Increased genetic variation of A(H3N2) virus from influenza surveillance at the end of the 2016/2017 season for Shanghai port, China

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Influenza A(H3N2) virus exhibited complex seasonal patterns to evade pre-existing antibodies, resulting in changes in the antigenicity of the virological surface protein hemagglutinin (HA). To monitor the currently imported influenza viruses as well as to assess the capacity of health emergencies at the Shanghai port, we collected respiratory specimens of passengers from different countries and regions including some of Europe with influenza-like illness at the Shanghai port during 2016/2017, examined amino acid substitutions, and calculated the perfect-match vaccine efficacy using the epitope model. Phylogenetic analysis of the HA genes revealed that influenza A(H3N2) viruses belonging to eight subclades were detected, and three amino acid substitutions in the subclade 3C.2a.4 were also added. Besides, two epidemic influenza virus strains were found in the 2016/2017 winter and 2016 summer. The results of lower predicted vaccine effectiveness in summer suggest that the imported A(H3N2) strains were not a good match for the A/Hong Kong/4801/2014 vaccine strain since the summer of 2017. Therefore, the Shanghai Port might stop the risk of the international spread of influenza for the first time, and curb the entry of A(H3N2) from overseas at the earliest stage of a probable influenza pandemic.

Influenza viruses in the Orthomyxoviridae family cause highly contagious respiratory diseases with potentially fatal outcomes. There are currently four types in this family, which are A, B, C and D. Type D viruses have not been reported to infect humans yet1. In contrast to type B and C viruses, type A viruses in humans evolve fast and spark a devastating pandemic2–4. In particular, H3N2 subtype viruses hold responsible for a major seasonal influenza epidemic. Additionally, H3N2 subtype viruses could escape host immunity through piecemeal recombination, antigen drift or antigen conversion, and finally induce a lethal new flu pandemic with a potential to kill millions5.

On the surface of H3N2 subtype viruses, hemagglutinin (HA) proteins are closely related to the antigen variation of the epidemic influenza virus, and the variants further trigger phylogenetic clade changes. Since the spring and the summer of 2009, there have been up to seven clades (clades 1 to 7) defined by phylogeny inference6. After 2011, the derivative of Clade 3C, 3C1, 3C2, and 3C3 were dominated in many regions7,8. Previous studies demonstrated similar rates for clades 3C.2a, 3C.3, and 3C.3a early in the season9. In 2014, more new genetic subclades with special HA mutation sites emerged, 3C.2a from 3C2, 3C.3a and 3C.3b from 3C36–10. Past research showed similar rates for clades 3C.2a, 3C.3, and 3C.3a early in the season, but 3C.2a dominated rapidly in the virus population for more than 70% by January 20157. The genetic subclade 3C.2a1 emerged at the end of the 2015/2016 season11 and has become predominant in the 2016/2017 season. Afterwards, A(H3N2) 3C.2a further divided into new genetic groups by genetic drift (3C.2a.2, 3C.2a.3 and 3C.2a.4)12–14.

Compared to former years, the influenza season 2016/2017 co-circulated earlier in China, particularly in the southern regions. The influenza cases continued to rise, and A(H3N2) viruses became the dominant strain. Hong
Kong SAR government continues to report a significant number of serious influenza-related cases and deaths. By June 1st 2017, the Hong Kong Center for Health Protection had confirmed 223 cases, with 155 deaths. A(H3N2) viruses were also dominant in Europe and North America. The laboratory experiments verified that the vaccine effect of influenza A was not ideal for people over 65 from Finland and Sweden. There were higher mortality and hospitalization rate in the United States in the 2016/2017 flu season. Therefore, it is extremely vital to evaluate whether the vaccine matches the strains in circulation.

Shanghai is one of the port cities of China to the world, from which H3N2 subtype data might indicate the worldwide trend of the viruses' evolution to some extent. Here, we analyzed virological surveillance data at the Shanghai Port, described the phylogenetic evolution, inspected the antigen variation characteristics from the molecular level of the currently circulating viruses, and compared them with the vaccine and WHO reference viruses representing various genetic clades. Our findings highlighted the structural implications for the understanding of the phenotypic changes, evolution, and epidemiological monitoring of A(H3N2) viruses.

