Epidemiology and Infection

cambridge.org/hyg

Original Paper

*These authors contribute equally.

Cite this article: Chen L-L *et al* (2019). Assessment of population susceptibility to upcoming seasonal influenza epidemic strain using interepidemic emerging influenza virus strains. *Epidemiology and Infection* **147**, e279, 1–7. https://doi.org/10.1017/S0950268819001717

Received: 20 June 2019
Revised: 6 August 2019
Accepted: 4 September 2019

Key words:
Influenza; respiratory tract infections; serology; population susceptibility

Author for correspondence:
Kelvin To, E-mail: kelvinto@hku.hk

CAMBRIDGE UNIVERSITY PRESS

Assessment of population susceptibility to upcoming seasonal influenza epidemic strain using interepidemic emerging influenza virus strains

Lin-Lei Chen[1,*], Wai-Lan Wu[1,*], Wan-Mui Chan[1], Carol H. Y. Fong[1], Anthony C. K. Ng[1], Jonathan D. Ip[1], Lu Lu[1], Thrimendra K. Dissanayake[1], Xixia Ding[2], Jian-Piao Cai[1], Anna J. X. Zhang[1], Sidney Tam[3], Ivan F. N. Hung[4,5], Kwok-Hung Chan[1,5,6], Kwok-Yung Yuen[1,5,6,7] and Kelvin K. W. To[1,5,6,7]

[1]Department of Microbiology, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Pokfulam, Hong Kong Special Administrative Region, China; [2]Laboratory of Emerging Infectious Diseases and Division of Laboratory Medicine, Zhujiang Hospital, Southern Medical University, Guangzhou, China; [3]Division of Clinical Biochemistry, Department of Pathology, Queen Mary Hospital, Hong Kong Special Administrative Region, China; [4]Department of Medicine, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Pokfulam, Hong Kong Special Administrative Region, China; [5]State Key Laboratory for Emerging Infectious Diseases, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Hong Kong Special Administrative Region, China; [6]Carol Yu Centre for Infection, The University of Hong Kong, Hong Kong Special Administrative Region, China and [7]Department of Clinical Microbiology and Infection Control, The University of Hong Kong-Shenzhen Hospital, Shenzhen, China

Abstract

Seasonal influenza virus epidemics have a major impact on healthcare systems. Data on population susceptibility to emerging influenza virus strains during the interepidemic period can guide planning for resource allocation of an upcoming influenza season. This study sought to assess the population susceptibility to representative emerging influenza virus strains collected during the interepidemic period. The microneutralisation antibody titers (MN titers) of a human serum panel against representative emerging influenza strains collected during the interepidemic period before the 2018/2019 winter influenza season (H1N1-inter and H3N2-inter) were compared with those against influenza strains representative of previous epidemics (H1N1-pre and H3N2-pre). A multifaceted approach, incorporating both genetic and antigenic data, was used in selecting these representative influenza virus strains for the MN assay. A significantly higher proportion of individuals had a ⩾four-fold reduction in MN titers between H1N1-inter and H1N1-pre than that between H3N2-inter and H3N2-pre (28.5% (127/445) *vs.* 4.9% (22/445), $P < 0.001$). The geometric mean titer (GMT) of H1N1-inter was significantly lower than that of H1N1-pre (381 (95% CI 339–428) *vs.* 713 (95% CI 641–792), $P < 0.001$), while there was no significant difference in the GMT between H3N2-inter and H3N2-pre. Since A(H1N1) predominated the 2018–2019 winter influenza epidemic, our results corroborated the epidemic subtype.

Introduction

Seasonal influenza virus infection has been associated with an estimated 9.4 million respiratory hospitalisations and an estimated 0.3 to 0.6 million deaths per year globally [1, 2]. During influenza epidemics, the sudden surge in the number of patients attending out-patient clinics and hospitals leads to overcrowded clinics and hospital wards, and increased workload of healthcare workers [3, 4]. The total healthcare and society cost has been estimated to be US $11.2 billion per year in the United States [5].

Seasonal influenza epidemics are caused by influenza A(H1N1), A(H3N2) and influenza B virus. There are important epidemiological differences between these influenza viruses [6]. Studies have shown that the median ages of patients with influenza A(H1N1) (20 years) and influenza B (16 years) virus infection are younger than those with influenza A(H3N2) (30 years) virus infection [7]. For influenza B virus, patients infected by the Victoria lineage are younger than those infected with the Yamagata lineage (median age: 20 years *vs.* 40 years) [8]. Influenza A(H1N1) virus has also been associated with higher incidence of intensive care unit admission [6]. After the 2009 pandemic, the mortality rate was higher for A(H3N2) virus than A(H1N1) virus for older patients born before 1946, but was higher for A(H1N1) virus for younger patients born after 1947 [9]. Vaccine effectiveness is much lower for influenza A (H3N2) virus than influenza A(H1N1) or influenza B virus [10]. These differences in

epidemiological characteristics and vaccine effectiveness have significant implication in healthcare resource and workforce planning for an influenza season.

