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Interim estimates of divergence date and vaccine strain match of human influenza A(H3N2) virus from systematic influenza surveillance (2010–2015) in Hangzhou, southeast of China

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SUMMARY

Objectives: In the post-pandemic period 2010–2015, seasonal influenza A(H3N2) virus predominated in Hangzhou, southeast of China, with an increased activity and semi-annual seasons. This study utilized *HA* virus gene segment sequences to analyze the divergence date and vaccine strain match of human influenza A(H3N2) virus from systematic influenza surveillance in Hangzhou.

Methods: Virological and serological analyses of 124 representative A(H3N2) viruses from prospective studies of systematic surveillance samples were conducted to quantify the genetic and antigenic characteristics and their vaccine strain match.

Results: Bayesian phylogenetic inference showed that two separate subgroups 3C.3 and 3C.2 probably diverged from group 3C in early 2012 and then evolved into groups 3C.3a and 3C.2a, respectively, in the 2014/15 influenza season. Furthermore, high amino acid substitution rates of the HA1 subunit were found in A(H3N2) group 3C.2a variants, indicating that increased antigenic drift of A(H3N2) group 3C.2a virus is associated with a vaccine mismatch to the 2015/16 vaccine reference strain Switzerland/9715293/2013 (group 3C.3a).

Conclusions: A portion of the group 3C.2a isolates are not covered by the current A(H3N2) vaccine strain. These findings offer insights into the emergence of group 3C.2a variants with epidemic potential in the imminent influenza seasons.

1. Introduction

Influenza viruses are important causative pathogens of respiratory tract infections in humans and animals globally and occasionally give rise to human pandemics. Currently circulating subtypes of human seasonal influenza viruses are A(H1N1)pdm09, A(H3N2), and B-Yamagata lineage, which are the three components of the annual trivalent vaccine recommended by the World Health Organization (WHO) Collaborating Centers for Reference and Research on Influenza (WHOCCs).[1] A(H3N2) evolves significantly faster than the other subtypes,[2] and thus could spread more quickly; it usually causes more severe outcomes among risk groups.[3] Season by season, continuous antigenic drift occurs in these viruses, altering their ability to cause infection and be transmitted among hosts. The circulating viruses escape the human immune response by having differences from the vaccine strains, and this can lead to vaccine failure.[4] In response to the threats posed by variants, the recommended seasonal A(H3N2) vaccine strain for use in the northern hemisphere seasons has been replaced by the WHO four times in the last 5 years.[1]

Hangzhou is a city of national tourism with a registered population of 8.9 million. It is located on the south wing of the Yangtze River Delta and has a humid, subtropical climate, facilitating the airborne survival and transmission of influenza viruses.[5] A(H3N2) viruses emerged in the post-pandemic period and have predominated in Hangzhou since August 2010. Previous genetic analysis of the very recent Hangzhou A(H3N2) strains indicated the emergence of escape variants with antigenic drift in

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the HA1 domain.[6] However, the lack of a comprehensive comparison of prevalent isolates with vaccine strains has hampered attempts to estimate the potential vaccine efficacy (VE) and to reconstruct the origins of these epidemics in the locality. This study was performed to quantify the genetic diversity and antigenic dynamics of seasonal A(H3N2) viruses in Hangzhou using a dataset of 124 isolates from more than 60 months of systematic surveillance. This was done in order to determine the possibility of more severe outbreaks in the near future.

2. Materials and methods

2.1. Systematic influenza surveillance

The surveillance was conducted from January 2010 to March 2015 among patients with an influenza-like illness (ILI) admitted to two tertiary hospitals in Hangzhou, China. Nasal swabs, oropharyngeal swabs, and/or tracheal aspirate samples were collected (N = 8958) with the informed consent of the patients or their spouses (Figure 1A). These were sent to the Hangzhou Center for Disease Control and Prevention for diagnosis within 24 h.

2.2. Virus detection and subtype identification

Viral RNA was extracted directly from the supernatant of the clinical sample using the RNeasy Mini Kit, as per the manufacturer's instructions (Qiagen, Germany). This was tested for the presence of seasonal influenza virus using a diagnostic real-time reverse transcription PCR (RT-PCR) on an ABI 7500 instrument (Applied Biosystems, USA) following the WHO guidelines.[7] Swab materials of positive samples were then inoculated into Madin–Darby canine kidney (MDCK) cells for virus isolation.