Results

Virological influenza surveillance during flu season 2016/2017. Virological influenza surveillance data in the Shanghai port were collected weekly. From February 2016 to September 2017, a total of 64 swab samples were collected from passengers of different countries, including 41 passed through Asia (25 from Hong Kong and 12 from Southeast Asia, especially), 16 passed through Europe, 7 passed through the America, and 7 passed through Oceania.

A(H3N2) virus activity increased from the 44th week of 2016, peaked in the 1st week of 2017 and decreased afterwards. The highest proportion of A(H3N2) was observed in summer (28/64, 43.7%), followed by winter (22/64, 34.3%) which outnumbered by that in spring and fall (11/64, 21.8%).

Phylogeny relationships of imported A(H3N2) viruses during the flu season 2016/2017. Of the 610 genetically characterized viruses, 546 were provided from GISAID EpiFlu databases. All 64 HA genes sequenced by the Shanghai Port belonged to the H3N2 3C.2a clade. This clade also included the vaccine strain A/HongKong/4801/2014, supporting the vaccine recommendation in the 2016–2018 northern hemisphere influenza season by WHO. Among the 64 viruses, the majority (n = 20, 31.2%) belonged to the subclade 3C.2a.1 represented by A/Singapore/INFIMH-16-0019/2016. The proportions for other subclades were 26.5% (3C.2a.2, n = 17), 25% (3C.2a.3, n = 16) and 6.2% (3C.2a.4, n = 4) (Fig. 1).

Individual clades of A(H3N2) are typically defined by amino acid substitutions that occur as they diversify from parental strains. Analysis of HA sequences indicated co-circulation of multiple variants in clade 3C.2a. All variants within subclade 3C.2a.1 shared four substitutions N121K, N171K, I406V and G484E. Three additional substitutions were observed in the 3C.2a.1 subcluster: S92R and H311Q in cluster I, G479E in cluster II. Variants 3C.2a.3 shared N121K/E and S144K (158V and S219R in cluster I and T135K and R150K in cluster II), Variants 3C.2a.2 were characterized by T131K and R142K substitutions and variants 3C.2a.4 were characterized by D53N, R142G, S144R, I182T and Q197H (Fig. 2).

There were more 3C.2a.1 variants identified from samples collected in the 2017 summer (n = 11) than in the 2016/2017 winter (n = 7). This subclade was further divided into two homogenous sub-clusters (cluster I and II). The strains from cluster I were concentrated in winter, and the cluster II strains were persisted more common in the summer months. Most viruses in the subgroup 3C.2a.3 happened in summer. And we also found that there was no prominent summer or winter trend of viruses clustered in 3C.2a.2.

The clade pattern of imported A(H3N2) influenza viruses, 2016/2017. To analyze the geographical distribution of A(H3N2) in China, 31 provinces were classified into six regions based on geographic proximity: North (Beijing), East-coastal (Shanghai), East-inland (Anhui), South-coastal (Guangdong), South-inland (Guizhou), Northwest (Jilin), North-west (Shanxi) and West (Sichuan). According to our phylogenetic analysis, the A(H3N2) number of the above six regions be counted (Fig. 3A). The Proportions for A(H3N2) in these regions were 5%, 43%, 7.2%, 19%, 4% and 7% respectively. Interestingly, higher epidemic waves of A(H3N2) were observed in Eastern and Southern in China coastal areas, and we presumed that convenient transportation and dense population contributed to it.

The genetic diversity results (Fig. 3B) indicated that the diversity increased in the East and South, especially coastal cities, Shanghai and Guangzhou. All clades and subclades of the current A(H3N2) were detected in both cities, 3C.2a.3 (60%) was dominant in Guangzhou, with a small proportion of 3C.2a.4 (10%), 3C.2a.3 (3%), 3C.1 (2%), 3C.3 (5%), 3C.2a.1-I (10%) and 3C.2a.1 (5%). In contrast, 3C.2a.1, 3C.2a.1-I and 3C.2a.2 were the major subcluster in Shanghai, with proportions of 19.21%, 32.36% and 30.34%, respectively, 3C.2a.3-II (4%), 3C.1 (1%), 3C.3 (2%) and 3C.2a (1%) were also detected in this region. The diversity of the clade pattern and the dominant clade in these two coastal cities matched well with the trends of the current global A (H3N2), likely because of the higher density of migration and subtropical monsoon climate.