An antibody titer against an influenza virus strain correlates with protection against antigenically similar strains [11]. As influenza virus strains evolve, the population antibody titer against the new strains may be reduced, and these new strains will emerge as the predominant strain [12]. For example, the influenza virus A (H1N1)pdm09 has quickly spread around the world because most people, except the elderly born near the 1918 pandemic, do not have protective antibody against the new virus [13–15].

The aim of this study is to determine the population susceptibility to influenza viruses that are newly emerging in the interepidemic period. We used a human serum panel consisting of individuals from all age groups as we described previously [12, 16]. We hypothesise that the influenza subtype with a greater reduction in the antibody titer would signify an increased susceptibility of the population to that subtype.

Methods

Patient samples

We screened 445 random anonymised archived serum samples from the clinical biochemistry laboratory of Queen Mary Hospital in Hong Kong as we described previously [12]. The serum samples consisted of 50 samples of each 10-year age cohort from 10–19 year-old to ⩾80 year-old cohorts. For the 0–9 year-old cohort, 45 serum samples were retrieved. These serum samples were collected from April to June 2018, which is after the 2017/2018 winter influenza season. This study was approved by the HKU/HA HKW Institutional Review Board (UW 18–141).

Choosing influenza A virus strains for microneutralisation assay

Influenza A strains representative of previous epidemics (H1N1-pre and H3N2-pre) and those representative of emerging influenza strains collected during the interepidemic period before the 2018/2019 winter influenza season (H1N1-inter and H3N2-inter) were chosen based on genetic and antigenic data that are publicly available. These include the antigenic data published by the World Health Organisation [17], and the genetic information available at the Global Initiative on Sharing All Influenza Data (GISAID) [18]. The amino acid sequences were aligned using FAMSA [19]. The nucleotide sequences of A/HK/412489/2016, A/HK/439315/2018 and A/HK/417610/2018 have been deposited on the GISAID EpiFlu database under accession numbers EPI1331036-EPI1331038.

Microneutralisation assay

A microneutralisation (MN) assay was performed and interpreted according to the 2-day enzyme-linked immunosorbent assay protocol of the World Health Organisation [20, 21]. Serum samples were serially diluted by two-fold from 1:20 to 1:2560. Viral antigen was detected using anti-nucleoprotein antibody [22]. All viruses used in the MN assay were cultured in Madin Darby canine kidney cells as we described previously [12], to avoid mutations that may arise during egg passage. The haemagglutinin (HA) gene of the virus stocks used for the MN assay was sequenced.

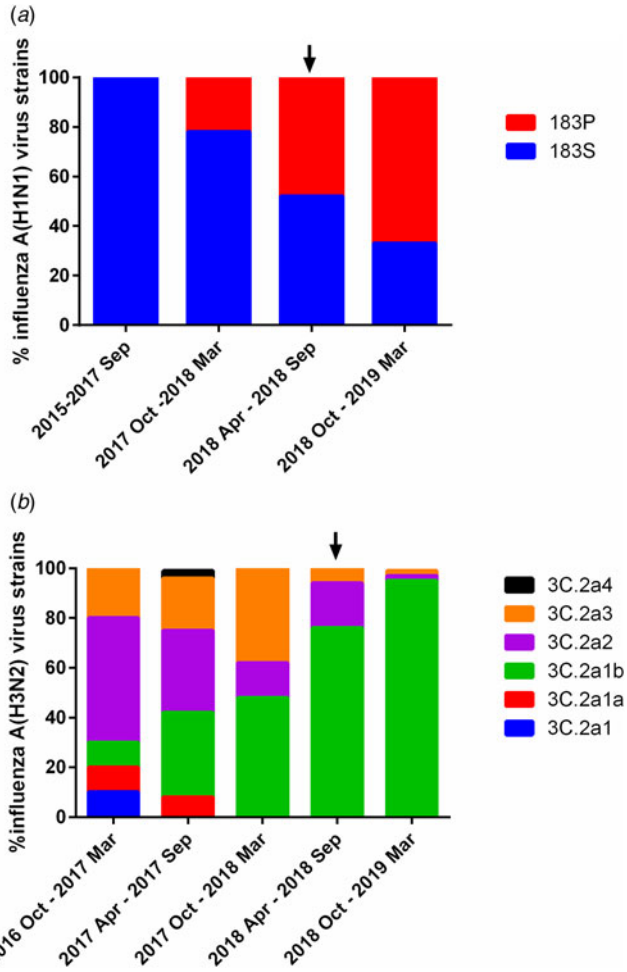

Fig. 1. Influenza A strains emerging in Hong Kong. (a) Emergence of influenza A (H1N1) strains with HA S183P substitution. (b) Emergence of influenza A(H3N2) strains belonging to lineage 3C.2a1b. Amino acid sequences were downloaded from GISAID (Supplementary Table S1). Serum samples in this study were collected from April to June 2018 and are indicated by the black arrows.

