

# Contribution of low population immunity to the severe Omicron BA.2 outbreak in Hong Kong

**Lin-Lei Chen**

The University of Hong Kong

**Syed Muhammad Umer Abdullah**

The University of Hong Kong <https://orcid.org/0000-0002-7334-0722>

**Wan-Mui Chan**

University of Hong Kong

**Brian Pui-Chun Chan**

The University of Hong Kong

**Jonathan Daniel Ip**

University of Hong Kong

**Allen Wing Ho Chu**

University of Hong Kong

**Lu Lu**

The University of Hong Kong

**Xiaojuan Zhang**

The University of Hong Kong

**Yan Zhao**

The University of Hong Kong

**Vivien Wai-Man Chuang**

Hospital Authority

**Albert Ka-Wing Au**

Department of Health

**Vincent Chi-Chung Cheng**

The University of Hong Kong

**Siddharth Sridhar**

The University of Hong Kong

**Kwok-Yung Yuen**

The University of Hong Kong <https://orcid.org/0000-0002-2083-1552>

**Ivan Fan Ngai Hung**

University of Hong Kong <https://orcid.org/0000-0002-1556-2538>

**Kwok-Hung Chan**

The University of Hong Kong

**Kelvin Kai-Wang To** (✉ [kelvinto@hku.hk](mailto:kelvinto@hku.hk))

The University of Hong Kong <https://orcid.org/0000-0002-1921-5824>

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## Article

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## Abstract

Monitoring population protective immunity against SARS-CoV-2 variants is critical for risk assessment. In this serosurveillance study, older adults show much lower seropositive rates of neutralizing antibody (NAb) against ancestral virus than the younger population. The increase in NAb seropositive rate generally follows the population vaccination uptake rate, but older adults have a much lower NAb seropositive rate than vaccination uptake rate. For all age groups, the seropositive rates of NAb against Omicron variant are much lower than those against the ancestral virus. During the fifth wave of COVID-19 in Hong Kong which is dominated by Omicron sublineage BA.2, the case-fatality rate is exceptionally high in the  $\geq 80$  year-old age group (9.2%). Our study suggests that the severe BA.2 outbreak in Hong Kong can be attributed by the lack of protective immunity in the population, especially among the vulnerable older adults, and highlights the importance of continual surveillance of protective immunity against emerging variants of SARS-CoV-2.

## Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the causative agent of COVID-19, is characterized by efficient person-to-person transmission, propensity to cause severe disease, and frequent emergence of variants that escape population immunity<sup>1,2,3</sup>. The rapid surge of severe cases which require hospitalization, especially apparent during the emergence of novel variants, routinely overwhelms the healthcare systems. Governments have imposed stringent non-pharmaceutical public health interventions to curb the spread of COVID-19. While effective in limiting the spread of the virus, these measures have resulted in undesirable mental health and socioeconomic consequences. It has been estimated that COVID-19 was associated with an additional 53.2 and 76.2 million cases of depressive and anxiety disorders, respectively<sup>4</sup>. School closure has led to psychosocial issues in children<sup>5</sup>. Moreover, there is a significant economic impact due to COVID-19<sup>6</sup>. Lockdowns are also associated with disruptions in supply chains<sup>7</sup>.

Forecasting the severity of upcoming COVID-19 waves is a critical component in formulating public health preparedness plans. Knowledge of the population level immunity against SARS-CoV-2 can help to predict the impact of COVID-19 on the healthcare system. COVID-19 vaccination or SARS-CoV-2 infection induce protective immunity against SARS-CoV-2<sup>8</sup>. A population with high vaccination uptake rate or incidence of infection theoretically have a high level of population immunity. However, several factors can affect the humoral immunity elicited by vaccination or infection<sup>9</sup>. First, some COVID-19 patients or vaccine recipients do not develop sufficient protective antibody responses. Lower neutralizing antibody (NAb) titers are found in patients with asymptomatic or mild COVID-19, immunocompromised patients and older adults<sup>10,11,12,13,14,15,16</sup>. Second, the NAb titers elicited by inactivated whole virion or adenovirus-vectored vaccines are lower than those elicited by mRNA vaccines<sup>17,18,19</sup>. Third, NAb titers decline within a few months after infection or vaccination<sup>20,21,22,23,24,25,26</sup>. Fourth, NAb elicited from prior infection or vaccination may not be effective against new variants with mutations in the spike proteins, as in the case of Omicron variant<sup>17,26</sup>.

In view of the shortcomings of using history of vaccination or infection to predict population immunity, a more reliable method to determine population level immunity would be to assess known correlates of protection. NAb titers showed a good correlation with vaccine effectiveness against symptomatic infection<sup>18,27</sup>. In a clinical trial of the mRNA-1273 vaccine, the estimated vaccine efficacy decreased from 96% for a NAb titer of 1000 IU/ml to 78% for a NAb titer of 10 IU/ml<sup>28</sup>. Furthermore, individuals positive for anti-spike antibody, which correlates with NAb status, are less likely to have COVID-19 reinfection to cognate variants<sup>29</sup>.