Prediction of glycosylation sites in A(H3N2) viruses during flu season 2016/2017. There were two models of predicted glycosylation sites in the HA proteins of the A(H3N2) clade 3C.2a: 12 potential glycosylation sites (N9ST, N22GT, N38AT, N45SS, N63CT, N126WT, N133GT, N158YT, N165VT, N246ST, N285GS and N483GT) and 11 potential glycosylation sites (N8ST, N22GT, N38AT, N45SS, N63CT, N126WT, N133GT, N158YT, N165VT, N246ST and N285GS). All of virus strains detected at the Shanghai Port in the clade 3C.2a.1 had 11 potential glycosylation sites, and the rest in the other clades had 12 sites. Comparing to the vaccine strains 2016/2017 A/HongKong/4801/2014 (N9ST, N22GT, N38AT, N45SS, N63CT, N126WT, N133GT, N165VT, N246ST, N285GS and N483GT) and 11 potential glycosylation sites (N8ST, N22GT, N38AT, N45SS, N63CT, N126WT, N133GT, N158YT, N165VT, N246ST, N285GS and N483GT).
N483GT), the clade 3C.2a.1 virus did not have potential glycosylation site 483(N483GT), the viruses in the clade 3C.2a.2, the clade 3C.2a.3 and the clade 3C.2a.4 had the potential glycosylation site 158(N158YT).

Estimation of vaccine efficacy for A(H3N2). To assess the effect of the accumulated mutations in the HA1 domain on predicted vaccine efficacy in a given year, the p epitope method was used to evaluate how closely the vaccine strain resembles the imported strain (Table 1). Theoretically, when p epitope in the dominant epitope is higher than 0.19, the vaccine efficacy becomes negative²¹,²². For the 2016/2017 season, the average p
An epitope for all A(H3N2) strains was 0.090, which indicated the vaccine efficacy (VE) against those strains was 52.96% (E = 24.89% of 47%, p epitope = 0) of that of a perfect match with the vaccine strain. However, from the 2016/2017 winter to the 2017 autumn, the VE value fluctuated first and then decreased, with the highest value in spring (VE = 58.51%), the lowest value in autumn (VE = 58.51%), and the inflection point in summer (VE = 49.29%). These results suggest that the A(H3N2) strains circulating in 2017 were separated from the vaccine strain and effectively reduced the VE starting in the summer.

Figure 2. Schematic diagram demonstrating the shared amino acid changes between clades 3C.2a, 3C.2a.1, 3C.2a.2, 3C.2a.3 and 3C.2a.4 on HA gene.

Figure 3. The proportions of Influenza A(H3N2) in China six regions (A) and H3N2 clade patterns in China eight provinces (B).

Table 1. Calculated vaccine efficacy using $P_{\text{epitope}}$ model in dominant epitope A of influenza A(H3N2) circulating in Shanghai port during 2016/2017, winter, spring, summer and autumn.
Discussion

Human seasonal A(H3N2) virus epidemics in different zones have highly diverse patterns, especially in the northern hemisphere, where these patterns can exhibit semianual or annual epidemic cycles. Moreover, HA segments of A(H3N2) viruses have undergone considerable genetic differentiation and evolved in seven genetic groups and multiple clades/subclades since 2009. It is the result of H3N2 viruses circulating via the network of temporally overlapping epidemics and high rates of migration, rather than local persistence. It has been suggested that the global persistence of A(H3N2) virus is the result of a migrating meta-population in which multiple different localities may seed seasonal epidemics in temperate regions in a given year. Shanghai, the most developed and open city in China, attracts people around the world. So, the Shanghai Port has the characteristics of large passenger flow, high workload and wide international flight distribution. Around 44 million passengers enter and exit the Shanghai Port in 2017, accounting for 7.3 percent of the total number of people entering and exiting China's ports. In addition, the specimens involved in this study were all from international travelers, who pass through cities with high population density or dense traffic, such as Europe, the United States, and Hong Kong, China. These cities or regions provide convenient channels for the mutation, transmission and spread of influenza. Although surveillance for influenza at ports has been increasing, there have been few reports of interactions between ports and global epidemic trends. In this study, we performed genetic and evolutionary analyses for viruses obtained during 2016/2017 in order to investigate the evolution of the influenza virus during 2016/2017 at the Shanghai Port and predict the influenza (A(H3N2)) virus epidemic trends in the future.