Statistical analysis

Statistical analysis was performed using SPSS 23. For statistical analysis, a value of 2560 was assigned if the MN titer was ⩾2560. The McNemar test was used in comparing the proportion of serum specimens with ⩾four-fold reduction in the MN titer. The paired-sample *t* test was used in comparing the geometric mean titers (GMT). Log-transformed MN titers were used for the statistical analysis of the GMT and 95% confidence interval (CI) as we described previously [23, 24].

Results

Selection of influenza A(H1N1) strains

Before the 2018/2019 winter influenza season, the last A(H1N1) epidemic occurred in the 2015/2016 winter influenza season. S183P substitution in the HA, which was absent in A(H1N1) virus strains collected between 2015 and September 2017, was increasingly found among A(H1N1) strains collected in Hong Kong (Fig. 1a). HA S183P was highlighted as a marker of emerging A(H1N1) virus strains according to the World Health Organisation [17]. Hence, for H1N1-pre, we have chosen a strain

https://doi.org/10.1017/S0950268819001717 Published online by Cambridge University Press

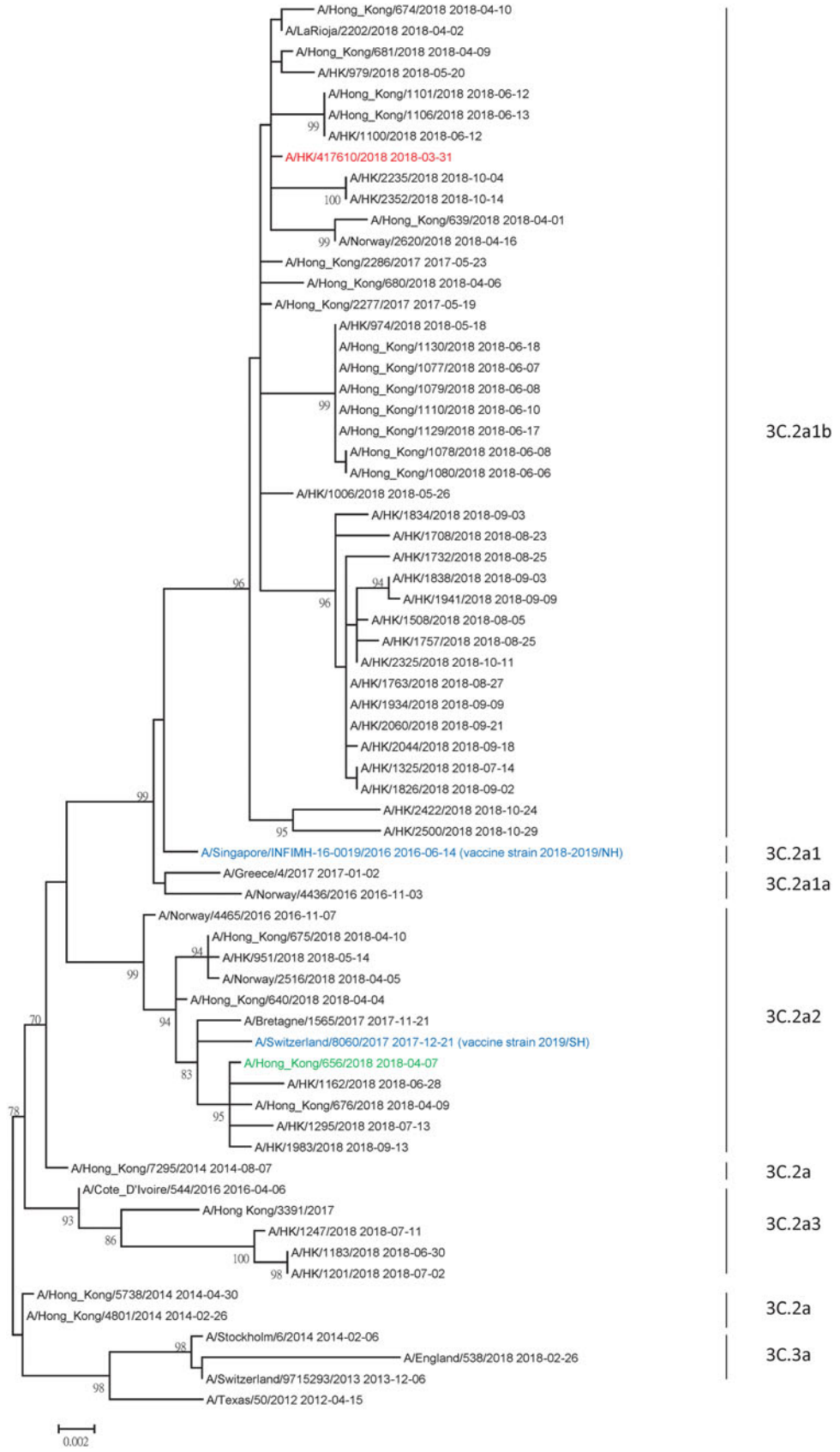

Fig. 2. Phylogenetic tree of HA showing the genetic relationship of influenza A(H3N2) in Hong Kong. Nucleotide sequences were downloaded from GISAID (Supplementary Table S2). All influenza A(H3N2) strains from Hong Kong available at GISAID as of 7 January 2019 are included. Vaccine strains recommended by the World Health Organisation are highlighted in blue. H3N2-pre and H3N2-inter used in the MN assay are highlighted in green and red, respectively. The phylogenetic trees were constructed using the maximum-likelihood method with the best-fit substitution model HKY + G. Bootstrap values were calculated from 1000 trees.

https://doi.org/10.1017/S0950268819001717 Published online by Cambridge University Press

Table 1. Comparison of microneutralisation antibody titer between influenza A virus strains representative of previous epidemics and those emerging during the interepidemic period

