panel C. The fully resolved tree with detailed information is depicted in Appendix Figure 7. C) Clades in the dashed box in panels A and B. Trees are drawn to the same scale. Blue indicates H3 avian influenza viruses sequenced in this study; violet indicates human H3N8 viruses. For HA (top) and NA (bottom) genes, branch tips are colored as in panels A and B. Blue node bars correspond to the 95% credible intervals of node heights. Arrows indicate the most recent common ancestors of HA and NA genes of H3N8 G25 viruses.

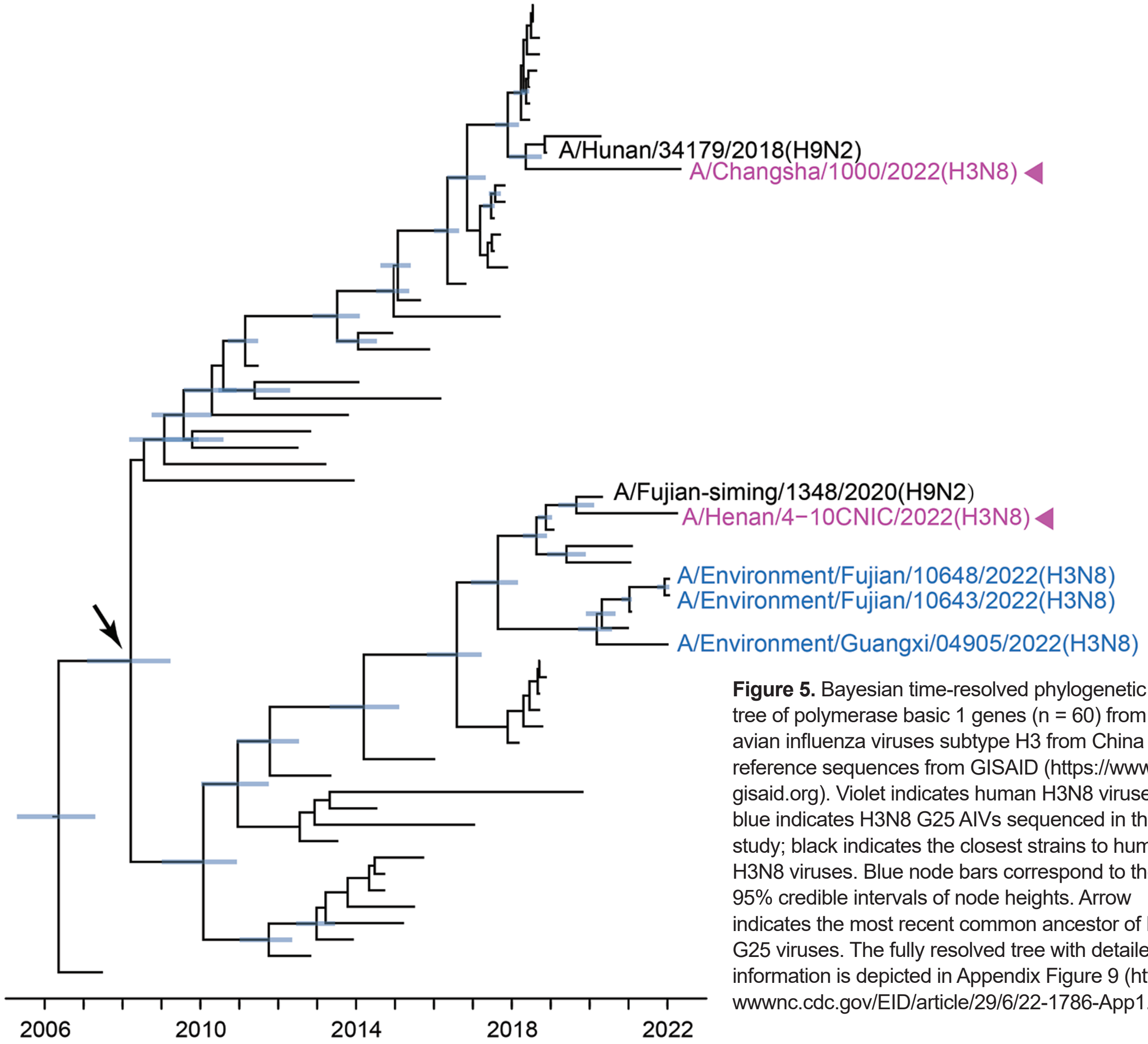

Figure 5. Bayesian time-resolved phylogenetic tree of polymerase basic 1 genes (n = 60) from avian influenza viruses subtype H3 from China and reference sequences from GISAID (https://www.gisaid.org). Violet indicates human H3N8 viruses; blue indicates H3N8 G25 AIVs sequenced in this study; black indicates the closest strains to human H3N8 viruses. Blue node bars correspond to the 95% credible intervals of node heights. Arrow indicates the most recent common ancestor of H3N8 G25 viruses. The fully resolved tree with detailed information is depicted in Appendix Figure 9 (https://wwwnc.cdc.gov/EID/article/29/6/22-1786-App1.pdf).

AIV isolated in Japan and H3N8 AIV isolated in the Russian Far East during 2019–2020 were closely related to H3N8 G25 viruses, specifically A/common teal/Sakhalin/OD17/2019 (H3N8) virus (Figure 4, panels B, C).

The internal genes of the H3N8 G25 viruses showed earlier tMRCAs than that estimated for HA and NA genes (Appendix Figure 8–13). The internal genes of H3N8 G25 viruses scattered within different subclades without forming a cluster alone. The closest H9N2 viruses to the human H3N8 viruses also differed. For example, the common ancestry of PB1 genes of the H3N8 G25 viruses could be dated back to March 2008 (95% HPD March 2007–May 2009). A/Fujian-siming/1348/2020 (H9N2) was closely related to human H3N8 virus A/Henan/4–10CNIC/2022, and A/Hunan/34179/2018 (H9N2) was close to human H3N8 virus A/Changsha/1000/2022 (Figure 5). Other internal genes of the H3N8 G25 viruses had been estimated to have tMRCAs tracing back to 2010–2018 (Appendix Figure 8–13). Those results further indicate that H3N8 G25 viruses dynamically reassorted with H9N2 viruses.

Molecular Characterization of the H3 AIVs

We investigated the molecular markers of H3 AIVs in China (Appendix Table 5). One human H3N8 isolate, A/Henan/4-10CNIC/2022, had 228G/S in the receptor binding site, which might alter the binding preference to human-type receptors (*26*). Three H3 AIVs previously sampled from poultry in 2014 had an aspartic acid at position 190, which might alter receptor specificity (*26*).

Key molecular markers associated with increased capacity for receptor binding, viral replication, and pathogenicity in mammals were found in the internal gene segments of avian H3 viruses in China (Appendix Table 5). E627K and E627V in PB2 genes were exclusively detected in human H3N8 viruses, suggesting adaptation of these viruses to mammals. Other mutations such as R389K, I292V, and A588V in PB2, which might be associated with increased polymerase activity and replication in mammalian and avian cells (*27*,*28*) and virulence in mice (*29*), were also found in 2 human isolates and several avian H3 viruses. All H3 AIVs contained N30D, T215A, and P41A in the M1 genes, which might alter the virulence in mice (*30*) and affect growth and transmission in the guinea pig model (*31*).

We identified host signature amino acids in PB2 and PA genes (PB2-702R, PA-356R, PA-409N) (*32*) in human H3N8 isolates and few H3 AIVs, except for A/Changsha/1000/2022, which had PB2-702K (Appendix Table 5, Figure 14). We also analyzed the substitutions related to antiviral drug resistance (Appendix Table 5). Two human H3N8 viruses contained an S31N mutation in the M2 gene, suggesting resistance to amantadine and rimantadine (*33*). In the M2 protein, 26 of 337 H3 AIVs contained drug-resistance mutation V27I/A and 15 contained S31N. Mutations, such as E119V/A/D and H274Y (N2 numbering) were not identified in NA gene, suggesting that all H3 viruses might be sensitive to NA inhibitors (e.g., oseltamivir) (*34*); however, 3 H3 AIVs possessed Q136L, E119G, or H274R, which might affect their drug sensitivity.

Discussion

The natural reservoir for AIVs is waterfowl; the viruses are spread worldwide by wild bird migration and introduced to domestic poultry across the wild bird–poultry interface (*35*). H3 AIVs have continuously circulated in poultry and wild birds across China (*14*). In China, 4 sublineages (China-1, China-2, China-3, and China-4) of HA genes evolved from the Eurasian lineage and became established in poultry, especially in domestic ducks, after introduction in recent decades. Currently, H3 viruses in China-1 and China-2 sublineages are cocirculating in poultry, with the China-1 sublineage predominating. Although frequent introductions from wild birds to poultry have been observed in other sublineages (e.g., worldwide-1), it is inevitable that continuous introductions will result in new sublineages in poultry (*36*). Our surveillance results also showed that H3N2 predominated among H3 AIVs in poultry-associated environments during 2009–2022. Consistent results for birds were revealed by the available avian strains in GISAID (Appendix Figure 15), although most were collected during 2013–2015 because of strengthened surveillance during the influenza (H7N9) outbreak (*37*–*39*).

Phylogenetic analyses revealed intense reassortment of the H3 AIVs, generating multiple genotypes. On the basis of the sublineage classification, we identified 126 genotypes from 284 H3 AIVs during 2009–2022. Most were transient, and the H3N2 G23 genotype seems to have stabilized in recent years, predominating in southern China. The H3N8 G25 viruses, which had caused human infection, contained complete internal gene cassettes originating from poultry H9N2 ZJ-HJ/07 sublineage, which has persistently circulated in chickens in China and named G57 genotype H9N2 AIVs (*40*). Similar to the pattern of H7N9 AIVs (*41*), H3N8 G25 AIVs might be adapted in chickens rather than ducks.

The H3N8 G25 viruses exhibited distinct tMRCAs among 8 segments. Molecular dating of HA and NA genes of the H3N8 G25 viruses implied that the ancestral virus might have been generated through reassortment between the H3N2 G23 virus and wild bird H3N8 virus before February 2021 (95% HPD October 2020–May 2021). However, the internal genes of the H3N8 G25 viruses showed much earlier tMRCAs than those of HA and NA, indicating that sequential reassortments underlie the emerging of H3N8 G25 viruses.

H3 AIVs have existed for a long time, but to our knowledge, no human infection had been reported until 2022. After reassortment with 6 internal genes of H9N2, current H3N8 AIVs seem to have the advantage of infecting humans (*42*). Ongoing adaptation in mammals after continuous human infections may underlie emergence of pandemic strains. The H3N8 G25 viruses had acquired human-adapted mutations after infecting humans (Appendix Figure 14), such as 228G/S in the HA gene and E627K/V in the PB2 gene, which were also present in 1968 H3N2 pandemic strains (*43*). This finding indicates the pandemic potential of the newly emerged H3N8 AIVs.