The genetic and phylogenetic analyses in different countries and regions indicate that there has been a similar pattern existed among all of the evolutionary trends of A(H3N2) viruses discussed above. Previous research was reported by either ethnically homogenous (Taiwan, Australian and Canada) or the GISAID EpiFlu databases (isolated in Japan, Bangladesh, Australia, Thailand and the USA). In the current study, 31.2% of A(H3N2) viruses of the 3C.2a clade clustered in a subgroup carrying N121K, N171K and G484E. In Europe and Canada, the majority of A(H3N2) viruses also belonged to the subclade 3C.2a.1 during the same season, whereas amino acid substitutions were used as clade markers just at N171K or N121K. Most of the rest viruses evolved away from the 3C.2a-A/Hong Kong/4801/2014-like clade by acquiring the genetic markers T131K, R142K and N121K/E, S144K which have been identified as being characteristic of clades 3C.2a.2 and 3C.2a.3. These markers were reported in most of other H3N2 influenza viruses isolated in the Danish, Finland, Israel, Korean, Yokohama, Taiwan and the UK, in 2016/2017. Additionally, clade 3C.2a.3 was further grouped into cluster I (carrying I58V and S219Y) and cluster II (T135K and R150K). The substitutions in cluster I has appeared in report from the UK, and in cluster II emerged in the Taiwan putative clades 3C.2a.3a, which were isolated from severe patients. Members of the subclade 3C.2a.4 were characterized by two amino acid substitutions at R142G and S144R. Three other variants (D53N, I182T and Q197H) were not reported previously, which is likely to increase the odds of implications including alterations of the antigenic epitopes and immune escape. These reports, together with our results, suggested that subclusters within clade 3C.2a and eight subclades have emerged and expanded during this recent influenza epidemic. These changes were continuing to diversify worldwide with complicated and rapid dynamics.

During 2016/2017, the following two influenza epidemics occurred in this study: one epidemic between December 2016 and February 2017 and one epidemic between June 2017 and August 2017. These epidemics were consistent with the influenza epidemics observed in Hong Kong, Southeast Asia and Europe in 2017. According to the previous research on influenza seasons, influenza cases usually appear between autumn and spring, with the influenza activity peaking after October. However, the A(H3N2) virus seemed to frequently result in larger summer epidemics, such as those in 2010 and 2012, most of which occurred in 2017. The number of 2017 summer season was over than the 2016/2017 winter season. Research has served region of the receptor-binding site in the antigenic epitope A and causes a loss of a glycosylation motif. Changes in glycosylation motifs may be relevant to antigenicity, viral fitness and/or pathogenicity. Amino acid 131 is located in the antigenic epitope A and conserved in 45% of all human H1, H2 and H3 viruses. VE studies from Stockholm and Finland show that the proportion of samples containing T131K (36%) increased may be responsible for viral antigenic change and part of the observed VE drop. Studies from northern Greece have provided evidence supporting indications that the specific T135K variant may be associated with vaccination failure. The T135K mutation was observed in 82% of the viruses originating from vaccinated patients. Based on the Canadian report, the higher VE estimates may be due to the relatively infrequent (15%) circulation of the T135K variant in Canada. T135K-R150K are also as genetic markers for clade 3C.2a.3, and viruses in this clade were isolated from patients with severe infections in Taiwan. This finding can be explained by the lower VE and
shorter protection time for these clades compared with other circulating clades\(^4\). As a result, decreasing influenza vaccine protection with increasing time, A/Hong Kong/4801/2014 may not be as effective in eliciting immunity against future circulating A(H3N2) in the next influenza season. If the emerging A(H3N2) virus subpopulation continues to diversify from vaccine components, VE may decrease further by the end of the 2016/2017 season in terms of its antigenic properties or a broader cycle of variant strains.