	No. (%) with ⩾four-fold reduction in MN titer between strains representative of previous epidemics and those emerging during the interepidemic period			No. (%) with ⩾eight-fold reduction in MN titer between strains representative of previous epidemics and those emerging during the interepidemic period		
Age group (years)[a]	H1N1	H3N2	*P* value[b]	H1N1	H3N2	*P* value[b]
0–9	6 (13)	0 (0)	0.031	1 (2.2)	0 (0)	1.000
10–19	6 (12)	2 (4)	0.289	2 (4)	0 (0)	0.500
20–29	14 (28)	4 (8)	0.013	2 (4)	1 (2)	1.000
30–39	29 (58)	1 (2)	<0.001	14 (28)	0 (0)	<0.001
40–49	11 (22)	5 (10)	0.146	4 (8)	1 (2)	0.375
50–59	25 (50)	3 (6)	<0.001	13 (26)	0 (0)	<0.001
60–69	10 (20)	4 (8)	0.180	5 (10)	2 (4)	0.453
70–79	17 (34)	1 (2)	<0.001	5 (10)	0 (0)	0.063
⩾ 80	9 (18)	2 (4)	0.065	0 (0)	1 (2)	1.000
Total ($n = 445$)	127 (28.5)	22 (4.9)	<0.001	46 (10.3)	5 (1.1)	<0.001

[a]$n = 50$ in each age group, except $n = 45$ for 0–9 year-old age group.
[b]*P* value calculated using the McNemar test.

with HA 183S (A/HK/412489/2016). For H1N1-inter, we have chosen a strain with HA 183P (A/HK/439315/2018), which was isolated from an adult patient with severe disease requiring extracorporeal membrane oxygenation. No mutations in the HA gene were found during virus passage for both H1N1-pre and H1N1-inter.

Selection of influenza A(H3N2) strains

Phylogenetic analysis showed that A(H3N2) clade 3C.2a2 and 3C.2a1b predominated in the 2017 summer epidemic in Hong Kong. However, only 3C.2a1b rapidly increased in 2018, accounting for 76% of the strains tested between April and September of 2018 (Fig. 1b). In the antigenic analysis by the World Health Organisation, A(H3N2) virus strains in the clade 3C.2a1b have different antigenic characteristic from strains in the clade 3C.2a2. Hence, for H3N2-pre, we have chosen a strain that belongs to clade 3C.2a2 (A/Hong Kong/656/2018; GISAID accession number EPI_ISL_312267). In the antigenic analysis by the World Health Organisation, A/Hong Kong/656/2018 is antigenically similar to egg-passaged A/Switzerland/8060/17, which is the recommended H3N2 vaccine strain [17]. For H3N2-inter, we have chosen a strain belonging to clade 3C.2a1b with 135K (A/HK/417610/2018) (Fig. 2). No mutations in the HA gene were found during virus passage for H3N2-pre. For H3N2-inter, one mutation (T160K) was found during passage.