For risk assessment of the pandemic potential, human population immunity to a newly emerged animal virus is a critical parameter. HA inhibition assays among poultry workers (*12*) and the general population (*44*) showed seropositivity for the human seasonal H3N2 virus but very low seroprevalence against the newly emerged H3N8 virus. Those results suggest little antigenic cross-reactivity between human seasonal H3N2 virus and the current H3N8 virus and that the human population has little or no preexisting immunity to emerging H3N8 viruses. No drug-resistance mutation to NA inhibitors was observed in H3N8 G25 viruses; therefore, vaccine and drug stockpiles are needed for the potential pandemic preparation.

H3 AIVs have been isolated from asymptomatic ducks (*45*). Recent studies indicate that the newly emerged H3N8 AIVs are pathogenic to chickens (*12*,*46*). Our samples were collected exclusively from avian-linked environments (including LPMs, poultry farms, backyards, and slaughterhouses), according to surveillance guidelines. Thus, we were unable to link the isolated H3 AIVs to specific host information. Poultry sampling might provide helpful information about H3 AIV activity in China. The species of poultry in the LPMs might be confounding factors for the spatiotemporal differences. In this study, the sampling sites were geographically dispersed, and the data were collected from a small number of LPMs. Considering the large number of LPMs in China, especially in rural areas, representativeness of the data might be biased.

AIV surveillance has greatly improved since HPAIV H5N1 infected humans in Hong Kong in 1997 (*47*). However, gaps still exist, and new virus is unpredictable. The AIVs circulating and evolving in poultry might have a preferential ability to transmit to humans directly across the poultry–human interface (*48*). The H3N8 G25 viruses, with increased human receptor binding and low population immunity (*12*), had raised concern for pandemic potential. Dual receptor-binding profiles (*49*,*50*) and mutations associated with enhanced virus replication and pathogenicity in mammals were also found in many H3 AIVs. Surveillance and research of H3 AIVs, as well as the drugs and vaccine capacity, should be strengthened for pandemic preparedness.

Acknowledgments

We thank the authors and laboratories for sharing the AIVs sequences in the GISAID database.

This study was supported by the National Key Research and Development Program of China (2022YFC2303800, 2021YFC2300100) and the National Nature Science Foundation of China (81961128002, 31970643).

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Article DOI: https://doi.org/10.3201/eid2906.221786

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) 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 ≥97% of the length of coding region and ≤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 ≥ 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 ucld.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

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	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

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	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

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	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	*EPI2211435~EPI2211436*
	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	***A/Environment/Guangdong/40751/2017***	Guangdong	2017-10-16	feces	A	EPI1590009~EPI1590016
	EPI_ISL_390761	***A/Environment/Chongqing/36536/2017***	Chongqing	2017-08-17	surface swab of cages	A	EPI1590017~EPI1590024
	EPI_ISL_390762	***A/Environment/Guangxi/32828/2017***	Guangxi	2017-07-04	sewage	A	EPI1590025~EPI1590032
	EPI_ISL_390763	***A/Environment/Hunan/34019/2017***	Hunan	2017-04-07	sewage	A	EPI1590033~EPI1590040
	EPI_ISL_390764	***A/Environment/Hubei/34289/2017***	Hubei	2017-01-23	sewage	C	EPI1590041~EPI1590048
	EPI_ISL_390765	***A/Environment/Guangxi/14185/2017***	Guangxi	2017-01-13	surface swab of chopping boards	A	EPI1590049~EPI1590056
	EPI_ISL_390766	***A/Environment/Guangdong/60350/2016***	Guangdong	2016-11-23	feces	A	EPI1590057~EPI1590064
	EPI_ISL_390767	***A/Environment/Anhui/33167/2016***	Anhui	2016-04-30	sewage	A	EPI1590065~EPI1590072
	EPI_ISL_390768	***A/Environment/Chongqing/38160/2016***	Chongqing	2016-03-18	feces	A	EPI1590073~EPI1590080
	EPI_ISL_390769	***A/Environment/Jiangsu/44006/2016***	Jiangsu	2016-03-09	feces	A	EPI1590081~EPI1590088
	EPI_ISL_390770	***A/Environment/Chongqing/22909/2016***	Chongqing	2016-01-19	feces	B	EPI1590089~EPI1590096
	EPI_ISL_390771	***A/Environment/Chongqing/22907/2016***	Chongqing	2016-01-14	surface swab of cages	A	EPI1590097~EPI1590104
	EPI_ISL_390772	***A/Environment/Hunan/11043/2016***	Hunan	2016-01-18	sewage	A	EPI1590105~EPI1590112
	EPI_ISL_390773	***A/Environment/Sichuan/32281/2016***	Sichuan	2016-01-08	sewage	A	EPI1590113~EPI1590120
	EPI_ISL_390774	***A/Environment/Inner Mongolia/02114/2015***	Inner Mongolia	2015-12-04	others	C	EPI1590121-EPI1590128
	EPI_ISL_390775	***A/Environment/Yunnan/01457/2015***	Yunnan	2015-11-27	surface swab of cages	D	EPI1590129~EPI1590136
	EPI_ISL_390776	***A/Environment/Guangdong/15241/2015***	Guangdong	2015-09-15	surface swab of cages	A	EPI1590137~EPI1590144
	EPI_ISL_390777	***A/Environment/Hunan/39684/2015***	Hunan	2015-04-01	sewage	A	EPI1590145~EPI1590152
	EPI_ISL_390778	***A/Environment/Jiangxi/27771/2015***	Jiangxi	2015-03-05	feces	A	EPI1590153~EPI1590160
	EPI_ISL_390779	***A/Environment/Guangdong/21134/2015***	Guangdong	2015-01-20	surface swab of cages	A	EPI1590161~EPI1590168
	EPI_ISL_390780	***A/Environment/Hunan/98839/2014***	Hunan	2014-10-14	feces	A	EPI1590169~EPI1590176
	EPI_ISL_390781	***A/Environment/Guangxi/79509/2014***	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	***A/Environment/Chongqing/79459/2014***	Chongqing	2014-09-15	surface swab of cages	A	EPI1590185~EPI1590192
	EPI_ISL_390783	***A/Environment/Guangxi/28753/2014***	Guangxi	2014-04-14	surface swab of chopping boards	A	EPI1590193~EPI1590200
	EPI_ISL_390784	***A/Environment/Hunan/26067/2014***	Hunan	2014-03-27	surface swab of cages	A	EPI1590201~EPI1590208
	EPI_ISL_390785	***A/Environment/Hunan/18323/2014***	Hunan	2014-02-27	drinking water	A	EPI1590209-EPI1590216
H3N3	EPI_ISL_15723483	A/Environment/Guangxi/29409/2018	Guangxi	2018-01-23	surface swab of chopping boards	A	EPI2210728~EPI2210735
	EPI_ISL_15723901	A/Environment/Guangdong/34076/2019	Guangdong	2019-03-13	feces	A	EPI2211144~EPI2211151
	EPI_ISL_15723927	A/Environment/Hunan/13561/2020	Hunan	2020-06-30	surface swab of cages	D	EPI2211352~EPI2211359
	EPI_ISL_15723928	A/Environment/Fujian/14131/2020	Fujian	2020-10-28	feces	D	EPI2211360~EPI2211367
	EPI_ISL_390786	***A/Environment/Fujian/02754/2016***	Fujian	2016-11-11	drinking water	A	EPI1590217~EPI1590224
	EPI_ISL_390787	***A/Environment/Hunan/03259/2015***	Hunan	2015-12-22	feces	A	EPI1590225~EPI1590232
	EPI_ISL_390788	***A/Environment/Fujian/85141/2014***	Fujian	2014-10-20	feces	A	EPI1590233~EPI1590240
H3N6	EPI_ISL_15720732	A/Environment/Zhejiang/7/09	Zhejiang	2009-01-01	others	D	EPI2210281~EPI2210288
	EPI_ISL_15723336	A/Environment/Sichuan/03397/2015	Sichuan	2015-12-09	surface swab of chopping boards	A	EPI2210521~EPI2210528
	EPI_ISL_390789	***A/Environment/Sichuan/03404/2015***	Sichuan	2015-12-24	feces	B	EPI1590241~EPI1590248
H3N8	EPI_ISL_15720734	A/jilongtumo/Jiangxi/29/09	Jiangxi	2009-10-22	surface swab of cages	A	EPI2210297~EPI2210304
	EPI_ISL_15720735	A/wushui/Chongqing/2/2010	Chongqing	2010-01-27	sewage	A	EPI2210305~EPI2210312
	EPI_ISL_15720736	A/jixue/Zhejiang/04/2010	Zhejiang	2010-04-01	chicken blood	A	EPI2210313~EPI2210320
	EPI_ISL_15723505	A/Environment/Guangxi/32891/2018	Guangxi	2018-10-29	drinking water	A	EPI2210904~EPI2210911
	EPI_ISL_15723924	A/Environment/Guangxi/03561/2019	Guangxi	2019-12-23	sewage	D	EPI2211328~EPI2211335
	EPI_ISL_15723972	A/Environment/Guangxi/04905/2022	Guangxi	2022-01-11	others	A	EPI2211469~EPI2211476
	EPI_ISL_15723974	A/Environment/Fujian/10643/2022	Fujian	2022-01-25	surface swab of cages	A	EPI2211485~EPI2211492
	EPI_ISL_15723975	A/Environment/Fujian/10648/2022	Fujian	2022-01-25	surface swab of cages	A	EPI2211493~EPI2211500
	EPI_ISL_390790	***A/Environment/Hunan/46780/2015***	Hunan	2015-11-18	drinking water	A	EPI1590249~EPI1590256
	EPI_ISL_390791	***A/Environment/Hunan/37939/2015***	Hunan	2015-04-09	sewage	A	EPI1590257~EPI1590264
H3N0	EPI_ISL_15723964	A/Environment/Guangxi/01993/2021	Guangxi	2021-01-27	sewage	A	*EPI2211416~EPI2211418*

*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

	Sample site, n (%)				
Subtype	Live poultry markets	Poultry farms/backyards	Slaughterhouses	Unknown*	Total
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

	Sublineage, n(%)				
Host	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_SZLGWL009/2015_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

				Avian					Human
Protein	Biological Effect	Mutations	Amino Acids	H3N2	H3N3	H3N6	H3N8	H3N0/N1 /N9#	H3N8
HA	Altered receptor specificity	E190D	E	289	19	32	54	80	2
			D	2	0	0	0	1	0
		Q226L	Q	291	19	32	54	81	2
		G228S	G	291	19	32	54	81	1
			S/G	0	0	0	0	0	1
NA	Reduced susceptibility to neuraminidase inhibitors	E119V/A/D *	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
			R	1	0	0	0	-	0
PB2	Increase polymerase activity in avian and mammalian cell line	I292V	I	214	14	29	36	2	0
			V	19	2	3	7	0	2
			M	0	0	0	1	0	0
			T	0	0	0	0	1	0
		R389K	R	221	16	32	39	3	0
			K	11	0	0	5	0	2
	Efficient replication in mammalian and avian cells, and higher virulence in mice	A588V	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†
			V	0	0	0	0	0	1‡
		D701N	D	232	16	32	44	3	2
	Host signature amino acids (avian to human)	K702R	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
	Host signature amino acids (avian to human)	K356R	K	0	15	31	41	2	0
			R	6	0	1	3	0	2
		S409N	S	225	15	28	40	2	0
			N	5	0	4	4	0	2
M1	Altered virulence in mice	N30D	D	237	17	33	49	2	2
	Impacts growth and transmission in the guinea pig model	P41A	A	237	17	33	49	2	2
	Altered virulence in mice	T215A	A	237	17	33	49	2	2
	Enhanced infectivity	T37A	T	237	17	31	44	2	0
			A	0	0	2	5	0	2
		R95K	R	237	16	33	43	2	0
			K	0	1	0	6	0	2
		S224N	S	237	16	31	42	2	0
			N	0	1	2	7	0	2
		K242N	K	237	16	33	46	2	0
			N	0	1	0	3	0	2
M2	Enhanced infectivity	D21G	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
		S31N/G	S	226	16	33	46	2	0