In addition, compared to A/Hong Kong/4801/2014, the 3C.2a strains in our surveillance data had at least eleven potential N-glycosylation sites and had similar glycosylation patterns. Clade 3C.2a.1-like strains lost one potential glycosylation sites: 483(N\(^{483}\)GT), Clade 3C.2a.2-like, 3C.2a.3-like, and 3C.2a.4-like strains gained one potential glycosylation site: 158(N\(^{158}\)YT). Research shows that the mechanisms of glycosylation and deglycosylation in virus fall into three general classes: (i) mask antigenic epitopes and thus block binding to neutralizing antibodies\(^4\), (ii) adjust receptor-binding affinity\(^5\), or (iii) modulate the virulence of the Influenza viruses in mammals\(^6\). It’s could be concluded that the virus would make itself to elude the existed antibodies by changing herd immunity or increasing the viral fitness in an unknown mechanism.

In summary, our study indicates that new mutations and derivations of clade 3C.2a emerged continuously and rapidly during 2016/2017, which can reflect the trend of the current global influenza A(H3N2), and also has a paramount impact on the viral adaptation and transmission. So Shanghai might stop the risk of the international spread of influenza for the first time, and curbing the entry of A(H3N2) from overseas at the earliest stage of the influenza pandemic. Strengthening the entry monitoring of ports in coastal areas and improving the ability of ports health quarantine to deal with public health emergencis are required to investigate the antigenic effects and to avoid the next pandemic.

Methods

Ethics statements. This study was performed with the residual samples collected for the detection of influenza in the Shanghai Port. All the samples used for research have got the informed consent of passengers. The sample for the informed consent was attached as follows.

The study protocols were approved by the Institutional Review Board of Bio-X Life Science Research Center of Shanghai Jiao Tong University (IRB No. M202007). Informed consent was obtained from all subjects, and all methods were carried out in accordance with relevant guidelines and regulations.

Surveillance and data collection. The Shanghai port located on the south wing of the Yangtze River Delta Region. It is a key transportation hub combing water, land, and air transportation, facilitating the airborne survival and transmission of influenza viruses. During 2016/2017, a total of 64 throat swabs were collected from influenza-like illness cases in the Shanghai port. The basics epidemiological data including patients’ age, stopover sites and sampling dates were collected. The detailed list of viruses is provided in Table 2.

RNA purification and HA gene sequencing. Viral RNA was obtained from 140 μl of the sample by the QIAMP viral RNA extraction kit (Qiagen, Hilden, Germany) according to the manufacturer’s instructions. RNA was eluted in 100 μl RNase/DNase elution buffer provided in the kit and stored at –40 °C. Samples were subtyped based the Real-Time RT-PCR Diagnostic assay (Shanghai Biogerm Influenza H1/H3 typing assay) for detection of influenza virus in an ABI Prism 7500 thermocycler (Applied Biosystems, Foster City, California, USA). The HA genes were subsequently amplified by the samples with a Ct value of less than 30, and were subjected to reverse transcription and amplification using a PrimeScript One-step RT-PCR Kit Ver.2 (TaKaRa, Japan) as previously described\(^6\). RT-PCR products with the correct band size were selected by agarose gel electrophoresis and sequenced using ABI PRISM Dye Deoxy Terminator cycle sequencing kit (Life Technology, Foster City, CA, USA). These HA gene sequences were assembled using the SeqMan Pro software (DNASTAR, Madison, WI) and deposited in the National Center for Biotechnology Information (https://www.ncbi.nlm.nih.gov/) with the accession numbers (OM956220–OM956283). Finally we obtained 64 full-length HA sequences.

Sequence alignment and phylogenetic analysis. Reference sequences of known clades and vaccine strains (the accession numbers in Supplementary Table S2) as recommended by WHO\(^7\) included in the evolutionary analysis were retrieved from GISAID EpiFlu databases. All HA gene sequences aligned by MAFFT program (MAFFT-7.220-WIN64 version). Protein translation were performed on the basis of nucleotide sequences using Molecular Evolutionary Genetics Analysis software (MEGA, version 6.0; http://www.megasoftware.net/). Phylogenetic tree was constructed using the maximum-likelihood method with a Hasegawa-Kishino-Yano (HKY) + gamma nucleotide substitution model and 1000 bootstrap replications. The ability to perform clade designations based on signature amino acids as compared to A/Hong Kong/4801/2014-like A/H3N2-like clade 3C.2a viruses and A/Singapore/INFIMH-16-0019/2016-like A/H3N2-like clade 3C.2a.1 viruses was confirmed with the 64 isolates depicted in the final tree, and extended to the other 346 isolates.