With zero-case strategy, Hong Kong had a very low incidence of COVID-19, with a cumulative count of just 12,655 cases (0.17% of the population) as of December 2021. Local transmission of SARS-CoV-2 was almost absent from May to December 2021. The COVID-19 vaccination program was launched in Hong Kong in February 2021 (Table 1). Our previous serosurveillance studies showed that <1% of sera collected from the Hong Kong population before March 2021 tested positive for SARS-CoV-2 IgG, but the seropositive rate started to increase from April 2021<sup>30,31</sup>. In the current study, we first screened 1800 serum specimens collected between January and December 2021 with a surrogate virus neutralization test (sVNT), which has been

shown to have a good correlation with conventional live virus neutralization test (cVNT) against the ancestral virus<sup>26</sup>. For sera tested positive by sVNT, we assessed the NAb against the ancestral virus and Omicron sublineages BA.1 and BA.2 using a cVNT. We interpreted our serosurveillance findings with the epidemiological and genomic information of the fifth wave of COVID-19 in Hong Kong, which exceeded 1 million cases by March 18, 2022<sup>32</sup>.

## Results

### Sampled population

We retrieved a total of 1800 archived serum specimens collected between January and December 2021 from Queen Mary Hospital, with 300 serum specimens collected once every two months. We retrieved approximately the same number of serum specimens from each age group from 0-9 years old to  $\geq 80$  years old depending on availability (Supplementary Table S1).

### COVID-19 vaccination in Hong Kong

The COVID-19 vaccination program was launched in Hong Kong in February, 2021<sup>33</sup> (Table 1). BNT162b2 and CoronaVac are the only vaccines available. Overall, 63.1% (4,662,005/7,394,700) of the population in Hong Kong has received the second dose of COVID-19 vaccines by December 31, 2021 (Figure 1A). The two-dose vaccination rate was highest for the 20-29 year-old age group (76.3%; 616,982/808,700), and lowest for the  $\geq 80$  year-old age group (18.4%; 73,772/401,800) (Figure 1B-1J). The highest number of second-dose vaccination occurred in July/August, with a much lower number of people receiving the second dose in September/October and November/December (Supplementary Figure S1).

Overall, 63.0% (2,936,358 / 4,662,004) and 37.0% (1,725,646 / 4,662,004) received BNT162b2 and CoronaVac, respectively. However, the proportion of individuals receiving BNT162b2 was higher among the younger age groups than older age groups. For the 10-19 year-old age group, 94.0% (241,813 / 257,242) received BNT162b2, while there was a decreasing trend for older age groups (Figure 2). For the 80 years or above age group, only 35.1% (25,869 / 73,772) received the BNT162b2 vaccine.

### Surrogate virus neutralization test

We first screened all 1800 serum specimens with a sVNT (iFlash-2019-nCoV NAb assay, YHLO), a competitive chemiluminescence immunoassay which measures antibodies that block the binding of ancestral virus receptor binding domain (RBD) and human angiotensin-converting enzyme 2 (ACE2). Previous studies showed good correlation between the results of this sVNT and cVNT against ancestral virus<sup>34,35</sup>. The sVNT NAb result between January and April 2021 was reported previously<sup>30</sup>. Overall, there was an increase in the proportion of individuals tested positive in the sVNT in March/April after the launch of the COVID-19 vaccination program. The sVNT seropositive rate increased from 1.7% (5/300) in March/April to a peak of 34.3% (103/300) in September/October, with a slight decline to 30.3% (91/300) in November/December 2021 (Figure 1A). The geometric mean sVNT levels also peaked in September/October, and declined in all age groups in November/December except for the 10-19 year-old age group (Supplementary Figure S2).

Subgroup analysis of different age groups revealed stark differences in the positive rate in the sVNT among different age groups (Figure 1B-J). For sera collected from the 0-9 year-old individuals, who were not eligible for COVID-19 vaccines in 2021, all specimens tested negative in the sVNT (Figure 1B). For the 10-19 year-old age group, the seropositive rate rose sharply after July 2021, which coincided with the commencement of COVID-19 vaccination program for adolescents aged 12-17 years old on 14<sup>th</sup> June 2021 (Figure 1C)<sup>36</sup>. In November/December, the 10-19 year-old age group had the highest sVNT seropositive rate, and there was a statistically significant trend towards lower seropositive rate for older age groups (age trend by Cochran-Armitage trend test: seropositive rate  $P < 0.0001$  for age groups between 30-39 and  $\geq 80$  years) (Figure 1K). Notably, none of the sera from the  $\geq 80$  year-old age group had tested positive in the sVNT in November/December. The 10-19 year-old age group also had the highest sVNT activity, and there was a statistically significant trend towards lower sVNT activity for older adults ( $P = 0.0014$ ; excluding the 0-9 year-old age group) (Figure 3).

### Conventional live virus neutralization tests

The commercial sVNT assay in this study was designed based on the ancestral virus. Our previous study showed that this assay has a high concordance with the result of cVNT NAb against the ancestral virus, but poor correlation with cVNT NAb against the Omicron sublineage BA.1 due to immune escape<sup>26</sup>. Hence, for specimens tested positive in the sVNT, we used a cVNT to determine the NAb activity against the Omicron sublineages BA.1 and BA.2 and the ancestral virus. Overall, 86.0% (227/264) of sVNT positive specimens had detectable NAb against the ancestral virus (Figure 1A). In contrast, only 7.2% (19/264) and 30.3% (80/264) had detectable NAb against BA.1 and BA.2, respectively. Live virus NAb against BA.1 was only found in sera from individuals between 10 and 69 years old, whereas live virus NAb activity against BA.2 could be found in sera from all age groups (Figure 1B-J). Assuming that all sVNT negative sera did not have detectable NAb against BA.1 or BA.2, the proportion of individuals with detectable NAb against BA.1 and BA.2 in November/December 2021 were 2.3% (7/300) and 7.3% (22/300), respectively.

