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Human infection with avian influenza A(H3N8) viruses shed light on the public health concern

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Dear editor,

We read with interest that a recent article published in the *Journal of Infection*, described that the H3N8 avian influenza viruses (AIVs) that caused human infection

had originated from chickens (1). Avian influenza H3N8 viruses were widely circulating in multiple avian hosts, including wild birds, chickens, and ducks (2). Of particular note is that sporadic cross-species transmission events of the H3N8 viruses had been reported for multiple mammalian species including dogs, pigs, horses, seals, donkeys, and humans (2, 3). Although H3N8 viruses were low pathogenic in birds with mild symptoms, H3N8 viruses could cause severe respiratory disease in mammals with mammalian adaptive mutation (4). Strikingly, during 2022, human infections with H3N8 influenza viruses had been reported in China (5), highlighting that H3N8 influenza viruses had resulted in spillover events and posed a new threat to public health. Therefore, we have performed the genomic analysis to explore the epidemiology, evolutionary dynamics, and adaptive mutations of H3N8 viruses.

To understand the global prevalence and evolution of H3N8 viruses, all H3N8 resources available from GISAID's database sampled at different times and locations were analyzed. We found that except for the sharp increase in the number of H3N8 viruses isolated from 1993, there were two stages of H3N8 viruses during the period (from 1960 to 2002 and from 2003 to now). After 2003, a significant increase in the number of H3N8 viruses occurred since then (Figure 1D). In addition, we observed that H3N8 viruses were mostly distributed in North American, and East Asian countries (Figure 1C). In China, the H3N8 viruses were mainly prevalent in Eastern part of China. However, H3N8 viruses were distributed in only sporadically countries including Central and South American, African, and Oceania countries (Figure 1C),

indicative of regional distribution particularity of H3N8 viruses.

We then conducted a phylogenetic analysis of the HA and NA genes of global H3N8 viruses. In the maximum likelihood (ML) phylogenetic trees of HA and NA genes of H3N8 viruses, H3N8 viruses can be divided into three lineages. Following a previous study, three lineages were designated with North American lineage, Eurasian lineage, and Canine & equine lineages (6). We found that before 2015, H3N8 viruses were mainly distributed in three lineages; however, a sharp decrease had occurred in the number of Canine & equine lineage since 2015 (Figure 1A and Supplementary Figure 1-2). In addition, the main animal hosts of H3N8 viruses from North American and Eurasian lineage were aquatic birds including ducks and wild birds. However, H3N8 viruses from the Canine & equine lineage mainly originated from mammalian species including canine and equine. We also found that human-origin H3N8 viruses originated from Eurasian lineage, and the genomes of human-origin H3N8 viruses were genetically closely related to the poultry-origin H3N8 viruses persisting in China (Figure 1B and Supplementary Figure 1-2), indicating that a spillover event. Aquatic birds are regarded as natural reservoirs that contribute to the dissemination of AIVs through long-distance migration (7-9). The reassortment of H3N8 viruses derived from wild aquatic birds, domestic poultry, and mammals accelerated the evolutionary process of influenza viruses (10), highlighting the persistent threat that posed to humans. Using a root-to-tip regression, the temporal structure revealed the aspects of the clock-like structure of HA genes of H3N8 viruses (Figure 2A). We found that the

epidemic H3N8 viruses were classified into three lineages. We estimated the times of origin of H3N8 viruses in each lineage with 95% highest probability density (HPD) as follows: North American lineage (January 1856—March 1932), Eurasian lineage (May 1921—January 1950), Canine & equine lineage (October 1940—May 1960) (Figure 2B), indicating that the time of recent common ancestor (tMRCA) of North American lineage for HA was earlier than the counterpart in Canine & equine lineage. In this study, we found that H3N8 viruses isolated from China is prevalent in Eurasian lineage, and the tMRCA of human-origin H3N8 viruses were June 2019 (95% HPD, August 2017—March 2021), indicative of the recently emerged spillover events.

To investigate the molecular changes that occurred in H3N8 viruses after interspecies transmission to a new host, changes in the amino acid residues of the eight viral proteins, including PB2, PB1, PA, HA, NP, NA, M1, and NS1 were analyzed using Datamonkey (http://covid19.datamonkey.org). Sixteen residues of the HA protein in H3N8 viruses exhibited a positive selection. Residues 11 and 205 in the HA protein, residues 125, 376, 450, and 452 in the NA protein, residue 201 in the PA protein, residues 205, 207, 209, 212, 213, 216, 223, and 226 in the NS protein were under positive selection according to the SLAC, FUBAR, MEME, and FEL. All of the residues were polymorphic, indicating that these positively selected sites had been undergoing continuous evolution. It is noteworthy that the percentage of novel A154S substitution of HA protein is increasing in recent years, and the 154 site of HA protein is located at the head of the HA1 subunit and the receptor-binding site, which requires

further investigation of the function of this mutation. In sum, our study offers novel insights into the evolutionary dynamics and adaptive mutation of H3N8 viruses. With the continuous evolution and human infection with H3N8 viruses, comprehensive influenza surveillance in birds and mammals should be taken immediately performed to prevent H3N8 viruses and other novel subtype influenza to infect humans.