Prediction of glycosylation sites. The prediction of potential N-liked glycosylation sites was performed with an online server: NetNGlyc 1.0\(^8\). This server considers the amino acid alignment N-X-S/T, where X can be any amino acid except Asp or Pro. A threshold value of > 0.5 suggests glycosylation.

Prediction of vaccine efficacy. The predicted VE of A(H3N2) was estimated using the Pepitope model, which is a measure of the antigenic distance between vaccine and circulating strains. Antigenic distance was calculated from the fraction of substituted amino acid residues in the dominant HA epitope\(^9\). The amino acid residues in the HA epitopes of A(H3N2) were pre-defined in the p epitope calculator to respectively possess 19, 21,
| Name            | Patients' age | Nationality | Stopover sites                  | Sampling dates | Accession numbers   |
|-----------------|---------------|-------------|----------------------------------|----------------|---------------------|
| 201602161010.seq | 9             | China       | Hong Kong                        | 2016.02.16     | OM956282            |
| 201608081702.seq | 29            | China       | Australia, Hong Kong              | 2016.08.08     | OM956281            |
| 201609171121.seq | 29            | China       | Hong Kong, Australia              | 2016.09.17     | OM956280            |
| 201610051316.seq | 31            | China       | Hong Kong                        | 2016.10.05     | OM956279            |
| 201610241504.seq | 44            | China(Taiwan)| Japan, the USA, Hong Kong, Taiwan| 2016.10.24     | OM956257            |
| 201610251519.seq | 24            | China       | Hong Kong                        | 2016.10.25     | OM956278            |
| 201611031787.seq | 25            | China       | Hong Kong                        | 2016.11.30     | OM956277            |
| 201612141923.seq | 2             | China       | Hong Kong                        | 2016.12.14     | OM956276            |
| 201612261085.seq | 20            | China       | Hong Kong                        | 2016.12.26     | OM956275            |
| 201701051269.seq | 49            | Australia   | Italy                            | 2017.01.05     | OM956256            |
| 201701071301.seq | 63            | The Netherlands | The Netherlands                  | 2017.01.07     | OM956255            |
| 201701111341.seq | 65            | China       | Indonesia                        | 2017.01.11     | OM956228            |
| 201701121362.seq | 23            | China       | The United Arab Emirates(UAE)    | 2017.01.12     | OM956227            |
| 201701121363.seq | 62            | China       | Vietnam                          | 2017.01.12     | OM956224            |
| 201701151395.seq | 38            | China       | The USA                          | 2017.01.15     | OM956254            |
| 201701211465.seq | 13            | China       | Britain                          | 2017.01.21     | OM956253            |
| 201701211466.seq | 53            | China       | France                           | 2017.01.21     | OM956252            |
| 201701241505.seq | 5             | China       | Italy                            | 2017.01.24     | OM956223            |
| 201701251535.seq | 36            | Germany     | Germany                          | 2017.01.26     | OM956221            |
| 201701291611.seq | 30            | China       | Macao                            | 2017.01.29     | OM956225            |
| 201702051704.seq | 10            | China       | The USA, Hawai                      | 2017.02.06     | OM956231            |
| 201702101743.seq | 58            | China       | Iceland, Norway                   | 2017.02.10     | OM956230            |
| 201702112178.seq | 34            | China       | Canada                           | 2017.02.12     | OM956249            |
| 201702131787.seq | 1             | The USA     | The USA                           | 2017.02.13     | OM956248            |
| 201702211287.seq | 45            | China       | Canada                           | 2017.02.22     | OM956247            |
| 201702211974.seq | 43            | Indonesia   | Indonesia                         | 2017.02.21     | OM956258            |
| 201702221782.seq | 64            | Netherlands | Netherlands, France              | 2017.02.22     | OM956246            |
| 201703261906.seq | 30            | China       | The Netherlands                   | 2017.02.26     | OM956245            |
| 201703041965.seq | 30            | China       | Germany, South Korea              | 2017.03.06     | OM956244            |
| 201703081006.seq | 55            | Russia      | Russia, Indonesia                  | 2017.03.08     | OM956243            |
| 201705116550.seq | 32            | Indonesia   | Indonesia, Malaysia               | 2017.05.11     | OM956224            |
| 201705191715.seq | 33            | China       | Hong Kong                         | 2017.05.19     | OM956272            |
| 201706081022.seq | 67            | China       | Turkey                            | 2017.06.08     | OM956242            |
| 201707231849.seq | 12            | China       | Hong Kong                         | 2017.07.23     | OM956283            |
| 201707231857.seq | 59            | China       | Hong Kong                         | 2017.07.23     | OM956271            |
| 201707241890.seq | 54            | China       | Hong Kong, Thailand               | 2017.07.24     | OM956270            |
| 201707261936.seq | 69            | China       | Hong Kong                         | 2017.07.26     | OM956269            |
| 201707271994.seq | 21            | China       | Macao, Hong Kong                  | 2017.07.27     | OM956268            |
| 201707271996.seq | 54            | China       | Hong Kong                         | 2017.07.27     | OM956267            |
| 201707301041.seq | 8             | China       | Indonesia                         | 2017.07.30     | OM956230            |
| 201707301044.seq | 72            | China       | Russia                            | 2017.07.30     | OM956241            |
| 201708061243.seq | 21            | China       | Japan                             | 2017.08.06     | OM956266            |
| 201708061245.seq | 26            | China       | The USA, Canada                   | 2017.08.07     | OM956240            |
| 201708071293.seq | 38            | China       | Hong Kong, Thailand               | 2017.08.07     | OM956265            |
| 201708101387.seq | 20            | China       | Hong Kong                         | 2017.08.10     | OM956264            |
| 201708111416.seq | 71            | China       | France, Switzerland, Italy        | 2017.08.11     | OM956239            |
| 201708111419.seq | 9             | China       | Hong Kong, Macao                  | 2017.08.11     | OM956263            |
| 201708121441.seq | 5             | China       | Hong Kong                         | 2017.08.12     | OM956262            |
| 201708131464.seq | 24            | China       | South Korea                       | 2017.08.13     | OM956226            |
| 201708151516.seq | 76            | China       | Russia                            | 2017.08.15     | OM956238            |
| 201708191682.seq | 16            | China       | South Korea                       | 2017.08.19     | OM956223            |
| 201708221788.seq | 18            | China       | Germany, France, Switzerland, Italy| 2017.08.22     | OM956237            |
| 201708241888.seq | 5             | China       | Taiwan                           | 2017.08.24     | OM956221            |
| 201708251901.seq | 24            | China       | Philippines, Hong Kong             | 2017.08.25     | OM956220            |
| 201708251923.seq | 8             | China       | France, Italy, Turkey              | 2017.08.25     | OM956236            |
| 201708261954.seq | 22            | China       | Hong Kong, Thailand               | 2017.08.26     | OM956261            |
Table 2. Influenza A(H3N2) virus strains sequenced in this study.