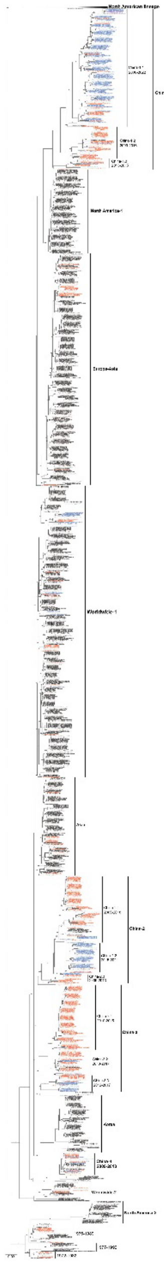
				Avian					Human
Protein	Biological Effect	Mutations	Amino Acids	H3N2	H3N3	H3N6	H3N8	H3N0/N1/N9#	H3N8
			N	11	1	0	3	0	2
NS1	Altered virulence in mice	P42S	S	237	13	31	35	5	2
			A	10	3	2	11	1	0
		L103F	F	234	12	30	32	5	0
			Y	10	3	2	11	1	0
			L	3	1	1	3	0	2
		I106M	M	246	15	32	43	6	0
			I	1	1	1	3	0	2
	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

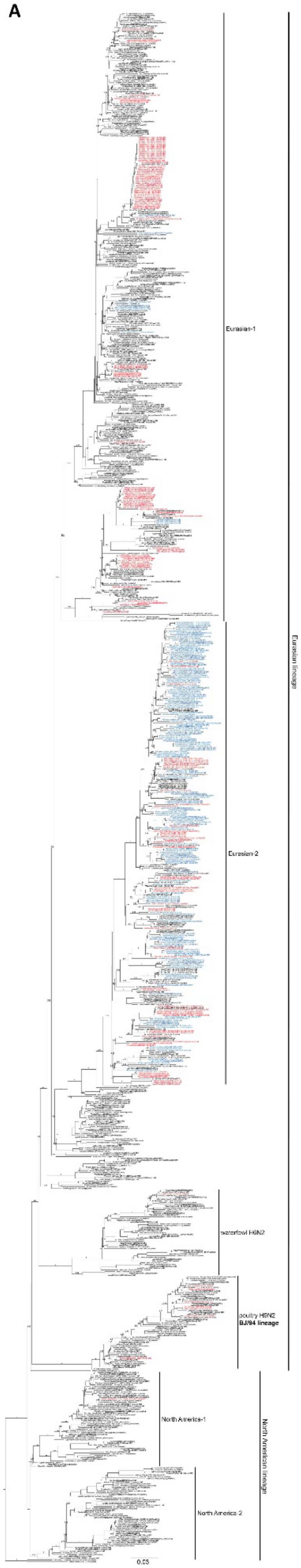
†A/Henan/4-10CNIC/2022(H3N8).

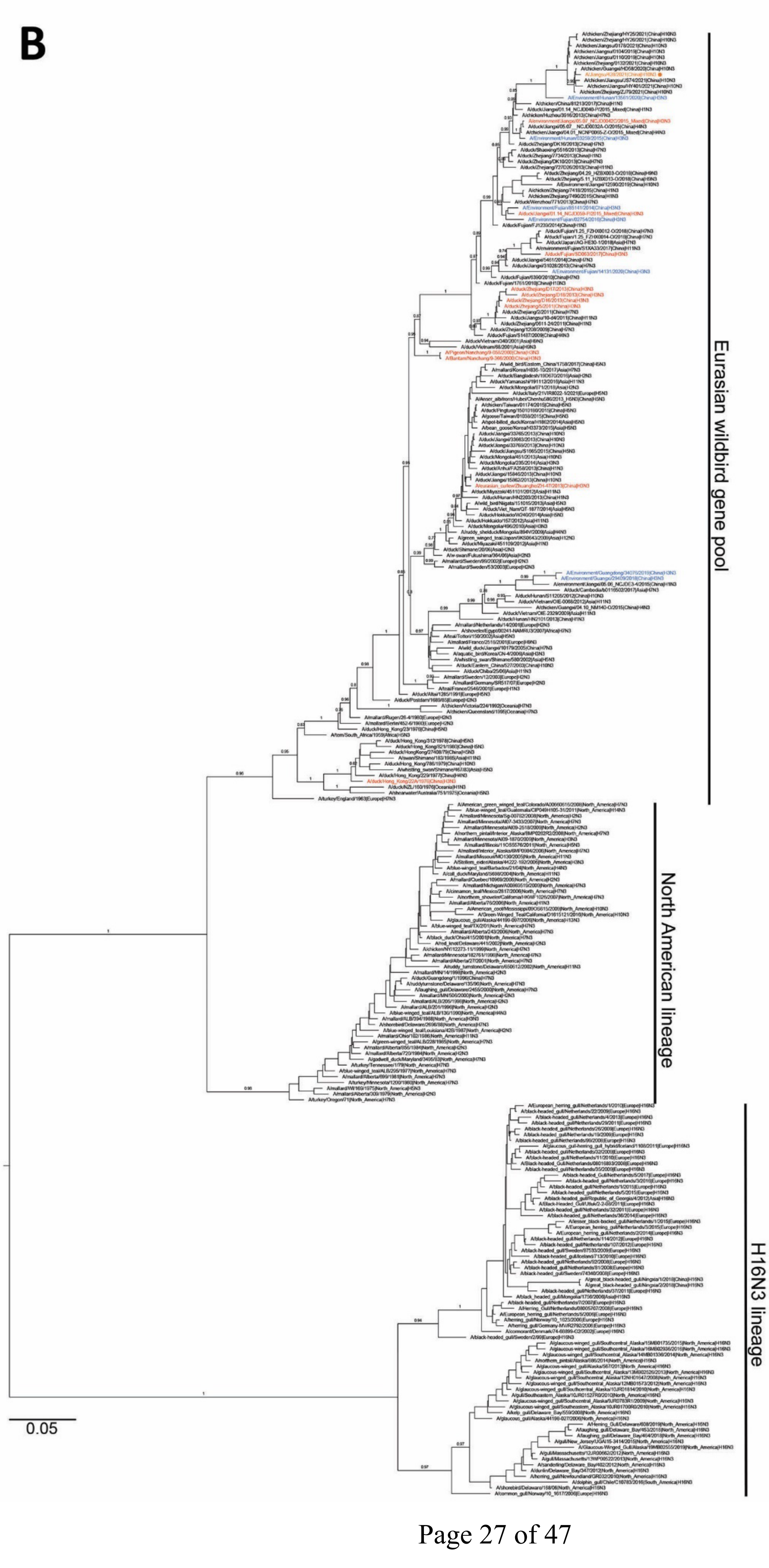
‡A/Changsha/1000/2022(H3N8).

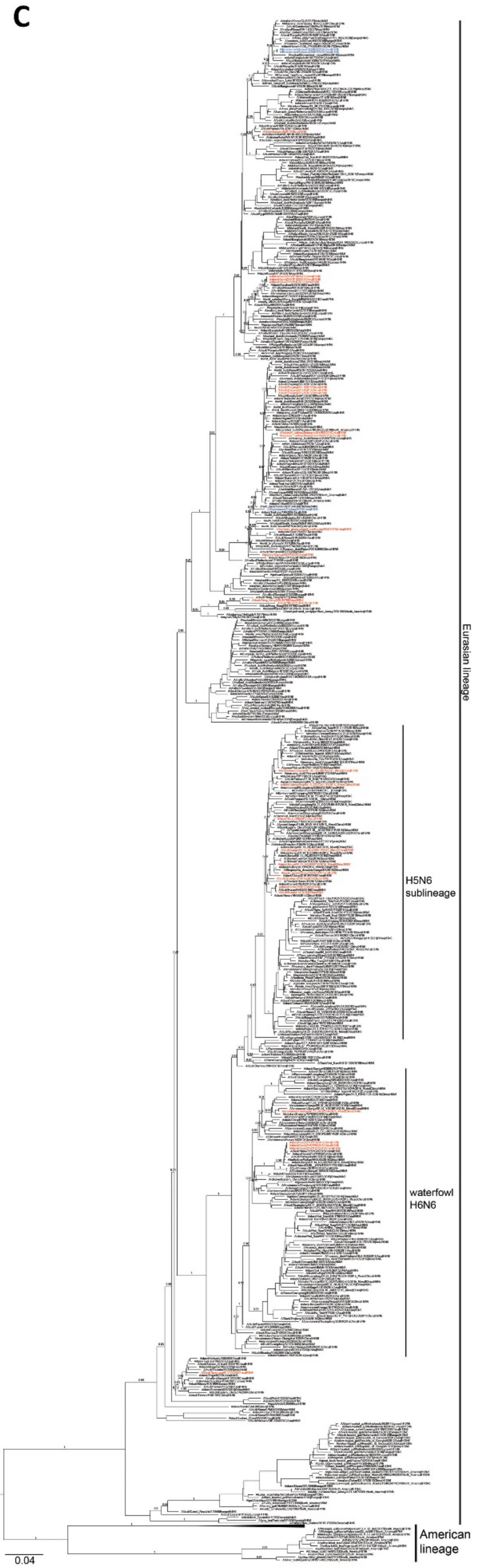


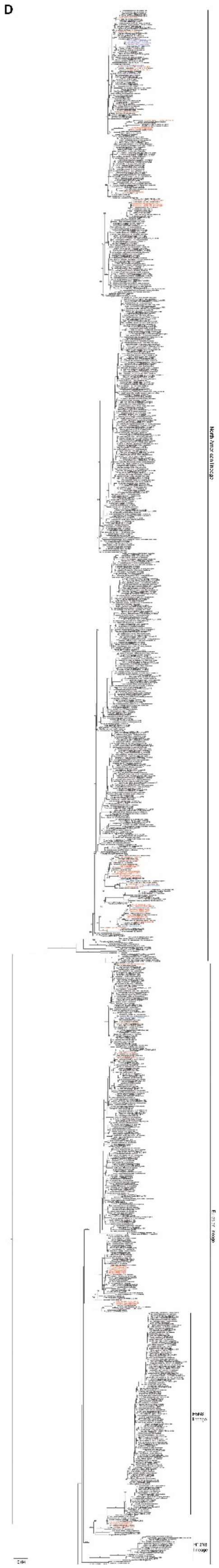
Appendix Figure 1. Phylogenetic tree of H3 hemagglutinin (HA) genes. Maximum likelihood tree (n=2871). H3 strains sequenced in this study are in steel blue. Other H3 AIVs in China are in red. Two human viruses are in violet and marked with triangles. A mini group containing two human and three environmental 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