2.3. Hemagglutinin (HA) gene sequencing

Of 664 clinical specimens from laboratory-confirmed A(H3N2) infections, 124 (18.7%) with a high viral load (cycle threshold (Ct) value <30) were selected randomly from each epidemic for sequencing (E1, n = 8; E2, n = 9; E3, n = 8; E4, n = 5; E5, n = 54; E6, n = 22; E7, n = 18). The HA segment was subsequently amplified by RT-PCR with the primers described previously, using a PrimeScript One Step RT-PCR Kit Ver.2 (TaKaRa, Japan).[8,9] The amplicons were sequenced in both directions at Sangon (Shanghai, China). All sequences were assembled and edited with Lasergene v. 7.1.0 (DNASTAR). The full-length sequences obtained were then deposited in Global Initiative on Sharing Avian Influenza Data (GISAID) databases under accession numbers **EPI622591**–**EPI622714**.

2.4. Phylogenetic analysis

Multiple sequence alignment was conducted using Clustal X v. 1.8 combined with reference sequences of A(H3N2) viruses available in the GISAID databases (the accession numbers are listed in Table 1). Phylogenetic trees were generated by maximum likelihood method with bootstrap analysis (1000 replicates) using the MEGA v. 5.0 program.[10] The group numbers were assigned to achieve consistency with earlier studies.[11]

In order to assess the extent of divergence between different groups or clusters, these data were then used to infer the temporal phylogenies, viral evolution rates, and divergence dates of A(H3N2) viruses employing a relaxed clock model with uncorrelated log-normal rate distribution in a Bayesian Markov chain Monte Carlo (MCMC) framework implemented in BEAST package v. 1.7.4[12] and Tracer v. 1.5.0 software. Bayesian phylogenetic inference was presented graphically using FigTree v. 1.4.2 application.

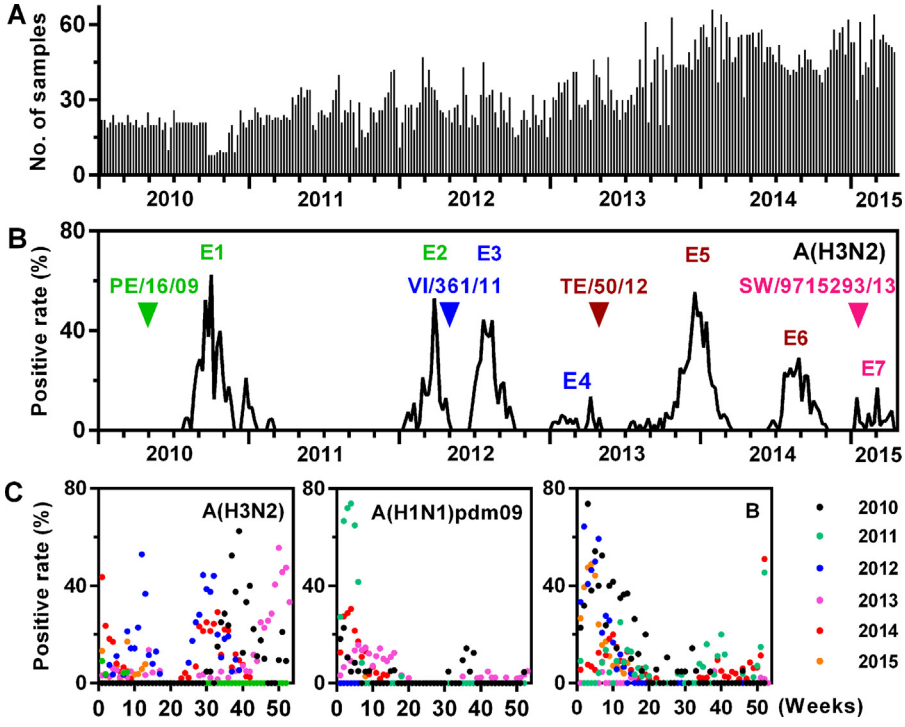

Figure 1. The prevalence of seasonal influenza virus in Hangzhou, China. (A) The number of patients with an influenza-like illness (ILI) sampled weekly during a 5-year surveillance period, from January 2010 to March 2015. (B) The positive rate of seasonal influenza A(H3N2) virus; seven A(H3N2) epidemics are seen (E1, August 2010 to December 2010; E2, January 2012 to March 2012; E3, July 2012 to September 2012; E4, January 2013 to March 2013; E5, July 2013 to February 2014; E6, July 2014 to October 2014; E7, January 2015 to March 2015). The annual vaccine candidates recommended by the WHO during these seasons are also shown, in the same color as the associated epidemics. (C) Overlapping images of the positive rates indicate the seasonal distribution of the currently circulating subtypes of human seasonal influenza viruses.