The absence of seropositive sera in the 0-9 year-old age group, the temporal association of seropositive rate and two-dose vaccination rate, and the low incidence of locally acquired COVID-19 cases after April 2021, suggested that NAb were elicited by COVID-19 vaccination in most of the seropositive individuals in our population. While the two-dose vaccination rate and sVNT positive rates were very similar in the younger age groups, there was a greater discrepancy between the vaccination and sVNT seropositive rates in the older age groups (Figure 1). The greater discrepancy between vaccination and sVNT seropositive rates may be related to the lower proportion of individuals in the older age group receiving BNT162b2 (Figure 2), as the seropositive rate and antibody titer has been shown to be higher for BNT162b2 than CoronaVac<sup>17,37,38,39</sup>.

### **The fifth wave of COVID-19 in Hong Kong**

In Hong Kong, the fourth wave occurred between November 2020 and April 2021. Only five cases were reported between May 12 and October 8, 2021, and no locally acquired cases were reported between October 9 and Dec 30, 2021. The first local case of the fifth wave was reported on Dec 31, 2021. There was limited local transmission in January 2022 due to several Omicron variant-related clusters which could be traced to imported cases (n=836), including a BA.1 outbreak in a restaurant and a large Omicron BA.2 outbreak in a housing estate which we reported previously<sup>40,41</sup> (Figure 4A). There was also a cluster of pet-store-related Delta variant AY.127<sup>42</sup>. Since early February, 2022, there was an exponential increase in cases from <200 cases per day before February 4 to a peak of 76,991 cases (56,827 confirmed by nucleic acid test and 20,164 confirmed by self-collected rapid antigen test on March 3, 2022<sup>43,44</sup>. As of March 24, 2022, there were 1,087,610 cases reported during the fifth wave. The age specific rate for COVID-19 hospitalization was highest among older adults ( $\geq 80$  years: 0.62% [2,485/401,800]; 70-79 years old: 0.25% [1,381/560,500] and children (0-9 years old: 0.19%) [1,013/538,500] (Figure 4B). For the age groups from 10-59, the hospitalization rate was between 0.08-0.12%. The overall case-fatality rate was 0.60%, but the case-fatality rates were 15.3-fold and 2.4-fold higher for the  $\geq 80$  (9.19%) and 70-79 (1.46%) year-old age groups, respectively<sup>45</sup> (Figure 4C).

### **Genomic epidemiology of the fifth wave from January to March 2022**

We have previously reported our viral genome sequencing results for specimens collected up to February 2, 2022, including those from an Omicron BA.1 restaurant outbreak<sup>41</sup>, an Omicron BA.2 housing estate outbreak<sup>40</sup>, and a Delta AY.127 outbreak related to pet stores<sup>42</sup>. Here, we have performed additional sequencing for specimens collected in Hong Kong up to March 11, 2022 (Supplementary Table S2).

In total, 545 specimens collected between December 1, 2021 and March 11, 2022, including 383 specimens collected during the fifth wave (on or after December 31, 2021), were analyzed in a time-resolved phylogenetic tree. During the fifth wave, 80.2% (307/383) of viral genome sequences belonged to Omicron sublineages BA.2 or related lineage (BA.2.2 or BA.2.3), 15.4% (59/383) belonged to Omicron sublineage BA.1 or related sublineages (BA.1.\*), and 4.4% (17/383) belonged to Delta variant AY.127 (Figure 4D). Notably, all 127 viral genomes from specimens collected on or after February 12, 2022, belonged to the sublineages BA.2 or BA.2.2 (Supplementary Table S2), and the majority of (98.4%; 125/127) belonged to BA.2.2, which is characterized by the mutations C4893T (ORF1a T1543I), T10696C (synonymous), C12525T (ORF1a T4087I), and T25224C (Spike I1221T).

## Discussions

Serosurveillance is an effective tool that reveals the hidden burden of infection, especially during the beginning of the COVID-19 pandemic<sup>31</sup>. In addition, serosurveillance using NAb assays is useful in assessing the level of protective immunity in a population. Our study found that although the cumulative two-dose vaccination uptake rate exceeded 60% in Hong Kong by November 2021, only 30% of our study population had a positive sVNT; and only 2.3% and 7.3% had detectable NAb against the Omicron sublineages BA.1 and BA.2, respectively. In particular, older adults had the lowest levels of protective immunity. None of the  $\geq 80$  year-old age group and only 12% of the 70-79 year-old age group had a positive sVNT, while none of the individuals  $\geq 70$  years old had detectable NAb against BA.1 or BA.2. The lack of protective immunity against the Omicron BA.2 sublineage, especially among older adults, likely contributed to the severe fifth wave in Hong Kong which exponentially increased in February 2022 and overwhelmed the healthcare system in Hong Kong<sup>46</sup>.