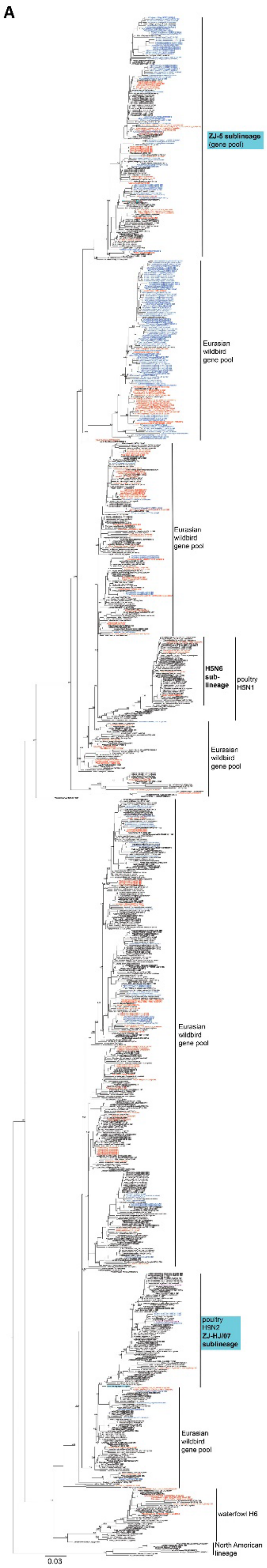

Eurasian-1
Eurasian-2
Eurasian lineage
waterfowl H6N2
poultry H6N2
BJ/94 lineage
North America-1
North America-2
North American lineage
0.03

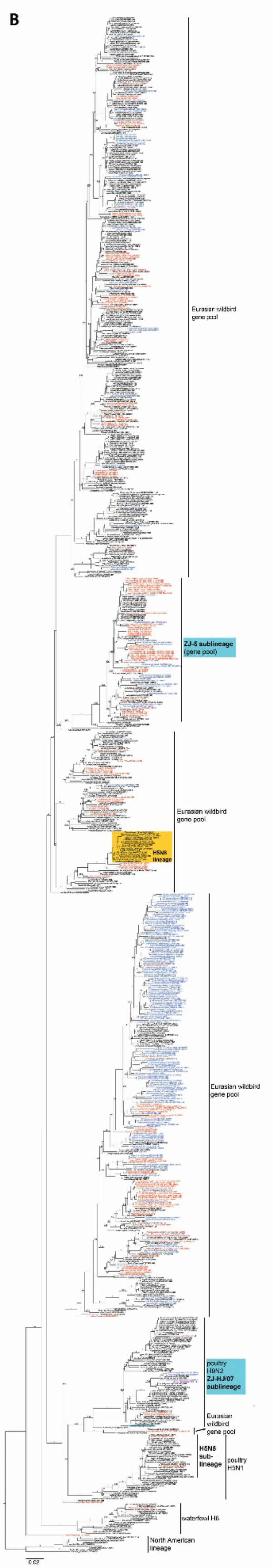

B
Eurasian wildbird gene pool
North American lineage
H16N3 lineage
0.05

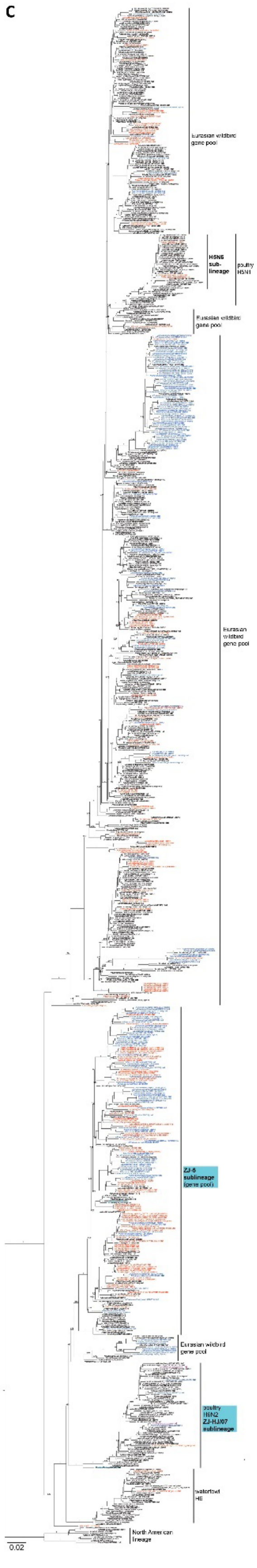

C
Eurasian lineage
H5N6 sublineage
waterfowl H6N6
American lineage
0.04


D
North American lineage
Eurasian lineage
H5N8 lineage

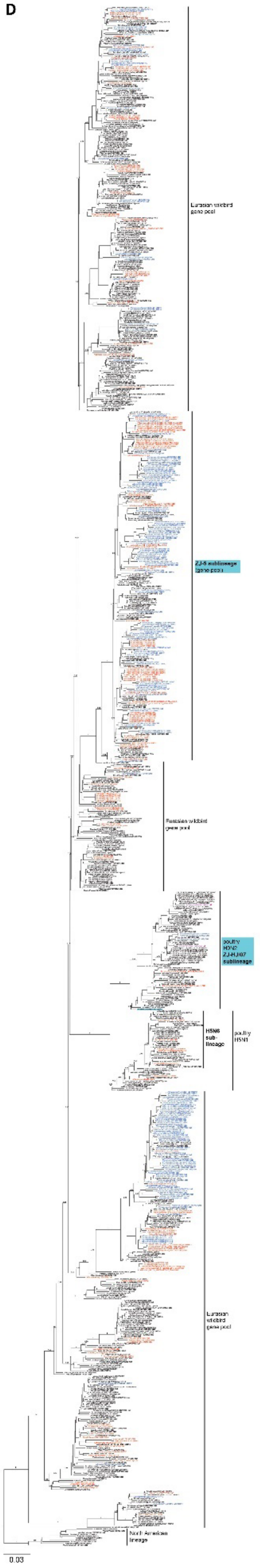
Appendix Figure 2. Phylogenetic trees of neuraminidase (NA) genes. Maximum likelihood tree of N2 (n=876, A), N3 (n=253, B), N6 (n=941, C), and N8 (n=1106, D) genes. H3 strains sequenced in this study are in steel blue. Other H3 AIVs in China are in red. For N3 gene (B), human H10N3 virus is indicated 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.

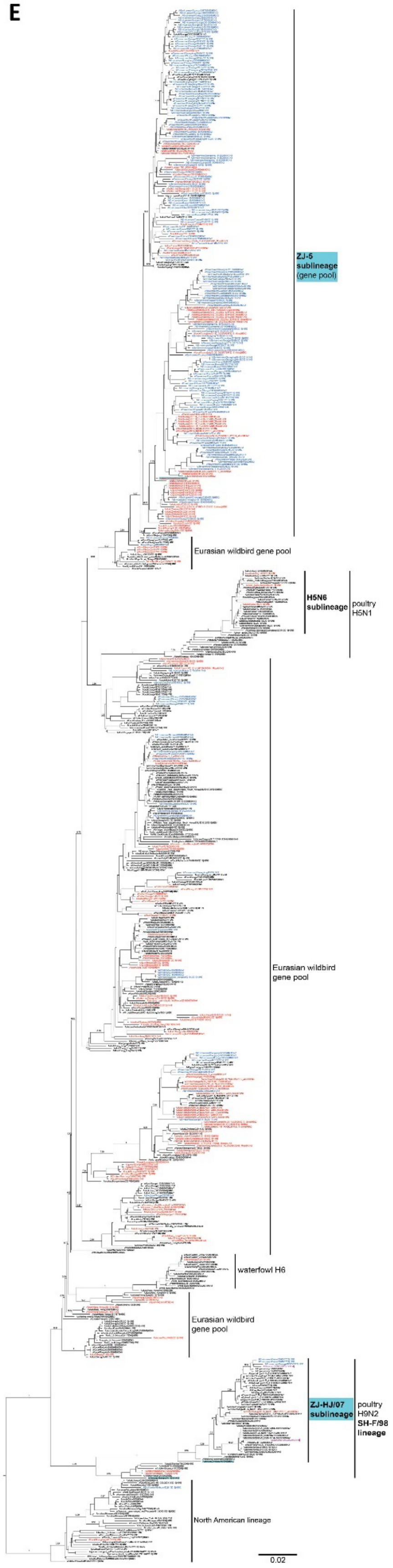

A
ZJ-5 sublineage
(gene pool)
Eurasian
wildbird
gene pool
Eurasian
wildbird
gene pool
H5N6
sub-
lineage
poultry
H5N1
Eurasian
wildbird
gene pool
Eurasian
wildbird
gene pool
poultry
H9N2
ZJ-HJ/07
sublineage
Eurasian
wildbird
gene pool
waterfowl H6
North American
lineage
0.03


B
Eurasian wildbird gene pool
ZJ-5 sublineage (gene pool)
Eurasian wildbird gene pool
H5N8 lineage
Eurasian wildbird gene pool
poultry H9N2
ZJ-HJ07 sublineage
Eurasian wildbird gene pool
H5N8 sub-lineage
poultry H5N1
waterfowl H6
North American lineage
0.02