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Conflicts of interest

The authors declare no conflict of interest.

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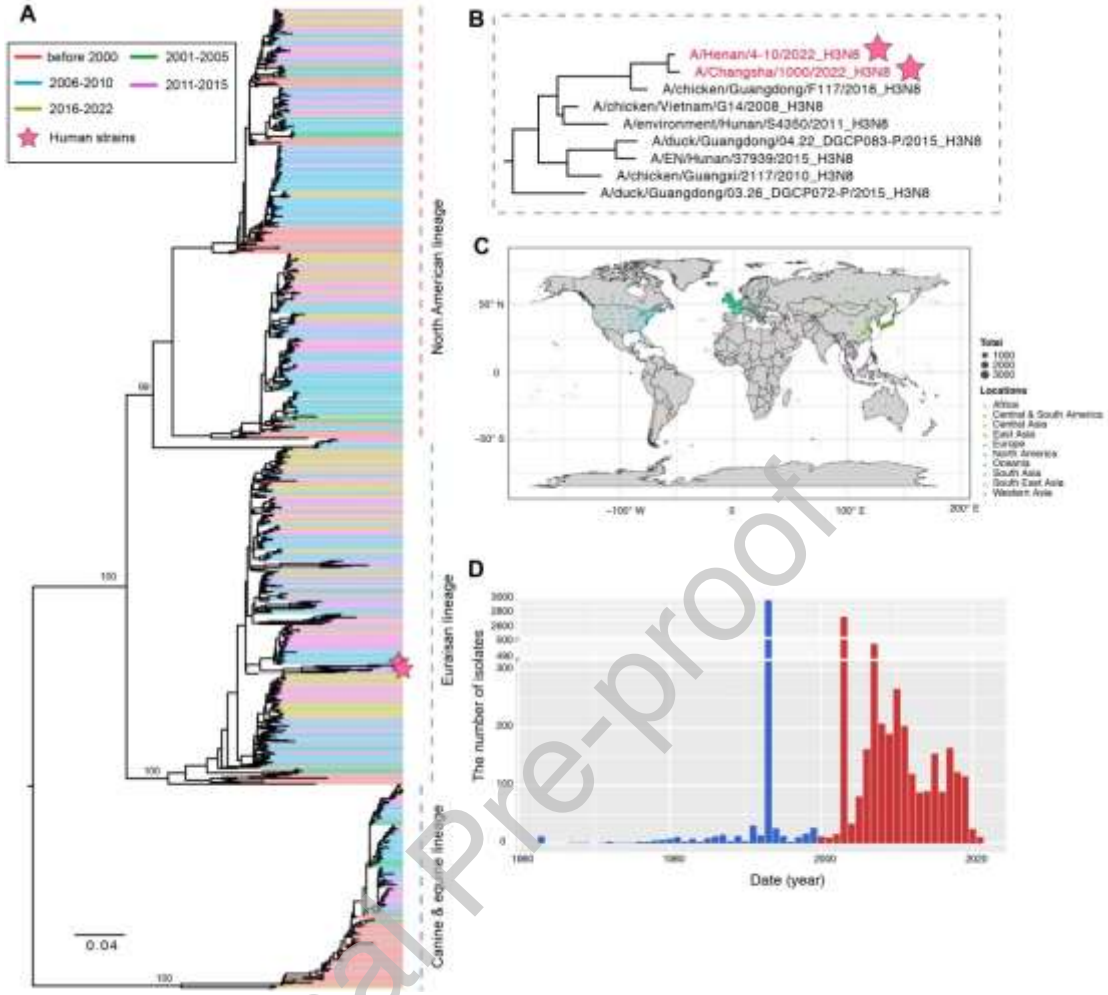

Figure 1. Evolutionary history of H3N8 avian influenza viruses. (A) Phylogenic tree of the hemagglutinin gene of H3N8 viruses. Colors indicate reference H3N8 viruses from each period (**B**) Red star on the right of the tree indicates isolates from humans. All branch lengths are scaled according to the numbers of substitutions per site. (C) Global distribution of H3N8 viruses in different continents. The map was drawn by R software. **(D)** The number of global H3N8 viruses from each year.