Comparison of MN titers between previous epidemic strain and emerging strain

The MN titers for H1N1-inter were ⩾four-fold or ⩾eight-fold lower than those of H1N1-pre for 28.5% (127/445) and 10.3% (46/445) of individuals, respectively (Table 1). In comparison, the MN titers for the H3N2-inter were ⩾four-fold or ⩾eight-fold lower than those for H3N2-pre for only 4.9% (22/445) and 1.1% (5/445), respectively. Overall, the proportion of individuals with ⩾four-fold or ⩾eight-fold reduction in MN titers between the previous epidemic strains and the interepidemic emerging strains was significantly higher for A(H1N1) than that of A (H3N2) ($P < 0.001$). Subgroup analysis also showed that the proportion of individuals with ⩾four-fold reduction in MN titers between the previous epidemic strains and the interepidemic emerging strains of A(H1N1) was higher than that of A(H3N2) for all nine different age groups. The difference is most striking for the 30–39 year-old age group, in which 58% of individuals had ⩾four-fold reduction in H1N1 titer *vs.* 2% for H3N2.

The GMT for H1N1-inter was significantly lower than that for H1N1-pre (381 (95% CI 339–428) *vs.* 713 (95% CI 641–792), $P < 0.001$). Conversely, there was no significant difference in the GMT between H3N2-pre and H3N2-inter (523 (95% CI 462–592) *vs.* 523 (95% CI 469–583), $P = 1.000$). The GMT for H1N1-inter was significantly lower than that for H1N1-pre for all age groups (Table 2). For H3N2, only the 20–29 year-old age group had lower GMT for the H3N2-inter than H3N2-pre.

Use of pooled serum specimens for comparing MN titers

The testing of MN titers of individual serum is labour intensive and time consuming. Hence, we determined whether serum specimens from different individuals can be pooled together for MN testing. From each age group, we have pooled serum specimens from all individuals and the MN titers against H1N1-pre and H1N1-inter were determined. The difference of MN titers between H1N1-pre and H1N1-inter for all age groups was within one dilution, except for the age group 60–69 for which the MN titer of H1N1-inter was four-fold lower than that of H1N1-pre (Table 3).

https://doi.org/10.1017/S0950268819001717 Published online by Cambridge University Press

Table 2. Geometric mean microneutralisation antibody titer against influenza A virus of each age group

	Geometric mean microneutralisation titer					
Age group (years)[a]	H1N1-pre	H1N1-inter	*P* value[b]	H3N2-pre	H3N2-inter	*P* value[b]
0–9	819 (565–1188)	621 (416–925)	0.004	470 (325–681)	630 (454–875)	<0.001
10–19	1512 (1213–1884)	1162 (887–1522)	0.005	1554 (1201–2012)	1372 (1087–1732)	0.071
20–29	696 (509–951)	383 (277–529)	<0.001	868 (616–1223)	686 (496–948)	0.020
30–39	715 (526–972)	220 (165–293)	<0.001	316 (225–442)	348 (262–462)	0.279
40–49	411 (296–570)	197 (148–263)	<0.001	334 (222–502)	320 (224–457)	0.690
50–59	549 (396–762)	194 (142–266)	<0.001	246 (185–328)	239 (190–302)	0.749
60–69	389 (277–545)	253 (179–357)	0.001	338 (229–499)	348 (256–474)	0.811
70–79	957 (739–1239)	513 (372–707)	<0.001	676 (484–946)	725 (525–1001)	0.471
⩾80	931 (700–1237)	589 (429–809)	<0.001	766 (522–1124)	745 (544–1021)	0.749
Total	713 (641–792)	381 (339–428)	<0.001	523 (462–592)	523 (469–583)	1.000

H1N1-inter, A(H1N1) interepidemic strain; H1N1-pre, A(H1N1) strain representative of previous epidemic.
Data are geometric mean microneutralisation titer (95% CI).
[a]$n = 50$ in each age group, except $n = 45$ for 0–9 year-old age group.
[b]*P* value calculated using the paired sample *t*-test with a log-transformed MN titer.

Table 3. Microneutralisation antibody titer of pooled serum against influenza A H1N1 virus of each age group

	Microneutralisation antibody titer		
Age group (years)[a]	H1N1-pre	H1N1-inter	H1N1-pre/H1N1-inter
0–9	1280	1280	1
10–19	2560	2560	1
20–29	1280	640	2
30–39	320	320	1
40–49	320	320	1
50–59	640	320	2
60–69	1280	320	4
70–79	1280	640	2
⩾80	1280	640	2

H1N1-inter, A(H1N1) interepidemic strain; H1N1-pre, A(H1N1) strain representative of previous epidemic.
[a]$n = 50$ in each age group, except $n = 45$ for 0–9 year-old age group.