The sVNT seropositive rate was very similar to the two-dose cumulative vaccination rate among the 10-19 and 20-29 year-old age groups. However, we observed a greater discrepancy between two-dose vaccination rate and sVNT seropositive rate for older adults, which may be related to the weaker immune response in this age group. Previous studies showed that older adults have poorer antibody response after vaccination compared to younger adults. Saure *et al* showed that the IgG seropositive rate was lower among adults aged  $\geq 60$  years than those aged 18-39 years<sup>20</sup>. Wei *et al* showed that seropositivity after vaccination reduced faster for vaccine recipients aged over 75 years<sup>15</sup>. Another possible reason for low seropositive rate among older adults is the vaccine preference among these age groups. Over 50% of adults aged  $\geq 60$  years have chosen CoronaVac. Head-to-head comparison showed that CoronaVac recipients had lower seropositive rates than BNT162b2 recipients<sup>16,17</sup>. Furthermore, seropositive rate declined more rapidly for the CoronaVac than BNT162b2 recipients<sup>20</sup>. Our results support the World Health Organization's recommendation that older adults should receive three doses of CoronaVac<sup>47</sup>.

The incidence of COVID-19 is very low in Hong Kong as a result of elimination strategy. Hence, the NAb detected by sVNT or cVNT for most individuals is likely elicited by vaccination rather than by infection. For the 0-9 year-old age group, none of the serum specimens tested positive in the sVNT, which is expected as the vaccination program for this age group was not yet started in 2021.

There was a decrease in seropositive rates in November/December. This coincided with a low vaccination update rate since September (Supplementary Figure S1), suggesting that there is waning of immunity. Our previous study showed that for natural infection, waning of NAb level occurs most rapidly in the first 4 months<sup>26</sup>. For vaccination, there is also evidence of waning immunity within the first few months<sup>48</sup>.

The spike protein of Omicron sublineage BA.2, especially the RBD, differs from that of BA.1. There were conflicting data regarding the difference in NAb titer against BA.1 and BA.2 for post-vaccine sera. In our previous study using serum from vaccine recipients or COVID-19 patients<sup>49</sup> and in this current study, we showed that BA.2 NAb titers were generally higher than those of BA.1. Iketani *et al* found that the NAb titers were similar between BA.1 and BA.2<sup>50</sup>, while Yamashobe *et al* and Yu *et al* showed a slightly lower NAb titer against BA.2<sup>51,52</sup>. The difference may be related to the assay used. Both Yamashobe and Yu *et al* used pseudovirus assay, while our studies and those of Iketani *et al* used cVNT.

sVNT is a convenient assay which can be conducted in clinical or research laboratories without biosafety-level 3 facilities. sVNT is a competitive assay which measures antibodies that prevent the binding between SARS-CoV-2 RBD and the human receptor ACE2. Our previous study showed that sVNT has good correlation with the cVNT against the ancestral virus<sup>26</sup>. Our current study also showed a good correlation between sVNT and live virus NAb test against the ancestral virus for sVNT seropositive individuals. The sVNT can be easily modified to measure antibodies against the RBD of Omicron sublineages, and this would allow a more convenient assay for surveillance in the future.

There are several limitations in this study. First, as we are using anonymized serum specimens, we do not have clinical information or vaccination history from these individuals. Therefore, we are not able to perform further analysis to study factors that affect NAb response. In a population study conducted in UK, Wei *et al* showed that ethnicity, sex, social deprivation, and

long term health conditions were associated with seropositivity after vaccination<sup>15</sup>. Second, this study included hospital patients instead of the “general population”. Therefore, our data may not be representative of people without comorbidities. However, those with underlying diseases would have a much greater risk of having severe disease, and therefore our estimate would be better in predicting the burden to the healthcare systems. Third, there were fewer specimens in the pediatric age groups as the volume of archived serum specimens were not sufficient for testing. Fourth, we did not test T cell immunity. Future studies are required to understand the relationship between population T cell immunity and susceptibility to infection.

Our study highlights the value of NAb surveillance in assessing population immunity. We demonstrated that the level of protective immunity, especially against antigenically distinct variants, may not be reliably predicted by vaccination history. The low level of protective immunity among older adults was associated with an exceptionally high case-fatality rate. Booster doses can improve the vaccine response, but it has been shown that waning of NAb also occurs after vaccine boosting<sup>53</sup>. Vaccine studies should put more focus on the older adults. Improving the immunogenicity among older adults should be a priority in vaccine research.

## Methods

### Patients

Anonymised archived serum samples from the clinical biochemistry laboratory of Queen Mary Hospital in Hong Kong were used for serological surveillance as we described previously<sup>30,31</sup>. The archived specimens encompassed all age groups from 0-9 years old to  $\geq 80$  years old. A total of 300 serum specimens were randomly selected and tested in each two-month period from January/February to November/December 2021, with an approximately equal number of archived specimens in each 10-year age group depending on availability (Supplementary Table S1). Specimens were excluded if there was an insufficient volume of serum. Results of the surrogate NAb and live virus NAb against the ancestral virus for serum specimens collected between January and April 2021 was reported in our previous publication<sup>30</sup>, but the result of NAb against the Omicron sublineages BA.1 or BA.2 has not been reported before. For viral genome sequencing, 382 sequences were reported in our previous publications<sup>40,41,42</sup>. In the current study, we have performed genome sequencing for 163 randomly selected archived respiratory specimens collected from patients admitted to Queen Mary Hospital. This study was approved by the Institutional Review Board of the University of Hong Kong/Hospital Authority Hong Kong West Cluster (UW 21-313 and UW 18-141). Written informed consent was waived since archived anonymized specimens were used.