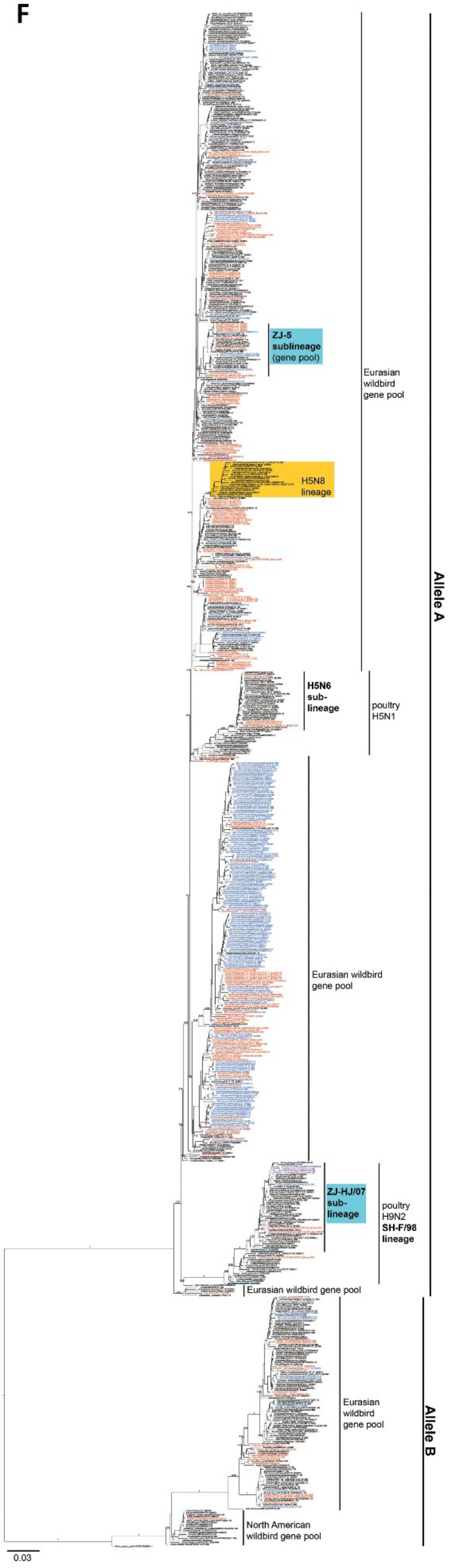

C
Eurasian wildbird gene pool
H5N6 sub-lineage
poultry H5N1
Eurasian wildbird gene pool
Eurasian wildbird gene pool
ZJ-6 sublineage (gene pool)
Eurasian wildbird gene pool
poultry H9N2 ZJ-HJ/07 sublineage
waterfowl H6
North American lineage
0.02

D

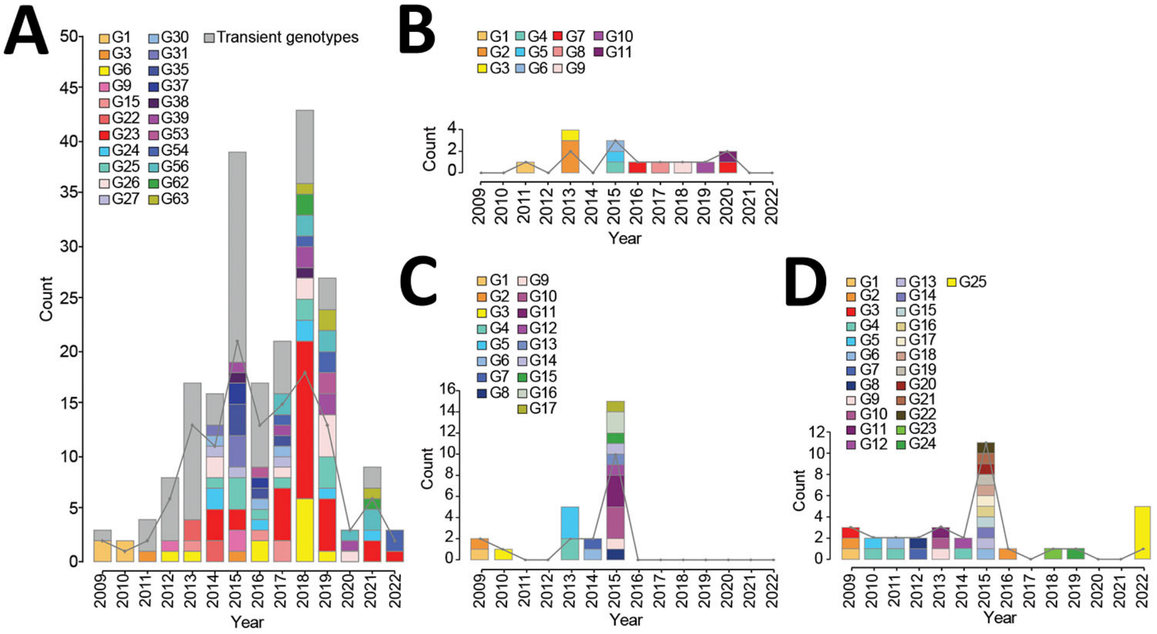

Eurasian wildbird gene pool
ZJ-5 sublineage gene pool
Eurasian wildbird gene pool
poultry H9N2 ZJ-HJ/07 sublineage
H5N6 sub-lineage
poultry H5N1
Eurasian wildbird gene pool
North American lineage
0.03


E
ZJ-5 sublineage (gene pool)
Eurasian wildbird gene pool
H5N6 sublineage
poultry H5N1
Eurasian wildbird gene pool
waterfowl H6
Eurasian wildbird gene pool
ZJ-HJ/07 sublineage
poultry H9N2 SH-F/98 lineage
North American lineage
0.02

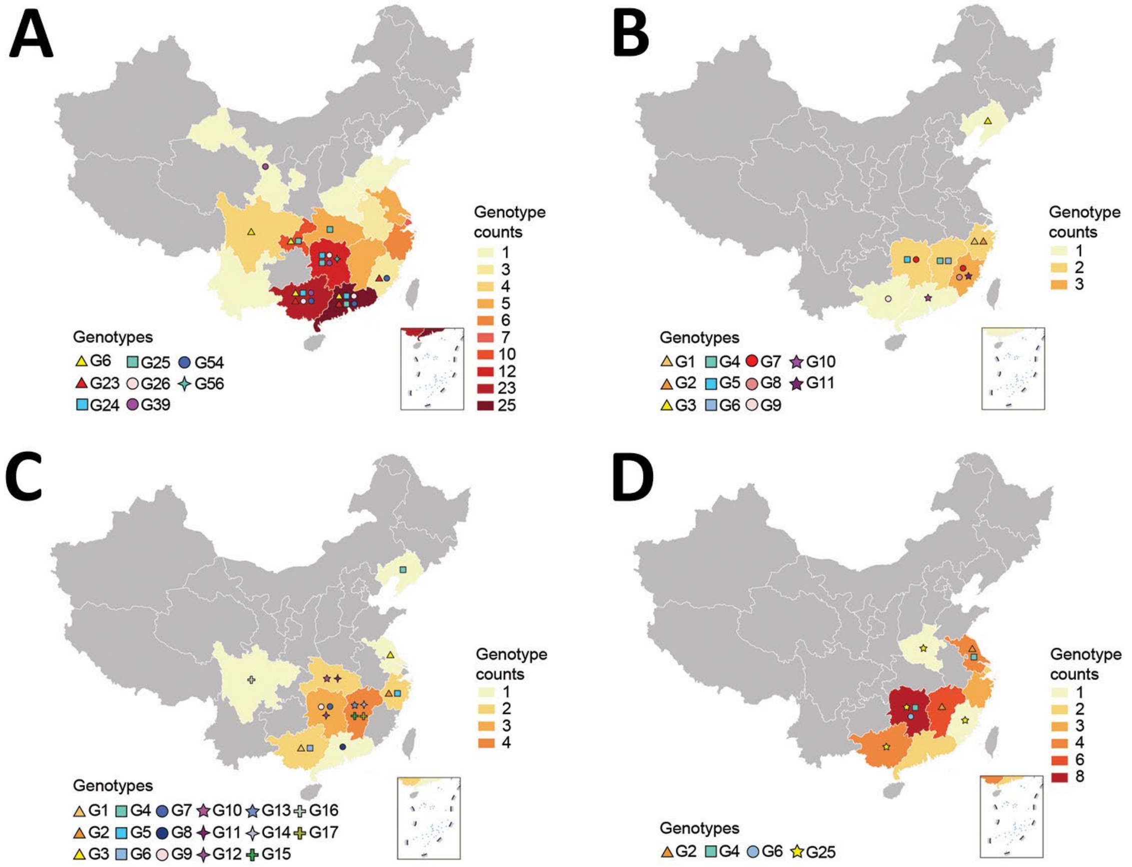
F


ZJ-5 sublineage (gene pool)
Eurasian wildbird gene pool
H5N8 lineage
Allele A
H5N6 sub-lineage
poultry H5N1
Eurasian wildbird gene pool
ZJ-HJ/07 sub-lineage
poultry H9N2 SH-F/98 lineage
Eurasian wildbird gene pool
Eurasian wildbird gene pool
Allele B
North American wildbird gene pool
0.03

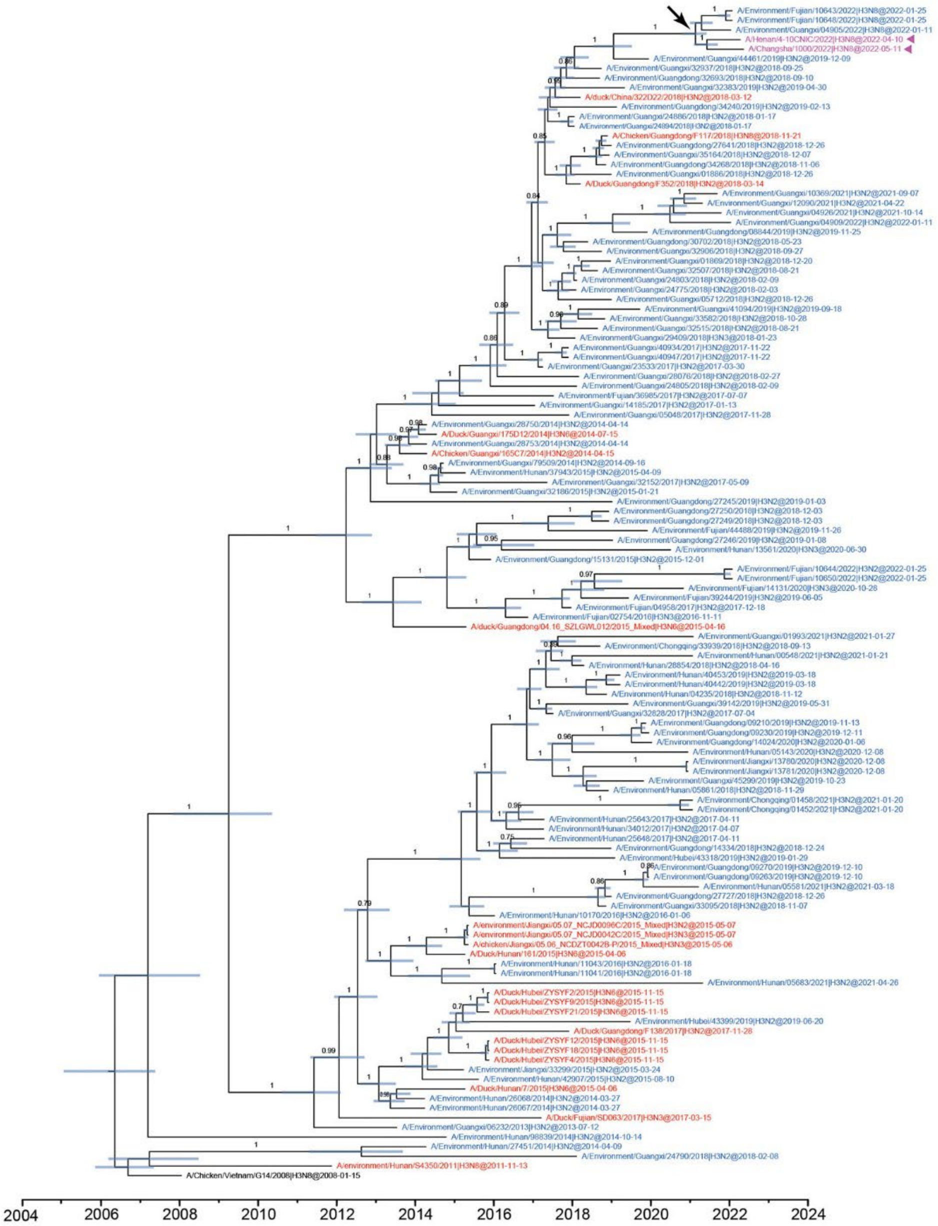
Appendix Figure 3. Phylogenetic trees of internal genes. Maximum likelihood trees of PB2 (n=929, A), PB1 (n=841, B), PA (n=841, C), NP (n=856, D), M (n=654, E), and NS (n=776, F) genes. H3 strains sequenced in this study are in steel blue. Other H3 AIVs in China are in red. The human H3N8 viruses are 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.