Discussion

Influenza virus causes seasonal epidemics worldwide every year, putting a significant burden on the healthcare system. Assessing the population susceptibility to the upcoming epidemic influenza strain is one of the important components in preparing for influenza epidemics. In this study, we determined the population susceptibility by comparing the antibody titers against representative influenza virus strains that emerge during the interepidemic period with influenza virus strains representative of those in the previous epidemic. From a human serum panel from 445 patients encompassing all age groups from <10 to ⩾80 years of age, 28.5% of individuals had ⩾four-fold lower MN titers against H1N1-inter compared with H1N1-pre, while only 4.9% had ⩾four-fold lower MN titers against H3N2-inter compared with H3N2-pre. For the influenza season 2018/19 winter influenza season in Hong Kong, A(H1N1) was the predominant subtype affecting Hong Kong, and the epidemic peak in the current season is much more severe than the A(H1N1) 2017/2018 winter or 2017 summer peak [25]. Similarly, A(H1N1) subtype affects most hospitalised patients with laboratory-confirmed influenza virus infection in the 2018/2019 season in Europe [26]. A(H1N1) is also the most predominant influenza virus subtype affecting the United States [27]. Therefore, the findings from our serosurveillance of interepidemic influenza virus strains, which were collected before the 2018/2019 winter epidemic, corroborated with the predominant influenza virus subtype in the 2018/2019 winter epidemic.

Although the predominant influenza virus strains of a particular influenza subtype during an influenza season can only be ascertained after the influenza season has begun, these can be predicted by analysing the influenza virus strains collected during the interepidemic period. As seen in Figure 1a, HA S183P substitution, which was found in most A(H1N1) strains collected in the 2018/19 winter influenza season, showed a clear trend of increase since the last A(H1N1) predominant season in 2015/16 winter. Our approach, which used representative emerging influenza strains collected during the interepidemic period, provides population susceptibility data before an epidemic has been started. The population susceptibility data that are available before an epidemic would guide resource allocation.

Our serum panel consists of individuals from all age groups. This is important because antibodies from individuals of different ages have different antiviral properties. Xie *et al.* have shown that antigenic distance determined using sera from children does not correlate with that determined using sera from adults [28].

Some studies have tested post-vaccination human serum with emerging strains [17]. This approach is useful in predicting vaccine effectiveness. However, the data is not useful in predicting population susceptibility to emerging strains in areas with a low-vaccine uptake rate. In Hong Kong, the overall seasonal influenza uptake rate was only 14.8% as of 3 March 2019 [29]. The vaccination rate for those not eligible in the government vaccination

https://doi.org/10.1017/S0950268819001717 Published online by Cambridge University Press

program, such as young healthy adults without chronic medical illness, is likely to be lower. Since A(H1N1) disproportionately affects the younger population, our study approach is particularly relevant.

Our result is in stark contrast with the results using post-infection ferret antisera. According to the World Health Organisation and European Centre for Disease Prevention and Control, there was no significant antigenic difference between old and circulating strains of A(H1N1) as determined by ferret antisera [17, 30]; however, there was a significant reduction in ferret serum neutralisation titer against the circulating A(H3N2) genetic clade 3C.2a1b when compared with that clade 3C.2a2 [17, 31]. Several reasons may account for the difference. First, our study uses human serum panel instead of ferret panel. Many studies have demonstrated that the results from human and ferret may be different. Second we use microneutralisation assay instead of HA inhibition assay. Traditionally, antigenic characteristics of influenza viruses are determined by haemagglutination inhibition assay (HAI) using post-infection ferret antisera [15, 32, 33], and antigenic distance can be derived from the difference in HAI between strains [34]. However, recent A(H3N2) strains poorly agglutinate red blood cells, and therefore HAI cannot be performed for these viruses [17, 30].

Other groups have developed models to predict the predominant influenza virus subtype in the upcoming influenza season. These models are based on the evolution rate, or specific mutations in the HA [35, 36]. The addition of serosurveillance data using emerging strains in the interepidemic period may strengthen these models.

Pooled serum panels have been used by some groups in determining the antibody titer against a particular virus [37]. However, our data showed that the use of pooled serum may mask the difference between two viruses. Hence, it is important to test and compare the titer of individual serum specimens.

There are several limitations in this study. First, since all the serum comes from individuals in Hong Kong, this may not reflect the situation in other places. For example, in some parts of Europe, A(H3N2) was the predominant subtype in the 2018/2019 winter influenza season. Second, we have used a limited number of influenza virus strains. Third, one mutation arose during the virus passage for H3N2-inter. However, for this mutation, the MN titer was the same against T160 or K160 strain when ferrets were infected with a natural strain (T160) of H3N2 [38]. Since most of the Hong Kong population has not been vaccinated, this should not affect our results substantially.