### Surrogate neutralization test and live virus neutralization test

sVNT test was performed using iFlash-2019-nCoV neutralization antibody assay according to manufacturer’s instructions (Shenzhen YHLO Biotech Co. Ltd., Shenzhen, China)<sup>54</sup>. The manufacturer’s cutoff for seropositivity is 15 AU/ml, while the maximum measurable result is 800 AU/ml.

cVNT was performed on VeroE6/TMPRSS2 cells (JCRB cell bank of Okayama University; Cat#JCRB1819). The cVNT NAb titer was determined by an end-point dilution and cytopathic effect as we described previously<sup>17,31</sup>. Briefly, serum specimens were heat inactivated at 56°C for 30 min and were serially diluted in 2-folds with MEM containing 1% fetal calf serum (FBS) (Gibco). Duplicates of each diluted serum were mixed with 100 TCID<sub>50</sub> of an ancestral virus (GISAID accession number: EPI\_ISL\_434571), an Omicron sublineage BA.1 virus (GISAID accession number: EPI\_ISL\_7138045)<sup>49</sup>, an Omicron sublineage BA.2 virus (GISAID accession number: EPI\_ISL\_9845731)<sup>49</sup> at 37°C for 1 hour. SARS-CoV-2 virus isolate to reach 100 TCID<sub>50</sub> final concentration and incubated at 37°C for 1 hour. After incubation, 100 mL of the serum-virus mixture was then added to VeroE6/TMPRSS2 cells that were seeded in 96-well plates 24 hours before infection. The cells were incubated with the mixture at 37°C. After incubation for 3 days, cytopathic effect was examined. The live virus neutralizing antibody titer was determined as the highest dilution with 50% inhibition of cytopathic effect. A live virus neutralizing antibody titer  $\geq 10$  was considered positive. For statistical analysis, a value of 5 was assigned if the live virus neutralizing antibody titer is  $< 10$ .

### Whole genome sequencing and genome data analysis

Whole genome sequencing was performed using the Oxford Nanopore MinION device (Oxford Nanopore Technologies) as we described previously<sup>17</sup>. For the determination of viral lineage, nanopore sequencing was performed following the Nanopore protocol - PCR tiling of COVID-19 (Version: PTC\_9096\_v109\_revH\_06Feb2020) according to the manufacturer's instructions with minor modifications (Oxford Nanopore Technologies) as we described previously<sup>55,56</sup>. Briefly, extracted RNA was first reverse transcribed to cDNA using SuperScript<sup>TM</sup> IV reverse transcriptase (ThermoFisher Scientific, Waltham, MA, USA). PCR amplification was then performed using the hCoV-2019/nCoV-2019 Version 3 Amplicon Set (Integrated DNA Technologies, Coralville, IA, USA) with the Q5® Hot Start High-Fidelity 2X Master Mix kit (New England Biolabs, Ipswich, Massachusetts, United States) according to the Nanopore protocol. PCR products were purified using 1x AMPure XP beads (Beckman Coulter, Brea, CA, USA) and quantified using Qubit dsDNA HS Assay Kit (Thermo Fisher Scientific, Waltham, Massachusetts, United States). The purified DNA was then normalized for end-prep and native barcode ligation reactions according to the PCR tiling of COVID-19 virus protocol with Native Barcoding Expansion 96 (EXP-NBD196, Oxford Nanopore Technologies). Barcoded libraries were then pooled, purified with 0.4x AMPure XP beads and then quantified using Qubit dsDNA HS Assay Kit. Purified pooled libraries were ligated to sequencing adapters and sequenced with the Oxford Nanopore MinION device using R9.4.1 flow cells for 24-48 hours.

For bioinformatics analysis, the recommended ARTIC bioinformatics workflow was used with minor modifications applied as described previously<sup>55,56</sup>. The modifications include reducing the minimum length at the guppyplex step to 350 to allow potential small deletions to be detected and increasing the “-normalise” value to 999999 to incorporate all the sequenced reads and the high accurate mode was used for basecalling with an increased QC passing score from 7 to 10. The sequence NC\_045512.2 obtained from NCBI was used as the reference and the alignment files produced by Medaka were inspected using Integrative Genomics Viewer (IGV) to verify the mutations called by the ARTIC pipeline. SARS-CoV-2 lineage were assigned using the PANGOLIN software suite<sup>57</sup>. All sequences are deposited onto the GISAID database (Supplementary Table S2).

### **Time-resolved phylogenetic tree**

Time-resolved phylogenetic tree was constructed using TreeTime program as we described previously<sup>55</sup>. In addition to the described homoplastic position masking, we also removed the highly diverged sequences suggested by the TreeTime program. Sequences collected from December 1, 2021 to March 11, 2022 were aligned using MAFFT aligner. The maximum-likelihood whole genome phylogenetic tree construction and phylodynamic analysis were performed using IQ-TREE and TreeTime. For the construction of the phylogenetic tree, 100 standard nonparametric bootstrap replicates were used, and the option -czb was used to mask the unrelated substructure of the tree with branch length representing mutation count of less than 1. The resulting tree, sample collection date and the aligned sequences were used as an input for the treetime pipeline to create the phylodynamic tree. The outlier sequences (flagged by the treetime program) were removed when visualizing the tree.