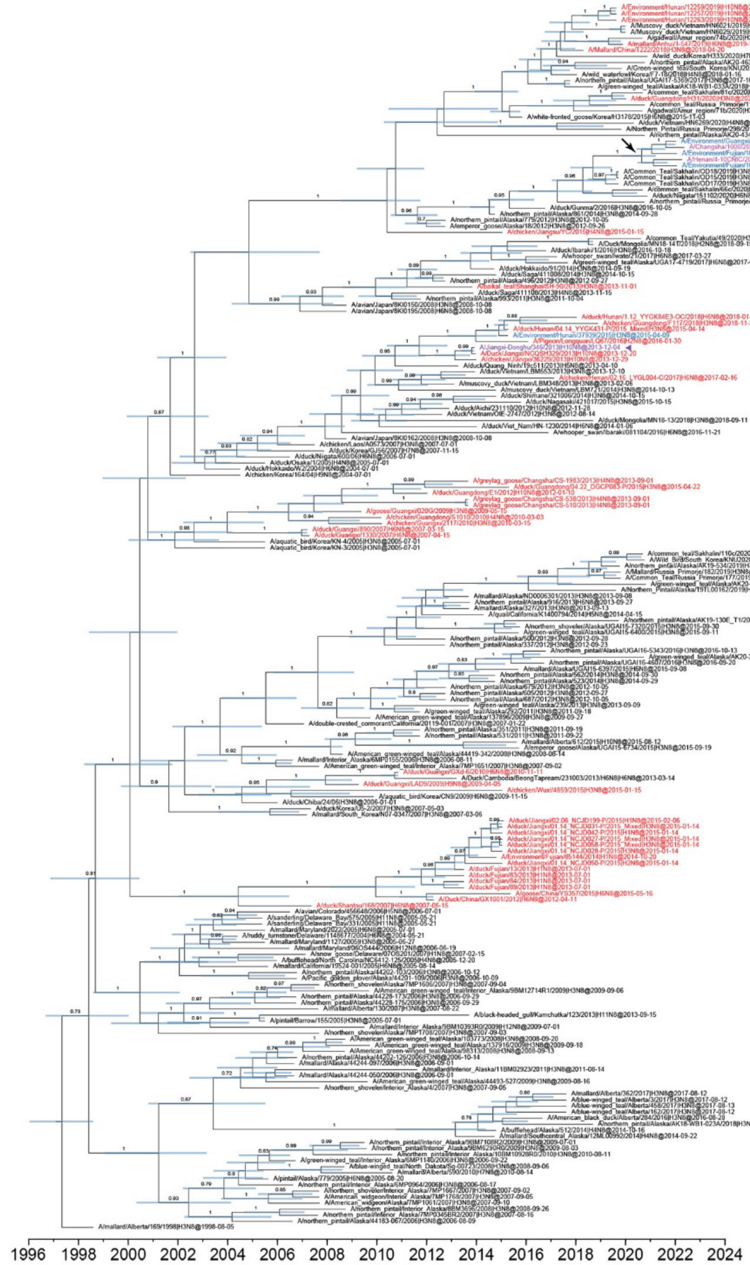
Appendix Figure 4. Diversity of genotypes of H3 subtype viruses sampled in China during 2009–2022. (A-D) Temporal distribution of genotypes of H3N2(A), H3N3(B), H3N6 (C), and H3N8 (D) AIVs. The counts of genotypes (line) and isolates of each genotype (column) are shown. For the H3N2 AIVs (A), grey column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



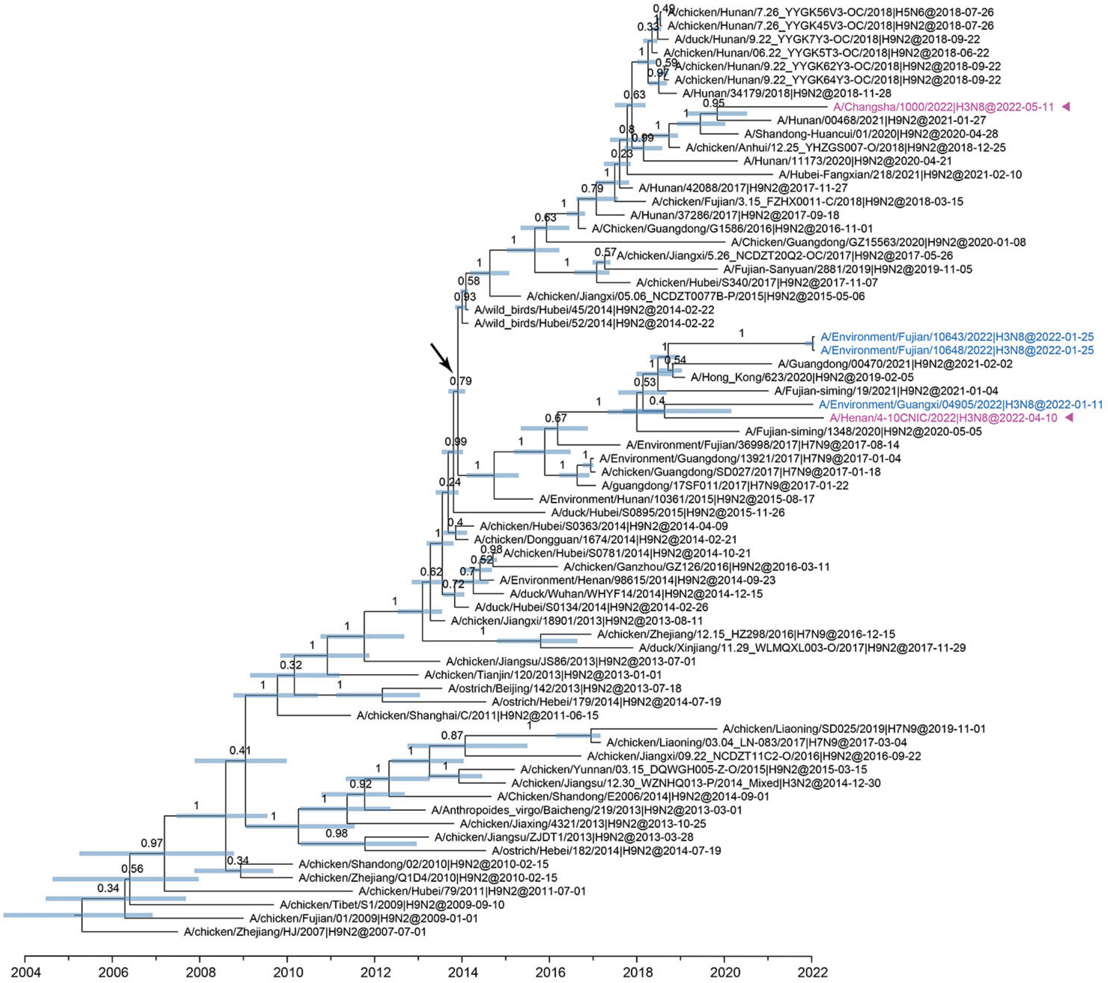
Appendix Figure 5. Geographical distribution of genotypes of H3 subtype viruses sampled in China during 2009–2022. (A-D) Geographical distributions of genotypes of H3N2 (A), H3N3 (B), H3N6 (C), and H3N8 (D) AIVs. The color of each province represents the number of genotypes. Provinces without applicable 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.



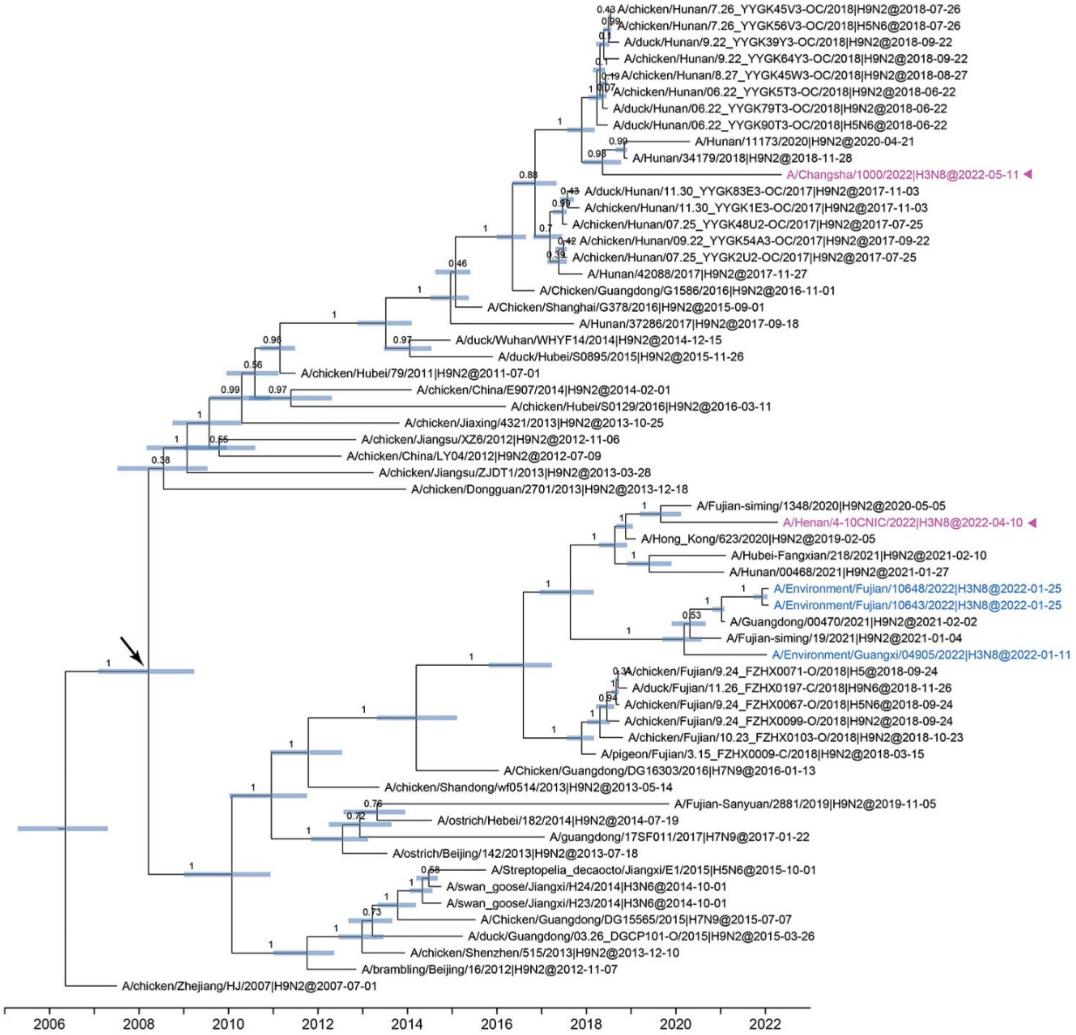
Appendix Figure 6. Maximum clade credibility tree of H3 hemagglutinin (HA) sequences of the China-1.1 subgroup viruses (n=122). H3 strains sequenced in this study are in steel blue. Other H3 AIVs in China are in red. Two human H3N8 viruses are indicated by violet and triangles. The timescale bar is provided 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.