In summary, our results have demonstrated significant antigenic changes in the interepidemic emerging A(H1N1) virus, which was the virus subtype that predominated the 2018/2019 influenza season in Hong Kong. Our results support the use of human serum panels and MN assay in determining antigenic changes which are relevant to the human population, but further studies are required to assess whether this method is generalisable.

Supplementary material. The supplementary material for this article can be found at https://doi.org/10.1017/S0950268819001717.

Acknowledgements. The authors gratefully acknowledge the Public Health Laboratory Services of Hong Kong in providing the influenza strain A/Hong Kong/656/2018; the division of Clinical Chemistry, Department of Pathology, Queen Mary Hospital, for providing the archived serum specimens and the originating and submitting laboratories who contributed sequences to GISAID EpiFlu database.

Financial support. This study is supported by Consultancy Service for Enhancing Laboratory Surveillance of Emerging Infectious Diseases and Research Capability on Antimicrobial Resistance for Department of Health of the HKSAR Government, donations from the Shaw Foundation Hong Kong, Richard Yu and Carol Yu, Michael Seak-Kan Tong, the Respiratory Viral Research Foundation Limited, the Hui Ming, Hui Hoy and Chow Sin Lan Charity Fund Limited.

Conflict of interest. None.

Ethical standards. This study was approved by the HKU/HA HKW Institutional Review Board (UW 18-141).

References

1. **Iuliano AD *et al.*** (2018) Estimates of global seasonal influenza-associated respiratory mortality: a modelling study. *Lancet* **391**, 1285–1300.
2. **Collaborators GBDI** (2019) Mortality, morbidity, and hospitalisations due to influenza lower respiratory tract infections, 2017: an analysis for the Global Burden of Disease Study 2017. *Lancet Respiratory medicine* **7**, 69–89.
3. **Beysard N *et al.*** (2018) Impact of the 2014–2015 influenza season on the activity of an academic emergency department. *Internal and Emergency Medicine* **13**, 251–256.
4. **Poon CM *et al.*** (2019) Management decision of hospital surge: assessing seasonal upsurge in inpatient medical bed occupancy rate among public acute hospitals in Hong Kong. *QJM* **112**, 11–16.
5. **Putri W *et al.*** (2018) Economic burden of seasonal influenza in the United States. *Vaccine* **36**, 3960–3966.
6. **Caini S *et al.*** (2018) Clinical characteristics and severity of influenza infections by virus type, subtype, and lineage: a systematic literature review. *Influenza and Other Respiratory Viruses* **12**, 780–792.
7. **Bedford T *et al.*** (2015) Global circulation patterns of seasonal influenza viruses vary with antigenic drift. *Nature* **523**, 217–220.
8. **Skowronski DM *et al.*** (2017) Age-related differences in influenza B infection by lineage in a community-based sentinel system, 2010–2011 to 2015–2016, Canada. *Journal of Infectious Diseases* **216**, 697–702.
9. **Budd AP *et al.*** (2019) Birth cohort effects in influenza surveillance data: evidence that first influenza infection affects later influenza-associated illness. *Journal of Infectious Diseases* **220**, 820–829.
10. **Belongia EA *et al.*** (2016) Variable influenza vaccine effectiveness by subtype: a systematic review and meta-analysis of test-negative design studies. *Lancet Infectious Diseases* **16**, 942–951.
11. **Monto AS *et al.*** (2015) Antibody to influenza virus neuraminidase: an independent correlate of protection. *Journal of Infectious Diseases* **212**, 1191–1199.
12. **Zhu H *et al.*** (2018) Low population serum microneutralization antibody titer against the predominating influenza A(H3N2) N121K virus during the severe influenza summer peak of Hong Kong in 2017. *Emerging Microbes and Infection* **7**, 23.
13. **To KK *et al.*** (2010) Concurrent comparison of epidemiology, clinical presentation and outcome between adult patients suffering from the pandemic influenza A (H1N1) 2009 virus and the seasonal influenza A virus infection. *Postgraduate Medical Journal* **86**, 515–521.
14. **Zhang AJ *et al.*** (2011) High incidence of severe influenza among individuals over 50 years of age. *Clinical and Vaccine Immunology* **18**, 1918–1924.
15. **Cheng VC *et al.*** (2012) Two years after pandemic influenza A/2009/H1N1: what have we learned? *Clinical Microbiology Reviews* **25**, 223–263.
16. **Chan KH *et al.*** (2011) Differences in antibody responses of individuals with natural infection and those vaccinated against pandemic H1N1 2009 influenza. *Clinical and Vaccine Immunology* **18**, 867–873.
17. **World Health Organization** (2018) Recommended composition of influenza virus vaccines for use in the 2019 southern hemisphere influenza season. Available at https://www.who.int/influenza/vaccines/virus/recommendations/201809_recommendation.pdf (Accessed 20 December 2018).
18. **Shu Y and McCauley J** (2017) GISAID: Global initiative on sharing all influenza data – from vision to reality. *Eurosurveillance* **22**, pii: 30494.