### **Data for two-dose vaccination rate and COVID-19 outbreak in HK**

The data for the two-dose vaccination uptake rate in Hong Kong was obtained from the Hong Kong government webpage<sup>33</sup>. The data of the Hong Kong population was obtained from Census and Statistics Department<sup>58</sup>.

### **Statistical analysis**

Statistical analysis was performed using SPSS 26.0 (IBM SPSS Statistics) and GraphPad PRISM 9.1.1 (GraphPad Software, San Diego CA, USA). The Cochran-Armitage trend test was used to assess the age trend in the seropositive rate of neutralization test. The simple linear regression was used to assess the age trend in the sVNT titer. For the purpose of statistical analysis, an MN titer of <10 was considered as 5. A P value of <0.05 was considered statistically significant.

## **Declarations**

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## AUTHOR CONTRIBUTION

LLC and KKWT had roles in study design, data collection, data analysis, data interpretation, literature search and writing of the manuscript. LLC, SMUA, WMC, BPCC, JDI, AWHC, LL, XZ, YZ, WMC, AKWA, VCC, SS, KYY, KHC had roles in performing the experiments, data collection, data analysis, and/or data interpretation. All authors interpreted the data, revised the manuscript critically for important intellectual content and approved the final version of the manuscript.

## COMPETING INTERESTS

All authors declare no conflicts of interest.

## DATA AVAILABILITY

Genome sequences have been deposited into the GISAID database (Supplementary Table S2). Raw data for the neutralizing antibody assays are available upon reasonable requests.

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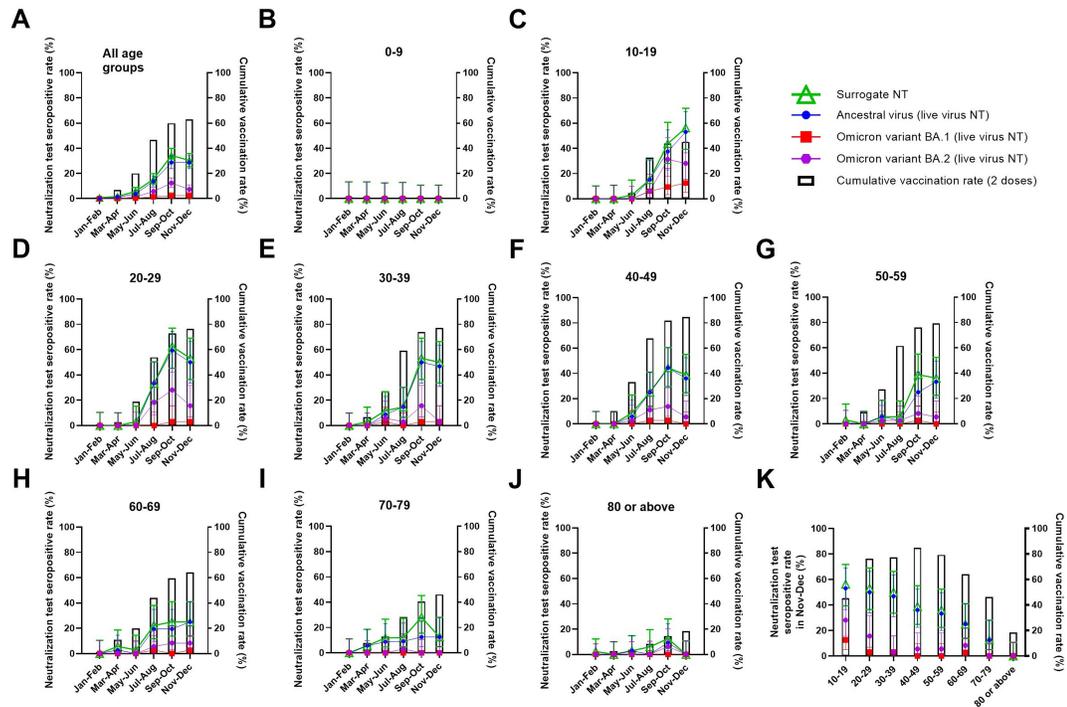
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## Table

**Table 1.** Timeline of COVID-19 vaccination program in Hong Kong

Date	Event
26 <sup>th</sup> February 2021	Administration of CoronaVac (Sinovac) vaccine officially started for adults aged 18 years or above
10 <sup>th</sup> March 2021	Administration of BNT162b2 (Pfizer-BioNTech) vaccine started for adults aged 18 years or above
14 <sup>th</sup> June 2021	Administration of BNT162b2 (Pfizer-BioNTech) vaccine started for adolescents aged 12 to 17 years old
2 <sup>nd</sup> December 2021	Administration of CoronaVac (Sinovac) vaccine started for adolescents aged 12-17 years old