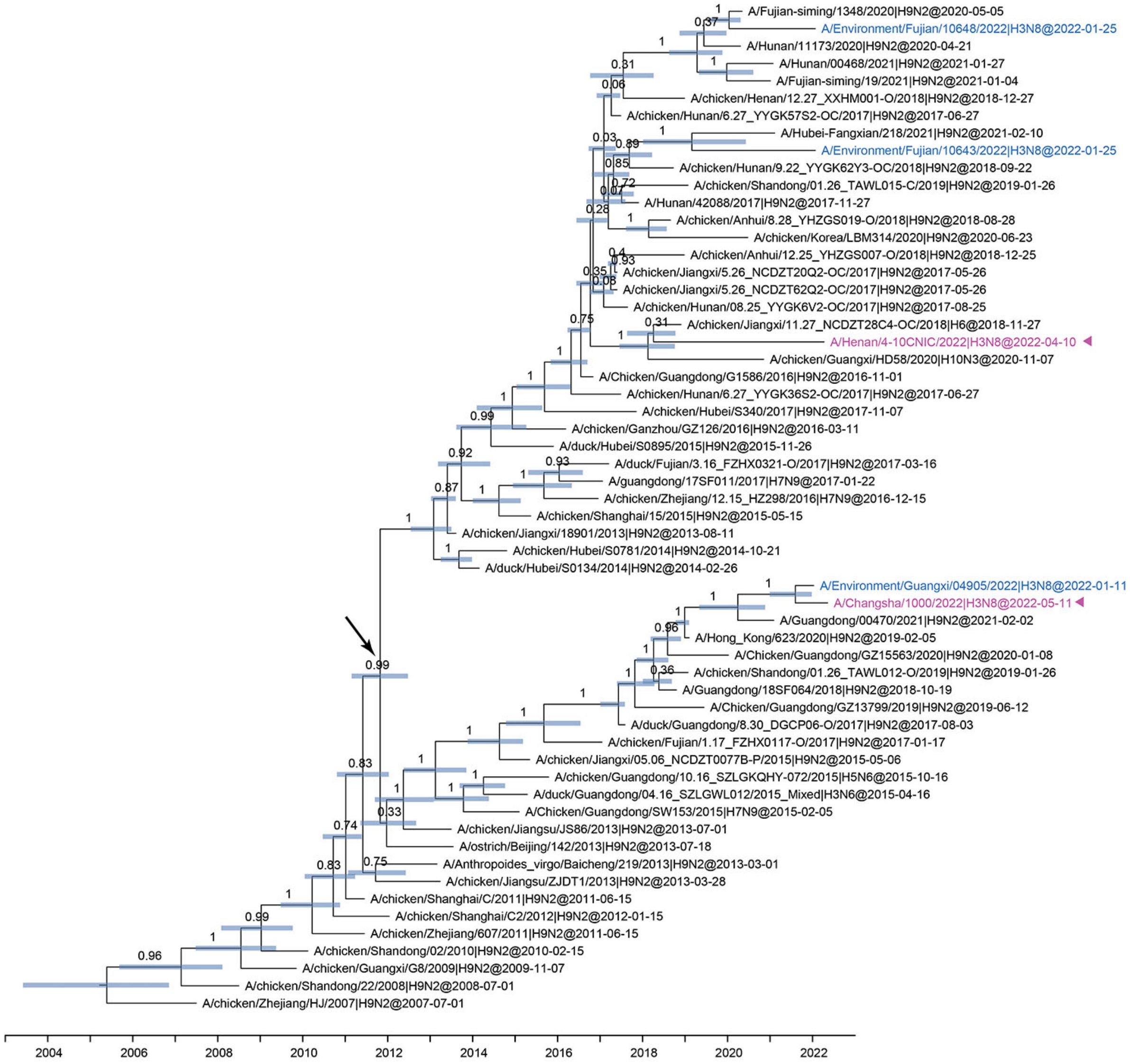
Appendix Figure 7. Maximum clade credibility tree of N8 neuraminidase (NA) sequences (n=202). H3 strains sequenced in this study are in steel blue. Other H3 AIVs in China are in red. Human H3N8 and H10N8 viruses are indicated by violet and purple, respectively. The timescale bar is provided at the bottom. 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.



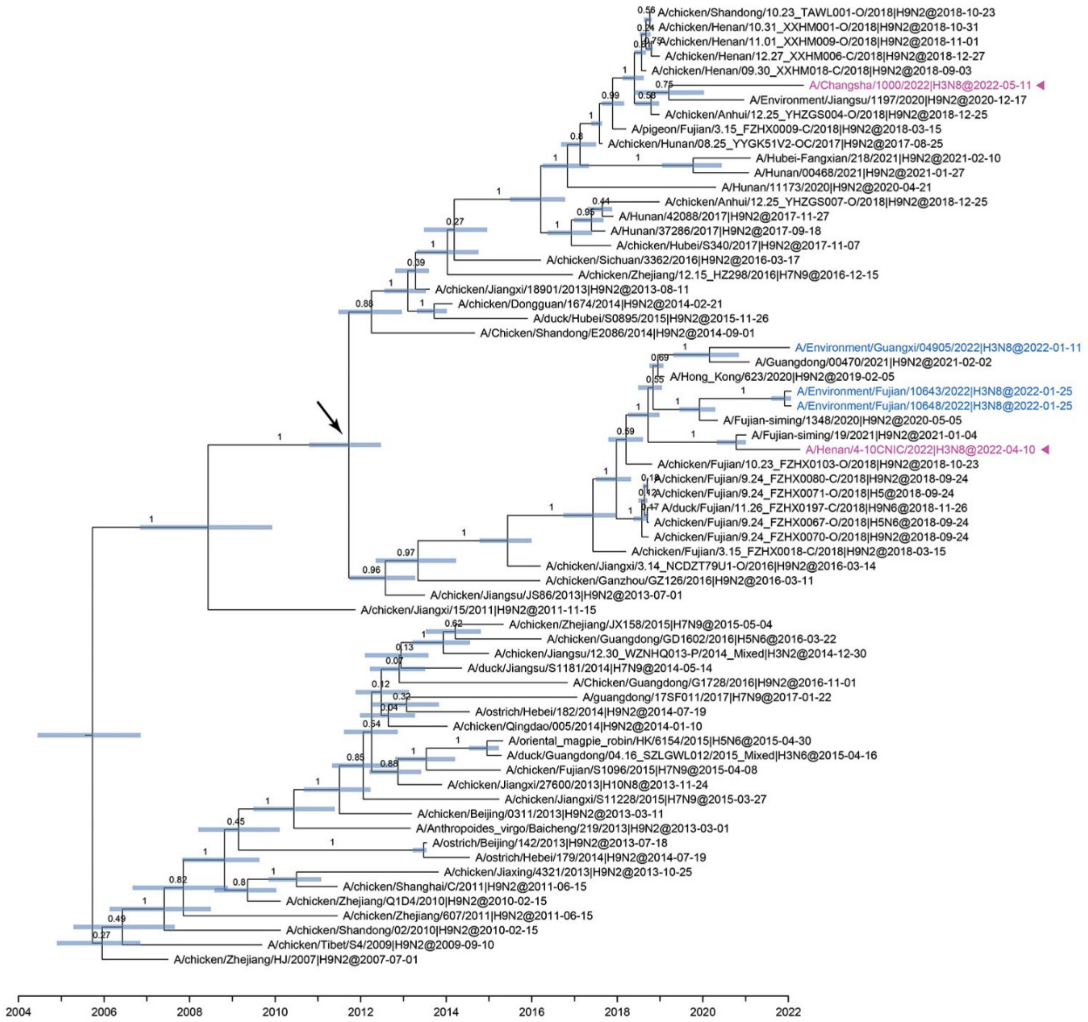
Appendix Figure 8. Maximum clade credibility tree of PB2 gene (n=69). H3 strains sequenced in this study are in steel blue. Two human H3N8 viruses are indicated by violet and triangles. The timescale bar is provided at the bottom. Clade posterior probabilities and 95% highest posterior density (HPD) 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.