19. **Deorowicz S, Debudaj-Grabysz A and Gudys A** (2016) FAMSA: Fast and accurate multiple sequence alignment of huge protein families. *Scientific Reports* **6**, 33964.
20. **WHO Global Influenza Surveillance Network** (2011) *Manual for the Laboratory Diagnosis and Virological Surveillance of influenza*. Geneva, Switzerland: WHO Global Influenza Surveillance Network.
21. **Laurie KL *et al.*** (2015) International laboratory comparison of influenza microneutralization assays for A(H1N1)pdm09, A(H3N2), and A(H5N1) influenza viruses by CONSISE. *Clinical and Vaccine Immunology* **22**, 957–964.
22. **Wang YD *et al.*** (2009) Development and application of monoclonal antibodies-based antigen capture ELISA for detecting nucleocapsid protein of Influenza A virus. *Guangdong Medical Journal* **30**, 703–705.
23. **Hung IF *et al.*** (2016) Topical imiquimod before intradermal trivalent influenza vaccine for protection against heterologous non-vaccine and antigenically drifted viruses: a single-centre, double-blind, randomised, controlled phase 2b/3 trial. *Lancet Infectious Diseases* **16**, 209–218.
24. **To KK *et al.*** (2012) High titer and avidity of nonneutralizing antibodies against influenza vaccine antigen are associated with severe influenza. *Clinical and Vaccine Immunology* **19**, 1012–1018.
25. **Centre for Health Protection** (2019) Local situation of influenza activity (as of Jan 9, 2019). *Flu Express* **16**, 1–9.
26. **European centre for Disease Prevention and Control** (2019) Flu News Europe. Available at http://flunewseurope.org/ (Accessed 17 January 2019).
27. **Centers for Disease Control and Prevention** (2019) Weekly U.S. Influenza Surveillance Report, 2018–2019 Influenza Season Week 1 ending January 5, 2019. Available at https://www.cdc.gov/flu/weekly/#S1 (Accessed 17 January 2019).
28. **Xie H *et al.*** (2017) Differential effects of prior influenza exposures on H3N2 cross-reactivity of human postvaccination sera. *Clinical Infectious Diseases* **65**, 259–267.
29. **The Government of the Hong Kong Special Administration Region** (2019) LCQ11: Seasonal influenza vaccination. Available at https://www.info.gov.hk/gia/general/201903/20/P2019032000606.htm (Accessed 11 May 2019).
30. **European Centre for Disease Prevention and Control** (2018) Influenza virus characterisation, Summary Europe, December 2018. Available at https://ecdc.europa.eu/en/publications-data/influenza-virus-characterisation-summary-europe-december-2018 (Accessed 12 February 2019).
31. **European Centre for Disease Prevention and Control** (2018) Influenza virus characterisation, Summary Europe, July 2018. Available at https://ecdc.europa.eu/en/publications-data/influenza-virus-characterisation-summary-europe-july-2018 (Accessed 27 January 2019).
32. **Petrova VN and Russell CA** (2018) The evolution of seasonal influenza viruses. *Nature Reviews Microbiology* **16**, 60.
33. **Skowronski DM *et al.*** (2016) A perfect storm: impact of genomic variation and serial vaccination on low influenza vaccine effectiveness during the 2014–2015 season. *Clinical Infectious Diseases* **63**, 21–32.
34. **Smith DJ *et al.*** (2004) Mapping the antigenic and genetic evolution of influenza virus. *Science* **305**, 371–376.
35. **Du X *et al.*** (2017) Evolution-informed forecasting of seasonal influenza A (H3N2). *Science Translational Medicine* **9**, pii: eaan5325.
36. **Neher RA *et al.*** (2016) Prediction, dynamics, and visualization of antigenic phenotypes of seasonal influenza viruses. *Proceedings of the National Academy of Sciences* **113**, E1701–E1709.
37. **World Health Organization** (2019) Recommended composition of influenza virus vaccines for use in the 2019–2020 northern hemisphere influenza season. Available at https://www.who.int/influenza/vaccines/virus/recommendations/2019_20_north/en/ (Accessed 20 May 2019).
38. **Zost SJ *et al.*** (2017) Contemporary H3N2 influenza viruses have a glycosylation site that alters binding of antibodies elicited by egg-adapted vaccine strains. *Proceedings of the National Academy of Sciences* **114**, 12578–12583.

https://doi.org/10.1017/S0950268819001717 Published online by Cambridge University Press