# Figures

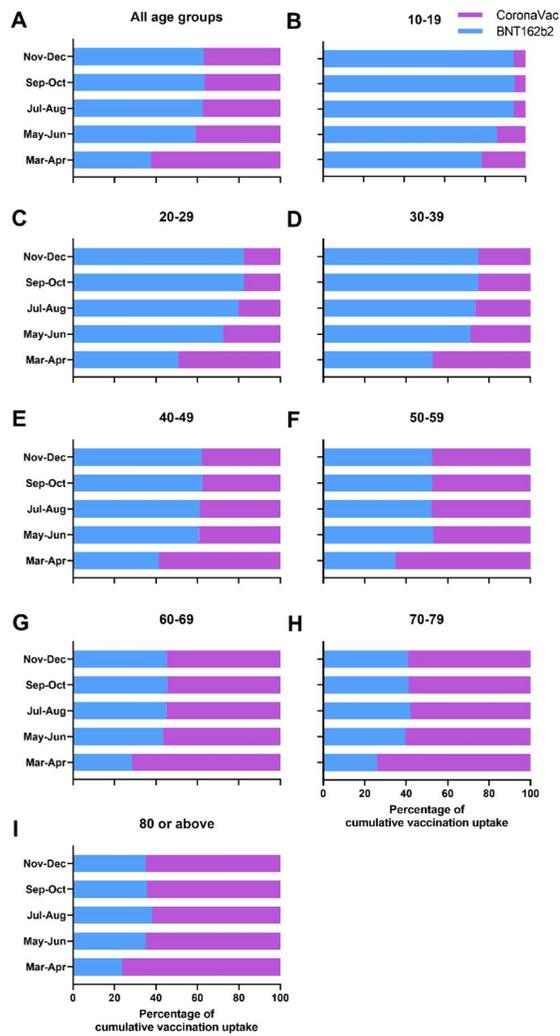


**Figure 1**

**Cumulative two-dose vaccination uptake rates and neutralization test seropositive rates for ancestral virus and Omicron variant sublineages in 2021.**

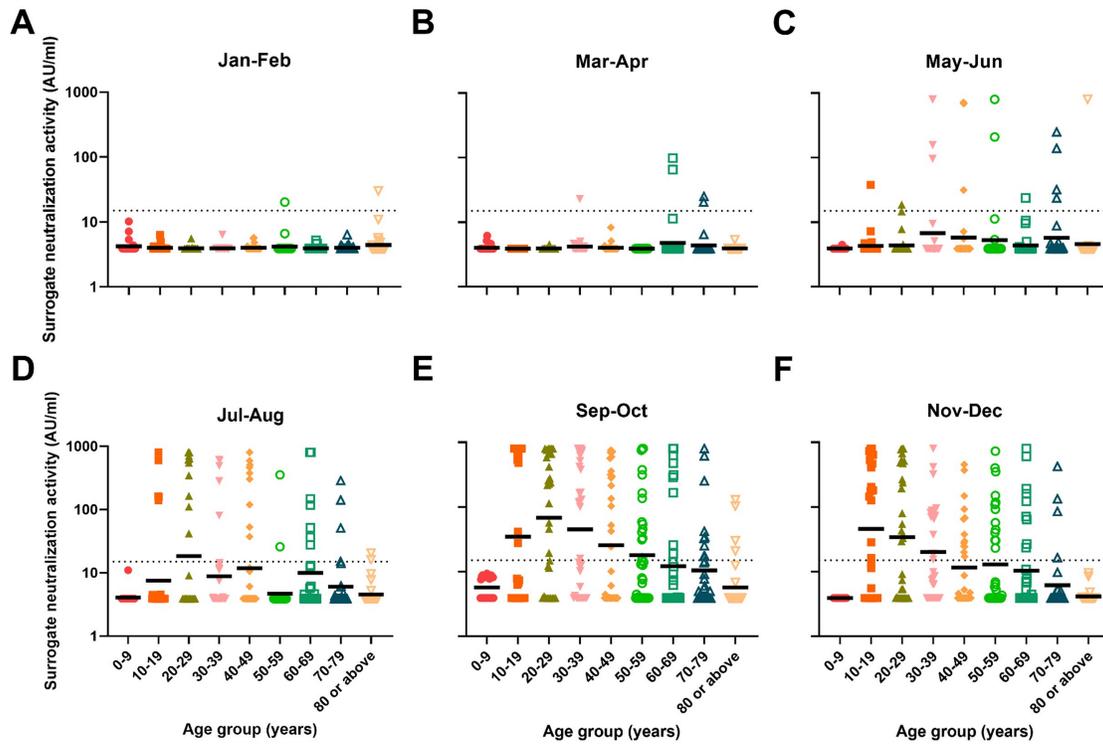
(A-J) Cumulative two-dose vaccination rates and neutralization test seropositive rates of different age groups. Cumulative vaccination rate represents the percentage of the population that have received 2 doses of COVID-19 vaccines at the end of each time period<sup>33</sup>. Neutralization test seropositive rates represent the percentage of sera tested positive in each time period. All serum specimens are tested with the surrogate virus neutralization test (sVNT) (iFlash-2019-nCoV neutralization antibody assay), which measures antibodies that block the interaction between ancestral virus receptor binding domain (RBD) and human angiotensin-converting enzyme 2 (ACE2). Serum specimens tested positive in the sVNT were then tested for NAb against ancestral virus, Omicron sublineage BA.1 and Omicron sublineage BA.2 using a conventional live virus neutralization test (cVNT). A serum specimen is considered to be seropositive in the sVNT if  $\geq 15$  AU/ml, and in the cVNT if the NAb titer is  $\geq 10$ . The number of serum specimens tested in each period is shown in Supplementary Table S1. Error bars indicate 95% confidence interval.

(K) Two-dose cumulative vaccination uptake rates and neutralization test seropositive rates in November/December 2021. Error bars indicate 95% confidence interval.