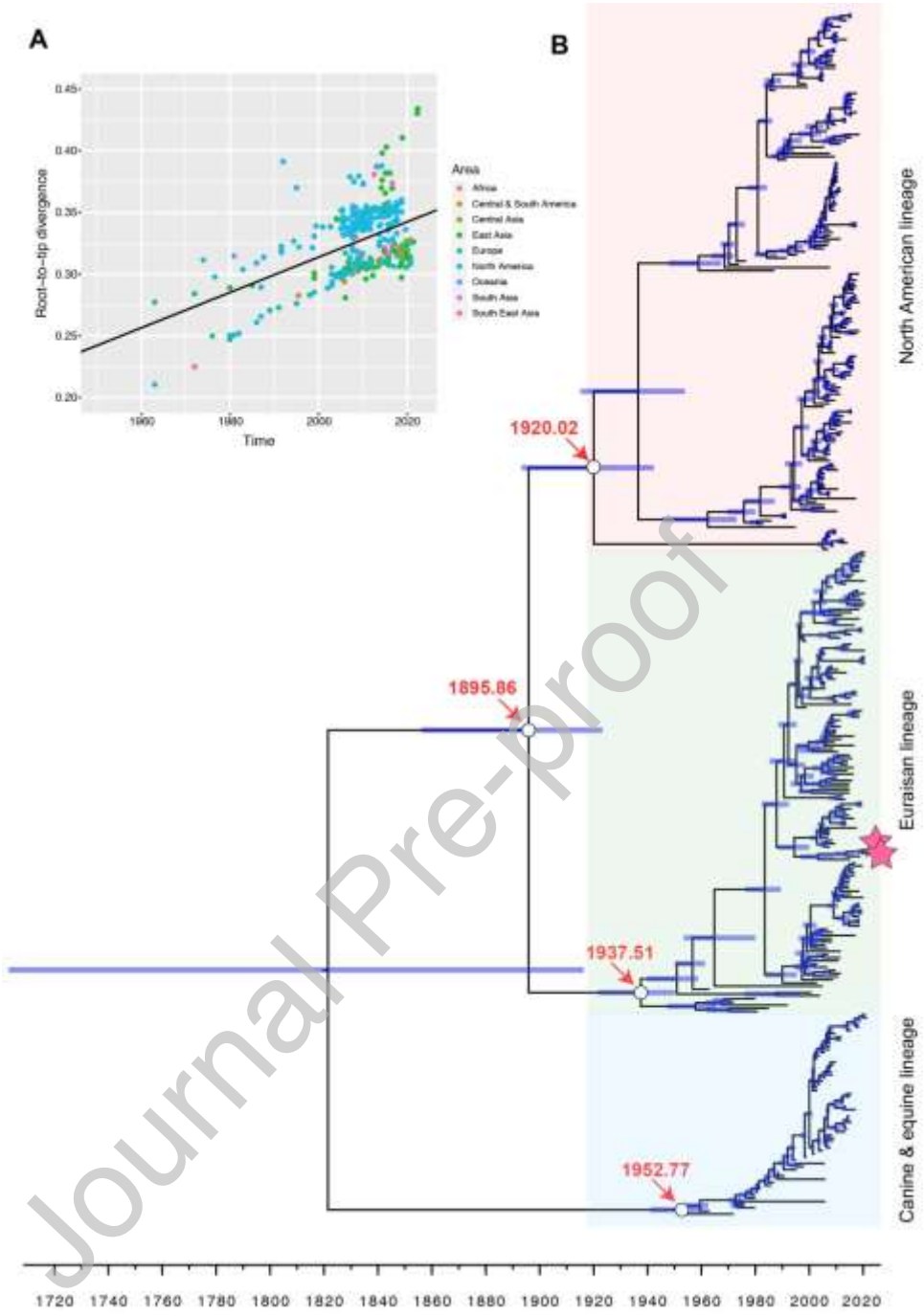

Figure 2. Time-scaled evolution of global influenza A(H3N8) viruses. **(A)** Analysis of root-to-tip divergence against sampling date for the hemagglutinin gene segment. **(B)** Maximum clade credibility tree of the hemagglutinin sequence of global H3N8 viruses. The human-origin H3N8 viruses are highlighted in a red star. Shaded bars represent the 95% highest probability distribution for the age of each node.

Supplementary Figure 1. The ML tree of HA gene of H3N8 viruses.

Supplementary Figure 2. The ML tree of NA gene of H3N8 viruses.

Supplementary Figure 3. The percentage of positive selective amino acid sites of H3N8 viruses in this study.