| Name                        | Patients' age | Nationality | Stopover sites | Sampling dates | Accession numbers |
|-----------------------------|---------------|-------------|----------------|----------------|------------------|
| 201708261955.seq            | 19            | China       | Hong Kong      | 2017.08.26     | OM956260         |
| 201708281998.seq            | 62            | China       | Russia         | 2017.08.28     | OM956235         |
| 201708301087.seq            | 18            | China       | Taiwan         | 2017.08.30     | OM956229         |
| 201709041192.seq            | 34            | China       | Hong Kong, Australia | 2017.09.04     | OM956259         |
| 201709113430.seq            | 46            | Poland      | Poland         | 2017.09.13     | OM956232         |
| 201709231691.seq            | 30            | South Korea | Indonesia, South Korea | 2017.09.25     | OM956222         |
| 201709261774.seq            | 33            | India       | Hong Kong, India | 2017.09.26     | OM956234         |
| 201709301835.seq            | 25            | China       | Hong Kong      | 2017.10.03     | OM956233         |

27, 41 and 22 amino acids. The mathematical formula linking VE and the \( p \) epitope is given by \( VE = -2.47 \times p + 0.47 \) in which VE is 47% when \( p \) epitope = 0.

**Data availability**
The datasets generated and analysed during the current study are available in the National Center for Biotechnology Information (https://www.ncbi.nlm.nih.gov/), ACCESSION NUMBER TO DATASETS: OM956220-OM956283.

Received: 6 February 2022; Accepted: 25 August 2022
Published online: 12 October 2022

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