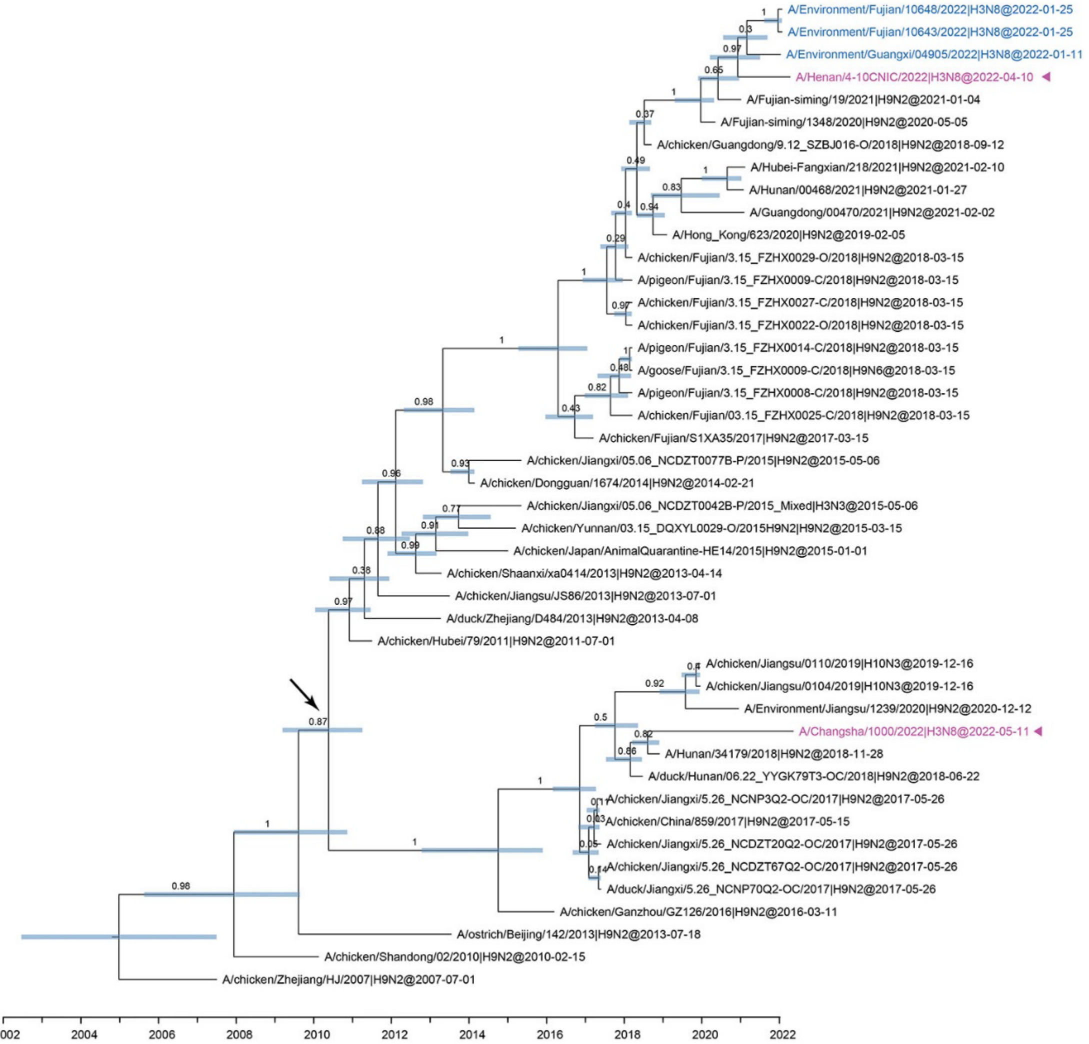
Appendix Figure 9. Maximum clade credibility tree of PB1 gene (n=60). Annotation as in Appendix Figure 8.



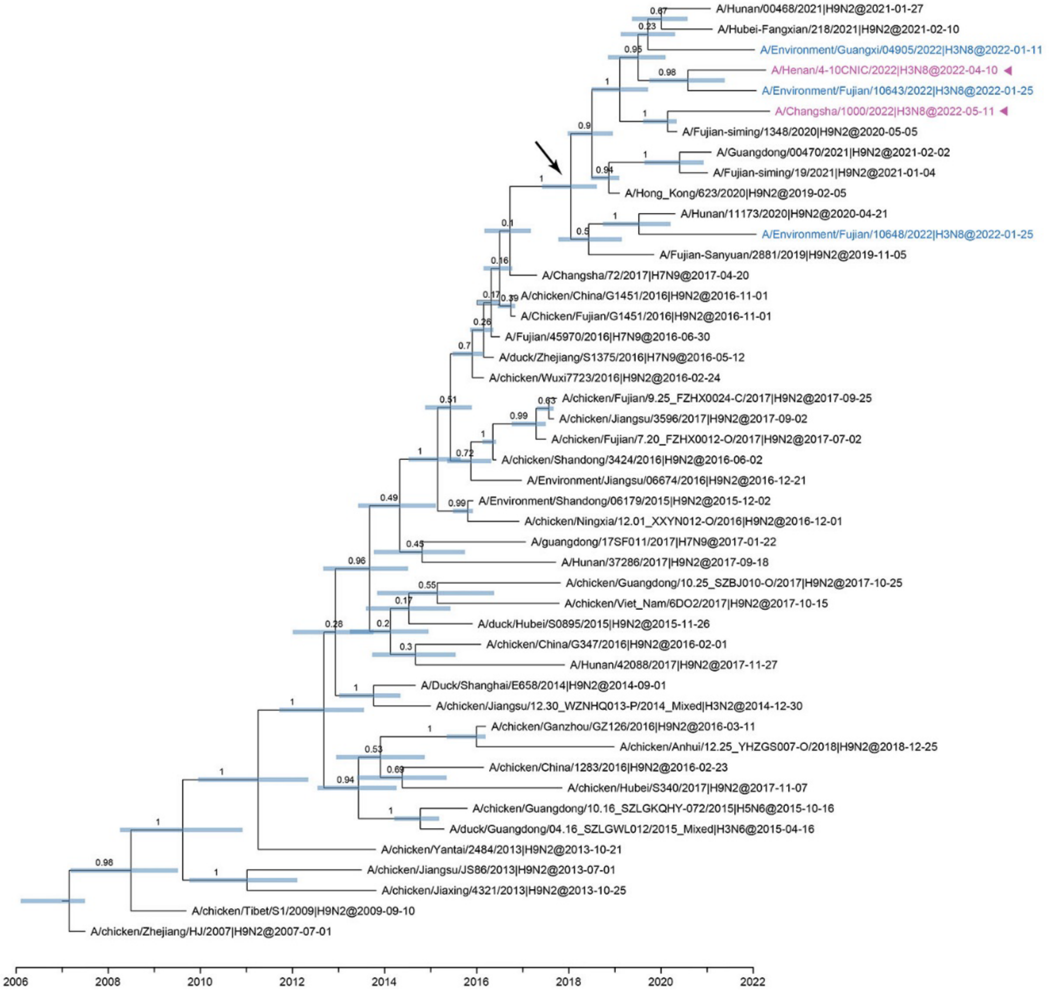
Appendix Figure 10. Maximum clade credibility tree of PA gene (n=58). Annotation as in Appendix Figure 8.



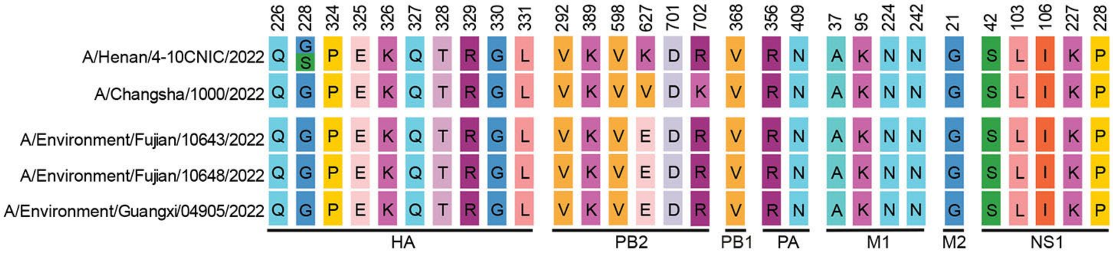
Appendix Figure 11. Maximum clade credibility tree of NP gene (n=66). Annotation as in Appendix Figure 8.



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

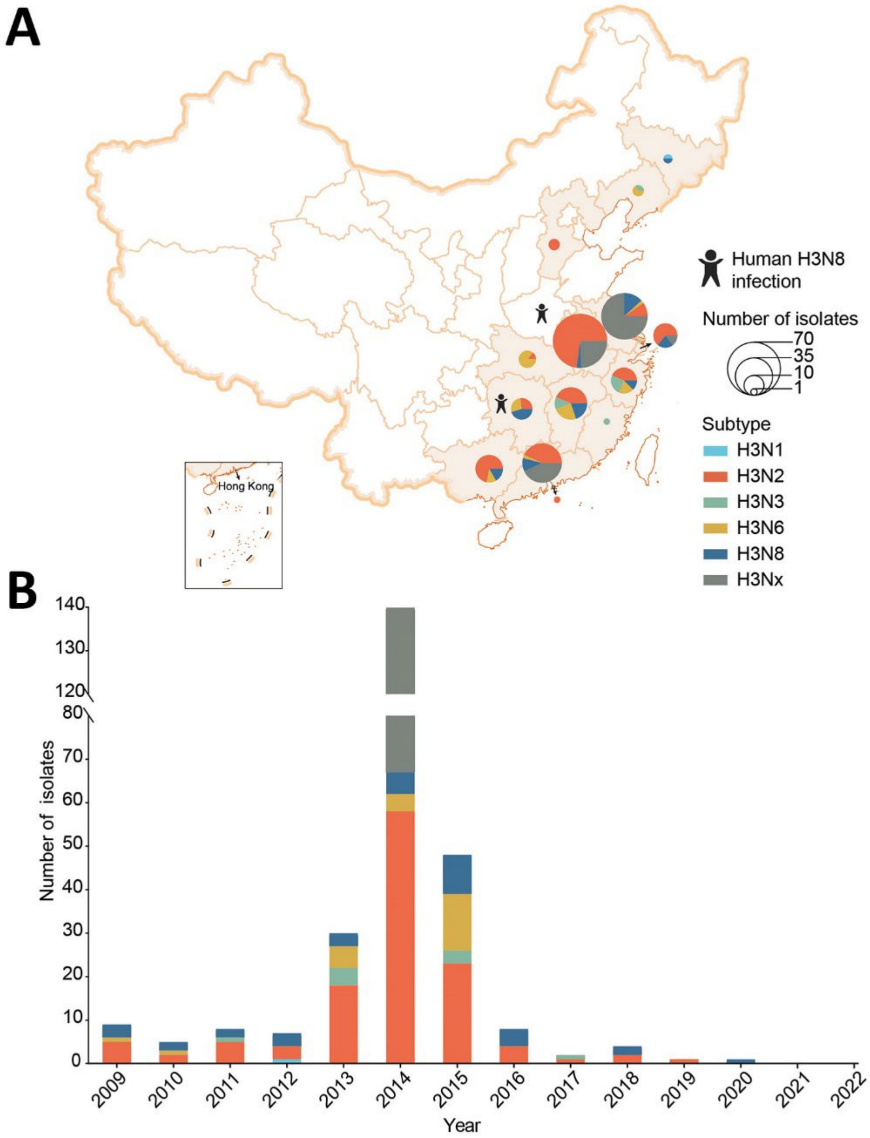


Appendix Figure 13. Maximum clade credibility tree of NS genes(n=46). Annotation as in Appendix Figure 8.



	226	228	324	325	326	327	328	329	330	331	292	389	598	627	701	702	368	356	409	37	95	224	242	21	42	103	106	227	228
A/Henan/4-10CNIC/2022	Q	G S	P	E	K	Q	T	R	G	L	V	K	V	K	D	R	V	R	N	A	K	N	N	G	S	L	I	K	P
A/Changsha/1000/2022	Q	G	P	E	K	Q	T	R	G	L	V	K	V	V	D	K	V	R	N	A	K	N	N	G	S	L	I	K	P
A/Environment/Fujian/10643/2022	Q	G	P	E	K	Q	T	R	G	L	V	K	V	E	D	R	V	R	N	A	K	N	N	G	S	L	I	K	P
A/Environment/Fujian/10648/2022	Q	G	P	E	K	Q	T	R	G	L	V	K	V	E	D	R	V	R	N	A	K	N	N	G	S	L	I	K	P
A/Environment/Guangxi/04905/2022	Q	G	P	E	K	Q	T	R	G	L	V	K	V	E	D	R	V	R	N	A	K	N	N	G	S	L	I	K	P
	HA										PB2						PB1	PA		M1				M2	NS1				

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).