**Figure 2**

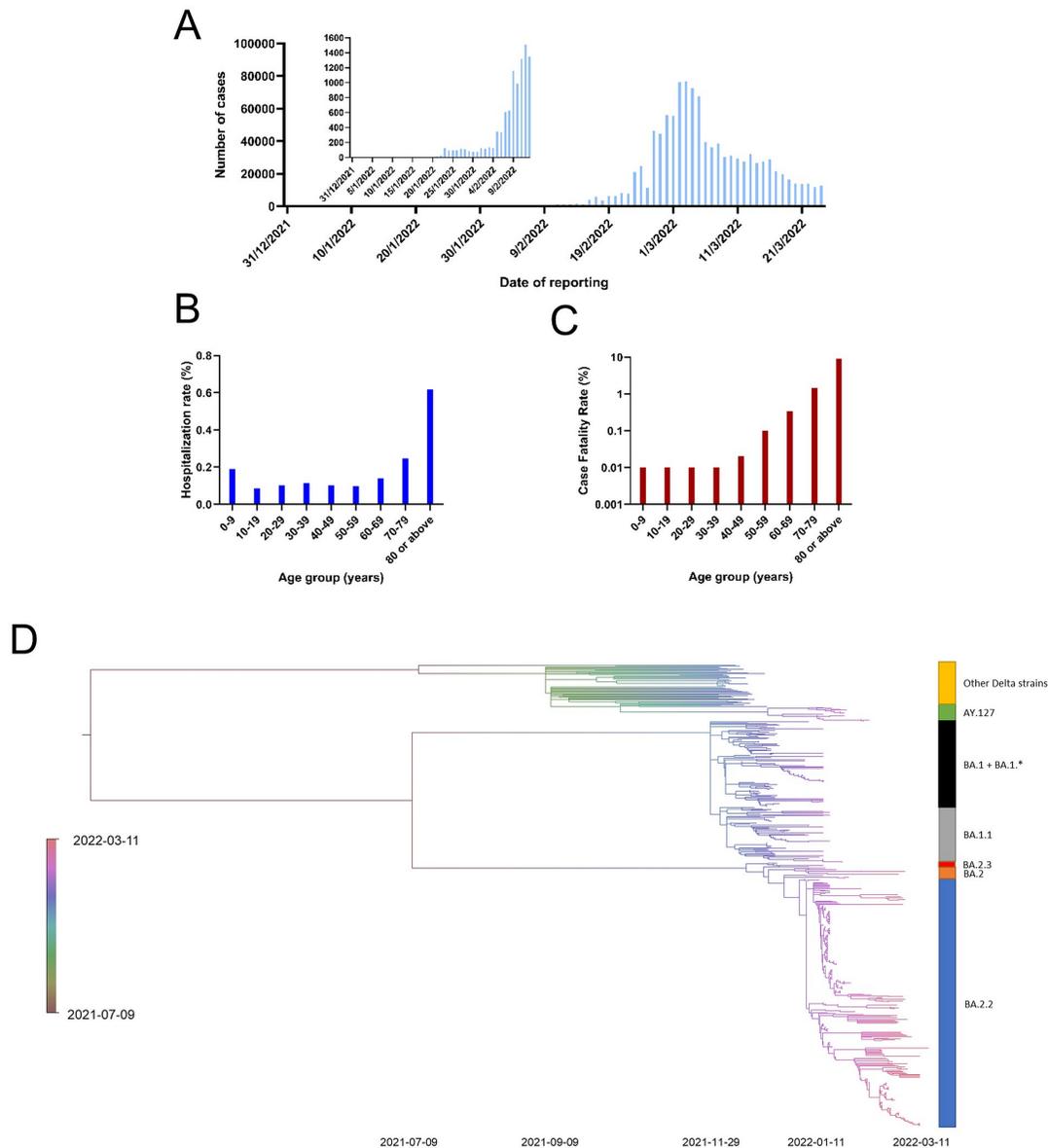
Percentage of individuals receiving BNT162b2 or CoronaVac in Hong Kong in 2021. The data were extracted from the website of the Food and Health Bureau of Hong Kong<sup>33</sup>.



**Figure 3**

**Comparison of surrogate neutralization activity among different age groups.**

(A-F) Surrogate neutralization activity in time periods. The horizontal bar indicates the geometric mean level. Dotted horizontal lines represent the manufacturer's seropositivity cutoff level (15 AU/ml).



**Figure 4**

**Epidemiology and genomic analysis of the fifth wave of COVID-19 in Hong Kong.**

(A) Epidemic curve showing the incidence of COVID-19 cases between December 31, 2021 and March 24, 2022.

(B) Age specific incidence rate of COVID-19 related hospitalization in Hong Kong as of February 24, 2022.

(C) Age specific case-fatality rate of COVID-19 as of March 24, 2022 <sup>45</sup>.

(D) Time-resolved phylogenetic tree of 545 viral genomes from Dec 1, 2021 to March 11 2022. BA.1.\* includes BA.1.1, BA.1.1.1, BA.1.1.10, BA.1.1.14, BA.1.1.16, BA.1.1.2, BA.1.14, BA.1.15, BA.1.15.1, BA.1.17. Details of the sequences used are shown in Supplementary Tables S2.

**Supplementary Files**

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